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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 Containing Isopoly-L-Lysine Derivative

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

Polymer J., in contribution.

CONTENTS

PREFACE i

CONTENTS iii

GENERAL INTRODUCTION 1

Chap. I Photochemical Reactions on Oligo- and Polyethyleneimines Which Contain Pendant Thymine Bases 4

- I-1. Introduction 4
- I-2. Results and Discussion 5
- I-3. Experimental 19
- I-4. Summary 21
- I-5. References 21

Chap. II Photochemical Reactions of Oligo- and Polyethyleneimines Containing Pendant Thymine Bases : Effect of Spacer 23

- II-1. Introduction 23
- II-2. Results and Discussion 24
- II-3. Experimental 41
- II-4. Summary 44
- II-5. References 45

Chap. III Photodimerization of Pendant Thymine Bases in Thymine Containing Poly-Lysine Derivatives 47

- III-1. Introduction 47
- III-2. Results and Discussion 47
- III-3. Experimental 67
- III-4. Summary 74

III-5. References 74

Chap. IV Photoinduced Conformational Change of Thymine Containing Poly-Lysine Derivatives 76

IV-1. Introduction 76

IV-2. Results and Discussion 77

IV-3. Experimental 94

IV-4. Summary 95

IV-5. References 96

Chap. V Photodimerization of Pendant Thymine Bases in Thymine Containing Isopoly-L-Lysine Derivative 97

V-1. Introduction 97

V-2. Results and Discussion 97

V-3. Experimental 104

V-4. Summary 105

V-5. References 105

Chap. VI Synthesis and Photochemical Reaction of Polymethacrylate Derivatives Containing 6-Cyanouracil 106

VI-1. Introduction 106

VI-2. Results and Discussion 107

VI-3. Experimental 118

VI-4. Summary 123

VI-5. References 123

CONCLUSION 125

SUPPLEMENTARY TREATISE 127

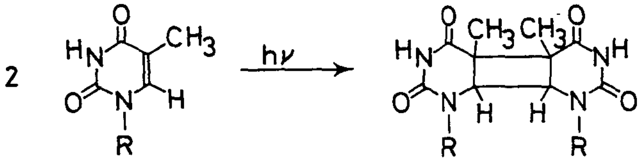
ACKNOWLEDGEMENT 128

GENERAL INTRODUCTION

In recent years, studies on the functional polymers have received much attention, and they have been becoming one of the most important field in chemical industry. The functional polymers cover many attractive ones, such as polymer catalysts, semiconductive polymers, 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 photoresponsible polymer[1], the photocoductive polymer[2] and so on have been exceedingly developed. It is known that the most popular photosensitive 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 photobiology 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



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 photodimerization, 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 development on a series of subjects, in order to clarify the relationship of the properties of polymer in paticular, molecular weight, molecular 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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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 intramolecular 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 sequence of thymine bases in the copolymers[7].

Another factor that may influence the photodimerization of thymine bases in the polymer structure is the degree of polymerization. This chapter deals with the reactions of the oligo- and polyethyleneimine 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 corresponding model compounds (Fig.I-1) were carried out in aqueous solution. 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 intermolecular 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 corresponding 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 increased 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].

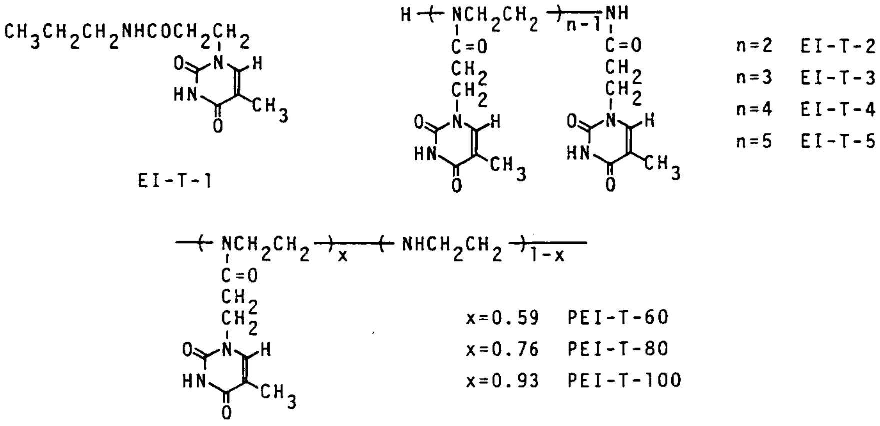


Fig.I-1. Thymine derivatives.

