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STUDIES ON
THE PHOTOSENSITIVE POLYMERS CONTAINING PYRIMIDINE BASES

(ピリミジン塩基を有する感光性高分子に関する研究)

YASUO SUDA

PREFACE

The work of this dissertation was done since 1978 under the guidance by Professor Kiichi Takemoto and many other members of Prof. Takemoto Laboratory at Department of Petroleum Chemistry, Faculty of Engineering, Osaka University.

The content of this dissertation is composed of the following papers:

(1) Photochemical Reactions on Oligo- and Polyethyleneimines Which Contain Pendant Thymine Bases

Y.Inaki, Y.Suda, Y.Kita, and K.Takemoto

J. Polymer Sci. Polymer Chem. Ed., 19, 2519 (1981).

(2) Photodimerization of Pendant Thymine Bases in Thymine Containing Poly-Lysine Derivatives

Y.Suda, Y.Inaki, and K.Takemoto

J. Polymer Sci. Polymer Chem. Ed., 21, 2813 (1983).

(3) Photoinduced Conformational Change of Thymine Containing Poly-Lysine Derivatives

Y.Suda, Y.Inaki, and K.Takemoto

J. Polymer Sci. polymer Chem. Ed., 22, (1984) in press.

(4) Photoreaction of Poly(amino acid)s Containing Thymine Moieties as Side Groups

Y.Suda, Y.Inaki, and K.Takemoto

Nucleic Acid Research Suppl., 12, 169 (1983).

(5) Photodimerization Reaction on Oligo- and Polyethyleneimines
    Containing Pendant Thymine Bases : Effect of Spacer
        Y.Suda, M.Kono, Y.Inaki, and K.Takemoto
            J. Polymer Sci. Polymer Chem. Ed., in contribution.
(6) Synthesis and Photochemical Reaction of Polymethacrylate
    Derivatives Containing 6-Cyanouracil
        Y.Suda, S.Fukunaga, Y.Inaki, and K.Takemoto
            J. Polymer Sci. Polymer Chem. Ed., in contribution.
(7) Photodimerization of Pendant Thymine Bases in Thymine Contain-
    ing Isopoly-L-Lysine Derivative
        Y.Suda, Y.Inaki, and K.Takemoto
            Polymer J., in contribution.

CONTENTS

GENERAL INTRODUCTION

In recent years, studies on the functional polymers have recei-
ved much attention, and they have been becoming one of the most im-
portant field in chemical industry.   The functional polymers cover
many attractive ones, such as polymer catalysts, semiconductive poly-
mers, photosensitive polymers, polymeric drugs, biomedical polymers
and so on, in which a variety of functional groups are incorpolated.

So far as seen in the field of photosensitive polymers, many
studies seem to have been done, and particularly, the photorespon-
sible polymer[1], the photocoductive polymer[2] and so on have been
exceedingly developed.   It is known that the most popular photo-
sensitive material is a photoresist as the image-forming material
by use of photocrosslinking or photodisintegration reaction[3].
Especially for the nega-type photoresist using the photocrosslinking
reaction, the polyvinylcinnamate or bisazide-rubber system is useful.

On the other hand, the studies on photochemistry and photobio-
logy of nucleic acid have been developed extensively.   Exposure
of nucleic acids to UV irradiation in vivo and in vitro lead to the
formation of photodimers with cyclobutane ring of thymine or other
pyrimidine bases as shown below[4].   This fact suggests that the

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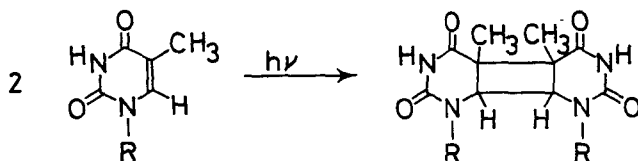
pyrimidine base can serve as the useful photosensitive group and that it is possible to apply the pyrimidine-containing polymer for the new type of photosensitive polymer.   Moreover, after the photo-dimerization, some new properties of the polymer may be realized, and in this sence a new functional polymer may be developed.

The object of my study is to develop the new functional polymers by use of such the photodimerization reaction of pyrimidine bases. The present study concerns with both the chemistry and the develop-ment on a series of subjects, in order to clarify the relationship of the properties of polymer in paticular, molecular weight, mole-cular structure, and the conformation with the reactivity of the photodimerization of thymine or other pyrimidine bases present in the side chain of the synthetic polymer, the following discussions are done from chapters I to VI.

Chapters I and II deal with photochemical reactions on oligo-and polyethyleneimines which contain pendant thymine bases. Effects of the molecular weight, the electron density of thymine bases, and the structure of the side chain of the compounds on the photodimerization reaction are discussed.

Chapters III, IV, and V deal with photoreaction of poly(amino acid)s containing thymine moieties as side groups.   The effect of the conformation of the polymer main chain on the photodimerization is discussed, and the photoinduced conformational change of thymine containing poly-lysine derivatives is described.

Chapter VI deals with synthesis and photochemical reaction of

polymethacrylate derivatives containing 6-cyanouracil derivatives.
The reactivity for the photodimerization of 6-cyanouracil and the
evaluation of pyrimidine-containing polymers for the photoresist
are discussed.

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    ducting Polymeric Materials ", H.Sasabe, Ed., CMC Co. Ltd.,
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Chapter I. Photochemical Reactions on Oligo- and Polyethyleneimines

Which Contain Pendant Thymine Bases

I-1. Introduction

There has been extensive discussion on the intermolecular and
intramolecular interactions on a series of both natural and synthetic
polynucleotide[1]. Concerning the subjects, Overberger and Inaki
prepared polyethyleneimine derivatives containing grafted nucleic
acid base moieties, and made a comprehensive study on their specific
interactions[2,3]. In a series of our studies about intermolecular
and intramolecular interactions of the nucleic acid analogs, the
interactions in the ground state have been discussed thouroughly in
terms of hypochromicity[4]. For the estimation of the intramolecular
interaction of thymine bases in the synthetic polymers, the intra-
molecular photodimerization study is one of the interesting subjects.
In this concern, the photodimerization was studied on thymine bases
present in the side chains of the synthetic polymers and oligomers.
By that study, following facts have been clarified; that is, the
rate of photodimerization was accelerated in the cases of polymers[5],
the reaction was found to depend on the solvent used[6], and the sequ-
ence of thymine bases in the copolymers[7].

Another factor that may influence the photodimerization of thy-
mine bases in the polymer structure is the degree of polymerization.
This chapter deals with the reactions of the oligo- and polyethylene-
imine derivatives. Because oligoethyleneimine derivatives that

contain pendant thymine bases are soluble in water, the effect of
pH on these systems could be studied for photodimerization reaction.

I-2. Results and Discussion

I-2-1. Photodimerization in aqueous solution

The photodimerization reactions of the polymers and correspond-
ing model compounds (Fig.I-1) were carried out in aqueous solu-
tion.   The concentration of the thymine unit disappearence was
plotted against the irradiation time at pH 9.7 in Fig.I-2, which
shows that the concentration of thymine in a sample (EI-T-1) did
not change with a lapse in time; this suggests that the intermole-
cular photodimerization did not occur under the conditions used here.
Intramolecular photodimerization occurred in the dimeric, oligomeric,
and polymeric moldel compounds and the rates increased as the thymine
units increased in the identical molecules.

From the slope of the line in Fig.I-2 the quantum yield at the
beginning photodimerization reactions of the polymer and the corres-
ponding model compounds was obtained and tabulated in Table I-1.
This table also contains the data obtained at pH 10.3 and 11.3.
The quantum yield of the photodimerization for model compounds inc-
reased as follows: dimeric < oligomeric < polymeric.   The quantum
yield of the polymers tended to increase with increasing thymine
content.   This tendency was in agreement with the self-association
ability of the thymine bases in the polymers obtained by UV and
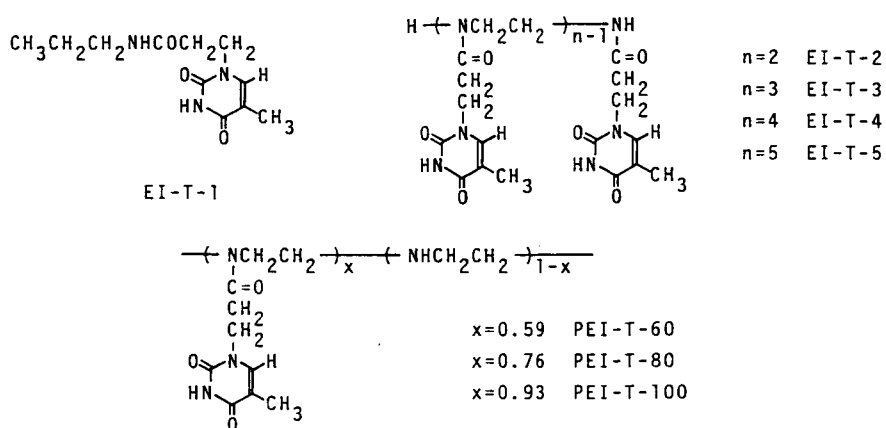NMR spectral studies[8].
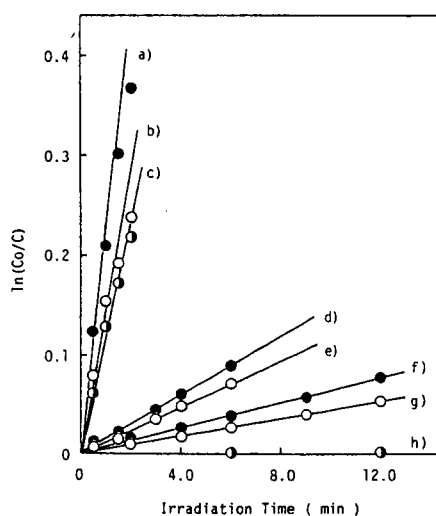
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Fig.I-1. Thymine derivatives.

Fig.I-2. Photolysis of thymine derivatives in aqueous solution at pH
9.7: (a) PEI-T-100; (b) PEI-T-80; (c) PEI-T-60; (d) EI-T-5; (e) EI-T-4;
(f) EI-T-3; (g) EI-T-2; (h) EI-T-1.

The quantum yield of the photodimerization was strongly influenced by the pH of the solution.   In Fig.I-3 the data for EI-T-3 in neutral and alkaline pH regions are shown and the values of the molar extinction coefficients of thymine base are plotted against the pH of the system.   The ε values of the thymine base of EI-T-3 changed dramatically at a pH range near 10.0, which suggests that the thymine bases were deprotonated at the N-3 position.   On the other hand, the quantum yield of the photodimerization of EI-T-3 tended to decrease as the pH of the system increased.   It was found that the tendency of the plots of quantum yield against pH was in agreement with that of ε of thymine base against pH.   From these results it seems that the negatively charged thymine bases formed by deprotonation at the N-3 position in the thymine structure lowers the reactivity of the bases in the photodimerization reaction.   A similar tendency was confirmed among the other oligomers.

The decrease in the reactivity could be the result of electrostatic repulsion between the negatively charged thymine bases or a decrease in the photochemical reactivity of the negatively charged thymine bases themselves[9].   To elucidate this problem the photolysis of EI-T-5 and PEI-T-100 was then studied in a solution of varied ionic strength at pH 12.1 by adding sodium chloride in concentrations of 1×10^{-3} - 1×10^{-1} mol/L.   At this pH it can be assumed that all the thymine bases of EI-T-5 and PEI-T-100 are deprotonated at the N-3 position to produce the anionic form.   If the decrease in quantum yield is caused by electrostatic repulsion between the negatively

Table I-1. Quantum yield for photodimerization of thymine derivatives in aqueous solution[a,b]

Compound	ϕ_{DF}		
	pH 9.7	pH 10.3	pH 11.3
EI-T-2	0.00068	0.00064	0.00056
EI-T-3	0.00088	0.00072	0.00060
EI-T-4	0.0020	0.0012	0.0010
EI-T-5	0.0022	0.0014	0.0010
PEI-T-60	0.017	0.0068	0.0025
PEI-T-80	0.018	0.0084	0.0032
PEI-T-100	0.038	0.015	0.0060

a) Sodium bicarbonate and sodium carbonate buffer system.

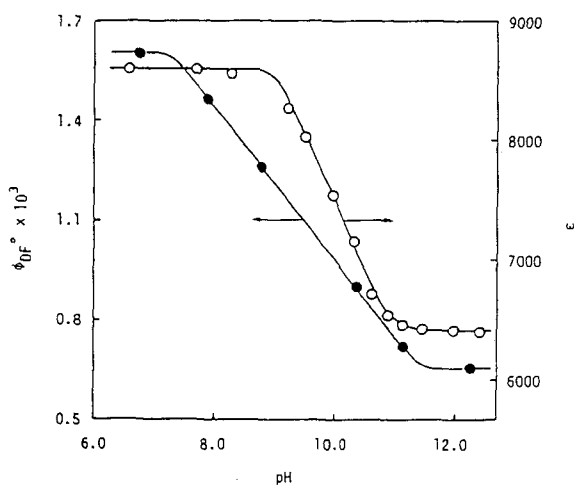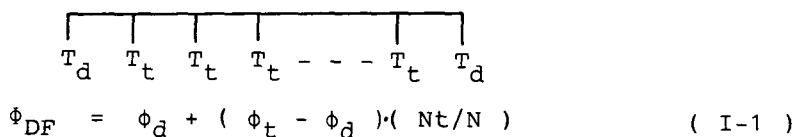b) All data for solution 1×10^{-4} mol/L of thymine unit.

Fig.I-3. Dependence of molar extinction coefficient at 270 nm and quantum yield for the photodimerization of EI-T-3 on pH of the system.

charged thymine bases, the quantum yield of the photodimerization of EI-T-5 and PEI-T-100 would be influenced by the change in ionic strength of the system because the electrostatic repulsion may be removed in a solution of high ionic strength.

Table I-2 shows the dependence of quantum yield of the photo-dimerization on the ionic strength. No remarkable differenc is apparent in the data (Table I-2). It appears that the yield decrease was not caused by electrostatic repulsion between the nega-tively charged thymine bases but by the decrease in the photochemi-cal reactivity of the negatively charged thymine bases.

Quantum yields in Table I-1 were plotted by using eq.(I-1) which was given by assuming Scheme I-1 (Fig.I-4).

<div align="center">(Scheme I-1)</div>

$$T_d \quad T_t \quad T_t \quad T_t \quad - \ - \ - \quad T_t \quad T_d$$

$$\Phi_{DF} \ = \ \phi_d \ + \ (\ \phi_t \ - \ \phi_d \ )\cdot(\ N_t/N\ ) \qquad\qquad (\ I\text{-}1\ )$$

where ϕ_d and ϕ_t are the quantum yields of thymine unit Td, which has a neighboring thymine unit on one side, and that of thymine unit Tt, which has the same units on both sides (Scheme I-1). N and Nt are the content of total and Tt thymine units, respectively.

At pH 11.3, where almost all thymine bases are anionic, the quantum yields of the oligomers resulted in straight line, whereas the data of the copolymers were never fitted on the line. Further study of the photodimerization in the system in which all thymine bases are absolutely neutral, as in organic solvent, is necessary.

Table I-2. Effect of ionic strength on photodimerization of
EI-T-5 and PEI-T-100 in aqueous solution at pH 12.1

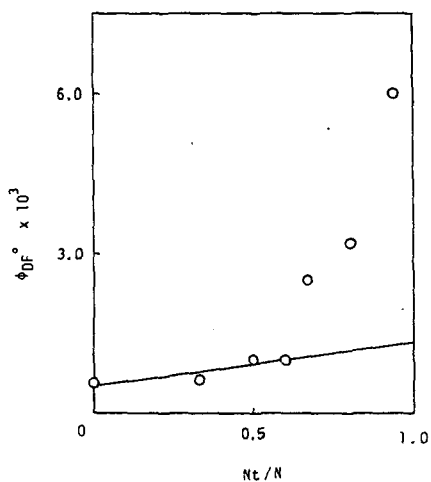| Compound	Conc. of NaCl (mol/L)	ϕ_{DF}
EI-T-5	None	0.00078
	1×10^{-3}	0.00080
	1×10^{-2}	0.00084
	1×10^{-1}	0.00093
PEI-T-100	None	0.0030
	1×10^{-3}	0.0027
	1×10^{-2}	0.0029
	1×10^{-1}	0.0032

Fig.I-4. Relationship between quantum yield of photodimerization in
aqueous solution at pH 11.3 and Nt/N value.

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I-2-2. Photodimerization in DMF Solution

The photodimerization reaction of copolymers and oligomers, with

the exception of EI-T-2 and EI-T-4, which are insoluble in N,N-dime-

thylformamide (DMF), was carried out in DMF solution (1×10^{-4} mol/L).

The thymine base of EI-T-1 did not react; this suggests that the

intermolecular photodimerization does not succeed under the condi-

tions used here.   For oligomer and polymer model compounds, however,

intramolecular photodimerization occurred and the quantum yields of

the photodimer formation beween thymine bases were measured.   The

data obtained are listed in Table I-3.   As shown in this table, the

quantum yield of the photodimerization for copolymers was found to

be greater than those for oligomers.   The values increased with

increasing thymine content in the copolymers and with incresing

molecular weight of the oligomers.   This tendency was in agreement

with the data obtained in aqueous solution.

The data in Table I-3 were plotted with eq.(I-1) to produce

Fig.I-5, which shows clearly two linear relationships.   One is a

straight line with a gentle slope for the oligomers, the other, a

straight line with a sharp slope for the copolymer.   The ϕ_d values

obtained from the intercept and slope were similar for both compounds.

Whereas the ϕ_t values were different.   The ϕ_t value for the copolymer

was about three times greater than that for the oligomers; this may

suggest that the singlet energy of thymine bases in the copolymers

migrates to the other thymine bases in the ground state and that the

migrated energy is effectively consumed for the photodimerization

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Table I-3. Quantum yield for photodimerization of thymine
derivatives in DMF solution[a]

Compound	ϕ_{DF}^{o}
EI-T-3	0.0068
EI-T-5	0.0089
PEI-T-60	0.022
PEI-T-80	0.025
PEI-T-100	0.029

a) All data for solution 1×10^{-4} mol/L of thymine unit.
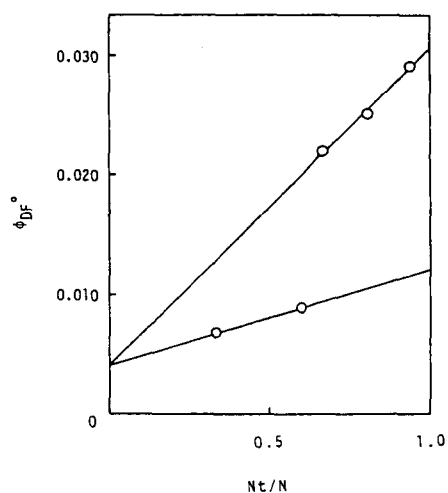

Fig.I-5. Relationship between quantum yield of photodimerization in DMF
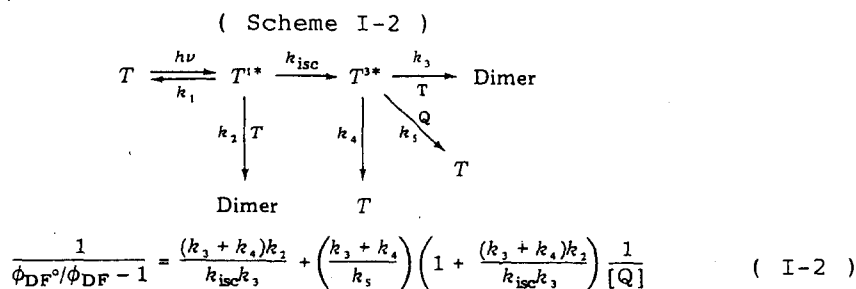solution and Nt/N value.

of the thymine bases in the copolymers. Migration of the singlet

energy in DNA should be recalled in this connection.

I-2-3. Quenching of the Photodimerization in DMF Solution

    The photodimerization reaction of the thymine derivatives in

DMF solution was studied next in the presence of isoprene as the

triplet quencher. The data obtained were plotted by using the usual

Stern-Volmer treatment (i.e., (Φ_{DF}^0/Φ_{DF}) versus [Q], where ϕ_{DF}^0 re-

presents the quantum yield at zero isoprene concentration). The

plots are shown in Fig.I-6. The Stern-Volmer plots of polymer and

oligomer models gave no straight lines. In particular, only a small

amount of quenching of the photodimerization was observed in PEI-T-100.

    For the photodimerization of these model compounds it can be

assumed that the excited singlet and triplet state were involve

(Scheme I-2):

(Scheme I-2)

$$T \underset{k_1}{\overset{h\nu}{\rightleftarrows}} T^{1*} \overset{k_{isc}}{\longrightarrow} T^{3*} \overset{k_3}{\underset{T}{\longrightarrow}} \text{Dimer}$$

$$\left. k_2 \right| T \qquad k_4 \left| \begin{array}{c} k_5 \\ \end{array} \right. Q$$

$$\text{Dimer} \qquad T \qquad T$$

$$\frac{1}{\phi_{DF}^\circ/\phi_{DF} - 1} = \frac{(k_3 + k_4)k_2}{k_{isc}k_3} + \left(\frac{k_3 + k_4}{k_5}\right)\left(1 + \frac{(k_3 + k_4)k_2}{k_{isc}k_3}\right)\frac{1}{[Q]} \qquad (\text{ I-2 })$$

    Equation (I-2), derived in an earlier article[5] was based on

Scheme I-2. The data for the polymers and corresponding model

compounds were plotted with eq.(I-2), which gave straight lines

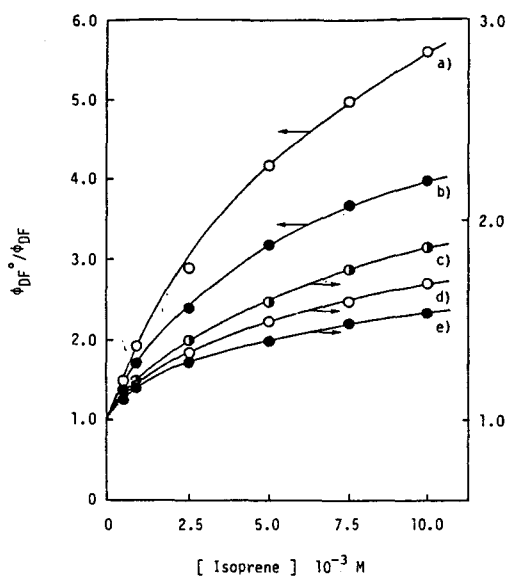(Fig.I-7). The values at 1/[isoprene] = 0 were obtained from

Fig.I-6. Stern-Volmer plots in DMF solution: (a) EI-T-3; (b) EI-T-5;
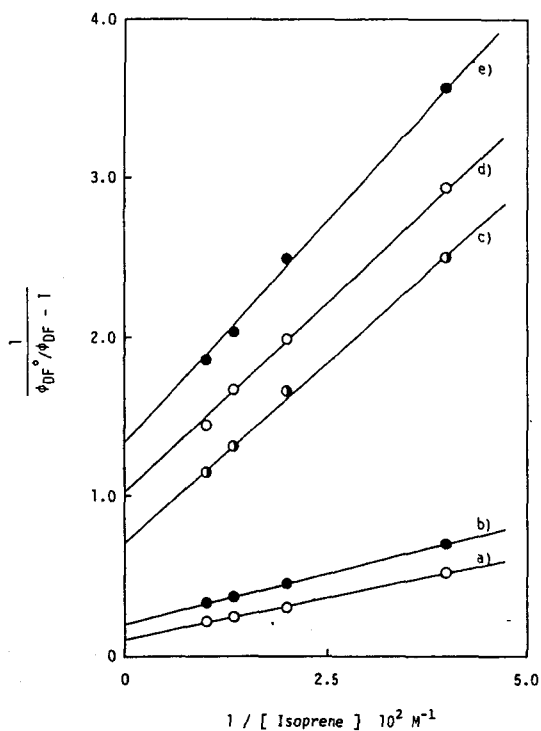(c) PEI-T-60; (d) PEI-T-80; (e) PEI-T-100.

Fig.I-7. Relationship between $1/(\phi_{DF}^0/\phi_{DF}-1)$ and $1/[\text{isoprene}]$ in DMF
solution: (a) EI-T-3; (b) EI-T-5; (c) PEI-T-60; (d) PEI-T-80; (e) PEI-T-100.

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the intercepts of the lines shown in Fig.I-7. The quantum yield

for the photodimerization from the singlet state was calculated by

using the values obtained above and listed in Table I-4. The photo-

dimerization from the singlet state was a minor reaction of EI-T-3

and EI-T-5, but most of the quantum yield for the photodimerization

of PEI-T-100 was derived from the singlet state.

Equation (I-3) is derived by assuming Scheme I-1 :

$$S = S_d + (S_t - S_d)\cdot(Nt/N)$$ (I-3)

where S, Sd, and St are the percentages of the singlet-derived photo-

dimerization in the oligomer, Td, Tt in Scheme I-1, respectively.

The data in Table I-4 were then plotted by eq.(I-3) to give Fig.I-8.