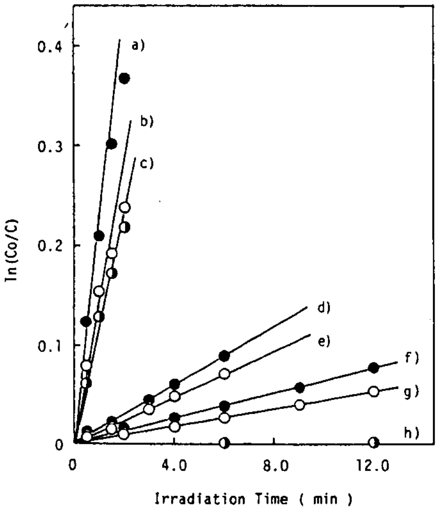


Fig.I-2. Photolysis of thymine derivatives in aqueous solution at pH 9.7: (a) PEI-T-100; (b) PEI-T-80; (c) PEI-T-60; (d) EI-T-5; (e) EI-T-4; (f) EI-T-3; (g) EI-T-2; (h) EI-T-1.

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 x 10^{-4} mol/L of thymine unit.

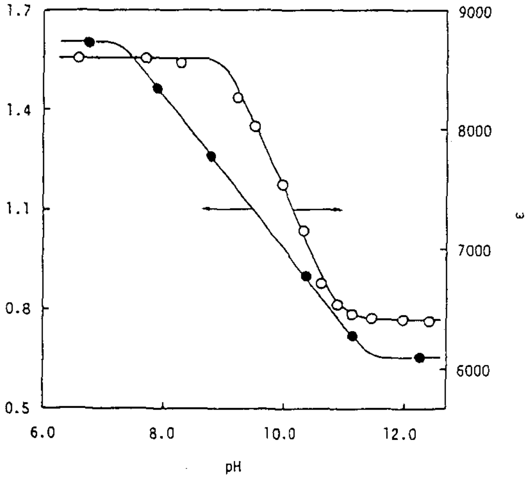


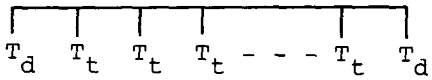
Fig.I-3. Dependence of molar extinction coefficient at 270 nm and quantum yield for the photodimerization of EI-T-3 on pH of the system.

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 photodimerization 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 negatively charged thymine bases but by the decrease in the photochemical 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).

(Scheme I-1)



$$\phi_{DF} = \phi_d + (\phi_t - \phi_d)\cdot(Nt/N) \qquad (I-1)$$

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

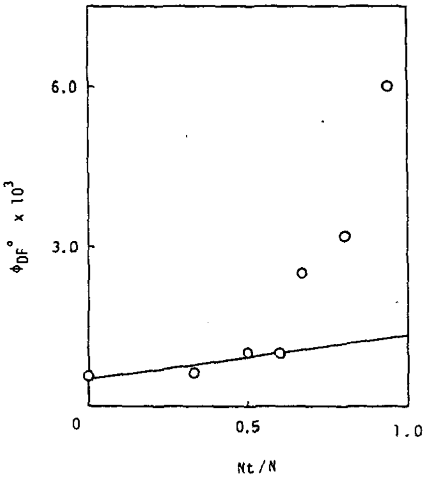


Fig.I-4. Relationship between quantum yield of photodimerization in aqueous solution at pH 11.3 and Nt/N value.

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-dimethylformamide (DMF), was carried out in DMF solution ($1x10^{-4}$mol/L). The thymine base of EI-T-1 did not react; this suggests that the intermolecular photodimerization does not succeed under the conditions 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

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

Compound	Φ^{o}_{DF}
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 x 10^{-4} mol/L of thymine unit.

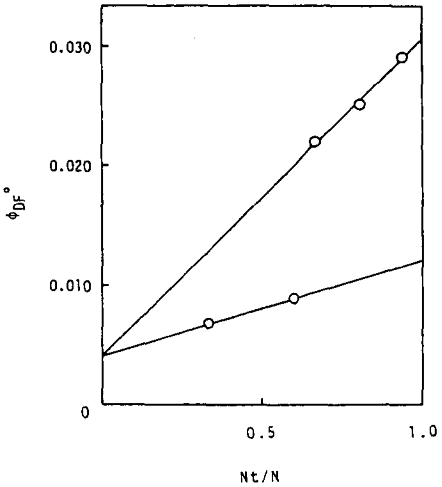


Fig.I-5. Relationship between quantum yield of photodimerization in DMF solution and Nt/N value.

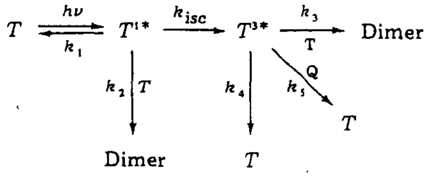
of the thymine bases in the copolymers. Migration of the singlet energy in DNA should be recalled in this connection.

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^0_{DF}/Φ_{DF}) versus [Q], where Φ^0_{DF} represents 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)



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

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

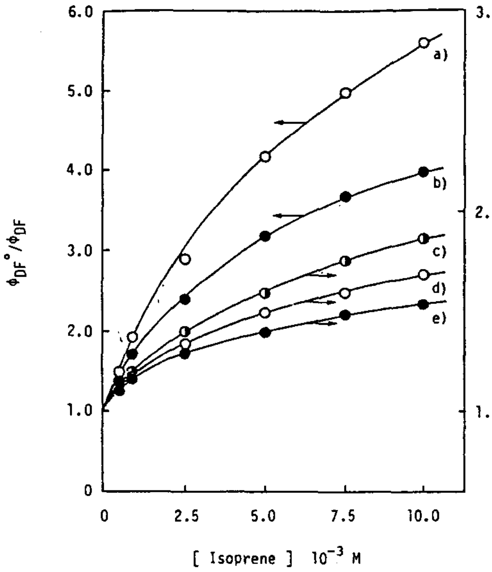


Fig.I-6. Stern-Volmer plots in DMF solution: (a) EI-T-3; (b) EI-T-5; (c) PEI-T-60; (d) PEI-T-80; (e) PEI-T-100.

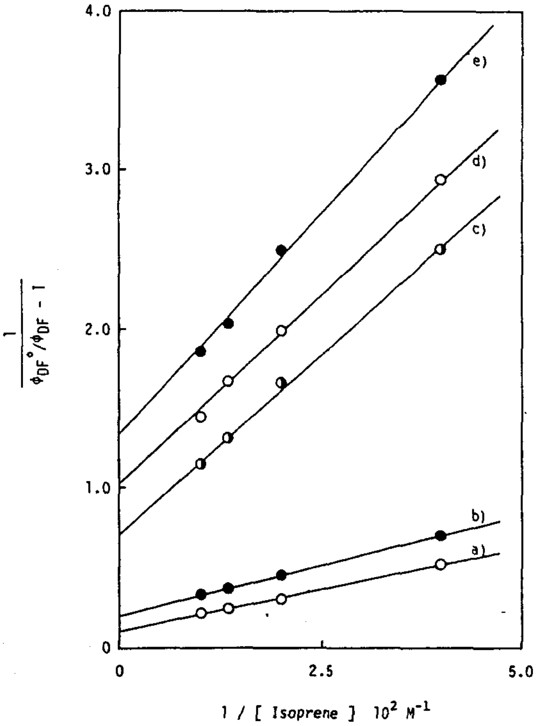


Fig.I-7. Relationship between 1/(ϕ^{0}_{DF}/ϕ_{DF}-1) and 1/[isoprene] in DMF solution: (a) EI-T-3; (b) EI-T-5; (c) PEI-T-60; (d) PEI-T-80; (e) PEI-T-100.