As in Fig.I-7, two different straight lines are shown for the oligo-

mers and copolymers. The S value of EI-T-5 was small in relation to

the S values for the copolymers, but the Nt/N values were comparable,

which suggests that the singlet energy of the thymine base in the

copolymer migrates intramolecularly to another thymine bases in the

ground state and the migrated singlet energy is effectively used for

the photodimerization reaction in the copolymer. Migration of the

singlet energy of the thymine bases in DNA is known[10].

The Sd value of the oligomers was zero, but the Sd value of the

copolymer obtained from Fig.I-8 was not. A similar results was

obtained for polyacrylate and polymethacrylate with different thymine

units[7]. The St value of the copolymer was about twice that of the

oligomers. These facts also suggest singlet energy migration in

Table I-4. Photodimerization of thymine derivatives in DMF solution

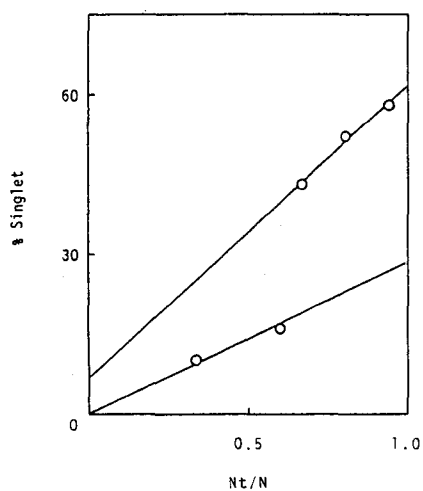| Compound	Singlet (%)	Triplet (%)
EI-T-3	10	90
EI-T-5	16	84
PEI-T-60	43	57
PEI-T-80	52	48
PEI-T-100	58	42

Fig.I-8. Relationship between S value and Nt/N value.

the copolymers. Although singlet energy migration was not observed

in the oligomer system, the migration apparently occurs in polymers

over a certain molecular weight. It is of interest to note how the

intramolecular singlet energy migration in polymers depends on the

molecular weight and to determine the weight in where the singlet

energy can migrate in the polymer intramolecularly.

It was reported that the hypochromicity, pKa values, and NMR

spectra for oligoer and copolymer systems gave linear relationships

with N_d/N (= 1 - N_t/N) values; this suggests the effect of the

nearest neighboring thymine units[8]. For the photodimerization, how-

ever, the quantum yield and percentage of the singlet-derived photo

dimerization produced two straight lines for both compounds. These

facts also support singlet energy migration for photodimerization

in the copolymer system.

I-2-4. Photodimerization in PolyMMA Film

By irradiating UV light onto polyMMA film containing oligomers

and copolymers and absorption of thymine bases to decrease rapidly

(Fig.I-9). The quantum yield for photodimer formation of

thymine bases in polyMMA film was measured from the initial rates

and listed in Table I-5. The quantum yield in polyMMA film for

PEI-T-100 shows higher value than those for polymethacrylate and

polyacrylate derivatives of thymine which have 100% thymine units

in the polymer[11]. Polyethyleneimine derivatives have more stacke

thymine units, which are favored for the photodimerization, than

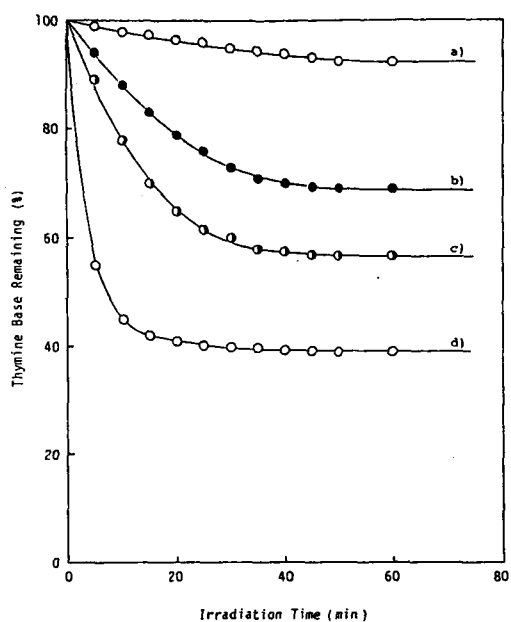Fig.I-9. Photolysis of thymine derivatives in polyMMA film: (a) EI-T-1;
(b) EI-T-3; (c) EI-T-5; (d) PEI-T-100.

Table I-5. Quantum yield for photodimerization of thymine
derivatives in polyMMA film

Compound	ϕ_{DF}	Maximum conversion (%)
EI-T-1	0.013	7.6
EI-T-3	0.062	30.8
EI-T-5	0.13	43.3
PEI-T-100	0.52	61.1

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polyacrylate and polymethacrylate derivatives.   The greater stacked

conformation in the polyethyleneimine derivatives is shown by the

higher hypochromicity values and the higher quantum yields in solu-

tion, compared with those of polyacrylate and polymethacrylate deri-

vatives.

The quantum yield for polyethyleneimine derivatives in polyMMA

film was about 18 times higher than that in solution, but, the maxi-

mum conversion of photodimerization in film was lower than that in

solution.   A possible reason for the high quantum yield and low

maximum conversion in film appears to be that the thymine bases in

stacked conformation, which is favorable for photodimerization, are

fixed in the film below Tg (101-105°C) .

I-3. Experimental

I-3-1. Materials

The thymine derivatives used here are oligoethyleneimine which

contains grafted thymine bases and polyethyleneimine with different

amounts of grafted thymine bases (Fig.I-1).   These compounds were

prepared according to the method reported earlier[8].   All reagents

and solvents were used after purification in the usual manner.

Poly(methyl methacrylate) (polyMMA) films with thymine deriva-

tives were prepared by the thermal polymerization of a mixed solu-

tion of MMA monomer and methanol or dimethyl sulfoxide (DMSO) which

dissolved thymine derivatives with AIBN as initiator[11].

I-3-2. Photolysis

Photochemical reactions in solution were carried out in a 10-mm quartz cell filled with 4 mL of solution at 20°C. A 1-kW superhigh-pressure mercury lamp filtered through a Pyrex filter was used as the light source. The filter showed 0% transmittance below 260 nm and 50% transmittance at 281 nm. Light intensities were determined by potassium ferrioxalate actinometry. The quantum yield for oxalic acid decomposition was 1.24 at 313 nm. The concentration of thymine derivatives was kept constant at 1×10^{-4} mol/L of thymine unit both in aqueous and DMF solution and a stream of purified nitrogen gas was passed through the solution to displace the air. All aqueous solutions were buffered with sodiun bicarbonate and sodium carbonate buffer systems. Quenching studies in DMF solution were performed in the manner mentioned above for photochemical reactions with isoprene (0.1 - 0.001 mol/L) as a triplet quencher.

Photolysis of polyMMA films that contain thymine derivatives was carried out by ultraviolet (UV) light at room temperature in the presence of air at atmospheric pressure. Concentration of the polyMMA film was kept at about 2×10^{-3} mol/L of thymine unit. At given time intervals the concentration of the thymine units remaining in the polyMMA films was determined by analysis at 275 nm, at which neither photodimers nor polyMMA absorb significantly.

I-4. Summary

The photodimerization reaction of oligo- and polyethyleneimine derivatives which contain pendant thymine bases in various amounts was studied in aqueous solution in a wide pH range and in N,N-dimethylformamide solution. The photodimerization reaction of these derivatives in poly(methylmethacrylate) was also studied. It was found that its quantum yield tended to decrease with incerasing anionized thymine in the compound, and tended to increase as thymine units were added to the oligomers and copolymers. The result is discussed in terms of the effect of the nearest neighboring thymine units, and it was assumed that the singlet energy migration affected on the photodimerization, parcularly in the polymer.

I-5. References

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Chapter II. Photochemical Reactions on Oligo- and Polyethyleneimines

Containing Pendant Thymine Bases : Effect of the Spacer

II-1. Introduction

In our extensive series of articles on base-base interaction between polymers with pendant nucleic acid bases[1], the photodimerization of thymine bases in the polymer chain has been one of the most important subjects. Studies were done in particular to estimate the intramolecular interaction among the thymine bases along the polymer chain[2-4].

As described in chapter I, the effects of the degree of the polymerization (molecular weight) and pH of thsystem on the photodimerization were investigated by using both oligo- and polyethyleneimine derivatives having grafted thymine moieties[5], in which it was assumed that the singlet energy migration can occur particularly in the case of copolymer.

The present chapter deals with the effect of side chain structure on the photodimerization of the grafted thymine bases, and the influence of the singlet energy migration on the reaction in more detail. The study was made by using modified oligo- and polyethyleneimine derivatives containing thymine and a spacer group. As the spacer group, α- or β-alanine unit was chosen, which was present between thymine base and the main chain in an incorporated form.

II-2. Results and Discussion
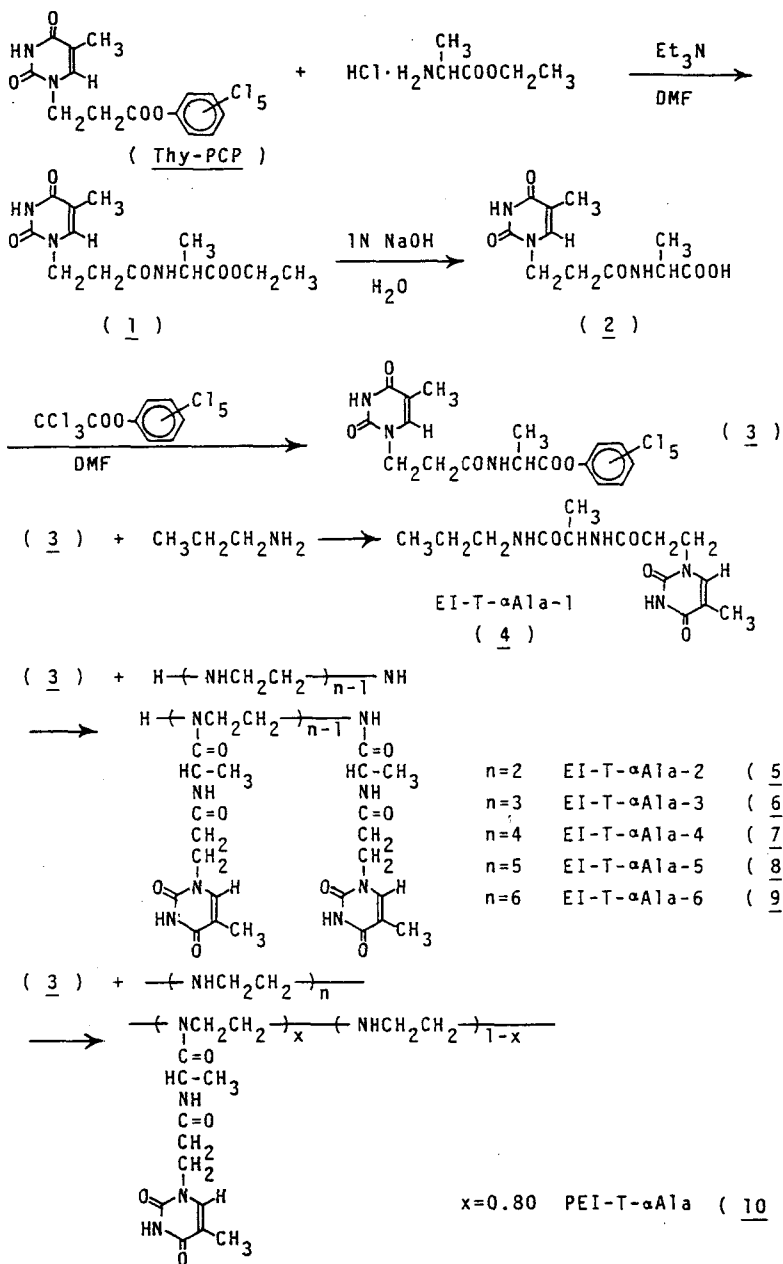
II-2-1. Preparation of α-Alanine Derivatives

The derivatives used here are three kinds of oligo- and poly-ethyleneimines having grafted thymine bases. One kind of compounds were abbreviated as EI-T-n and PEI-T-n, which have no spacer groups between the main chain and the grafted thymine moieties (Fig.II-1). Another kind of compounds were abbreviated as EI-T-βAla-n and PEI-T-βAla, which have β-alanyl unit as a spacer group between the main chain and the grafted thymine bases (Fig.II-2). The synthesis of these compounds was reported earlier[6,7]. The other compounds were abbreviated as EI-T-αAla-n and PEI-T-αAla, which have α-alanyl unit as a spacer group between the main chain and the grafted thymine bases. The α-alanine derivatives were synthesized according to Scheme II-1. Identification of the compounds was made by IR, NMR (with a JNM-PS-100 spectrometer; JEOL), UV spectra, and elemental analysis. The synthetic data are listed in Tables II-1 and II-2.

II-2-2. Intramolecular Interaction in the Ground State

The molar extinction coefficients as well as the hypochromicity values of thymine derivatives having α-alanine unit as a spacer in aqueous and in dimethyl sulfoxide (DMSO) solutions are listed in Table II-3. The hypochromicity values, h, at pH 12.7 and pH 6.0 were plotted against Nt/N values according to eq.(II-1) in Fig.II-3:

$$ h = h_d + (h_t - h_d)(Nt/N) \qquad (\text{II-1}) $$

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(Scheme II-1)

(Thy-PCP)

+ HCl·H₂NCHCOOCH₂CH₃ with CH₃

→ Et₃N / DMF

(1)

1N NaOH / H₂O →

(2)

CCl₃COO-⟨⟩-Cl₅ / DMF →

(3)

(3) + CH₃CH₂CH₂NH₂ → CH₃CH₂CH₂NHCOCHNHCOCH₂CH₂...

EI-T-αAla-1
(4)

(3) + H-(NHCH₂CH₂)ₙ₋₁-NH →

H-(NCH₂CH₂)ₙ₋₁-NH

n=2   EI-T-αAla-2   (5)
n=3   EI-T-αAla-3   (6)
n=4   EI-T-αAla-4   (7)
n=5   EI-T-αAla-5   (8)
n=6   EI-T-αAla-6   (9)

(3) + -(NHCH₂CH₂)ₙ- →

-(NCH₂CH₂)ₓ-(NHCH₂CH₂)₁₋ₓ-

x=0.80   PEI-T-αAla   (10)

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CH$_3$CH$_2$CH$_2$NHCOCH$_2$CH$_2$

EI-T-1

H$-$(NCH$_2$CH$_2$)$_{n-1}$NH

n=2	EI-T-2
n=3	EI-T-3
n=4	EI-T-4
n=5	EI-T-5

$-$(NCH$_2$CH$_2$)$_x$(NHCH$_2$CH$_2$)$_{1-x}$

x=0.59 PEI-T-60
x=0.76 PEI-T-80
x=0.93 PEI-T-100

Fig.II-1. Thymine base derivatives having no spacer group.

CH$_3$CH$_2$CH$_2$NHCOCH$_2$CH$_2$NHCOCH$_2$CH$_2$

EI-T-βAla-1

H$-$(NCH$_2$CH$_2$)$_{n-1}$NH

n=2	EI-T-βAla-2
n=3	EI-T-βAla-3
n=4	EI-T-βAla-4
n=5	EI-T-βAla-5
n=6	EI-T-βAla-6

$-$(NCH$_2$CH$_2$)$_x$(NHCH$_2$CH$_2$)$_{1-x}$

x=0.95 PEI-T-βAla
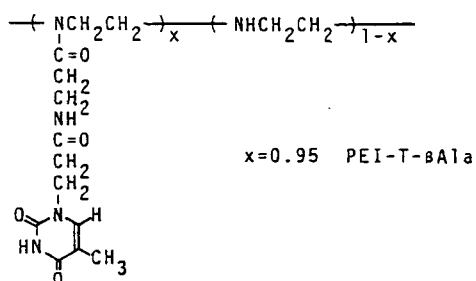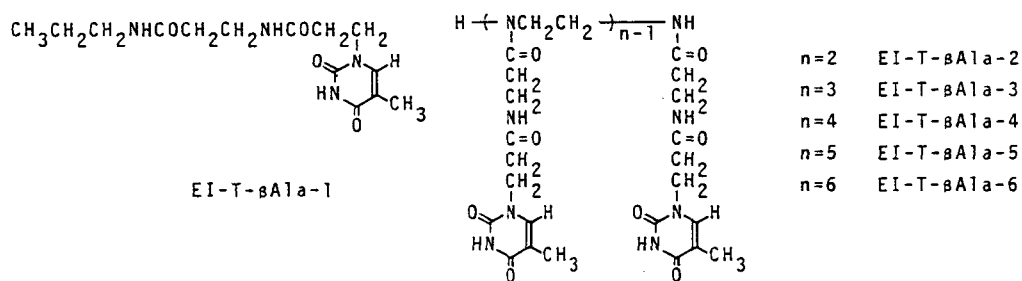
Fig.II-2. Thymine base derivatives having β-alanine unit as a spacer
unit between the main chain and the grafted thymine base.

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Table II-1. Yield, melting points, and infrared spectral data for the compounds
having α-alanine unit as a spacer group .

Compound	Yield (%)	mp '(°C)	Infrared Absorption Band (cm^{-1})
EI-T-αAla-1	48	204.0-206.0	3320, 3000, 2960, 1700, 1620, 1550, 1480, 1250
EI-T-αAla-2	37	288.0-290.0	3300, 1650, 1540, 1450, 1360, 1220
EI-T-αAla-3	53	218.0-220.0	3400, 1660, 1540, 1450, 1360, 1220
EI-T-αAla-4	52	198.0-201.0	3400, 1655, 1530, 1460, 1360, 1220
EI-T-αAla-5	46	201.0-203.0	3400, 1660, 1530, 1470, 1360, 1220
EI-T-αAla-6	46	197.0-200.0	3400, 3000, 1660, 1540, 1460, 1360, 1220
PEI-T-αAla-80	50		3410, 1660, 1540, 1460, 1360, 1220

Table II-2. Analytical data for the compounds having α-alanyl unit as a spacer group

Compound	Calcd. for	Calcd. (%)			Found (%)		
		C	H	N	C	H	N
EI-T-αAla-1	$C_{14} H_{22} O_4 N_4$	54.18	7.14	18.05	53.88	7.03	18.11
EI-T-αAla-2	$C_{24} H_{34} O_8 N_8$	51.24	6.09	19.92	51.45	6.23	19.73
EI-T-αAla-3	$C_{37} H_{52} O_{12} N_{12}$	51.86	6.12	19.62	51.93	6.32	19.35
EI-T-αAla-4	$C_{50} H_{70} O_{16} N_{16}$	52.17	6.13	19.47	52.30	6.16	19.27
EI-T-αAla-5	$C_{63} H_{88} O_{20} N_{20}$	52.35	6.14	19.38	52.46	6.20	19.26
EI-T-αAla-6	$C_{76} H_{106} O_{24} N_{24}$	52.47	6.14	19.32	52.68	6.36	19.14
PEI-T-αAla-80	$C_{10.8} H_{15.4} O_{3.2} N_{3.4}$	53.15	6.36	19.51	52.85	6.68	19.61

Table II-3. Statistical and UV spectral data of the compounds having
α-alanine unit as a spacer group.

Compound	Nt/N [a]	ε at 270 nm			Hypochromicity (%) [b]			pK_a [c]
		pH 12.7	pH 6.0	DMSO	pH 12.7	pH 6.0	DMSO	
EI-T-αAla-1	—	7450	10180	9040	—	—	—	9.66
EI-T-αAla-2	0	7300	9670	8580	2.0	5.0	5.0	9.80
EI-T-αAla-3	0.33	7180	9310	8180	3.7	8.6	9.5	9.88
EI-T-αAla-4	0.50	6920	9150	8090	7.1	10.1	10.5	9.85
EI-T-αAla-5	0.60	6840	8890	8120	8.3	12.7	10.2	10.00
EI-T-αAla-6	0.67	6770	8730	7860	9.2	14.3	13.1	9.99
PEI-T-αAla-80	0.84	6480	8300 [d]	7550	13.1	18.5	16.5	10.21

a) The ratio of triad thymine unit to thymine base content in the compound.
b) Hypochromicity based on the monomeric model compound, EI-T-αAla-1.
c) The pK_a values were obtained from the UV titration curves with Henderson's equation.
d) Obtained in 6N hydrochloric acid solution, because the copolymer are insoluble in neutral water. The ε value of the monomeric model compound is almost identical either in 6N hydrochloric acid solution or in neutral water.
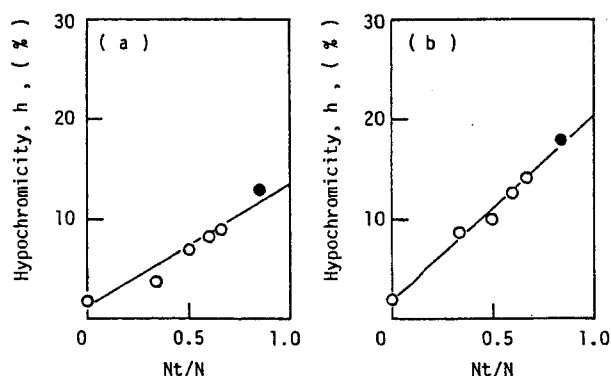

Fig.II-3. Relationships between hypochromicity values (h) of thymine derivatives and Nt/N values in aqueous solution for EI-T-αAla-n and PEI-T-αAla at pH (a) 12.7; (b) 6.0: (O) Oligomers; (●) Copolymers .

where h_d and h_t represent the hypochromicity values of Td and Tt

thymine units in Scheme II-2, respectively.   The linear relation-

(Scheme II-2)

Oligomer

T_d T_t T_t T_d

Copolymer

T_d T_t T_d T_d T_t T_t T_t T_d
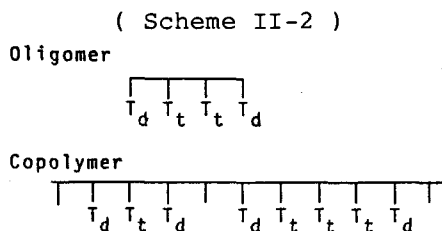
ship between h value and Nt/N values was found to be established

for the oligomers.   It was further recognized that h value of the

copolymer fitted on the line for the oligomers.   For the oligomers

and the copolymer having no spacer group, or β-alanine spacer,

similar relationships were observed[6,7].   From these facts, it was

suggested that the intramolecular interaction of the polymers in the

ground state was due to that between the nearest neighboring thymine

units along the main chain, even in the case of the copolymer.

The slope of the lines was compared with those of EI-T-n (PEI-

T-n) and EI-T-βAla-n (PEI-T-βAla) to give the following decreasing

order: EI-T-βAla-n > EI-T-n > EI-T-αAla-n.   The reason why the

slope of the line for EI-T-βAla was greater than that of EI-T-n can

presumably be explained as follows: The hydrogen bonding between

amide groups in β-alanine units at the side chain restricts a free

rotation of the side chains so as to take a preferable base confor-

mation for the intramolecular interaction; hypochromicity[6].   By

comparing EI-T-n with EI-T-αAla-n, the steric effect of methyl group

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in α-alanyl unit may be responsible for the difference of the slopes. In other wards, in the case of EI-T-αAla-n, methyl group at the side chain restricts the approach of the grafted thymine base to cause a week intramolecular interaction.

The results on the hypochromicity at the neutral pH region of EI-T-αAla-n and PEI-T-αAla have shown not to be similar to those of EI-T-n and EI-T-βAla-n. The hypochromicity values of EI-T-n or EI-T-βAla-n at pH 6.0 gave a straight line which was very similar to that given by the corresponding data at pH 12.7. On the other hand, the data of EI-T-αAla-n at pH 6.0 gave also a straight line which was somewhat different from that obtained at pH 12.7. This seems not to be clearly explained. However, it may be suggested that different kinds of interaction, for example, a hydrophobic interaction between the side chains, is present particularly in the case of EI-T-αAla-n in aqueous solution at neutral pH region. On the contrary, it was reported that there was no stacking interaction in the case of EI-T-n or EI-T-βAla-n[6,7].

The hypochromicity values of EI-T-n, EI-T-βAla-n, and EI-T-αAla-n in DMSO solution were also plotted against Nt/N values (Fig.II-4). As was shown already in the case of aqueous solution, linear relationships were also present in DMSO solution between hypochromicity and Nt/N values for all the thymine oligomers and copolymers. The slope for EI-T-βAla-n was slightly steeper than that for EI-T-n or EI-T-αAla-n. This might be also explained that the hydrogen bonding between amide groups in β-alanyl units at the side chain inhibits
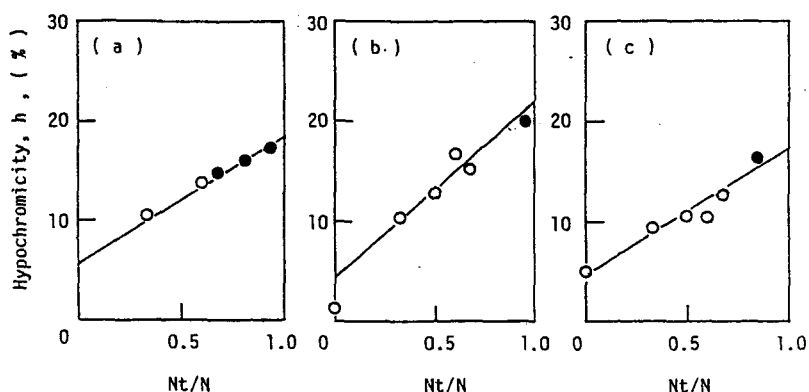
Fig.II-4. Relationships between hypochromicity values (h) of thymine derivatives and Nt/N values in DMSO solution for (a) EI-T-n and PEI-T-n; (b) EI-T-βAla-n and PEI-T-βAla; (c) EI-T-αAla-n and PEI-T-αAla: (O) Oligomers; (●) Copolymers.