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 photodimerization 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 :

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

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

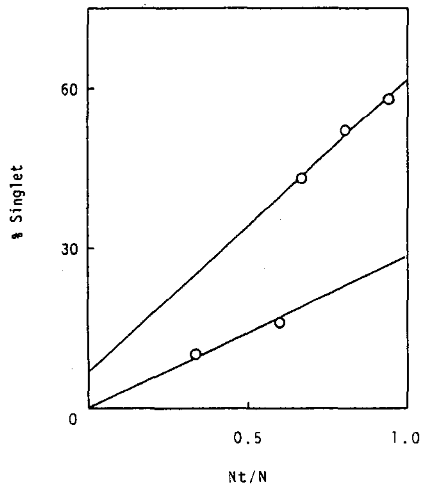


Fig.I-8. Relationship between S value and Nt/N value.

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 Nd/N (= 1 - Nt/N) values; this suggests the effect of the nearest neighboring thymine units.[8] For the photodimerization, however, 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

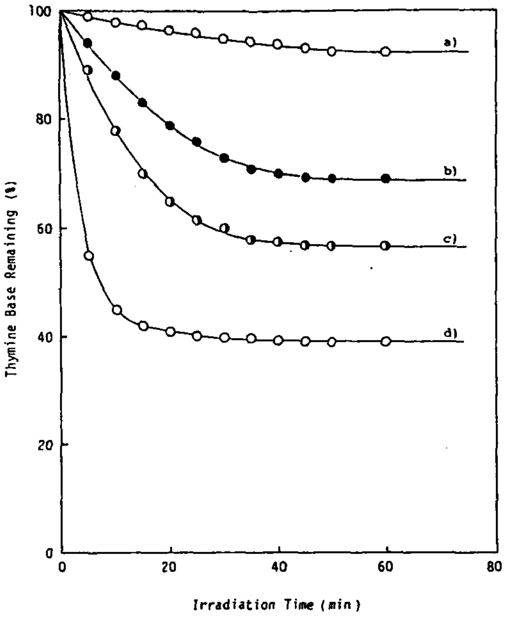


Fig.I-9. Photolysis of thymine derivatives in polyMMA film: (a) EI-T-1; (b) EI-T-3; (c) EI-T-5; (d) PEI-T-100.

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

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 solution, compared with those of polyacrylate and polymethacrylate derivatives.

The quantum yield for polyethyleneimine derivatives in polyMMA film was about 18 times higher than that in solution, but, the maximum 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 derivatives were prepared by the thermal polymerization of a mixed solution 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.

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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