Table II-4. Quantum yields for the photodimerization of thymine bases in the compounds[a]

Compound	ϕ_{DF} [b]		
	pH 11.3	pH 10.5	DMSO
EI-T-βAla-2	0.00054	0.00046	0.0051
EI-T-βAla-3	0.00070	0.0013	0.0057
EI-T-βAla-4	0.00087	0.0012	0.0065
EI-T-βAla-5	0.0014	0.0019	0.0067
EI-T-βAla-6	0.0014	0.0022	0.0089
PEI-T-βAla-95	0.0046	0.0089	0.018
EI-T-αAla-2	0.00043	0.00055	0.0033
EI-T-αAla-3	0.00070	0.00086	0.0036
EI-T-αAla-4	0.00075	0.0011	0.0048
EI-T-αAla-5	0.00088	0.0015	0.0058
EI-T-αAla-6	0.00088	0.0017	0.0052
PEI-T-αAla-80	0.0022	0.0035	0.013
EI-T-2	0.00056	0.00064[c]	——[d]
EI-T-3	0.00060	0.00072[c]	0.0068
EI-T-4	0.0010	0.0012[c]	——[d]
EI-T-5	0.0010	0.0014[c]	0.0084
PEI-T-60	0.0025	0.0068[c]	0.016
PEI-T-80	0.0032	0.0084[c]	0.017
PEI-T-100	0.0060	0.015[c]	0.019

a) The data of EI-T and PEI-T in aqueous solution were obtained from ref. (5).
b) All data for solution 1 x 10^{-4} mol/L of thymine unit.
c) pH 10.3
d) insoluble in DMSO.

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a free ratation of it even in DMSO solution, in which thymine bases

are solvated by the DMSO molecules.   However, the difference in the

slopes in Fig.II-4 was somewhat smaller than the case obtained in

aqueous solution.   It appears that the difference in the side chain

could not be discussed in terms of the intramolecular interaction

in the ground state.

II-2-3. Photodimerization

   The photochemical reaction of the thymine derivatives was carried

out in DMSO and in aqueous buffer solution.   The UV absorbance of

thymine base in the monomeric model compounds (EI-T-βAla-1 and

EI-T-αAla-1) did not decrease with UV irradiation under the reac-

tion condition used here.   This fact suggests that the intermole-

cular photodimerization or other photoreactions, for example, photo-

hydration reaction, can be neglected.   Therefore, only the intra-

molecular photodimerization is considered at least at the beginning

of the photolysis, in the cases of the oligomers and copolymers.

   The quantum yield for the intramolecular photodimerization of

thymine bases at the beginning of the reaction (Φ_{DF}) is listed in

Table II-4.   For all of the compounds listed in Table II-4, the

values of Φ_{DF} tended to increase as the pH of the system decreased.

These values were found to be larger in DMSO solution than those in

aqueous solution.   The result here obtained suggested that the

negatively charged thymine bases, which were formed by the deproto-

nation at N-3 position in thymine base (pKa ≒ 10), have signifi-

cantly lower quantum yield for the photodimerization than those of
the neutral thymine bases as descrived in chapter I .

The values of quantum yield in DMSO solution were plotted against
Nt/N values with eq.(II-2) in Fig.II-5[5]:

$$\Phi_{DF} \; = \; \phi_d \; + \; (\; \phi_t \; - \; \phi_d \;)(\; Nt/N \;) \hspace{2cm} (\; II-2 \;)$$

where ϕ_d and ϕ_t represent the quantum yields for the photodimeri-
zation of Td and Tt thymine bases, respectively (Scheme II-2).
This figure gave straight lines for the case of oligomers.   However,
it seems that there exists no marked difference in the slope of the
lines for EI-T-n, EI-T-βAla and EI-T-αAla, which may be related to
the observation in hypochromicity as shown in Fig.II-4.   That is,
in the case of the oligomers particularly in DMSO solution, effect
of the structural difference in the side chain on the intramolecu-
lar interaction could not be observed both in the ground and exci-
ted states.

The quantum yields of the copolymers, on the other hands, did
not fit on the lines obtained from the data of the oligomers, and
were always greater than those of oligomers (Fig.II-5).   This
result appears to be distinct from that of hypochromicity, where
the data of the copolymers fitted always on the lines obtained from
the data of the oligomers.   As the reason for such differences, it
may be suggested that the singlet energy of thymine bases migrates
to another bases in the ground state, and that the migrated energy
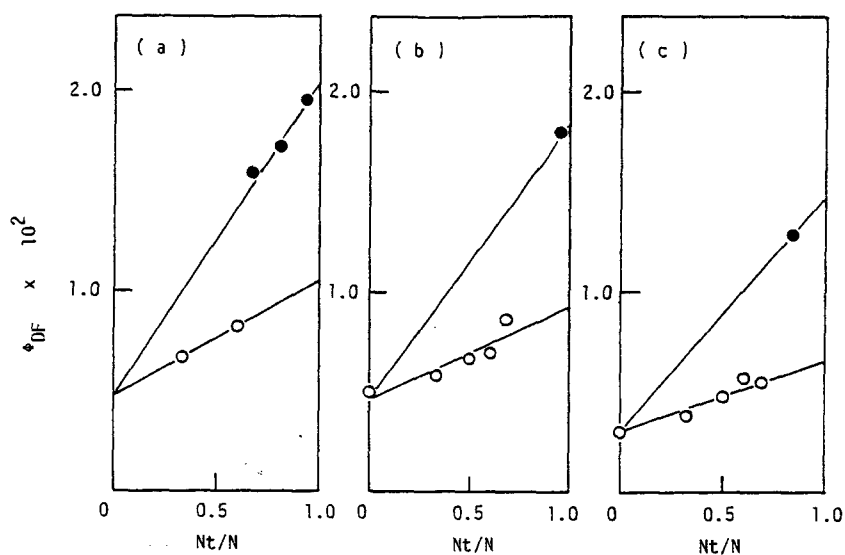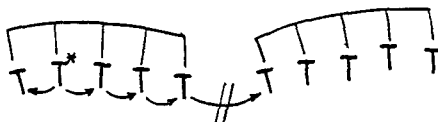is effective enough for causing the photodimerization of thymine

Fig.II-5. Relationships between quantum yields for the photodimerization of thymine bases (ϕ_{DF}) and Nt/N values in DMSO solution for (a) EI-T-n and PEI-T-n; (b) EI-T-βAla-n and PEI-T-βAla; (c) EI-T-αAla-n and PEI-T-αAla: (O) Oligomers; (●) Copolymers.
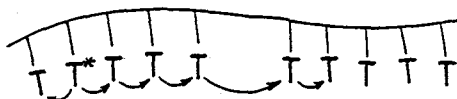

Fig.II-6. Singlet energy migration in the oligomers and copolymers.

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bases.   This was also assumed in chapter I.   As shown in Fig.II-6, such singlet energy migration may be possible to occur from one sequence to another sequence within a copolymer intramolecularly. On the other hand, it may be hardly possible for the case of the oligomers, because the concentration of thymine units is substantially low (1×10^{-4} mol/L) and the migration should occur intermolecularly.

As shown in Fig.II-7, two kinds of singlet energy migration can be considered for copolymers.   One is a short-range energy migration along the polymer chain (**b,c**) and the other is a long-range energy migration between distant units (**a**).   In DMSO solution, copolymers may exist in random (unextended) coil conformation, where the energy migrates effectively by both migrations.   Therefore, the quantum yield for the photodimerization of thymine bases in the copolymers, Φ_{DF}, can be given by eq.(II-3) from eq.(II-2):

$$\Phi_{DF} = f_d \phi_d + (f_t \phi_t - f_d \phi_d)(Nt/N) \qquad (\text{II-3})$$

where f_d and f_t represent the effects of both the short-range and long-range energy migrations.   With eq.(II-3), the data of PEI-T-n were plotted to give a straight line (Fig.II-5a).   The intercept of the line, $f_d \phi_d$, was found to be the same value of that obtained from the data for the oligomers (EI-T-n).   This may suggest that the value of f_d is unity.   Therefore eq.(II-3) can be rewritten as eq.(II-4):

$$\Phi_{DF} = \phi_d + (f_t \phi_t - \phi_d)(Nt/N) \qquad (\text{II-4})$$
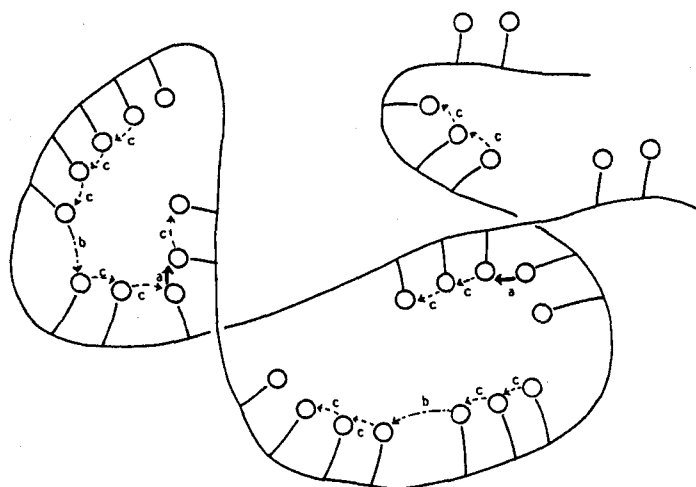
Fig.II-7. Singlet energy migration in a polymer: (a) a long range energy migration between remote units; (b) a short range energy migration along the polymer chain; (c) a energy migration in a sequence.
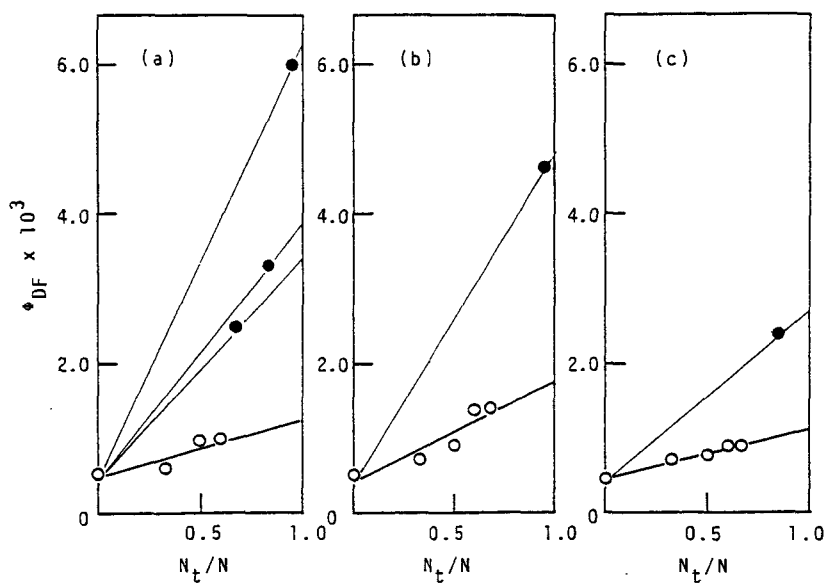

Fig.II-8. Relationships between quantum yields for the photodimerization of tymine bases (Φ_{DF}) and Nt/N values in aqueous solution at pH 11.3 for (a) EI-T-n and PEI-T-n; (b) EI-T-βAla-n and PEI-T-βAla; (c) EI-T-αAla-n and PEI-T-αAla: (O) Oligomers; (●) Copolymers.

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This equation was also derived from the data of PEI-T-n in DMF solu-
tion (Chapter I).   By using eq.(II-4), the data of PEI-T-βAla
and PEI-T-αAla were plotted as in Fig.II-5b and II-5c.   The diffe
rence of the slopes of the lines obtained from Fig.II-5 was essen-
tially small, but was in the decreasing order: PEI-T-n > PEI-T-βAla
> PEI-T-αAla.

The values of quantum yield in aqueous solution at pH 11.3 were
plotted against Nt/N values with eq.(II-2) in Fig.II-8.   In the
case of the oligomers, EI-T-βAla-n and EI-T-αAla-n, the values of
the quantum yield at pH 11.3 gave straight lines as shown in EI-T-n
(Chapter I).   The steepness of the slopes was found to be in the
following decreasing order: EI-T-βAla-n > EI-T-n > EI-T-αAla-n,
which was also parallel with the order of hypochromicity at pH 12.7
(Fig.II-3).   It was suggested from the fact that the intramole-
cular photodimerization of thymine bases was influenced by the
interaction of them in the ground state, particularly for the oligo-
meric compounds.

The quantum yields for copolymers did not fit on the lines formed
from the data of oligomers.   This also suggests the effect of the
singlet energy migration between sequences on the photodimerization
particularly in the case of copolymers.   However, the data of PEI
-T-n did not form the straight line against Nt/N (Fig.II-8a).
This fact may suggest that the way of the energy migration in aqueous
solution is not same as that in DMSO or DMF solution.

In aqueous solution, particularly at higher pH values, the co-

polymer has an extended conformation because of the electrostatic
repulsion between the anionic charges on the thymine bases, in which
case only a short-range energy migration along the polymer chain
(b,c in Fig.II-7) is possible and may be related to the sequence
of the chromophore in the copolymers[8].   Therefore eq.(II-4) may
be derived from eq.(II-4)[9]:

$$\Phi_{DF} = \phi_d + (gNt\phi_t - \phi_d)(Nt/N) \qquad (II-5)$$

where gNt represents the effect of the short-range singlet energy
migration of thymine base on the photodimerization.   In the data
of PEI-T-n in aqueous solution Φ_{DF} was plotted against Nt/N value in
Fig.II-8 and the slopes ($gNt\phi_t - \phi_d$) of straight lines were plotted
against the Nt value to give a straight line in Fig.II-9, which
supports the consideration that the short-range singlet energy mig-
ration is important to photodimeration in aqueous solution at higher
pH region.   From the intercept of the straight line at Nt = 1 the
quantum yield, that is $g\phi_t$ value of the homopolymer, which has 100
% content of thymine bases, can be estimated.

   According to eq.(II-4), the slopes of the line in Fig.II-8 of
PEI-T-βAla and PEI-T-αAla were also plotted against Nt value in
Fig.II-9, and the quantum yields of the homopolymers which have 100
% content of thymine bases were estimated.   It is understandable
that there exists a difference between the estimated values (the
intercept at Nt = 1 in Fig.II-9) for PEI-T-n, PEI-T-βAla and PEI-
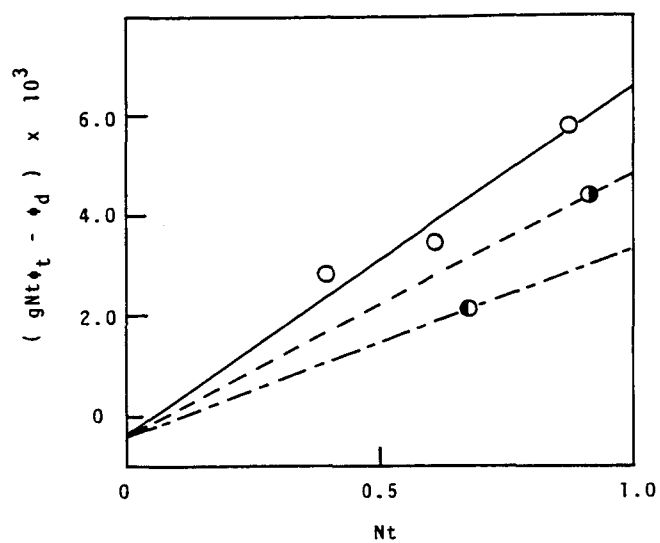T-αAla in the decreasing order: PEI-T-n > PEI-T-βAla > PEI-T-αAla.

Fig.II-9. Relationships between ($gNt\phi_t - \phi_d$) values and Nt values at pH 11.3:
(O) PEI-T-n; (◐) PEI-T-βAla; (◑) PEI-T-αAla.

This order was different from the result of hypochromicity and also from that of the photodimerization for the oligomers. In the case of PEI-T-αAla, the steric effect of methyl group in α-alanyl unit may restrict the singlet energy migration as well as the hypochromicity and photodimerization behavior for the oligomers. On the other hand, in the case of PEI-T-βAla, the reason why the estimated value for PEI-T-βAla was less steeper than that of PEI-T-n remained equivocal. However, the restriction of free rotation of the side chain by the hydrogen bonding of amide group in β-alanyl unit and the longer side chain of PEI-T-βAla may be responsible for causing negative effect for the energy migration.

Since the singlet energy migration is the Förster type one[10], it can be assumed that the structural difference in the side chain of the copolymer might give no drastic effect on the photodimerization behavior of the thymine bases in the copolymer, in which the photodimerization in question occurred mainly from the excited singlet state as mentioned previous chapter[5]. Actually in DMSO solution, the structural effect of the side chain on the photodimerization was not observed. On the other hand, in aqueous solution at higher pH region than pKa of thymine bases, where the singlet energy may migrate along the polymer chain because the main chain of the copolymer is present as an extended coil. Therefore, the effect of the structure of the side chain on the photodimerization can be observed.

II-3. Experimental

II-3-1. Materials

Oligo- and polyethyleneimine derivatives containing β-alanine and thymine moieties

The thymine derivatives of oligo- and polyethyleneimines containing β-alanine unit as a spacer group (EI-T-βAla-n and PEI-T-βAla) were prepared according to the method previously reported[6].

Ethyl-N-[3-(2,4-dihydroxy-5-methylpyrimidin-1-yl)propionyl]-2-amino propionate (1)

To a solution of 22.4g of pentachlorophenyl-3-(2,4-dihydroxy-pyrimidin-1-yl)propionate (Thy-PCP) (50mmol)[7] in 120mL of N,N-dimethylformamide (DMF) were added ethyl-2-aminopropionate hydrochloride (8.45g; 55mmol), which was prepared by the reaction of L-α-alanine with thioylchloride in ethanol solution according to the method of Brenner and Huber[11], and triethylamine (7.0mL; 50mmol). The reaction mixture was stirred for 20hr at room temperature. After the reaction, the salt precipitated, that is, triethylamine hydrochloride, was removed by filtration. The solvent was then distilled off under vacuum, and the residue was washed thoroughly with diethyl ether. Recrystallization from acetone gave colorless needle (1); yield 11.6g (78.1 %); mp 182-184°C; NMR (in DMSO-d_6 at 23°C): δ 1.18 (d, 3H), 1.34 (t, 3H), 4.04 (t, 2H), 4.20 (q, 2H), 4.56 (q, 1H), 7.23 (s, 1H), 7.50 (d, 1H), 10.35 (s, 1H); IR (KBr) 3360, 3180, 1750, 1660, 1540, 1480, 1370, and 1220 cm^{-1}. Anal. Calcd. for $C_{13}H_{19}O_5N_3$; C 52.51, H 6.44, N 14.13; Found C 52.57

H 6.50, N 14.06.

N-[3-(thymin-1-yl)propionyl]-L-α-alanine (2)

The compound 1 (4.5g, 15mmol) was dissolved in 30mL of 1N
aqueous sodium hydroxide solution, and stirred for several hours at
0°C. After the reaction, pH value of the solution was adjusted
to 3 with 1N aqueous hydrochloric acid. The solvent was evaporated
under reduced pressure, and excess amount of ethyl acetate was added
to the oily residue to give colorless needle as the precipitate.
Recrystallization from water gave colorless product (2); yield
3.6g (88.9 %); mp 234°C; IR (KBr) 3450, 3290, 3080, 1720, 1680,
1650, 1580, 1480, 1390, and 1250 cm^{-1}.
Anal. Calcd. for $C_{11}H_{15}O_5N_3$; C 49.07, H 5.62, N 15.61; Found C 48.90,
H 5.46, N 15.49.

N-[3-(thymin-1-yl)propionyl-L-α-alanyl]-propylamine (EI-T-αAla-1) (4)

To a solution of 2 (0.54g; 2mmol) in 15mL of dried DMF, penta-
chlorophenyl trichloroacetate (0.8g; 2mmol) and triethylamine
(0.15mL; 1.8mmol) were added in the order. The reaction mixture
was stirred for 30 min at room temperature. To the solution, in
which about 2 mmol of pentachlorophenyl-N-[3-(thymin-1-yl)propionyl]-
2-aminopropionate (3) formed was contained, n-propylamine (0.15ml;
1.8mmol) was then added, and further stirred for 1 day. After
the reaction, the solvent was distilled off under vaccum, and the
residue was washed with acetone thoroughly and filtered. Recry-
stallization from ethanol gave the product 4 (Table I and II).

N,N'-Bis[3-(thymin-1-yl)propionyl-L-α-alanyl]ethylenediamine (EI-T-αAla-2) (5)

The compound 5 was prepared in a similar way to the case of 4 by the reaction of 3 (2mmol) with ethylenediamine (0.54mL; 0.8 mmol). Reprecipitation from acetone-ethanol was repeated to give the product 5 (Table II-1 and II-2).

EI-T-αAla-3 (6), EI-T-αAla-4 (7), EI-T-αAla-5 (8), and EI-T-αAla-6 (9)

These compounds were prepared in the same way to the case of the compound 5 by the reaction with the corresponding oligomeric amines. Reprecipitation from ethanol-water-acetone was repeated to give the corresponding products (Table II-1 and II-2).

Poly-N-[3-(thymin-1-yl)propionyl-L-α-alanyl]ethyleneimine (PEI-T-αAla) (10)

Linear polyethyleneimine[12] (0.22g; 5.2mmol) was dissolved in 55mL of DMSO and dried under reduced pressure. To the solution, 30mL of DMSO solution of 3 (5.2mmol) was added and stirred for 1 day at 40°C. After the solvent was distilled off, the residue was washed with ethanol and filtered. Reprecipitation from DMSO-acetone was repeated to give the copolymer 10 (Table II-1 and II-2). The thymine base content in the copolymer was determined by measuring UV absorbance of the product after hydrolysis, which was carried out in 6N hydrochloric acid at 105°C for 24 hr. The quantitative calculation was made by using carboxyethylthymine as the standard sample. In this case, the content of thymine bases amounted to 80 unit mol%.

II-3-2. Photochemical Reaction

Photochemical reactions were carried out by using 10mm pathlength quartz cell filled with 4mL of solution at 20°C. A Philips SP lamp (500-w super high pressure mercury lamp) filtered through a combination of glass filters ($\lambda > 280$ nm) was used as the light source. Light intensities were determined by potassium ferrioxalate actinometry. The quantum yield for oxalic acid was 1.24 at 313 nm. The concentration of thymine derivatives was kept constant at 1 x 10^{-4} mol/L of thymine unit in aqueous or DMSO solution in order to avoid for occurring intermolecular reactions. A stream of purified nitrogen gas was passed through the solution for more than 15mL to displace air present before the reaction. All of aqueous solutions were buffered using 0.2mol/L of sodium bicarbonate and sodium carbonate buffer system. The rate of photodimerization of thymine bases was determined by measuring a decrease in UV absorbance of thymine bases at 270 nm by irradiating UV light by using UNION-SM-401 spectrometer at 25°C.

II-4. Summary

The photodimerization reaction of thymine bases incorporated in the modified oligo- and polyethyleneimines, in which α-alanine or β-alanine unit was inserted between the main chain and the grafted thymine bases, was studied both in DMSO and in aqueous solutions. It was found that the quantum yields for the photodimerization for the oligomers were closely related to the intramolecular interaction

in the ground state, while those of the copolymer were not related to the ground state interaction.   From the fact, it was concluded that the singlet energy migration affects on the photodimerization behavior of thymine bases in the polymer of certain degree of polymerization.   The structure of the side chain also gave some effect on the photodimerization of thymine bases in the copolymer, especially in aqueous solution at higher pH region, where singlet energy of the bases may migrate along the polymer chain.

II-5. References

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Chapter III. Photodimerization of Pendant Thymine Bases in Thymine

Containing Poly-Lysine Derivatives

III-1. Introduction

In chapters I and II, the effect of the degree of polymerization
on photodimerization was studied for the oligomers and copolymer
of ethyleneimine derivatives[1,2]. And it was suggested that the
singlet energy migration affected the photodimerization of thymine
bases especially in a polymer which has a certain degree of mole-
cular weight. The effect of pH and the influence of the structure
of the side chain of the compound were also discussed in those
chapters.

The present chapter concerns the effect of the conformation of
synthetic polymers on the photodimerization of their pendant thy-
mine bases. This has been studied for thymine that contained poly-
D-, poly-L-, and poly-DL-lysine derivatives. In a related study
intermolecular interactions were examined in detail for poly-L-lysine
derivatives that contained pendant thymine and adenine bases[3-6].