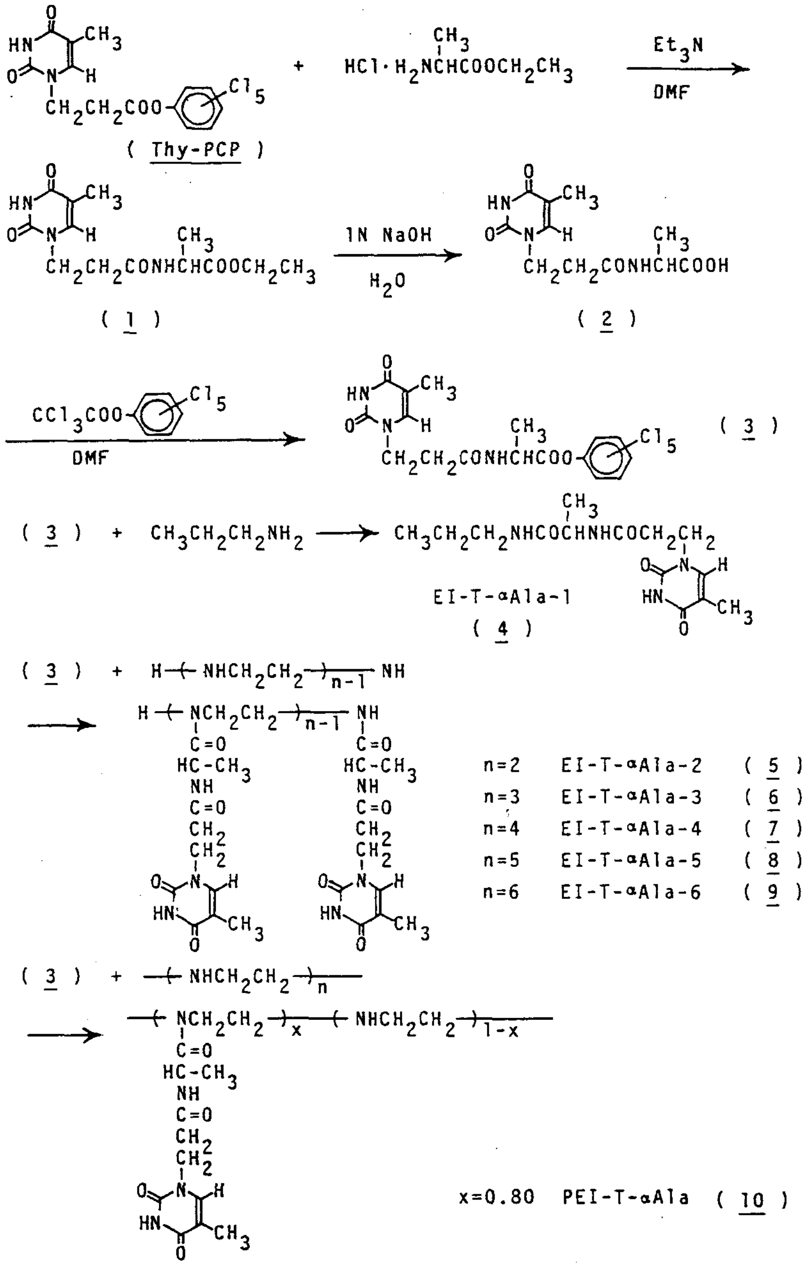
The derivatives used here are three kinds of oligo- and polyethyleneimines having grafted thymine bases. One kind of compounds were abbreviated as EI-T-n and PEI-T-n, which have no spacer groups between the main chain and the grafted thymine moieties (Fig.II-1). Another kind of compounds were abbreviated 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.

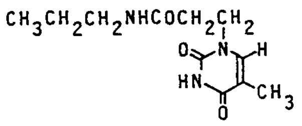
II-2-2. Intramolecular Interaction in the Ground State

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:

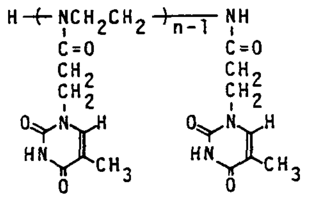
$$h = h_d + (h_t - h_d)(Nt/N) \qquad (II-1)$$

(Scheme II-1)

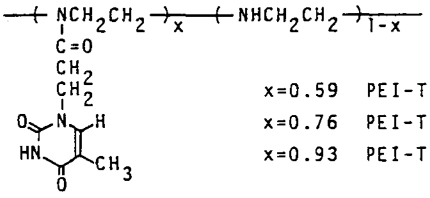

(Thy-PCP)
Et3N
DMF
(1)
1N NaOH
H2O
(2)
CCl3COO
DMF
(3)
EI-T-αAla-1
(4)
n=2 EI-T-αAla-2 (5)
n=3 EI-T-αAla-3 (6)
n=4 EI-T-αAla-4 (7)
n=5 EI-T-αAla-5 (8)
n=6 EI-T-αAla-6 (9)
x=0.80 PEI-T-αAla (10)