III-2. Results and Discussion

III-2-1. NMR and UV Spectra

The polymers used here were poly-D-, poly-L-, and poly-DL-lysines
that contained pendant thymine bases. These polymers were prepared
by the grafting reaction of thymine base, Thy-PCP, according to
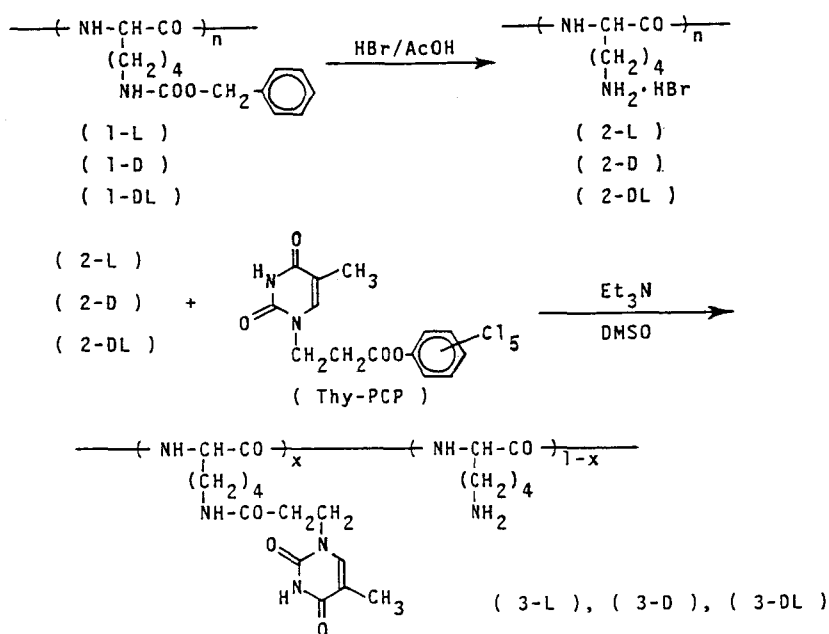Scheme III-1. Based on the absolute configuration of the asymmetric

carbon atoms in lysine residue, these polymers were abbreviated as poly(Thy-D-lys)-x, poly(Thy-L-lys)-x, and poly(Thy-DL-lys)-x, in which x represents the content of thymine bases in the polymers. The degree of polymerization ($\overline{DP}$) of these polymers was determined by viscometry of the corresponding N^{ε}-carbobenzoxy lysine polymers[7]. The content of thymine bases in the polymers was determined by measuring UV absorbances of the products after hydrolysis.   The synthetic data of the polymers are listed in Tables III-1 and III-2.

The oligomer of L-lysine that contained pendant thymine bases, oligo(Thy-L-lys), was obtained from the polymerization of N-carboxy anhydride of lysine in which the content of thymine bases was 100%[8]. The degree of polymerization, however, was low ($\overline{DP}$ = 10).   As the monomeric model compound for the present study, T-M was prepared according to Scheme III-2.
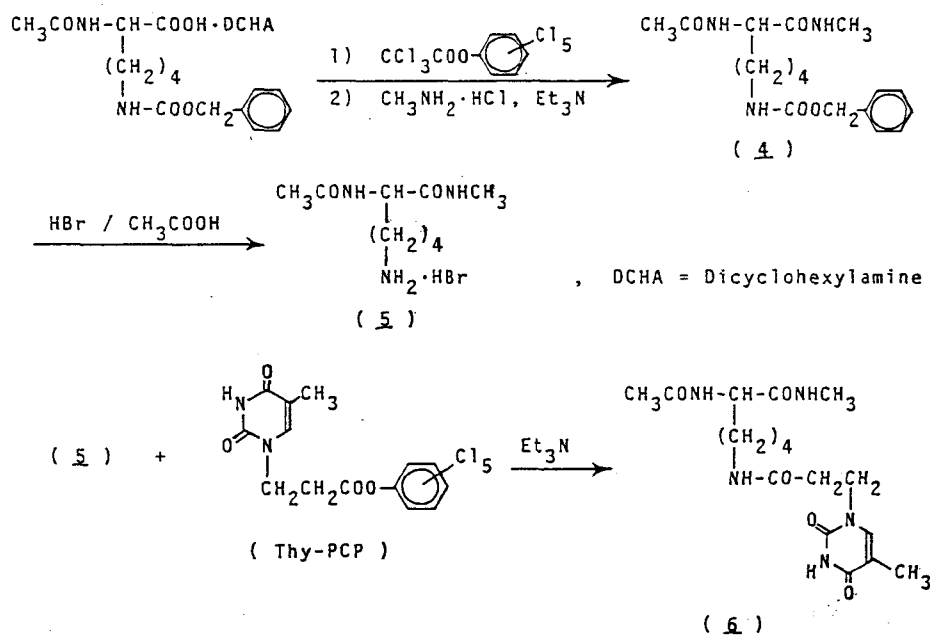
Nuclear magnetic resonance spectra of these compounds were measured in DMSO-d_6 at 90°C (Table III-3).   The NMR data for poly-(Thy-L-lys)s and oligo(Thy-L-lys) have been reported[8,9] and were exactly same as those for poly(Thy-D-lys)s.   It appears that no marked difference in NMR spectra could be detected among the three kinds of polymers, the oligomer, and the monomeric model compound. It was found that the peaks based on β, γ, δ-CH_2 of poly(Thy-DL-lys)s showed a slight shift to the lower magnetic field, compared with poly(Thy-D-lys)s.   The fact suggests that poly (Thy-DL-lys)s are present in an unordered comformation.

Ultraviolet spectra of the polymers and monomeric model compound

(Scheme III-1)

(1-L) (2-L)
(1-D) (2-D)
(1-DL) (2-DL)

$$\text{(2-L), (2-D), (2-DL)} + \text{(Thy-PCP)} \xrightarrow[\text{DMSO}]{\text{Et}_3\text{N}}$$

(3-L), (3-D), (3-DL)

(Scheme III-2)

$$\xrightarrow[\text{2) } CH_3NH_2 \cdot HCl, \ Et_3N]{\text{1) } CCl_3COO-C_6H\cdot Cl_5} \quad (\underline{4})$$

$$\xrightarrow{\text{HBr / CH}_3\text{COOH}} \quad (\underline{5})$$

, DCHA = Dicyclohexylamine

$$(\underline{5}) + \text{(Thy-PCP)} \xrightarrow{\text{Et}_3\text{N}} (\underline{6})$$

were measured in aqueous solution.   The thymine unit concentration

was low (1 x 10^{-4} mol/L) to avoid intermolecular interaction bet-

ween thymine moieties.   The values of λ_{max} for the polymers and

monomeric model compound were almost identical (270 nm in 0.5N

NaOH, 272.5 nm in 6N HCl).

From the spectroscopic titration curves for the polymers and

T-M, pKa values of the thymine base derivatives were determined by

eq.(III-1)[10] (Fig.III-1):

$$pH = pK_a + n \log \frac{\epsilon_A - \epsilon}{\epsilon - \epsilon_{HA}} \qquad\qquad (III-1)$$

where ϵ_A and ϵ_{HA} denote the molar extinction coefficients of anionic

and neutral thymine base, respectively.   The UV spectra for the

polymers could not be measured below pH 10 because of their insolu-

bility in this region, although the polymers were soluble in 6N

aqueous hydrochloric acid.   The ϵ values of the polymers in 6N

aqueous hydrochloric acid were used as their ϵ_{HA} values because the

ϵ value of T-M in 6N of aqueous hydrochloric acid was the same as

that in the neutral pH region.   Ultraviolet spectral data and pKa

values are listed in Table III-4.

The hypochromicity values of the thymine-grafted polymer in

Table III-4 were about 15%.   These values were smaller than those

of the polyethyleneimine derivatives[11].   The hypochromicity may

depend on the thymine base and helix content of the poly-lysine

derivatives.   However, the difference in the hypochromicity values

between the polymers was too small to discuss the dependency of the

Table III-1. Results of polymerization

Compound	NCA Conc. (mol/L)	[M]/[I][a]	[η] (dl/g)	$\overline{DP}$
Poly(N[ε]-carbobenzoxy-L-lysine)	0.25	400	0.63[b]	400
Poly(N[ε]-carbobenzoxy-D-lysine)	0.25	400	0.92[b]	500
Poly(N[ε]-carbobenzoxy-DL-lysine)	0.25	400	0.61[c]	400

[a] Monomer to initiator ratio (mol/mol).
[b] Measured in DMF at 25°C.
[c] Measured in m-cresol at 15°C.

Table III-2. Synthetic data of polymers

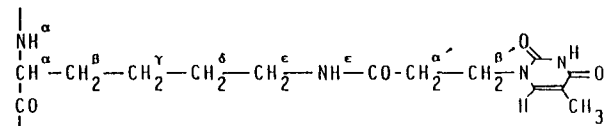| Compound	Ratio[a] (mol/mol)	Base[b] content	N_t/N[c]
Poly(Thy-D-lys)-88	1.30	0.88	0.91
Poly(Thy-D-lys)-72	0.80	0.72	0.78
Poly(Thy-L-lys)-93	1.44	0.93	0.95
Poly(Thy-L-lys)-79	0.80	0.79	0.84
Poly(Thy-DL-lys)-85	1.29	0.85	0.89
Poly(Thy-DL-lys)-79	0.81	0.79	0.84

[a] Molar ratio of the activated ester of thymine base (Thy-PCP) to the corresponding poly(lysine hydrobromide) in the grafting reaction.
[b] From UV spectra of the product after hydrolysis.
[c] The ratio of triad thymine units to thymine units in the polymer; see text.
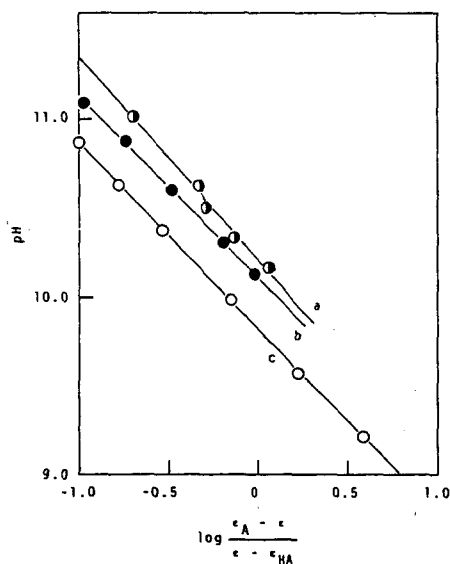
Fig.III-1. Relationship between pH and log($\varepsilon_A - \varepsilon$)/($\varepsilon - \varepsilon_{HA}$) of thymine derivatives: (a) poly(Thy-D-lys)-88; (b) poly(Thy-D-lys)-72; (c) T-M.

Table III-3.   NMR Spectral data of the polymers and monomeric model compound[a]

Compound	α,ϵ-NH	α-CH	β,γ,δ-CH$_2$	ϵ-CH$_2$	α'-CH$_2$	β'-CH$_2$	Thymine Base		
							5-CH$_3$	6-CH	3-NH
T-M[b]	7.60	4.16	1.32	3.02	2.40	3.84	1.72	7.36	10.82
Poly(Thy-D-lys)-88	7.64	4.2(b)	1.42	3.08	2.50	3.88	1.73	7.32	10.57
Poly(Thy-D-lys)-72	7.68	4.2(b)	1.40	3.02	2.50	3.84	1.72	7.32	10.78
Poly(Thy-DL-lys)-85	7.61	4.24	1.34	3.08	2.50	3.88	1.73	7.32	10.57
Poly(Thy-DL-lys)-79	7.61	4.24	1.33	3.08	2.50	3.89	1.73	7.32	10.56

a) δ in ppm from TMS (tetramethylsilane) at 90°C in DMSO-d$_6$.

$$\underset{\text{CO}}{\overset{\overset{\displaystyle\text{NH}^\alpha}{|}}{\underset{|}{\text{CH}^\alpha}}}-\text{CH}_2^\beta-\text{CH}_2^\gamma-\text{CH}_2^\delta-\text{CH}_2^\epsilon-\text{NH}^\epsilon-\text{CO}-\text{CH}_2^{\alpha'}-\text{CH}_2^{\beta'}-N\underset{\text{CH}_3}{...}$$

b) T-M: 1.84ppm (CH$_3$CONH-); 7.50ppm (CH$_3$NHCO-); 2.56ppm (CH$_3$NHCO-).

Table III-4. Ultraviolet spectral data and pKa values in aqueous
solution for polymers and the monomeric model compound

Compound	ϵ at 270 nm		Hypochromicity (%)[a]		pK_a
	0.5N NaOH	6N HCl	0.5N NaOH	6N HCl	
T-M	7350	9760	—	—	9.84
Poly(Thy-D-lys)-88	6240	8010	15.1	17.9	10.18
Poly(Thy-D-lys)-72	6560	8420	10.7	13.7	10.13
Poly(Thy-L-lys)-93	6170	8010	16.1	17.9	10.17
Poly(Thy-L-lys)-79	6530	8080	11.2	17.2	10.15
Poly(Thy-DL-lys)-85	6350	8270	13.6	15.3	10.12
Poly(Thy-DL-lys)-79	6440	8240	12.4	15.6	10.11

[a] Hypochromicity based on T-M.
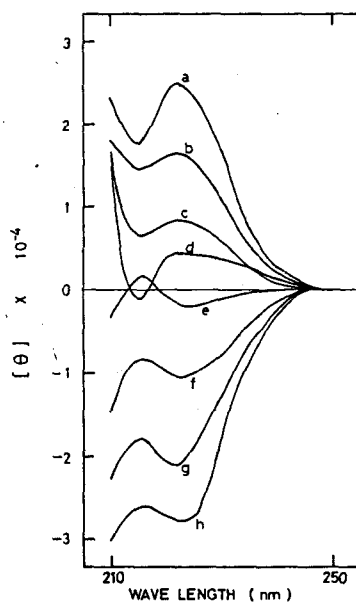
Fig.III-2.   CD Spectra of poly(Thy-D-lys)-88 at pH: (a) 10.18; (b) 10.63; (c) 11.02;
(d) 11.35;   CD spectra of poly(Thy-L-lys)-93 at pH: (e) 11.16; (f) 10.80; (g) 10.61; (h) 10.28.

hypochromicity on the thymine base and helix content.

III-2-2. CD Spectra

Poly(L-lysine) is known to exist in random coiled, helical, and β-sheet conformations, depending on the temperature, pH of the system, and solvent used. The side chain of the polymer has a significant effect on the conformation of the main chain. To determine how the thymine substituents affect the conformation of the polymer their CD spectra were measured in aqueous solution.

The CD spectra of poly(Thy-D-lys)-88 and poly(Thy-L-lys)-93 in aqueous alkaline solution are shown in Fig.III-2. The absolute value of the molar ellipticity at 222 nm ($[\theta]_{222}$) is known to be related to the helix content, that is, helicity of poly(α-amino acid). Fig.III-3 shows the plots of the $[\theta]_{222}$ value against he pH of the system; $[\theta]_{222}$ values for poly(Thy-D-lys)-72 and poly-(Thy-L-lys)-79 are also plotted in this figure. No CD bands were observed in poly(Thy-DL-lys)s. Fig.III-3 shows that poly(Thy-L-lys)s exist as right-handed helical or extended charged coiled structures because the $[\theta]_{222}$ values obtained are negative. On the other hand, poly(Thy-D-lys)s exist as left-handed helical or extended charged coiled structures from their positive values. Because no CD bands were observed in poly(Thy-DL-lys)s and the NMR peaks based on β, γ, δ-CH$_2$ were slightly shifted to the lower magnetic field, it can be assumed that poly(Thy-DL-lys)s exist as unordered structure compared with poly(Thy-D-lys)s and poly(Thy-L-lys)s.
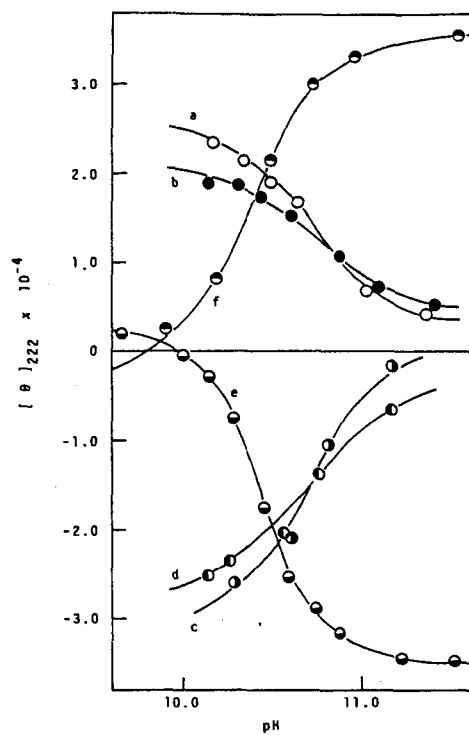

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Fig.III-3.   Dependence of molar ellipticity at 222 nm ($[\theta]_{222}$) on pH: (a) poly-
(Thy-D-lys)-88; (b) poly(Thy-D-lys)-72; (c) poly(Thy-L-lys)-93; (d) poly(Thy-L-lys)-79;
(e) poly(L-lysine); (f) poly(D-lysine).

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From CD spectra of oligo(Thy-L-lys) and poly(Thy-L-lys)-93 at
pH 10.2 in aqueous solution the $[\theta]_{222}$ value of poly(Thy-L-lys)-93
was about -30000, whereas the value of oligo(Thy-L-lys) was about
-1000.   This fact suggests that oligo(Thy-L-lys) is present as a
random coiled structure.   Because the degree of polymerization of
oligo(Thy-L-lys) is 10^8, it can be assumed that a certain degree
of polymerization is needed for the helical conformation.

Poly(L-lysine) exists as an extended coiled structure at pH 10
because ε-amino group is protonated at this pH value.   The absolute
value of $[\theta]_{222}$ for poly(Thy-D-lys)s and poly(Thy-L-lys)s is more
than 20000 at pH 10.   This suggests that poly(Thy-D-lys)s exist as
helical structures in this pH region.   When thymine bases are
grafted onto poly(D-lysine) or poly(L-lysine) the ε-amino groups
protonated are replaced by the neutral thymine base to cause helical
transition from the extended charged coiled structure.   This fact
suggests that some relationship exists between helix and thymine
base content in the polymer.

A CD titration was carried out for poly(L-lysine) to determine
how the helix content relates to the ratio of charged-to-neutral
ε-amino groups in the side chain for the simple case.   At the
neutral pH of the system each ε-amino group of lysine is protonated
and poly(L-lysine) exists in an extended charged coiled conformation.
At higher pH, above about 10, the ε-amino group of lysine becomes
a neutral form and the polymer undergoes a transition from the
charged coil to helical conformation.

For the ε-amino group of poly(L-lysine) the following equilibrium should be formed:

$$-NH_3^+ \rightleftharpoons -NH_2 + H^+ \qquad\qquad (\text{ III-2 })$$

$$pH = pK^1 + n \log \left(\frac{[NH_2]}{[NH_3^+]} \right) \qquad\qquad (\text{ III-3 })$$

$$[NH_2] + [NH_3^+] = 1 \qquad\qquad (\text{ III-4 })$$

where eq.(III-3) is a modified Henderson-Hasselbach equation[12]. For the neutral amino groups the following helix-coil equilibrium [eq. (III-5)] is formed:

$$-NH_2^{coil} \rightleftharpoons NH_2^{helix} \qquad\qquad (\text{ III-5 })$$

$$k^1 = \frac{[NH_2]^{helix}}{[NH_2]^{coil}} \qquad\qquad (\text{ III-6 })$$

$$[NH_2]^{helix} + [NH_2]^{coil} = [NH_2] \qquad\qquad (\text{ III-7 })$$

where k^1 is the equilibrium constant of eq.(III-5) and $[NH_2]^{helix}$ and $[NH_2]^{coil}$ are the concentrations of amino groups in the helical and charged coil conformation, respectively.   If these equilibria are established, the helix content of poly(L-lysine) can be written as eq.(III-8):

$$h = \frac{[NH_2]^{helix}}{([NH_2]^{helix} + [NH_2]^{coil} + [NH_3^+])}$$
$$= \left(\frac{k^1}{1 + k^1} \right) [NH_2] \qquad\qquad (\text{ III-8 })$$

where h represents the helix content.   If the pH is high, the helix

content tends to be unity (100%)[13]. The value of k^1 can then be assumed to be large enough compared with 1; $k^1 >> 1$. In this case eq.(III-8) can be rewritten as eq.(III-9):

$$h \doteqdot [NH_2] \qquad\qquad (III-9)$$

From eqs.(III-3), (III-4) and (III-9) eq.(III-10) can be obtained as follows:

$$pH = pK^1 + n \log \left(\frac{h}{1-h} \right) \qquad\qquad (III-10)$$

Because the helix content h can be calculated from $[\theta]_{222}$[13], the (h/1-h) value is obtained by using eq.(III-11):

$$\left(\frac{h}{1-h} \right) = \frac{([\theta]_{222} - [\theta]_{222}^{NH_3^+})}{([\theta]_{222} - [\theta]_{222}^{NH_2})} \qquad\qquad (III-11)$$

where $[\theta]_{222}^{NH_3^+}$ and $[\theta]_{222}^{NH_2}$, the molar ellipticity of poly(L-lysine) at 222 nm, have a quantitative amount of protonated ε-amino groups and neutral ε-amino groups, respectively. The log(h/1-h) values were plotted against pH to give the straight line in Fig.III-4. The result seems to support eq.(III-10); that is, the helix content is proportional to the content of the neutral amino units in poly-(L-lysine) [eq.(III-9)].

For the copolymers of lysine and its thymine derivatives, the equilibrium in eq.(III-12) can be established in addition to that of eq.(III-2):

$$T \rightleftharpoons T^- + H^+ \qquad\qquad (III-12)$$

$$pH = pK^T + n \log \left(\frac{[T^-]}{[T]} \right) \qquad\qquad (III-13)$$

$$[T] + [T^-] = N \qquad\qquad (\text{ III-14 })$$

$$[NH_3^+] + [\dot{N}H_2] + N = 1 \qquad\qquad (\text{ III-15 })$$

where T and T^- are neutral and anionic thymine units, respectively. For the equilibrium in eq.(III-12) the modified Henderson-Hasselbach equation, that is, eq.(III-13), can be applied. In eq.(III-14) N is the thymine content in the copolymers. For the copolymers the helix-coil equilibrium [eq.(III-16)] can be formed in addition to the equilibrium in eq.(III-5):

$$T^{coil} \rightleftharpoons T^{helix} \qquad\qquad (\text{ III-16 })$$

$$k^T = \frac{[T]^{helix}}{[T]^{coil}} \qquad\qquad (\text{ III-17 })$$

$$[T]^{helix} + [T]^{coil} = [T] \qquad\qquad (\text{ III-18 })$$

where T^{helix} and T^{coil} are the neutral thymine units in helical and coiled conformation, respectively. Also almost the neutral thymine units are assumed to exist in a helical conformation; that is, $k^T \gg 1$ [eq.(III-19)]:

$$[T]^{helix} = \left(\frac{k^T}{1 + k^T}\right)[T] \doteqdot [T] \qquad\qquad (\text{ III-19 })$$

The helix content of the copolymer can then be written as

$$h = [NH_2]^{helix} + [T]^{helix}$$
$$= [NH_2] + [T] = A + (B - A)N \qquad\qquad (\text{ III-20 })$$

$$A = \frac{K^1}{1 + K^1[H^+]} \qquad B = \frac{[H^+]}{K^T + [H^+]} \qquad\qquad (\text{ III-21 })$$
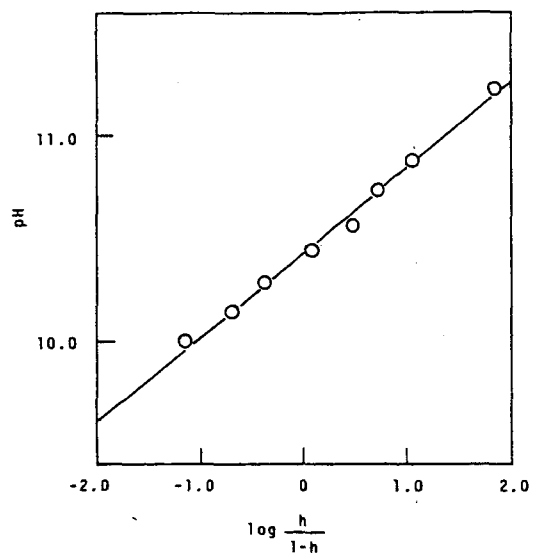
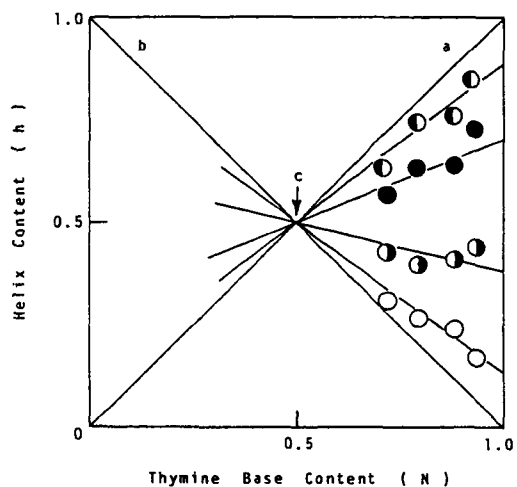Fig.III-4.　Relationship between pH and log(h/1-h) of poly(L-lysine)

Fig.III-5.　Relationship between hlix content (h) and thymine base content (N) of poly-lysine derivatives: pH = 10.4 (●); pH = 10.8 (◑); pH = 11.2 (○); pH = 10.0 (◐).

Equation (III-20) shows a linear relationship between the helix content h and the thymine base content N; A and B are constants at a given pH for the system.

The helix content for the poly-lysine derivatives were plotted against thymine base content by using eq.(III-20) in Fig.III-5. Line a in Fig.III-5 is theoretical at low pH (pH << pK^1, pK^T) and line b is at high pH (pH >> pK^1, pK^T). Fig.III-5 shows that all lines meet at point c, which is also the crosspoint of the lines a and b. By extrapolating the lines to N = 1 the helix content of the polymers that contained 100% thymine bases were estimated at three pH values (Table III-5).

The thymine-base-grafted poly-lysine have two kinds of dissociative group; thymine base and ε-amino group. The acid-base and helix-coil equilibria of both thymine and ε-amino groups make the CD spectra of the polymer complicated. The helix of the copolymers, however, had a linear relationship with the thymine base content at constant pH value [eq.(III-20)], and this equation gave the helix content of 100% thymine base grafted poly-lysine by extrapolation. This estimated value is useful in a discussion of the photodimerization of thymine bases in the polymer.

III-2-4. Photochemical Reactions

Photochemical reactions of the polymers, oligomers, and monomeric model compound were carried out in aqueous solution. The change in thymine unit concentration was plotted against the irradiation

Table III-5. Extrapolated values of poly-lysine derivatives with 100% content ot tymine bases

pH	Helix content[a]	$\Delta\Phi_{DF}$[b]
10.4	0.69	0.0031
10.8	0.39	0.0022
11.2	0.14	0.0008

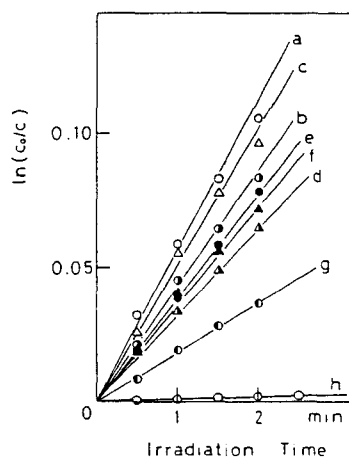a) From Fig.III-5.

b) From Fig.III-8.

Fig.III-6. Photolysis of thymine base derivatives at pH 10.8: (a) poly(Thy-D-lys)-88; (b) poly(Thy-D-lys)-72; (c) poly(Thy-L-lys)-93; (d) poly(Thy-L-lys)-79; (e) poly-(Thy-DL-lys)-85; (f) poly(Thy-DL-lys)-79; (g) oligo(Thy-L-lys); (h) T-M.

time at pH 10.8 (Fig.III-6). Fig.III-6 shows that the absorbance

of thymine units in the monomeric model compound (T-M) decreased

only slightly by UV irradiation, compared with the polymers and

the oligomer. Because the concentration of thymine units was sub-

stantially low (1 x 10^{-4}mol/L), photodimerization apparently does

not occur in T-M. The photochemical reaction in this case may be

a photohydration to thymine base[14], supported by the following facts:

a slight decrease in the absorbance of T-M, shown in Fig.III-6, was

almost recovered by heating at 60-70°C for a few minutes; the quan-

tum yield increased with an increase in the pH of the system. In

contrast, the quantum yields for polymers and oligomers decreased

with an increase in pH (Table III-6).

The rate of disappearence of thymine units was fairly large in

the polymers and the oligomer. The photochemical reaction in these

cases may involve photohydration and photodimerization. From the

slopes of the lines shown in Fig.III-6 quantum yields obtained at

the beginning of the photochemical reaction are tabulated in Table

III-6, which also contains the data obtained at pH 10.4 and 11.2.

On the basis of the results obtained at pH 10.4 the quantum yields

for the polymers and the oligomer were much larger than for the

monomeric model compound. Therefore the photohydration of thymine

bases could be neglected for the former cases. At pH 10.8 and 11.2,

however, the photohydration of thymine bases could not be neglected.

The quantum yield of photodimerization was then estimated by eq.

(III-22):

$$\Phi_{DF} = \Phi_0 - \Phi_0^M \qquad (\text{III-22})$$

Table III-6. Quantum yield of the photoreaction of thymine base (Φ_0) in the model compounds[a)]

Compound	Φ_0		
	pH 10.4	pH 10.8	pH 11.2
T-M	0.000067	0.00010	0.00014
Poly(Thy-D-lys)-88	0.0077	0.0049	0.0038
Poly(Thy-D-lys)-72	0.0055	0.0034	0.0029
Poly(Thy-L-lys)-93	0.0084	0.0061	0.0043
Poly(Thy-L-lys)-79	0.0063	0.0044	0.0028
Poly(Thy-DL-lys)-85	0.0053	0.0033	0.0030
Poly(Thy-DL-lys)-79	0.0046	0.0031	0.0025
Oligo(Thy-L-lys)	0.0035[b]	0.0019	0.0016

[a] All data for solution 1×10^{-4} mol/L of thymine unit.
[b] pH 10.2.

Table III-7. Quantum yields of the photodimerization of thymine base (Φ_{DF}) in polymers and oligomer

Compound	Φ_{DF}		
	pH 10.4	pH 10.8	pH 11.2
Poly(Thy-D-lys)-88	0.0077	0.0048	0.0037
Poly(Thy-D-lys)-72	0.0055	0.0033	0.0028
Poly(Thy-L-lys)-93	0.0084	0.0060	0.0042
Poly(Thy-L-lys)-79	0.0063	0.0043	0.0027
Poly(Thy-DL-lys)-85	0.0053	0.0032	0.0029
Poly(Thy-DL-lys)-79	0.0046	0.0030	0.0024
Oligo(Thy-L-lys)	0.0035[a]	0.0018	0.0015

[a] pH 10.2.

where ϕ_0 and ϕ_0^M are the quantum yield for the polymers or oligomer
and the monomeric model compound, respectively (Table III-6).
These values are tabulated in Table III-7, which shows that the
quantum yields of the photodimerization in the polymers and oligo-
mer tend to decrease with an increase in the pH of the system, as
discussed in earlier chapters[1]. The anionic thymine bases, which
are formed by the deprotonation of 3-NH in the thymine base, was
reported to have significantly lower quantum yield in photodimeri-
zation, compared with the neutral thymine bases[1]. For the polymers
used here the pKa values were obtained as 10.1-10.2 (Table III-4).
The ratio of the anionic thymine base to the neutral base increased
with an increase in the pH of the system. Therefore, the quantum
yield of the photodimerization decreased with the increasing pH.
In poly(Thy-D-lys)s and poly(Thy-L-lys)s the helix content also
decreased with an increase in pH (Fig.III-3). It should be
assumed that the decrease in the helix content affected the decrease
in the quantum yield of the photodimerization.

   Table III-7 also shows that the quantum yield was affected by
the content of thymine bases in the polymers. These yields were
next treated in terms of the sequence of thymine units along the
polymer chain with eq.(III-23) (Scheme III-3).

<p align="center">(Scheme III-3)</p>

<p align="center">T_d T_t T_t T_t T_d | | T_d T_d | T_d T_t T_t T_t T_d</p>
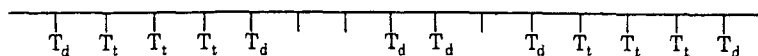
$$\sum T_d = N_d \; , \quad \sum T_t = N_t$$

$$N = N_d + N_t$$

$$\Phi_{DF} = \phi_d + (gN_t\phi_t - \phi_d)\left(\frac{N_t}{N}\right) \qquad (\text{ III-23 })$$

where Nt/N is the ratio of the content of triad thymine units (Nt)
to the thymine base content in the polymer (N), which was obtained
by the statistical calculation by the Monte Carlo method and listed
in Table III-2, and ϕ_d and ϕ_t are the quantum yields of diad thymine
unit (Td) and triad thymine unit (Tt), respectively.   Eq.(III-
23), which was derived in Chapter II, also involves the effect of
the singlet energy migration along the polymer chain in aqueous
solution at higher pH than pKa value of thymine base on photodimeri-
zation[15].

The quantum yields for the polymers and oligomer (Table III-7)
were plotted by using eq.(III-23); Fig.III-7 shows that the value
of the oligomer, oligo(Thy-L-lys) was smaller than those of the
polymers and on the line of the oligomers of ethyleneimine deriva-
tives (Chapter I).   This fact indicates that the molecular wei-
ght influences the energy migration between sequences.   In poly-
(Thy-D-lys)s and poly(Thy-L-lys)s, which can exist in a helical
conformation, the slopes in eq.(III-23), that is, $gNt\phi_t-\phi_d$, were
plotted against the Nt value, which produced straight lines at each
pH of the system (Fig.III-8).   The data for poly(Thy-DL-lys)s
also resulted in straight lines (Fig.III-8).   The slope of the
straight line for poly(Thy-DL-lys)s, however, was smaller than
those for poly(Thy-D-lys)s and poly(Thy-L-lys)s at each pH of the
system.   This suggests that the thymine bases of the polymer pre-

sent in a helical structure has higher reactivity to the photo-
dimerization than the thymine bases present in an unordered struc-
ture of poly(Thy-DL-lys)s.   The straight lines in Fig.III-8 were
extrapolated to Nt = 1 to give quantum yields of the photodimeri-
zation for the polymer that had a 100% content of thymine bases at
each pH of the system.   Differences in the values between poly-
(Thy-D-lys)s or poly(Thy-L-lys)s and poly(Thy-DL-lys)s with 100%
content of thymine bases ($\Delta\Phi_{DF}$) were obtained from Fig.III-8 and
are listed in Table III-5.   The values of $\Delta\Phi_{DF}$ were plotted against
the value of the helix content for the polymer with 100% of thymine
bases, obtained from Fig.III-5 (Fig.III-9).   This figure revealed
a linear relationship between $\Delta\Phi_{DF}$ value and the helix content.
At 100% helix content the $\Delta\Phi_{DF}$ value was given as 0.005, which
implies that the quantum yield of the photodimerization enhances
by 0.005 as the result of the conformational change from unordered
to helical conformation; for example, the quantum yield of photo-
dimerization for poly(Thy-L-lys)-93 at pH = 10.4 was 0.0084.
Because the helix content of this polymer was 0.72 at this pH re-
gion, about 30% of the quantum yield may be derived from the heli-
cal conformation.

III-3. Experimental
III-3-1. Materials
Poly(N^ε-Carbobenzoxy-L-Lysine) (1-L), Poly(N^ε-Carbobenzoxy-D-
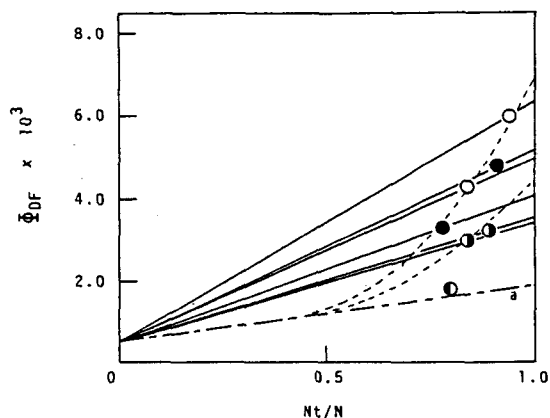Lysine) (1-D), and Poly(N^ε-Carbobenzoxy-DL-Lysine) (1-DL)

Fig.III-7. Relationship between quantum yield for the photodimerization (Φ_{DF}) and Nt/N value at pH 10.8: poly(Thy-L-lys)s (O); poly(Thy-D-lys)s (●); poly(Thy-DL-lys)s (◑); oligo(Thy-L-lys) (◉). The line a was obtained from the data of the oligomers of ethyleneimine derivatives (ref. 1).
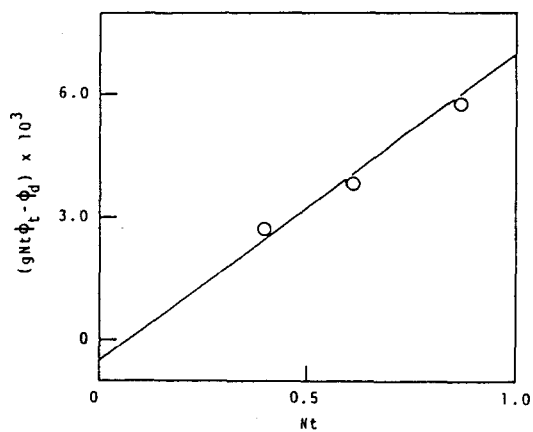

Fig.III-9. Relationship between Φ_{DF} and helix content (h) for poly-lysine derivatives with 100% content of thymine bases.
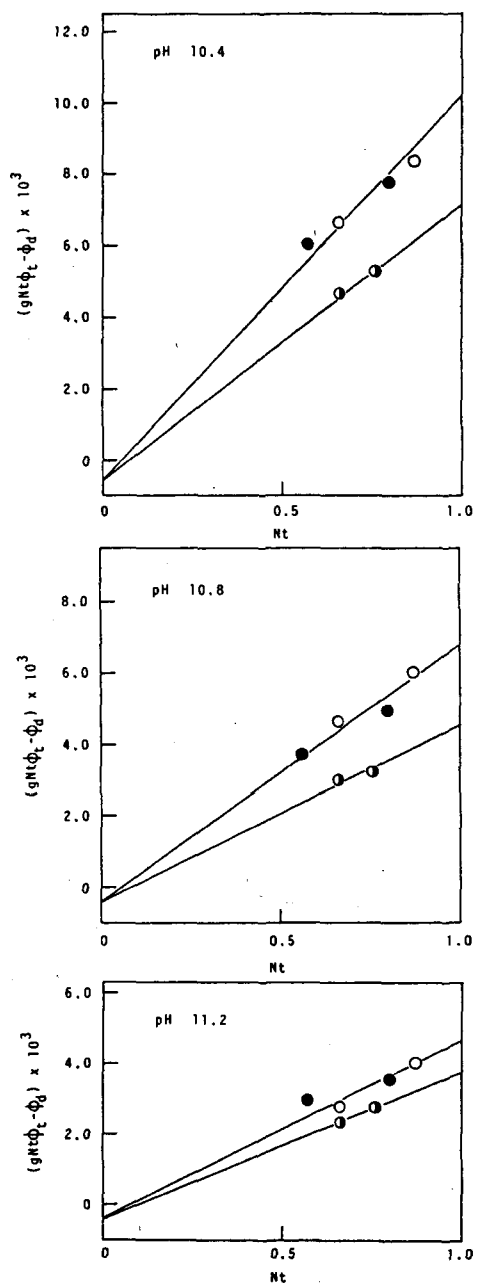
Fig.III-8.   Relationships between ($gNt\phi_t - \phi_d$) and Nt values at each pH
poly(Thy-L-lys)s (O); poly(Thy-D-lys)s (●); poly(Thy-DL-lys)s (◐).

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These polymers were parepared by polymerizing the corresponding
N-carboxyanhydrides in dry dioxane solution with triethylamine as
initiator[16].   Results of the polymerization are tabulated in Table
III-1.

Poly(N^ϵ-Carboxyethylthymin-1-yl-D-Lysine), Poly(Thy-D-lys)(3-D);

Poly(N^ϵ-Carboxyethylthymin-1-yl-L-Lysine), Poly(Thy-L-lys)(3-L); and

Poly(N^ϵ-Carboxyethylthymin-1-yl-DL-Lysine), Poly(Thy-DL-lys)(3-DL)

By deblocking the N^ϵ-carbobenzoxyl group with hydrogen bromide
in acetic acid solution from the corresponding N^ϵ-blocked polymers
poly(L-lysine hydrobromide) (2-L), poly(D-lysine hrobromide) (2-
D), and poly(DL-lysine hydrobromide) (2-DL) were prepared.
These polymers were confirmed by IR spectra by the disappearence
of carbobenzoxyl group.

Poly(D-lysine hydrobromide) (2-D) (0.45g; 2.2mmol) was dis-
solved in 10mL of dimethyl sulfoxide (DMSO).   To the solution
were added pentachlorophenyl 3-(thymin-1-yl)-propionate (Thy-PCP)
(0.98g; 2.2mmol) and triethylamine (0.62mL; 4.4mmol).   The
reaction mixture was stirred for one day at room temperature and
Thy-PCP (0.30g; 0.66mmol) was added.   The mixture was then stir-
red for two days at room temperature.   After the reaction DMSO was
distilled off under reduced pressure.   The addition of an excess
amount of methanol to the oily residue yielded the product as a
precipitate.   Reprecipitation from DMSO-acetone was repeated to
produce (3-D); yield, 0.69g (96 %); IR (KBr): 3320, 2920,
1660, 1550, 1480, 1360, and 1220 cm^{-1}.

Poly(Thy-D-lys), with a different thymine base content, was prepared in a similar procedure with ratios of (2-D) to Thy-PCP. Poly(Thy-L-lys)[4] and poly(Thy-DL-lys) were prepared in the same manner.

The content of thymine bases in the polymers was determined by measuring the ultraviolet (UV) absorbance of the product after hydrolysis[9]. The hydrolysis of the polymers was carried out in 6N aqueous hydrochloric acid at 105°C for one day. The quantitative calculation was made by using a standard sample of 1-carboxyethylthymine.

Oligo(N$^\varepsilon$-Carboxyethylthymin-1-yl-L-lysine), Oligo(Thy-L-lys)

Oligo(Thy-L-lys) was obtained by the polymerization of N$^\alpha$-carboxyanhydride of N$^\varepsilon$-carboxyethylthymin-1-yl-L-lysine with triethylamine as initiator, according to the method described by Ishikawa et al[8]. The degree of polymerization of oligo(Thy-L-lys) was estimated from the terminal amino units as 10 and determined by the trinitrobenzene sulfonate method.

N$^\alpha$-Acetyl-N$^\varepsilon$-Carboxyethylthymin-1-yl-L-Lysine Methylamide (T-M)(6)

N$^\alpha$-Acetyl-N$^\varepsilon$-carbobenzoxy-L-lysine dicyclohexylammonium (2.0g; 4.0mmol), prepared according to the literature[17], was dissolved in 40mL of chloroform. Pentachlorophenyl trichloroacetate (2.0g; 4.8mmol) was added to the solution at 0°C. After reaction for 4 h chloroform was distilled off and the residue was dissolved in 30 mL of DMF. To the solution were added methylamine hydrochloride (0.27g; 4.0mmol) and triethylamine (0.5mL; 4.0mmol) and stirred

for one day at room temperature.   After filtration of the salt the

solvent was distilled off in vacuo.   The addition of an excess

amount of diethyl ether to the residue yielded white needles.

Recrystallization from tetrahydrofuran (THF) produced 0.82g of

colorless crystals, N^{α}-acetyl-N^{ϵ}-carbobenzoxy-L-lysine methylamide

(<u>4</u>): yield 31%, mp 163°C; IR (KBr) 3320, 1690, 1640, 1550, and

1280 cm^{-1}.

The compound (<u>4</u>) (0.34g; 1.0mmol) was dissolved in 5mL of

acetic acid and 5mL of hydrogen bromide saturated acetic acid was

added to the solution.   The reaction was contined for 1 hr at room

temperature.   By adding an excess amount of diethyl ether the

product N^{α}-acetyl-N^{ϵ}-hydrobromide-L-lysine methylamide (<u>5</u>) was

precipitated, washed thoroughly with diethyl and petroleum ethers,

and dried in vacuo.

To a solution of the compound (<u>5</u>) in 15mL of DMF were added

Thy-PCP (0.45g; 1.0mmol) and triethylamine (0.3mL; 2.0mmol).

The reaction mixture was stirred for one day at room temperature.

After the solvent was distilled off in vacuo the residue reaming

was washed with diethyl ether.   Recrystallization from isopropyl

alcohol produced 0.1g of colorless needles of T-M (6); yield 26%,

mp 220°C, $[\alpha]_D^{25}$ = +5.0 (c = 0.159g/dl in H_2O); IR (KBr) 3320,

1660, 1550, 1470, and 1370 cm^{-1}.

Anal. Calcd. for $C_{17}H_{25}O_5N_5$: C 53.53; H 7.14; N 18.36. Found: C

53.15; H 7.05; N 18.01.

III-3-2. Instrumentation

Ultraviolet spectra of the compounds were measured with a UNION-SM-401 spectrophotometer at 25°C with a temperature controller in a 10-mm quartz cell. Nuclear magnetic resonance (NMR) spectra were measured with a JNM-PS-100 spectrometer (JEOL). The samples were dissolved in DMSO-d_6 to the concentration of apparently 5 % (w/v). Tetramethylsilane was used as the internal standard in the DMSO solution. Measurement of pH was done with a Hitachi-Horiba F-7-SS pH meter. A JASCO J-400 spectropolarimeter and a 10- or 1-mm quartz cell at ambient temperature were used for the circular dichroism (CD) spectra measurement.

III-3-3. Photochemical Reactions

Photochemical reactions were carried out with a 10-mm pathlength quartz cell filled with 4mL of solution at 20°C. A Philips SP lamp (500-w, superhigh pressure mercury lamp) filtered through a Corning 9-30 glass filter was used as the light source ($\lambda > 250$ nm). Light intensities were determined by potassium ferrioxalate actiometry. The quantum yield for oxalic acid decomposition was kept constant at 1 x 10^{-4} mol/L of thymine unit in aqueous solution. A stream of purified nitrogen gas was passed through the solution for more than 15 min to flush out air present before the reaction. All the aqueous solutions were buffered with 0.2 mol/L of sodium bicarbonate and a sodium carbonate buffer system.

III-4. Summary

The photodimerization reaction of pendant thymine bases in thymine-containing poly-lysine derivatives was studied over a wide range in aqueous solution. It was stated that the photodimerization of the grafted thymine bases was greatly affected by the conformation of the polymer main chain and that its helical structure brings about the photodimerization of grafted thymine bases. Thymine base may form the photodimer with the nearest thymine base. In thymine grafted poly-lysine α-helical structure the nearest thymine bases may be not those on the nearest neighbor units along the polymer chain but those on the next turn of the α-helix of the polymer.

III-5. References

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Chapter IV. Photoinduced conformational change of thymine contain-
           ing poly-lysine derivatives

IV-1. Introduction

    In a series of our studies on the synthetic nucleic analogues,
both intermolecular and intramolecular interactions have been ex-
tensively investigated[1].   On this line, thymine or adenine base
grafted poly-L-lysine derivatives were used particularly in order
to elucidate how the conformational change of the polymer main chain
affects their intermolecular interaction[2,3].   It has been concluded
that the polymer complex was formed by specific base pairing between
complementary poly-L-lysine derivatives, retaining their helical
conformation.   Another type of the intermolecular interaction bet-
ween a base grafted poly-L-lysine and a vinyl type polymer contain-
ing complementary bases was reported, and it was concluded also in
this case that the interaction was realized between the bases in a
double helical structure[4].

    For the estimation of the intramolecular interaction between
thymine bases along the polymer chain, photodimerization study on
the thymine bases has been one of the interesting subject as desc-
rived previous chapters.   In chapter III, the effect of the con-
formation of the polymer chain on the photodimerization reaction
was studied and it was clarified that the helical structure was
favorable to cause the photodimerization between the grafted thymine
bases[5].

In this chapter, the effect of the photodimerization of thymine bases on the conformational change of the polymer chain was studied[6], in order to see whether the helical structure was favorable compared with the unordered one for the photodimerization. The fact that CD spectrum was changed when the thymine grafted poly-L-lysine derivatives was irradiated by UV light can clearly be seen from the result shown in Fig.IV-1. This suggests a conformational change of the polymer chain by the photodimerization.

In this connection it is to be noted that the photoinduced conformational change of the polymers has been widely studied as a model of visual purple rhodopsin[7]. Most of the studies were done by the photoisomerization of photocleavage of chromophores in the polymers.

IV-2. Results and Discussion

IV-2-1. Determination of the Helix Content of the Polymers and
         the Conversion of their Thymine Bases

The helix content, that is, the helicity of poly(Thy-L-lys)-93 (Fig.IV-2) was obtained by using eq.(IV-1)[8]:

$$\text{helix content (\%)} = \frac{-[\Theta]_{222} + [\Theta]_{222}^{NH_3^+}}{-[\Theta]_{222}^{NH_2} + [\Theta]_{222}^{NH_3^+}} \times 100 \qquad (\text{ IV-1 })$$

where $[\Theta]_{222}$ is the molar ellipticity at 222 nm of poly(Thy-L-lys)-93 at each pH and $[\Theta]_{222}^{NH_3^+}$ and $[\Theta]_{222}^{NH_2}$ are those at 222 nm of poly(L-lysine) with 100% content of the protonated and neutral ε-amino groups, respectively[5]. Similarly, the helix content of poly(Thy-

Fig.IV-1.   CD Spectra of poly(Thy-L-lys)-93 at pH 10.2.   Photolysis with a glass filter: (a) before (b) after irradiation.

poly(Thy-D-lys)-88   x=0.88   (DP=500)
poly(Thy-L-lys)-93   x=0.93   (DP=400)

Fig.IV-2.   Thymine derivatives.
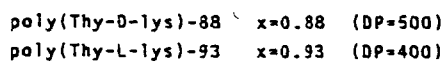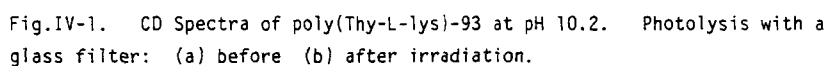
78

D-lys)-88 (Fig.IV-2) was obtained from the molar ellipticity of poly(D-lysine) at 222 nm[5] by using eq.(IV-2):

$$\text{helix content (\%)} = \frac{[\theta]_{222} - [\theta]_{222}^{NH_3^+}}{[\theta]_{222}^{NH_2} - [\theta]_{222}^{NH_3^+}} \times 100 \qquad (\text{ IV-2 })$$

The conversion of the photoreaction was obtained from UV absorbance at 270 nm by using eq.(IV-3):

$$\text{conversion (\%)} = \frac{A_0 - A}{A_0} \times 100 \qquad (\text{ IV-3 })$$

where Ao is the initial absorbance. As reported earlier[5], the photoreaction of thymine bases was regarded almost as their photodimerization. Therefore, it can be assumed that half the conversion corresponded (in %) to the formation of the photodimers of thymine bases.