EI-T-1

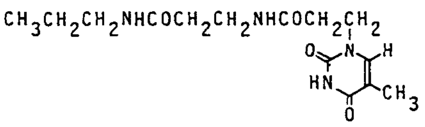


n=2 EI-T-2
n=3 EI-T-3
n=4 EI-T-4
n=5 EI-T-5

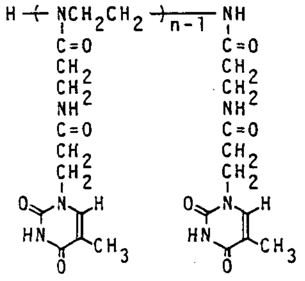


x=0.59 PEI-T-60
x=0.76 PEI-T-80
x=0.93 PEI-T-100

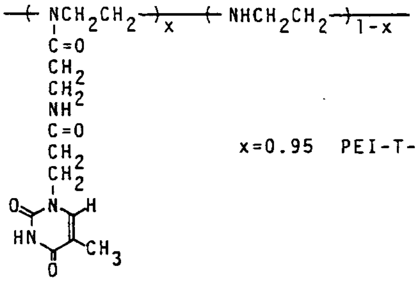
Fig.II-1. Thymine base derivatives having no spacer group.



EI-T-βAla-1



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



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.

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.

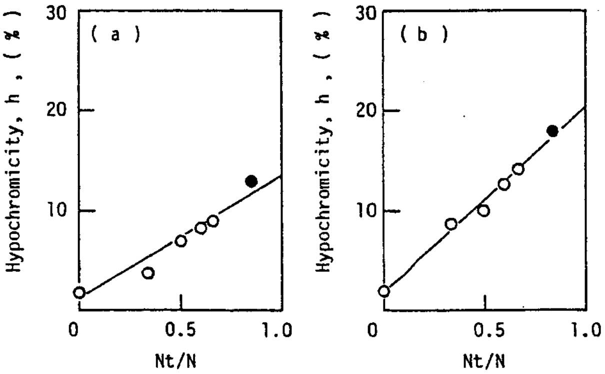
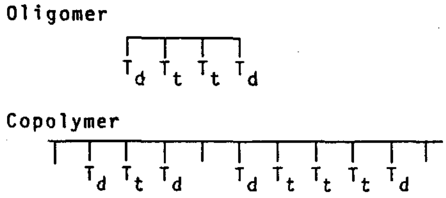


Fig.II-3. Relationships between hypochromicity values (h) of thymine derivatives and Nt/N values in aqueous solution for EI-T-αAla-n and PEI-T-αAla at pH (a) 12.7; (b) 6.0: (○) Oligomers; (●) Copolymers .

where h_d and h_t represent the hypochromicity values of Td and Tt thymine units in Scheme II-2, respectively. The linear relationship 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.

(Scheme II-2)



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 conformation for the intramolecular interaction; hypochromicity[6]. By comparing EI-T-n with EI-T-αAla-n, the steric effect of methyl group 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

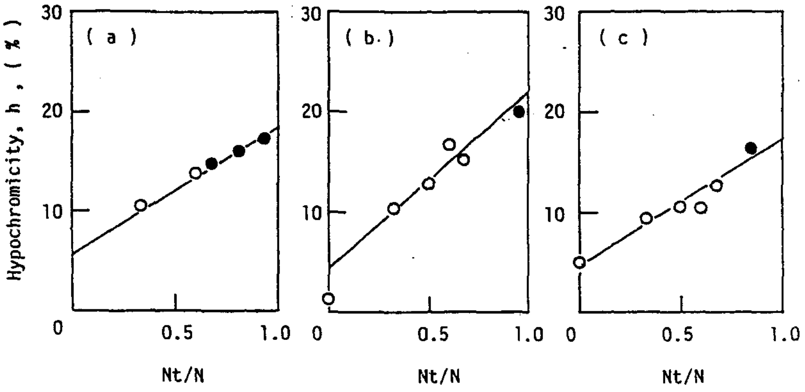


Fig.II-4. Relationships between hypochromicity values (h) of thymine derivatives and Nt/N values in DMSO solution for (a) EI-T-n and PEI-T-n; (b) EI-T-βAla-n and PEI-T-βAla; (c) EI-T-αAla-n and PEI-T-αAla: (○) Oligomers; (●) Copolymers.