IV-2-2. Photochemical Reaction of the Polymer

Photochemical reactions of the polymers were carried out in an aqueous solution. Fig.IV-3 shows the UV spectral change of poly-(Thy-L-lys)-93 at pH 11.0. From this figure it can be seen that the UV absorbance around 270 nm decreases with an increase in the UV irradiation, which shows that the thymine bases present on the polymer chain reacted by UV light. The reaction in question is regarded as the intramolecular photodimerization[5].

Fig.IV-4 shows CD spectra of poly(Thy-L-lys)-93 at pH 11.0. It also shows that they varied and that the absolute value of molar ellipticity at 222 nm, $\left|[\theta]_{222}\right|$, increased during UV irradiation.
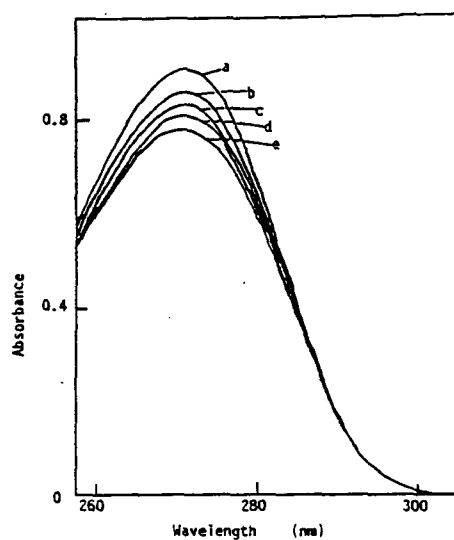
Fig.IV-3.   UV Spectra of poly(Thy-L-lys)-93 at pH 11.0.   Photolysis with a
glass filter: (a) 0 min, (b) 3.0 min, (c) 6.0 min, (d) 10.0 min, (e) 15.0 min.
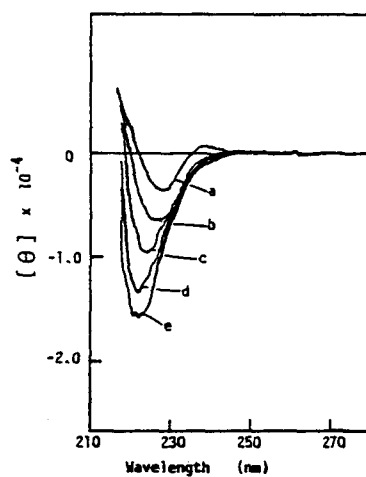

Fig.IV-4.   CD Spectra of poly(Thy-L-lys)-93 at pH 11.0.   Photolysis with a
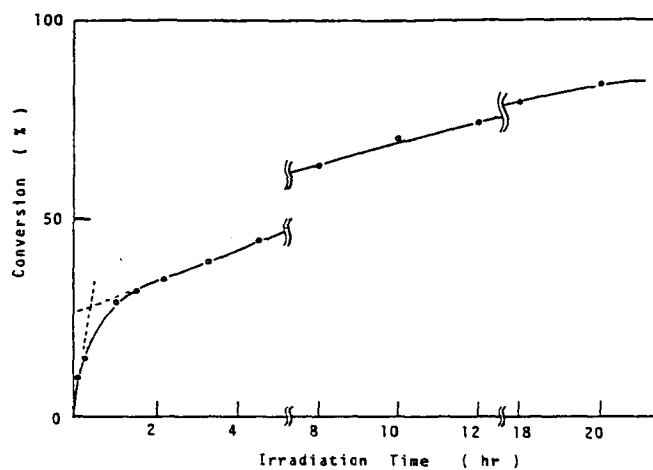glass filter: (a) 0 min, (b) 3.0 min, (c) 6.0 min, (d) 10.0 min, (e) 15.0 min.

Fig.IV-5. A time-conversion curve for poly(Thy-D-lys)-88 at pH 11.2.
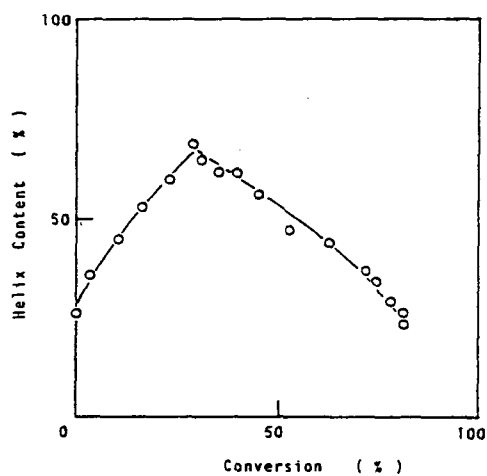Photolysis with a glass filter.

Fig.IV-6. The relationship between the conversion of thymine bases and the helix
content of the polymer for poly(Thy-D-lys)-88 at pH 11.2.   Photolysis with a glass filter

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This change in CD spectra, displayed in Fig.IV-1 as well, suggests that the helicity of the polymer main chain tends to increase with UV irradiation and that the photodimer formation between thymine bases affects the helicity of the polymer chain. Fig.IV-5 contains plots of the conversion calculated by eq.(IV-3) against irradiation time. It was found that photodimerization was extreamely fast at lower conversion and that the turning point of the reaction occurred near 30% conversion. Fig.IV-6 shows plots of the helicity of the polymer obtained from eq.(IV-2) against the conversion of thymine bases. It was clear that the helicity increased until a 30% conversion was reached and then decreased. The maximum value of the helicity was 70%. The relationship between the conversion and helicity of the polymer was observed not only for poly(Thy-D-lys)-88 in other pH regions, but also for poly(Thy-L-lys)-93.

For the experiments in photoreaction in Figs.IV-5 and IV-6 glass filters were used exclusively. The transmittance of these filters is shown in Fig.IV-7, from which it is assumed that the amide group in a lysine unit also absorbs UV light at λ_{max} ($n-\pi^{*}$) = 210-230 nm and that photoracemization of the polymer is induced. Fig.IV-8 shows the result of the photoreaction of poly(L-lysine) in which only a glass filter was used. Consequently, it was recognized that the value of $\left|[\theta]_{222}\right|$ tended to decrease during UV irradiation. When thymine bases are present in poly(L-lysine) solution, the rate of decrease in the value of $\left|[\theta]_{222}\right|$ is slower than when thymine bases are present. These facts suggest that the photoracemization
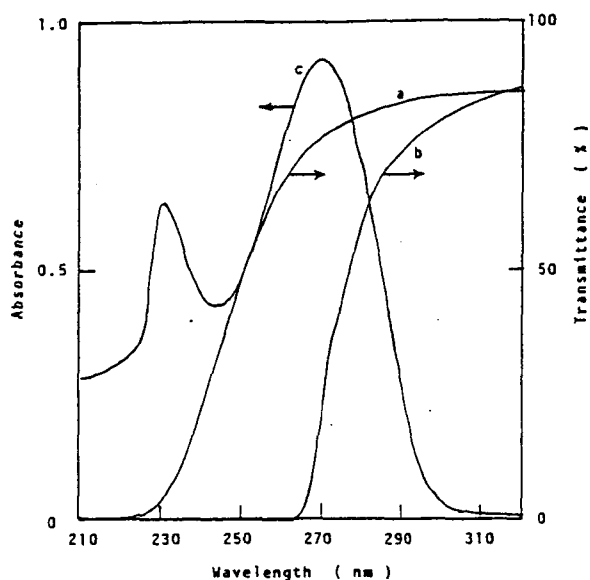
Fig.IV-7.   Percent transmittances of the filter: (a) a Corning 9-30 glass filter;
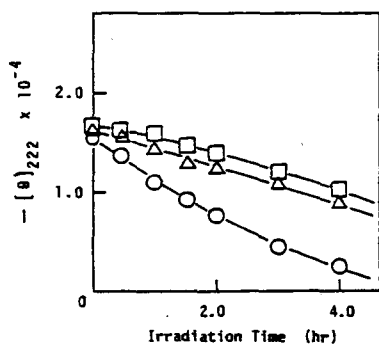(b) a 10-mm pathlength DMF solution filter; (c) UV absorbance of poly(Thy-L-lys)-93
at pH 11.2.

Fig.IV-8.   Photolysis of poly(L-lysine) with
a glass filter in the presence of thymine bases:
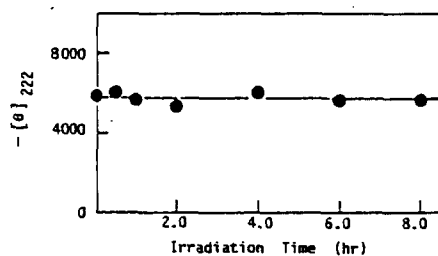[thymine]/[lysine unit] = 0 (O), 1.0 (▲), 5.0 (□).

Fig.IV-9.   Photolysis of poly(L-lysine) with
a DMF filter.

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reaction will occur and that the thymine bases quench the reaction.

To cut off the shorter wavelength of light a DMF solution filter (pathlength, 10 mm) was used next.   The transmittance of the solution filter appears in Fig.IV-7.   The value of $\left|[\theta]_{222}\right|$ of poly-(L-lysine) did not decrease during photolysis when DMF and a glass filter were used (Fig.IV-9), a fact that indicates that no photo-racemization occurred.   From these aspects, it can be considered that the photoracemization may be regarded as a factor in the dec-rease of the helicity of poly(Thy-D-lys)-88, as shown in Fig.IV-6.

By using the DMF filter, photochemical reactions of the polymers were then carried out in aqueous solution.   Figs.IV-10 and IV-11 give the results of poly(Thy-L-lys)-93 at pH 10.5.   In Fig.IV-10 the conversion was plotted against irradiation time.   It was found that the photodimerization reaction of thymine bases was fast at the outset and that its turning point occurred near 40%.   Fig.IV-11 suggests the relationship between helicity and conversion to photo-dimerization; the helicity increased first to 40% of conversion, which corresponded to the turning point on the time-conversion curve in Fig.IV-10; it then decreased.   The result is discussed in detail in the following section on the structure of the photoreacted polymers.

IV-2-3. The Structure of the Photoreacted Polymers

(a) Low Conversion

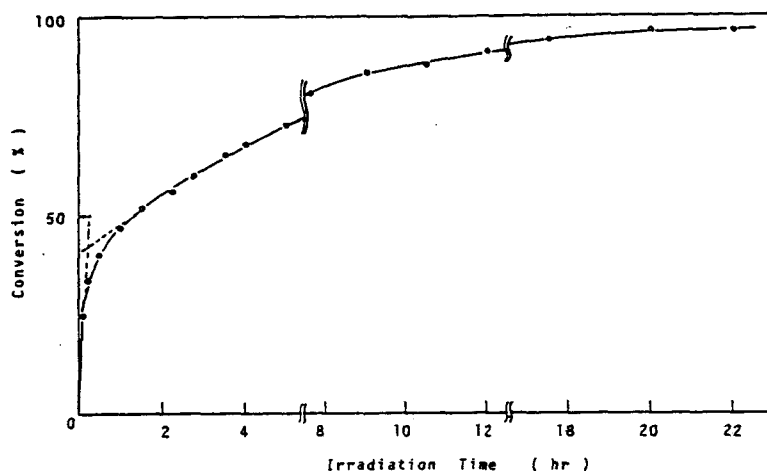It has already been reported that the helicity of poly(Thy-D-

Fig.IV-10.   A time-conversion curve for poly(Thy-L-lys)-93 at pH 10.5.   Photolysis
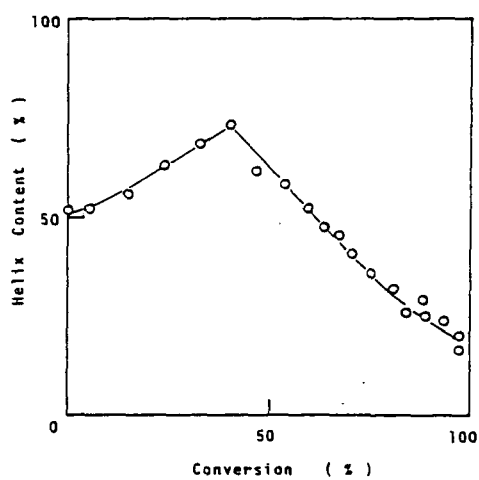with a DMF filter.

Fig.IV-11.   The relationship between the conversion of thymine bases and the helix
content of the polymer for poly(Thy-L-lys)-93 at pH 10.5.   Photolysis with a DMF filter.

lys)-88 and poly(Thy-L-lys)-93 is controlled by the pH of the solution[5]. In the present case, photoreaction of the polymers was carried out in several pH regions. Because of concerning with low conversion (< 20%), it can be assumed that only photodimerization occurs and that photoracemization can be almost entirely ignored. Fig.IV-12 plots conversion against helicity for poly(Thy-D-lys)-88 and poly(Thy-L-lys)-93 in several pH regions. It was found that the helicity of the polymer increased linearly according to eq. (IV-4):

$$h_x = a \cdot x + h_0 \qquad\qquad (\text{ IV-4 })$$

where X is the conversion of thymine bases, h_x and h_0 are the helicity at conversion X% and zero, respectively, and **a** is a parameter of the slope. Equation (IV-4) suggests that the helical structure of the polymer is fixed by photodimerization.

Scheme IV-1 illustrates the polymer under discussion. At a certain pH it is assumed that the following equilibrium between the helical and coiled units m_0 and n_0 exists:

$$m_0 \rightleftharpoons n_0 \qquad\qquad (\text{ IV-5 })$$

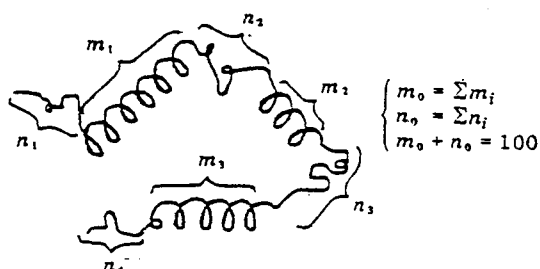$$\frac{n_0}{m_0} = K^0 \qquad\qquad (\text{ IV-6 })$$

$$m_0 + n_0 = 100 \qquad\qquad (\text{ IV-7 })$$

where K^0 denotes an equilibrium constant at the given pH. If this

equilibrium is established, the helical content of this polymer can be written as eq.(IV-8):

$$h_0 = \frac{m_0}{m_0 + n_0} \times 100(\%) \qquad (\ \text{IV-8}\ )$$

(Scheme IV-1)

$$\begin{cases} m_0 = \Sigma m_i \\ n_0 = \Sigma n_i \\ m_0 + n_0 = 100 \end{cases}$$

The photodimerization of thymine bases between the nearest neighboring units should cause fixation of the structure of 2 amino residues. On the other hand, in photodimerization between two remote thymine units the structure of $2f$ units of residues may be fixed, where f is a parameter. The structure of $f \cdot X$ units of residue may then be fixed at X% conversion of thymine bases for photodimerization. The following equilibrium should be achieved for the (100 $- f \cdot X$) units of residue:

$$m_x + n_x = 100 - f \cdot X \qquad (\ \text{IV-9}\ )$$

$$m_x \rightleftharpoons n_x \qquad (\ \text{IV-10}\ )$$

$$\frac{n_x}{m_x} = K^0 \qquad (\ \text{IV-11}\ )$$

where m_x and n_x are the number of the units in helical and coiled structure, respectively. The equilibrium constant K^0 is identical to that in eq.(IV-6) because the same pH systems exist. Eq.(IV-12) can be derived from eqs.(IV-6), (IV-8), and (IV-11):

$$\frac{m_x}{m_x + n_x} \times 100 = h_0(\%) \qquad (\text{ IV-12 })$$

The helicity of the polymer at X% of conversion for photodimerization (h_x) can then be written as eq.(IV-13):

$$h_x = \frac{m_x + f \cdot X}{m_0 + n_0} \times 100(\%) \qquad (\text{ IV-13 })$$

and eq.(IV-13) can be rewritten from eqs.(IV-7), (IV-9), and (IV-12):

$$h_x = \frac{h_0(100 - f \cdot X)}{100} + f \cdot X \qquad (\text{ IV-14 })$$

$$= h_0 + f\left(1 - \frac{h_0}{100}\right) \cdot X \qquad (\text{ IV-15 })$$

By comparing eq.(IV-15) with eq.(IV-4), slope a in eq.(IV-4) can be written as eq.(IV-16):

$$a = f - \frac{f}{100}h_0 \qquad (\text{ IV-15 })$$

By using eq.(IV-16) the slope of the straight lines in Fig.IV-12 was plotted against helicity at 0% of conversion (h_0), which resulted in the straight line shown in Fig.IV-13. This relationship apparently supports eq.(IV-16). In Fig.IV-13 the value of **f** is given as 2.25, which corresponds to 4.5 units of amino acid residue. This value suggests that 4.5 units of residue are fixed by a photo-dimer of thymine bases on the side chain of the polymer.
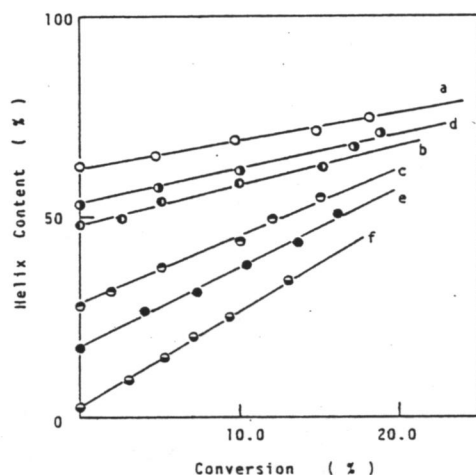
Fig.IV-12. The relationship between the conversion of thymine bases and the helix content of the polymer for poly(Thy-D-lys)-88 at pH: (a) 10.5, (b) 10.9, (c) 11.2. For poly(Thy-L-lys)-93 at pH: (d) 10.6, (e) 11.0, (f) 11.4. Photolysis with a glass filter.
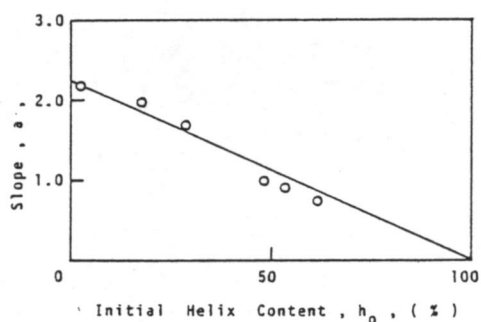

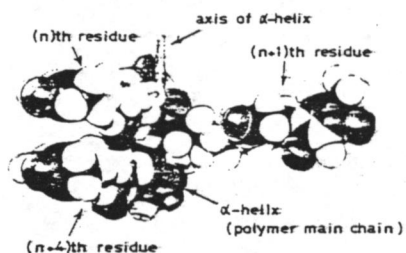Fig.IV-13. The relationship between slope a and the initial helix content h_0.

Fig.IV-14. A CPK model for poly(Thy-D-lys)-88 and/or poly(Thy-L-lys)-93.

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It has been established that a turn of the α-helix of poly(α-amino acid) is formed by 3.6 units of residue[9]. The CPK model of poly(Thy-D-lys)-88 and/or poly(Thy-L-lys)-93 (Fig.IV-14) reveals that the thymine base of the n-th residue in the polymer can be superimposed on that of the (n+4)th residue along the α-helix and that the distance between the two thymine bases is shorter than between the nearest neighboring thymine bases along the polymer chain. If the photodimer of thymine bases is formed with n-th and (n+4)th thymine bases, a turn of the α-helix (3.6 units of residue) can be fixed. The result appears to support this point; that is, that photodimerization occurs along the α-helix and the photodimer of the thymine bases fixes the helical structure of the polymer.

The results of the change in helicity of the polymer by photo-dimerization in terms of the initial pH of poly(Thy-D-lys)-88 are shown in Figs.IV-15 and IV-16 as 11.2 and 10.4, respectively. In Figs.IV-15(a) and IV-16(a), that is, before the photodimerization of the thymine bases, the extent of the change in helicity was about 30% in conjunction with the change in pH from 10.4 to 11.2 or from 11.2 to 10.4 . In Figs.IV-15(b) and (c) and IV-16(b), and (c) the results of photodimerization in which the conversion amounted to 12% [Fig.IV-15(b)], 42% [Fig.IV-15(c)], 16% [Fig.IV-16(b)], and 27% [Fig.IV-16(c)], respectively, are shown. In Figs.IV-15 (b) and (c) the helicity at pH 11.2 was high compared with that in Fig.IV-15(a) as a result of the fixation of the helical structure by processing the photodimerization reaction. In Fig.IV-15(b) and
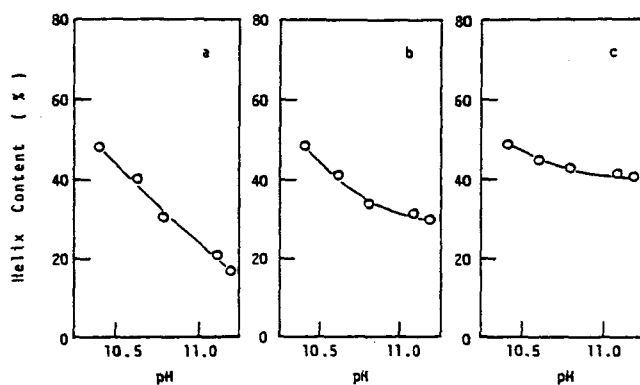
Fig.IV-15. Helix-coil equilibria for poly(Thy-D-lys)-88 irradiated at low helix content (pH 11.2).   The conversion of thymine bases for photodimerization: (a) 0%, (b) 12%, (c) 42%.
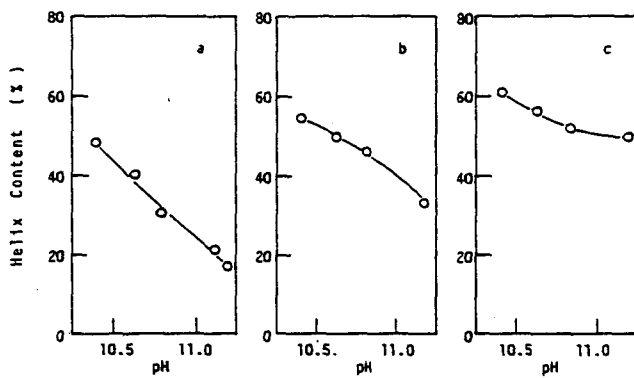

Fig.IV-16. Helix-coil equilibria for poly(Thy-D-lys)-88 irradiated at high helix content (pH 10.4).   The conversion of thymine bases for photodimerization: (a) 0%, (b) 16%, (c) 27%.

(c) the helicity also increased with decreasing pH in the order of 20 and 8%, respectively, whereas the degree of increase was smaller compared with zero conversion [Fig.IV-15(a)].   When the pH of the system increased [Fig.IV-16(b) and (c)] the helicity of the polymer decreased, whereas the degree of change in helicity decreased with an increase in conversion in the following order: Fig.IV-16(a) > Fig.IV-16(b) > Fig.IV-16(c).   From these facts it is suggested that the change in helicity coupled with the change in pH value was essentially reduced as the conversion increased.   This may give support for the establishment of eqs.(IV-9), (IV-10), and (IV-11); that is, the helicity of the polymer was assumed to be fixed by the formation of the photodimer and the helix-coil equilibrium was established in the unfixed part of the polymer at each pH region. The fixed portion of the helix is proportional to the conversion, and the total helix content depended on the initial helix content.

This may explain the fact that the photodimerization reaction proceeded with greater ease in a helical than in an unordered conformation[5].   As described earlier, the nearest bases in the polymer present in the helical structure may be those in the next turn of the α-helix, and with the photodimerization of the thymine bases the helical structure should be fixed.

(b) High Conversion

At high conversion of the photodimerization reaction (more than 20%) the helicity tended to decrease to about 70% the maximum value

even when photoracemization was not considered (Fig.IV-11).   From

the time-conversion curve shown in Fig.IV-10 it was found that the

turning point is present at 40% conversion, where the helicity has

the maximum value (Fig.IV-11).   Because the photodimerization

reaction was rapid below 40% conversion, compared with that of more

than 40%, it was assumed that the environment of the grafted thymine

bases at lower conversion is different from that at a higher conver-

sion; that is, at less than 40% the reaction may occur between the

nearest thymine bases in the next turn along the α-helix of the

polymer or in the nearest neighboring unit along the polymer chain,

where the photodimerization reaction may be fast and the photodimer

formed may fix the α-helical structure of the polymer.   On the

other hand, at a conversion of more than 40% the reaction may occur

between the remote grafted thymine bases.   The rate of photodimeri-

zation between the remote thymine bases should be slower than that

between the nearest thymine bases.

   When the photodimer of thymine bases is formed between remote

grafted bases, a distortion may occur in the α-helical structure

of the polymers.   As a result, this structure could assume a

strained helical form.   As mentioned above, the helicity of poly-

(Thy-L-lys)-93 was decreased at high conversion of thymine bases

to photodimerization (Fig.IV-11).   This may have been caused by

the formation of the strained helical structure in that polymer.

IV-3. Experimental

IV-3-1. Materials

The thymine-base-containing derivatives used here were poly-D-
and -L-lysine (Fig.IV-2).   These polymers were prepared by the
grafting reaction of the activated ester derivatives of thymine base
(Thy-PCP) with poly(D-lysine hydrobromide) and poly(L-lysine hy-
drobromide) according to the method reported earlier[5].   Thymine-
base content in the polymers was determined by measuring the UV ab-
sorbance of the product after hydrolysis[10].   From the absolute con-
figuration of the asymmetric units in the polymer these polymers
were abbreviated as poly(Thy-D-lys)-88 and poly(Thy-L-lys)-93, res-
pectively; 88 and 93 represent the thymine-base content (mol%) of
the polymers.   The degree of polymerization was determined by visco-
metry as 500 and 400, respectively[11].   All the reagents and organic
solvents were used after purification in the usual manner.

IV-3-2. Instrumentation

Ultraviolet spectra were measured at 25°C, with a UNION-SM-401
spectrophotometer equipped with a temperature controller in a 10-mm
pathlength quartz cell.   The pH measurement was carried out on a
Hitachi-Horiba F-7-SS pH meter.   For CD spectroscopy a JASCO J-400
spectropolarimeter was used.   The measurement was made with a 1.0-
mm pathlength quartz cell at ambient temperature.   The concentra-
tion of the polymer kept at 1×10^{-4} mol/L of the monomer unit.

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IV-3-3. Photochemical Reaction

    Photochemical reactions were carried out with a 10-mm pathlength
quartz cell filled with 4mL of solution at 20°C.   A Philips SP
lamp (500-w superhigh pressure mercury lamp) filtered through a
Corning 9-30 glass filter and/or a 10-mm pathlength dimethylforamide
(DMF) solution filter were used as the light source.   The glass
filter showed 0% transmittance below 225 nm, 50% transmittance at
250 nm, and 80% transmittance above 280 nm; the DMF filter showed
0% transmittance below 260 nm and 50% transmittance at 270 nm.
The concentration of thymine-base derivatives was kept constant at
1×10^{-4} mol/L of thymine unit in an aqueous solution.   A stream
of purified nitrogen gas was passed through the solution for more
than 15 min to displace the air present before the reaction.   All
the aqueous solution was buffered with 0.2 mol/L of sodium bicarbo-
nate in a sodium carbonate buffer system.

IV-4. Summary

    The photodimerization reaction between thymine bases in thymine-
grafted poly-D- and -L-lysine derivatives was studied in an alka-
line, aqueous, buffered solution.   The helix content of the poly-
mer was found to be changed by photodimerization.   And it was pro-
ved that the helical structure of the polymers was fixed by the
formation of the photodimer between thymine bases at the side chains.
The fixed part of the α-helix was not affected by the pH change in
the system; in other words, it was excluded from the helix-coil

equilibrium at a given pH region. It also became clear that the
photodimerization occurred between the remote grafted thymine bases
at high conversion and that the photodimer formed may cause a dis-
tortion of the α-helical structure and a decrease in the helicity.

IV-5. References

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Chapter V.   Photodimerization of Pendant Thymine Bases in Thymine

   Containing Isopoly-L-Lysine Derivatives

V-1.   Introduction

   In chapters III and IV, the effect of the conformation of the
polymer main chain on the photodimerization of the grafted thymine
bases was deeply investigated by use of poly-lysine derivatives
which was able to be existed as α-helical structure in aqueous
solution.   And it was reported that the photodimer was formed
much faster in α-helical structure than in extended coiled struc-
ture, because the photodimerization was occurred along the α-helix
and that the polymer main chain was fixed as helical structure
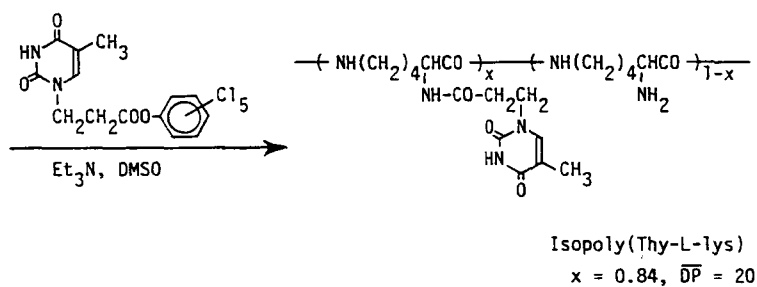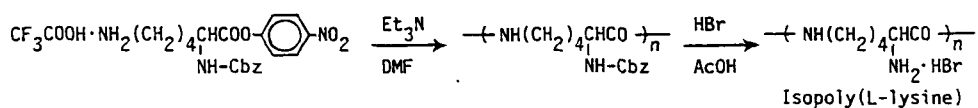by the photodimer of the grafted thymine bases[1,2].

   In this chapter, the effect of the β-sheet conformation of the
polymer main chain on the photodimerization of the grafted thymine
bases was mentioned by use of isopoly-L-lysine derivatives, which
was able to be existed as β-sheet structure.
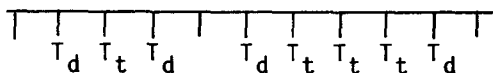

V-2.   Results and Discussion

V-2-1.   Thymine Derivative

   Thymine containing isopoly-L-lysine derivative used here was
prepared by the grafting reaction of Thy-PCP according to Scheme
V-1[3].   This compound was abbreviated as isopoly(Thy-L-lys).   The
degree of polymerization was determined as 20 by the vaper pressure
osmometry.   The content of thymine bases in the polymer was deter-

(Scheme V-1)

$CF_3COOH \cdot NH_2(CH_2)_4\underset{NH\text{-}Cbz}{CHCOO}\text{—}\langle\text{⬡}\rangle\text{—}NO_2$ $\xrightarrow[\text{DMF}]{Et_3N}$ $-\left(NH(CH_2)_4\underset{NH\text{-}Cbz}{CHCO}\right)_n$ $\xrightarrow[\text{AcOH}]{HBr}$ $-\left(NH(CH_2)_4\underset{NH_2\cdot HBr}{CHCO}\right)_n$

Isopoly(L-lysine)

[structure: thymine ring with CH_3, HN, O, and $CH_2CH_2COO\text{—}\langle\text{⬡}\rangle\text{—}Cl_5$]

$\xrightarrow{\quad\quad}$

$Et_3N, DMSO$

$-\left(NH(CH_2)_4\underset{NH\text{-}CO\text{-}CH_2CH_2}{CHCO}\right)_x\text{—}\left(NH(CH_2)_4\underset{NH_2}{CHCO}\right)_{1-x}$

[pendant thymine ring: N, O, HN, CH_3, O]

Isopoly(Thy-L-lys)

$x = 0.84, \overline{DP} = 20$

(Scheme V-2)

$T_d \quad T_t \quad T_d \quad\quad T_d \quad T_t \quad T_t \quad T_t \quad T_d$

mined as 84 mol% from its NMR spectra.

V-2-2. Photochemical Reaction

Photochemical reaction of the polymer was carried out in aque-
ous buffered solution.   The UV absorbance of the monomeric model
compound of poly-L-lysine derivative[1] (Chapter III) did not dec-
rease with UV irradiation under the reaction condition used here.
This fact suggests that only the intramolecular photodimerization
of thymine bases in isopoly(Thy-L-lys) is considered at least at
the beginning of the photolysis.

The quantum yields for the intramolecular photodimerization of
thymine bases in isopoly(Thy-L-lys) were 0.0033 at pH 10.4, 0.0022
at pH 10.8, and 0.0023 at pH 11.2, respectively.   The values were
then treated with eq.(V-1).   This equation was derived in Chapter
II, and involves both the influence of the sequence of the grafted
thymine bases in the polymer and the effect of the singlet energy
migration along the polymer chain on the photodimerization[1]:

$$\Phi_{DF} \ = \ \phi_d \ + \ (\ gNt\phi_t \ - \ \phi_d \ )(\ Nt/N\ ) \qquad\qquad (\ V\text{-}1\ )$$

where Nt/N is the ratio of the content of triad thymine units (Nt)
to the thymine base content (N) in isopoly(Thy-L-lys), and ϕ_d and
ϕ_t are the quantum yields of diad thymine unit (Td) and triad
thymine unit (Tt), respectively (Scheme V-2).   The slopes
obtained, $gNt\phi_t - \phi_d$, were plotted against Nt value in Fig.V-1.   In
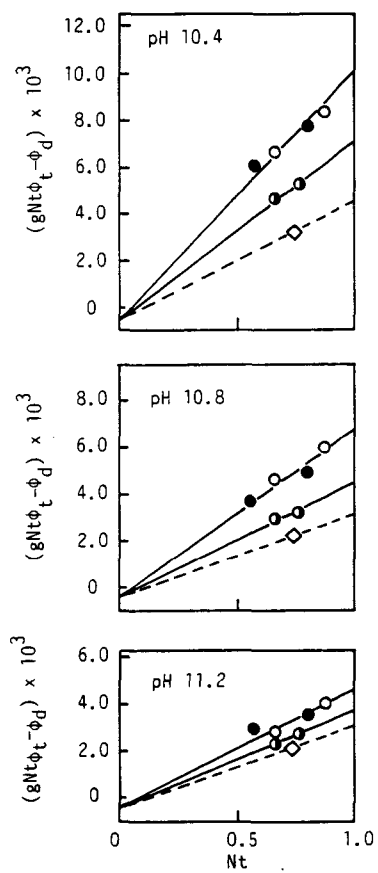this figure, the data of poly-lysine derivatives (Chapter III)

Fig.V-1. Relationships between ($gNt\phi_t - \phi_d$) values and Nt values at each pH: isopoly(Thy-L-lys) (◇); poly(Thy-DL-lys)s (◑); poly(Thy-L-lys)s (○); poly(Thy-D-lys)s (●).

were also plotted for the comparision. By the extrapolation of the straight lines in Fig.V-1 to Nt = 1, the estimated quantum yields of the polymer that had a 100% content of thymine bases were able to be obtained at each pH of the system.

The estimated value of isopoly(Thy-L-lys) was smaller than that of poly(Thy-DL-lys)s at each pH. And the differences between the values of isopoly(Thy-L-lys) and poly(Thy-DL-lys)s were increased with decreasing pH; pH = 11.2 < 10.8 < 10.4. At pH 11.2 isopoly-(Thy-L-lys) are present in an extended coiled conformation because of the electrostatic repulsion between the anionized grafted thymine bases (pKa = 10.07). CD Spectra of isopoly(Thy-L-lys) in ethy-leneglycol-water mixed solvent at higher pH (pH > 11) may support the extended coiled conformation of isopoly(Thy-L-lys) (Fig.V-2)[3]. Poly(Thy-DL-lys)s exist as extended coil at pH 11.2 because of the racemic polymer[1]. Therefore the conformational difference of isopoly(Thy-L-lys) and poly(Thy-DL-lys)s is not exist at pH 11.2. The small difference of the values between isopoly(Thy-L-lys) and poly(Thy-DL-lys)s at pH 11.2 may be derived from the structural formula of isopoly(Thy-L-lys); that is, the polymer has shorter side chains and longer distance between the side chains as compared with those of poly-lysine derivatives[1].

From CD specra of isopoly(Thy-L-lys) (Fig.V-2), the cross-over point near 260 nm and large peak at 216 nm ($[\theta]_{216} \fallingdotseq -10000$) were obtained at pH 10.4. These facts suggests that isopoly(Thy-L-lys) exists as ordered conformation: anti-parallel β-sheet struc-
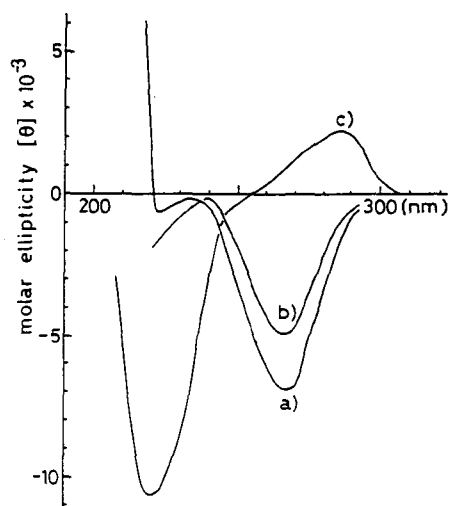
Fig.V-2.   CD Spectra of isopoly(Thy-L-lys)-95 (ref. 3) in ethylene-
glycol-water (1/1, v/v) mixed solvent at pH (a) 11.83; (b) 10.86;
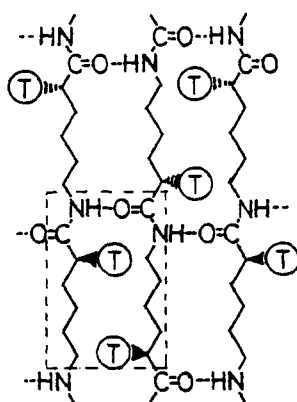(c) 10.31.

Fig.V-3.   Anti-parallel β-sheet structure of isopoly(Thy-L-lys):
Ⓣ = Thymine Base.

ture at lower pH. The β-sheet structure was reported for isopoly-(L-lysine)[3].

From this fact isopoly(Thy-L-lys) may exist as β-sheet conformation in aqueous solution when pH of the system is low and the content of β-sheet conformation of the polymer may increase with decreasing pH of the system. Therefore, at pH 10.4, isopoly(Thy-L-lys) exists partially in the anti-parallel β-sheet conformation as shown in Fig.V-3. In this conformation, the thymine bases of neighboring units are present on the opposite side of the β-sheet, and the nearest thymine bases are present on the neighboring chain of β-sheet as shown in FigV-3. When these thymine bases form the photodimer, a strain may be occurred in the β-sheet structure, because the length of the side chain of isopoly(Thy-L-lys) is too small to form the photodimer of the grafted thymine bases with ease. This situation was supported from CPK model for the anti-parallel β-sheet structure of isopoly(Thy-L-lys). This strain may lower the quantum yield for the photodimerization of thymine bases. The maximum conversion of the photodimer for isopoly-(Thy-L-lys) was about 60% at pH 10.4, and the CD peak below 230 nm in aqueous solution at pH 10.4 was decreased with increasing the conversion of the photodimer. These facts support that the photo-dimerization of thymine bases in isopoly(Thy-L-lys) gives the strain against the ordered β-sheet structure.

V-3. Experimental

V-3-1. Materials

The thymine-base-containing derivatives used here was isopoly-
L-lysine. This polymer was prepared by the grafting reaction of
the activated ester derivative of thymine base (Thy-PCP) with
isopoly(L-lysine hydrobromide) according to the method reported
ealier (Scheme V-1)[3]. Thymine-base content in the polymer was
determined as 84 mol% by measuring the NMR spectra of the polymer
in DMSO-d_6. The degree of polymerization was determined by the
data of vaper pressure osmometry (VPO) as 20^3. All the reagents
and organic solvent were used after purification in the usual man-
ner.

V-3-2. Instrumentation

Ultraviolet spectra were measured at 25°C, with a UNION-SM-401
spectrophotometer equipped with a temperature controller in a 10-mm
pathlength quartz cell. The pH measurement was carried out on a
Hitachi-Horiba F-7-SS pH meter. For CD spectroscopy a JASCO J-400
spectropolarimeter was used. The measurement was made sith a 1.0
mm pathlength quartz cell at ambient temperature. The concentra-
tion of the polymer kept at 1 x 10^{-4}mol/L of the monomer unit.

V-3-3. Photochemical Reaction

Photochemical reactions were carried out with a 10-mm pathlength
quartz cell filled with 4mL of solution at 20°C. A Philips SP

lamp (500-w superhigh pressure mercury lamp) filtered through a combination of the glass filters ($\lambda > 280$ nm) was used as the light source.   The concentration of thymine-base derivatives was kept constant at 1 x 10^{-4} mol/L of thymine unit in an aqueous solution.   A stream of purified nitrogen gas was passed through the solution for more than 15 min to displace the air present before the reaction.   All the aqueous solution was buffered with 0.2 mol/L of sodium bicarbonate in a sodium carbonate buffer system.

V-4. Summary

    The photodimerization reaction of grafted thymine bases in thymine-containing isopoly-L-lysine derivatives was studied over a wide range in aqueous solution.   It was stated that the photodimerization of the grafted thymine bases was greatly affected by the conformation of the polymer main chain and that its anti-parallel β-sheet structure inhibited the formation of photodimer.

V-5. References

(1) Y.Suda, Y.Inaki, and K.Takemoto, J. Polymer Sci. Polymer Chem. Ed., 21, 2813 (1983).

(2) Y.Suda, Y.Inaki, and K.Takemoto, J. Polymer Sci. Polymer Chem. Ed., 22, (1984) in press.

(3) T.Yamamoto, Y.Inaki, and K.Takemoto, Polymer Preprints, Japan, 31, 1597 (1982).

Chapter VI. Synthesis and Photochemical Reaction of Polymethacry-

late Derivatives Containing 6-Cyanouracil

VI-1. Introduction

For the estimation of the intramolecular interaction of thymine

bases in the synthetic polymers the photodimerization study of the

bases was utilized as described in the previous chapters. On the

other point of view, the intermolecular photodimerization of

thymine bases in the polymers makes the polymer insoluble, so that

the development of a new nega-type photoresist may be expected.

Because thymine bases dimerize effectively with irradiation of the

UV light at 280 nm (deep UV)[1], the high sensitivity at UV region

may be expected as compared with polyvinylcinnamate type photore-

sists which are allowed to react with the light at 320 nm[2].

In this concern, a study on the photodimerization of thymine

bases for the development of a new type of photoresist has been

done. From the results, the quantum yields for the photodimeri-

zation of thymine bases in the polymethacrylate derivative were

reported as 0.0074 in dimethyl sulfoxide (DMSO)[3] and 0.34 in

polymethylmethacrylate film[4], respectively. These quantum yields

may be low for the use as photoresist. On the other hand , it is

well known that orotic acid (6-carboxyuracil), which exists in

vivo, has much higher quantum yield for the photodimerization than

thymine base[1]. The reason for such high quantum yield for the

photodimerization is reported that orotic acid has electron-with-

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drawing group at 6-position of the pyrimidine ring[1]. The present

chapter concerns with the preparation of 6-cyanouracil, as a model

of orotic acid, in which the electron-withdrawing cyano group

exists at 6-position of the pyrimidine ring, and the photochemical

reaction of the 6-cyanouracil after its incorporation in polymetha

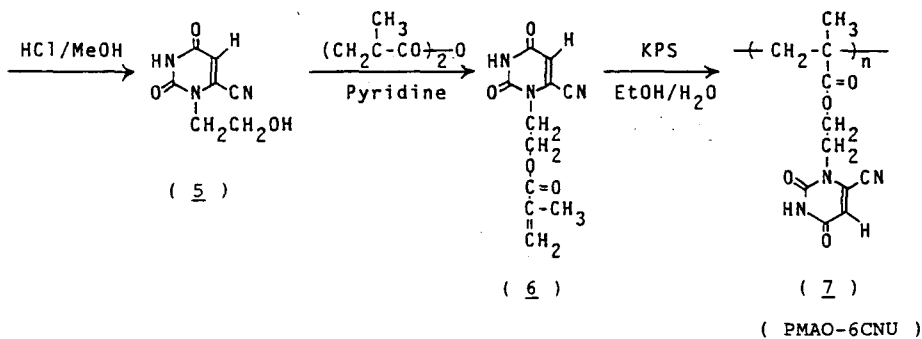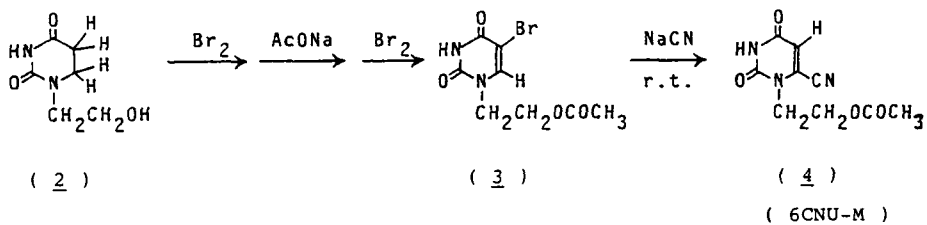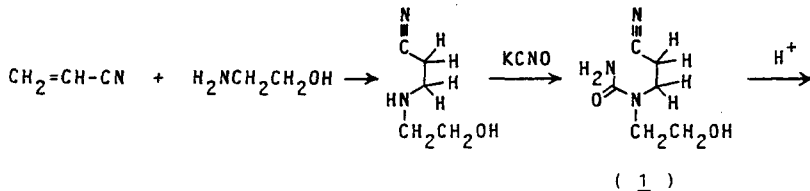crylate derivatives as well as its monomeric model compound.

VI-2. Results and Discussion

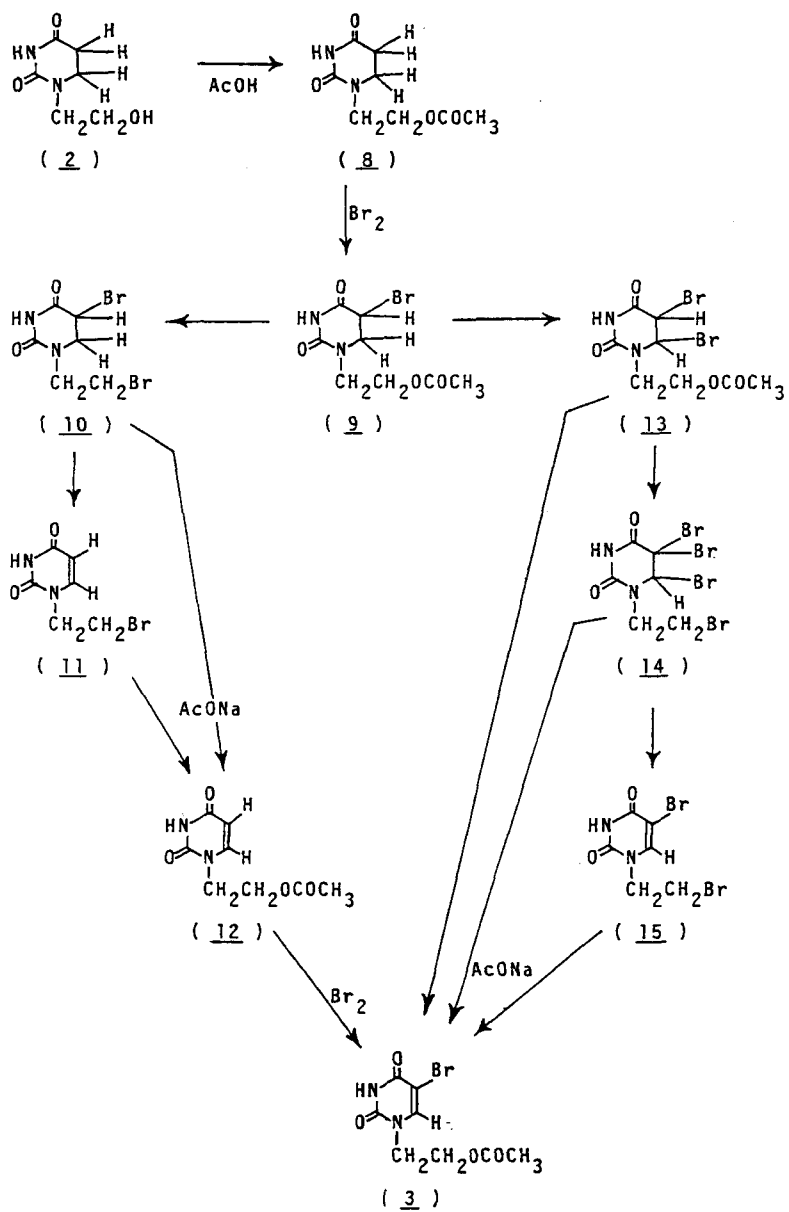VI-2-1. Preparation of the Polymer Containing 6-Cyanouracil

Polymethacrylate derivative having 6-cyanouracil moieties in

the side chain (7) (PMAO-6CNU) was prepared by a free radical

polymerization of the corresponding monomer according to Scheme VI-1.

The monomer, (6), 1-(2-methacryloyloxyethyl)-6-cyanouracil, was

obtained by the reaction of 1-(2-hydroxyethyl)-6-cyanouracil with

methacrylic anhydride. The reaction of 1-(2-acetoxyethyl)-5-

bromouracil (3) with sodium cyanide was carefully carried out to

give 1-(2-acetoxyethyl)-6-cyanouracil. It was reported that,

with exess amount of sodium cyanide and/or at higher temperature,

the corresponding 5-cyanouracil was obtained[5]. 1-(2-Acetoxyethyl)-

-5-bromouracil was prepared according to Scheme VI-1. The compound

(4), 1-(2-acetoxyethyl)-6-cyanouracil (6CNU-M), was used as

the monomeric model compound for the photoreaction.

It was confirmed that the reaction from 1-(2-hydroxyethyl)-5,6-

dihydrouracil (2) to compound (3) in Scheme VI-1 was occurred

according to Scheme VI-2. In acetic acid, 2-hydroxyethyl group of

(Scheme VI-1)

$CH_2=CH-CN$ + $H_2NCH_2CH_2OH$ $\longrightarrow$ [structure] $\xrightarrow{KCNO}$ [structure (1)] $\xrightarrow{H^+}$

(1)

[structure (2)] $\xrightarrow[\text{AcONa}]{Br_2}$ $\xrightarrow{Br_2}$ [structure (3)] $\xrightarrow[\text{r.t.}]{NaCN}$ [structure (4)]

(2)   (3)   (4)

(6CNU-M)

$\xrightarrow{HCl/MeOH}$ [structure (5)] $\xrightarrow[\text{Pyridine}]{(CH_2\overset{CH_3}{C}-CO)_2O}$ [structure (6)] $\xrightarrow[\text{EtOH/H}_2\text{O}]{KPS}$ [structure (7)]

(5)   (6)   (7)

(PMAO-6CNU)

(Scheme VI-2)

(2) was changed to 2-acetoxyethyl group (compound (8)), which was confirmed by its thin layer chromatography.   By the first bromination, 1-(2-acetoxyethyl)-5-bromo-6-hydrouracil (9) may be formed.   However, the acetyl group was substituted to the bromo group by the formed hydrogen bromide and 1-(2-bromoethyl)-5-bromo-6-hydrouracil (10) was isolated with yield of 48%.   At the same time, the uracil derivatives having 5,6-double bond was existed in the pyrimidine ring; 1-(2-bromoethyl)-uracil (11) and 1-(2-bromoethyl)-5-bromouracil (15), were obtained with yield of 11% and 7%, respectively.   The compound (11) is derived from compound (10), because (11) was obtained by refluxing the DMF solution of (10).   The fact that (15) was obtained suggests that the dibromination is also occurred at the first bromination and that the reaction via compounds (13) and (14) is given. Compound (10) was derived to 1-(2-acetoxyethyl)-uracil (12) with sodium acetate in the presence of small amount of DMF with refluxing.   Since (11) was obtained with refluxing the DMF solution of (10), the reaction from (10) to (12) may be the reaction via (11).   Compound (15) was reacted with sodium acetate in refluxing acetic acid to give (3) with high yield of 83%.   This fact may suggest that it is possible to obtain (3) from (2) with the first bromination and following treatment of sodium acetate.   However this direct path may not be the main reaction because the yield of (15) from (2) was low (< 10%). It might be possible that (3) was derived directly from compound

(13) or (14).   By the second bromination at room temperature,
(3) was obtained from (12) at high yield of 80%.

The UV spectral data of the polymer and the monomeric model
compound were measured in DMF and DMSO solution at 25°C.   The UV
spectral data are listed in Table VI-1.

VI-2-2.   Isolation and Identification of the Photodimer

Photolysis of the monomeric model compound of 6-cyanouracil
gave a photoproduct in a high yield, while that of thymine deriva-
tives was hardly reacted[3].   To confirm the photoproduct of 6-cyano-
uracil derivatives, the acetone sensitized photolysis was carried
out for 1-(2-acetoxyethyl)-6-cyanouracil (6CNU-M).   The photo-
lysis was carried out in acetone solution (9.0 x 10^{-3}mol/L) in a
Pyrex reaction vessel with light from a 500-w high pressure mercury
lamp for 2 hr.   After the photolysis, the solvent was distilled off,
which gave oily residue.   The oily residue was washed with diethyl
ether and recrystallized from acetone-chloroform to give rose colored
crystal.   The product obtained was a single product without accom-
panying any by-product.   The data of elemental analysis suggest
that the photoproduct was a cyclobutane type photodimer.   From the
NMR spectrum of the photoproduct in DMSO-d$_6$, it was found that the
peak of 5-H in 6-cyanouracil (6.60 ppm) disappeared and a new
peak appeared at 3.55 ppm.   The chemical shift of this new peak
was almost identical to that of 5-methine proton of the cyclobutane
type photodimer of orotic acid (6-carboxyuracil)[6].   In the UV

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spectrum of the photoproduct in methanol solution, the absorbance

near 290 nm, which was observed for the 6-cyanouracil derivative,

disappeared. From these facts, it was also suggested that the

photoproduct of 6-cyanouracil derivative was the cyclobutane type

photodimer.

The photodimer, however, was not able to be detected by mass

spectroscopy and only the starting material (6CNU-M) was given.

From this fact, it was well suggested that this photodimer is

fairly unstable at higher temperature and the reverse reaction

was proceeds easily. This was supported by the fact that the

starting material was recovered during the purification of the

photoproduct on heating. Both for NMR and for UV spectra, the

similar phemonemon was observed. In NMR spectra, after heating

the DMSO-d_6 solution of the photoproduct at 100°C for a few

minutes, the peak of 5-H originated from 6-cyanouracil appeared

at 6.60 ppm and the methine proton of the photodimer (3.55 ppm)

disappeared. Also in UV spectra, when the solution was heated at

60°C for a few minutes, the absorbance of 6-cyanouracil (290 nm)

again appeared. The structure of the photodimer might be predomi-

nantly the trans-syn type photodimer as was reported in the case

of orotic acid[6].

VI-2-3. Photodimerization Reaction

The photochemical reactions of 6-cyanouracil bases in the

polymer and the monomeric model compound were carried out in DMSO

and DMF solution at concentration of 1 x 10^{-4} mol/L of base unit. The absorbance of 6-cyanouracil (290 nm) in the polymer and the monomeric model compound decreased with irradiating UV light.   As the photoproduct obtained in DMSO or DMF solution was the same as that obtained in acetone solution, the decrease of absorbance at 290 nm may correspond to the formation of the photodimer.   The conversions of the photodimer for PMAO-6CNU and 6CNU-M are plotted in Fig.VI-1.   In the case of the monomeric model compound of thymine base, the formation of photodimer was not observed at the low concentration (1 x 10^{-4} mol/L)[3,7].   On the other hand, the photodimerization reaction of 6-cyanouracil in the monomeric model compound occurred at the low concentration.   This marked difference in reactivity between 6-cyanouracil and thymine might be derived from the stability of the excited state of the bases.   It is known that the life time of the triplet state of orotic acid (0.1 msec)[8] is much longer than that of thymine base (0.5 μsec)[9].

The rate of photodimer formation of 6-cyanouracil in the polymer (PMAO-6CN), was found to be about 10 times greater than that in the monomeric model compound (6CNU-M) (Fig.VI-1).   It is to be noted that the intramolecular photodimerization may occur predominantly in the photolysis of the polymer under this condition. It is reported that the intramolecular photodimerization proceeds much faster than the intermolecular photodimerization for the thymine derivatives[3].

In Table VI-2, quantum yields for the photodimerization of

6-cyanouracil in the polymer and the monomeric model compound in

DMSO and DMF solutions, by irradiating the monochromatic light at

290 nm are listed.   The effect of the solvent on the quantum

yield appears not to be observable.   The quantum yield for the

monomeric model was similar to that of uracil base but smaller

than that of orotic acid obtained in aqueous solution[1].   The

quantum yield for PMAO-6CN was about 5 times greater than that of

the polymethacrylate derivative of thymine base in DMF solution[3].

In Fig.VI-2, dependencies of the quantum yield for the photo-

dimerization on the wavelength of the irradiating monochromatic

light are shown.   It was found that the quantum yield tended to

increase with irradiating shorter wavelength of light.   This fact

suggests that the intersystem crossing proceeds probably from the

higher vibrational levels of the singlet state, and thus the

photodimer of 6-cyanouracil was formed from the triplet state.

That is, for almost all of the organic compounds, vibrational

levels are so short lived (10^{-13} sec) relative to the lowest

level of the singlet ($> 10^{-9}$ sec) that their contribution to the

intersystem crossing is negligible.   However, for pyrimidine base,

the singlet lifetime is much shorter (10^{-12} sec) at room tempera-

ture, so that the excited molecule spends relatively more time in

higher vibrational levels, and the excitation by higher energy

photons to these levels increases in intersytem crossing[1].   Then,

the photodimerization from a singlet state is independent on the

wavelength, while the photodimerization from a triplet state is
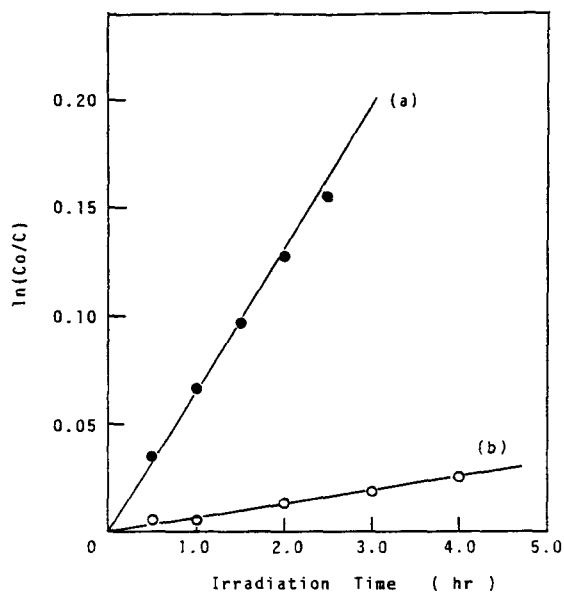
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Fig.VI-1. Photolysis of 6-cyanouracil derivatives in DMSO solution:
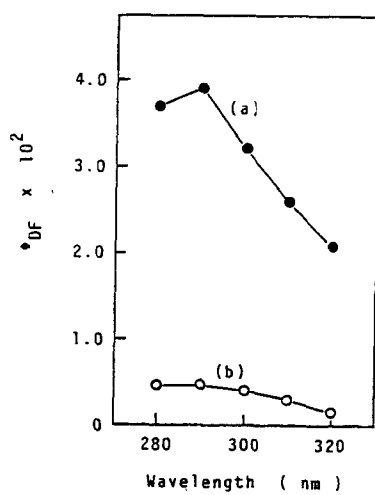(a) PMAO-6CNU; (b) 6CNU-M.

Fig.VI-2. Relationships between the quantum yield for the photodimerization of 6-cyanouracil and the wavelength of the irradiating monochromatic light in DMSO solution: (a) PMAO-6CNU; (b) 6CNU-M.

dependent on the wavelength of the irradiation light.   In the
case of thymine derivatives, the photodimer from the monomeric or the
dimeric model compounds was derived from the triplet state, and
the photodimer produced from the polymer was predominantly derived
from the singlet state[3,7].   For the 6-cyanouracil derivatives,
however, the dependency of the quantum yield for the photodimeri-
zation on the irradiation wavelength was observed even in the case
of the polymer.   This fact may suggest that the photodimerization
of 6-cyanouracil in the polymer is predominantly derived from the
excited triplet state.   High reactivity of 6-cyanouracil deriva-
tives for the photodimer formation mentioned above should be
caused by the stability of the triplet state.

VI-2-4. Lithographic Characteristics

    For the evaluation of the polymers for the photoresist, the
sensitivity test was carried out.   Results of test are listed in
Table VI-3.   In this table, the result for thymine containing
polymer is also listed for the comparision.   The value of the
minimum energy for keeping the membrane of 6-cyanouracil containing
polymer was found to be one third less compared with thymine con-
taining polymer.   This fact suggests that the 6-cyanouracil con-
taining polymer is more desirable than the thymine containing poly-
mers for the photoresist.

Table VI-1. UV Spectral data of 6-cyanouracil derivatives

Compound	λ_{max} (nm)		ϵ_{max}		Hypochromicity (%)[a]	
	DMSO	DMF	DMSO	DMF	DMSO	DMF
6CNU-M	292	292	7860	8550	—	—
PMAO-6CNU	292	292	7230	7510	8.7	12.2

a) Hypochromicity based on 6CNU-M.

Table VI-2. Quantum yields for the photodimeri-
zation of 6-cyanouracil in the compounds[a]

Compound	Φ_{DF}[b]	
	DMSO	DMF
6CNU-M	0.0042	0.0048
PMAO-6CNU	0.041	0.039

a) All data for solution 1×10^{-4} mol/l of
   base units.
b) Irradiation at 290 nm

Table VI-3. Lithographic sensitivity test for the photoresist

Compound	λ (nm)[a]	λ_{max} (nm)[b]	E (mj/cm^2)[c]
PMAO-6CNU	255 – 355	305	1.95×10^{-2}
PEI-T-βAla[d]	210 – 310	275	6.49×10^{-2}

a) The range of the photosensitive wavelength.
b) The maximum absorption wavelength.
c) The minimum energy for keeping the membrane with irradiation
   at the maximum absorption wavelength.
d) Polyethyleneimine derivatives having grafted thymine bases
   and β-alanine units; see chapter II.

VI-3. Experimental

VI-3-1. Materials

1-(2-Hydroxyethyl)-5,6-dihydrouracil (2)

Acrylonitrile (55ml, 0.84mol) was added to ethanolamine (42 ml, 0.70mol) at 0°C, and the mixture was allowed to reflux for 30 min. The obtained viscous oily product was dissolved in 3N HCl (270ml), and this solution was added dropwise to a aqueous solution (200ml) of potassium cyanate (70g). After stirring for 12hr at room temperature, the solvent was evaporated to give oily residue (1). The residue was dissolved in methanol (600ml), and precipitated KCl was filtered off. To the filtrate was added aqueous methanolic hydrochloric acid (conc. HCl; 100ml, water; 300ml, methanol; 300ml), and refluxed for 3 hr. Then the solvent was evaporated to dryness. The product was extracted from the residue with hot ethanol, the filtrate was cooled to give (2) as colorless crystals; yield 82.7g (75%); mp 143-145°C. IR (KBr) 3330, 3200, 3050, 2930, 1720, 1670, 1500, 1380, 1290, 1245, 1210, 1065, 885, 780, and 750 cm^{-1}. NMR (D_2O) δ 2.75 (d, 2H), 4.60 (m, 6H).

1-(2-Acetoxyethyl)-5-bromouracil (3)

The compound (2) (78.8g, 0.5mol) was dissolved in 100ml of acetic acid. Then, 30ml (0.58mol) of bromine was added dropwise under refluxing, which was refluxed for additional 2hr. Then, to the solution, 5ml of N,N-dimethylformamide (DMF), 10ml of acetic anhydride, and 82g (1.0mol) of sodium acetate was added

in the order. The reaction mixture was further refluxed for 2hr.

After the filtration, the reaction system was cooled at 0 °C. To

the solution, 28ml (0.54mol) of bromine was added dropwise at 0 °C

and the reaction system was stirred for 1 hr at room temperature.

After the solvent was evaporated, the residue was recrystallized

from ethanol to give a product ($\underline{3}$); yield 80.3g (58%). mp 195-

198 °C. IR (KBr) 3190, 3020, 2820, 1750, 1710, 1650, 1620, 1460,

1435, 1370, 1355, 1255, 1235, 1165, 1040, 965, 880, and 770 cm^{-1}.

Anal. Calcd. for $C_8H_9O_4N_2Br$: C 30.59; H 2.98; N 11.86; Br 34.13.

Found C 30.64; H 3.01; N 11.91; Br 34.04.

1-(2-Acetoxyethyl)-6-cyanouracil (4) (6CNU-M)

To 100ml of DMF solution of compound (3) (17.4g, 63mmol)

sodium cyanide (3.3g, 67mmol) was added and the reaction mixture

was stirred for 1 day at room temperature. After the solvent was

distilled off under vacuum at room temperature, the residue was

recrystallized from water to give 8.2g of colorless needles; yield

59%, mp 206-209°C. IR (KBr) 3020, 2800, 2250, 1750, 1715, 1690,

1600, 1480, 1420, 1360, 1260, 1060, 1030, 890, and 760 cm^{-1}. NMR

(DMSO-d$_6$) δ 2.05 (s, 3H), 4.20 (q. 4H), 6.40 (s, 1H),

11.95 (s, 1H).

Anal. Calcd for $C_9H_9O_4N_3$: C 48.43; H 4.04; N 18.83: Found C 48.19;

H 4.06; N 18.77.

1-(2-Methacryloyloxyethyl)-6-cyanouracil (6)

To 100ml of methanol solution of compound (4) (3.0g, 13.4

mol), 3ml of 12N hydrochloric acid was added.   The reaction

solution was refluxed for 1 hr.   After the solvent was evaporated

off, recrystallization from ethanol gave 2.2g of colorless crystal,

1-(2-hydroxyethyl)-6-cyanouracil ($\underline{5}$); yield 2.2g (90%), mp

249-251°C.   IR (KBr) 3370, 3100, 2990, 2820, 2225, 1710, 1600,

1480, 1440, 1400, 1365, 1250, 1165, 1055, 1020, 960, 900, 865, and

760 cm^{-1}. NMR (DMSO-d$_6$) δ 3.60 (t, 2H), 3.83 (t, 2H), 6.51

(s, 1H), 11.70 (b, 1H).

The compound ($\underline{5}$) (2.2g, 12mmol) was dissolved again in

15ml of pyridine, and 3.7ml (24mmol) of methacrylic anhydride

was added.   The reaction solution was then stirred for 1 day at

room temperature.   After the solvent and the unreacted

methacrylic anhydride were distilled off under vacuum, the residue

was recrystallized from ethanol to give 2.5g of colorless needles

($\underline{6}$); yield 82.6%, mp 193-194°C.   IR (KBr) 3200, 3090, 3020,

2800, 2225, 1730, 1600, 1470, 1420, 1370, 1325, 1160, 1020, 960,

880, 860, and 765 cm^{-1}.   NMR (DMSO-d$_6$) δ 1.80 (s,3H), 4.20

(q, 4H), 5.90 (d, 2H), 6.60 (s, 1H), 11.95 (b, 1H).

Anal. Calcd. for C$_{11}$H$_{11}$O$_4$N$_3$: C 53.01; H 4.45; N 16.86.   Found   C

52.65; H 4.50; N 16.73.

Poly[1-(2-methacryloyloxyethyl)-6-cyanouracil] (7)(PMAO-6CNU)

Polymerization of the compound ($\underline{6}$) was carried out at 60°C

for 18 hr in water-ethanol (1/1, v/v) mixed solvent with

potassium persulfate (KPS) as the initiator.   The conversion

was 60%.   The molecular weight of the polymer obtained was 10000

by vapor pressure osmometry (VPO).   IR (KBr) 3200, 3040, 2800,

2225, 1710, 1610, 1470, 1400, 1360, 1240, 1160, 1020, 840, and 760 cm^{-1}. NMR (DMSO-d$_6$) δ 0.85 (b, 3H), 1.83 (b, 2H), 4.12 (m, 4H), 6.50 (s, 1H), 11.60 (s, 1H).

VI-3-2. Preparation of the Photoirradiate product of 6-Cyanouracil
        Derivative

    Preparative photolysis was carried out in a single Pyrex reac-
tion vessel. The 6-cyanouracil derivative (3) (6CNU-M) (1.0g,
45mmol) was dissolved in 500ml of acetone (9.0 x 10^{-3} mol/L) and
irradiated with a 500-w high pressure mercury lamp for 2 hr.   The
vessel was cooled by immersing it in ice water.

    After the irradiation, the solvent was distilled off under
vacuum at room temperature.   The residue was washed with diethyl
ether and chloroform.   Recrystallization from acetone-chloroform
gave slightly rose-colored crystal; yield 0.8g (80%); mp 200°C.
IR (KBr) 3220, 3080, 2950, 2840, 2220, 1730, 1690, 1470, 1420,
1400, 1370, 1340, 1310, 1220, 1060, 820, and 760 cm^{-1}. NMR (DMSO
-d$_6$) δ 2.06 (s, 6H.), 2.55 (b,2H), 4.30 (b, 8H) 11.50 (s, 2H).
Anal. Calcd. for C$_{18}$H$_{18}$O$_8$N$_6$: C 48.43; H 4.06; N 18.83. Found C
48.22; H 4.15; N 18.57.

VI-3-3. Measurement of the Quantum Yield for the Photodimerization
    Photolysis was done in a 10-mm quartz cell filled with 4mL of
solution at 20 C.   A JASCO SS-25 monochrometer coupled with a xenon
arc lamp was used as the light source.   Light intensity was deter-

mined by potassium ferrioxalate actinometry. The quantum yield
for oxalic acid decomposition was 1.24 at 313 nm. The concentra-
tion of 6-cyanouracil derivatives was kept constant at 1×10^{-4} mol/L
of base unit either in DMSO or in DMF solution. The solvent, DMSO
and DMF, was degassed with purified nitrogen before use.

VI-3-4. Instrumentation

   UV Spectra were measured with a UNION SM-401 spectrophotometer
at 25°C. NMR Spectra were measured with a Hitachi R-600 high
resolution NMR spectrometer at 25°C; tetramethylsilane (TMS) or
sodium-2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as
an internal standard.

VI-3-5. Lithographic Evaluation

   The sensitivity test in question was carried out by Japan
Synthetic Rubber Co. Ltd.. A JASCO CT-40 monochrometer was used
as the light source for the test. The polymer was dissolved in
DMF (5% w/v). The DMF solution was casted to give a membrane
of the polymer (d = 1 μm) on the aluminum spinner plate. After
the photolysis, the membrane was soaked in DMF for 30 sec to dis-
solve out the unreacted part of the membrane. The minimum energy
value for keeping the membrane was computed in account of the
jointing property of the polymer to the aluminum plate.

VI-4. Summary

Polymethacrylate derivative containing 6-cyanouracil moieties and its monomeric model compound were synthesized, and the photochemical reaction of these compounds was studied in dimethyl sulfoxide and N,N-dimethylformamide solution. The monomeric model compound was found to react by irradiating UV light even its low concentration. The product from the monomeric model compound was readily prepared and was identified as the cyclobutane type photodimer. The stability of the photodimer against the temperature was relatively lower than that of thymine bases. It was also shown that the photodimerization reaction was predominantly derived from the excited triplet state even in the case of the polymer. The quantum yield for the photodimerization of 6-cyanouracil was much greater than that of thymine bases. With the results of the sensitivity test for the photoresist, it was suggested that the 6-cyanouracil containing polymer has good property for the photoresist.

VI-5. References

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CONCLUSION

The purpose of the present study was to be cleared the rela-
tionships between the properties of the polymers such as molecular
weight, molecular structure and the conformation and the photo-
dimerization of thymine or other pyrimidine bases present in the
side chain of the synthetic polymers.

In Chapter I, the effects of the degree of polymerization and
pH of the system on the photodimerization of the grafted thymine
bases were investigated by using oligo- and polyethyleneimine
derivatives containing pendant thymine bases.   It was found that
the anionized thymine base has lower quantum yield of photodimeri-
zation than that of neutral thymine base, and it was assumed that
the singlet energy migration was able to occur particularly in the
case of copolymer.

In Chapter II, the effect of the singlet energy migration on
the photodimerization was confirmed by use of the modified oligo-
and polyethyleneimines having grafted thymine bases and α- or β-
alanine units as a spacer groups.   And the influence of the
structure of the side chain of the compound was also reported.

In Chapter III, the effect of the conformation of polymer main
chain on the photodimerization of grafted thymine bases was inves-
tigated for thymine-containing poly-lysine derivatives.   It was
stated that the photodimerization was greatly affected by the con-
formation of the polymer main chain and that its helical structure

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brought about the photodimerization of grafted thymine bases.

In Chapter IV, the photoinduced conformational change of thymine containing poly-lysine derivatives was investigated in order to see whether the helical structure was favorable compared with the unordered one for the photodimerization. It was proved that the helical structure of the polymer was fixed by the photodimer between thymine bases at the side chain and that the fixed part was not affected by the pH change in the system. That is, it was succeeded to realize the fixed conformation of the polymer. This fixed conformation should be useful property for a new functional polymer.

In Chapter V, the effect of conformation of the polymer main-chain on the photodimerization was investigated in addition to chapter III, by using isopoly-L-lysine derivatives. It was found that anti-parallel β-sheet conformation of the polymer inhibited the photodimerization of the grafted thymine bases.

In Chapter VI, the photodimerization reaction of 6-cyanouracil of polymethacrylate derivatives and the monomeric model compound was investigated. It was found that 6-cyanouracil has high reactivity for the photodimerization compared with thymine base. Also in this chapter, the lithographic evaluation of the pyrimidine-containing polymers for the photoresist was done and it was suggested that the pyrimidine-containing polymer has good property for the photoresist.

SUPPLEMENTARY TREATISE

The supplementary treatise is as follows:

Synthesis of Oligomer Models of Polyethyleneimine Derivatives
Containing Pendant Thymine Bases

Y.Inaki, Y.Sakuma, Y.Suda, and K.Takemoto

J. Polymer Sci. Polymer Chem. Ed., <u>20</u>, 1917 (1982).

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