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

	Φ_{DF} b)		
Compound	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	0.00064c)	——d)
EI-T-3	0.00060	0.00072c)	0.0068
EI-T-4	0.0010	0.0012c)	——d)
EI-T-5	0.0010	0.0014c)	0.0084
PEI-T-60	0.0025	0.0068c)	0.016
PEI-T-80	0.0032	0.0084c)	0.017
PEI-T-100	0.0060	0.015c)	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.

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 reaction condition used here. This fact suggests that the intermolecular photodimerization or other photoreactions, for example, photohydration reaction, can be neglected. Therefore, only the intramolecular 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 deprotonation at N-3 position in thymine base (pKa ≒ 10), have significantly 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]:

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

where ϕ_d and ϕ_t represent the quantum yields for the photodimerization 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 intramolecular interaction could not be observed both in the ground and excited 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

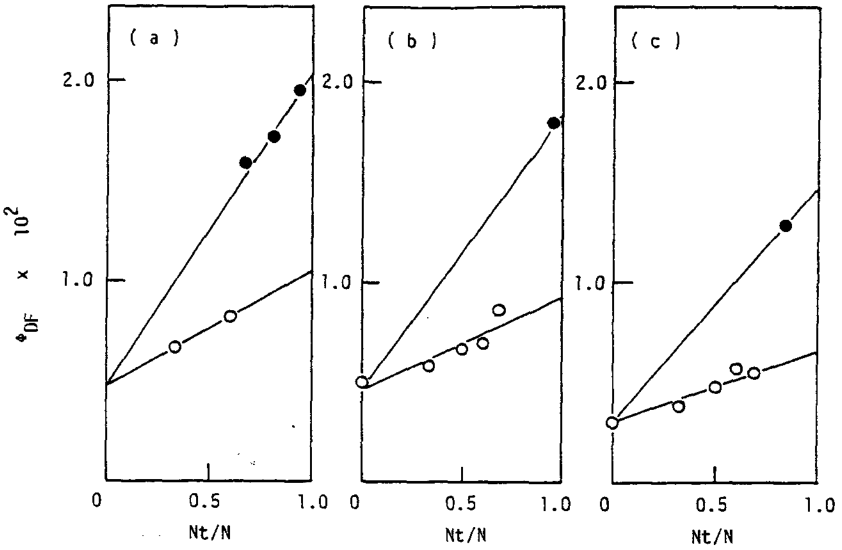


Fig.II-5. Relationships between quantum yields for the photodimerization of thymine bases (Φ_{DF}) and Nt/N values in DMSO solution for (a) EI-T-n and PEI-T-n; (b) EI-T-βAla-n and PEI-T-βAla; (c) EI-T-αAla-n and PEI-T-αAla: (○) Oligomers; (●) Copolymers.

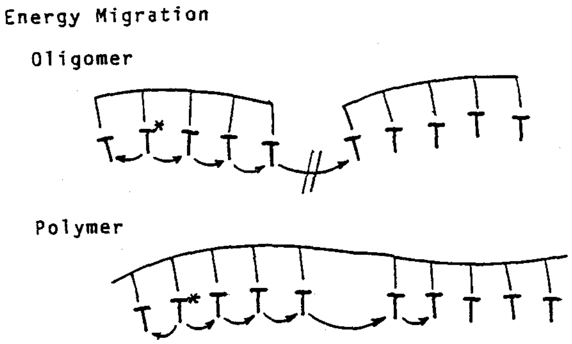


Fig.II-6. Singlet energy migration in the oligomers and copolymers.

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):

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

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):

$$\Phi_{DF} = \phi_d + (f_t\phi_t - \phi_d)(Nt/N) \qquad (II-4)$$

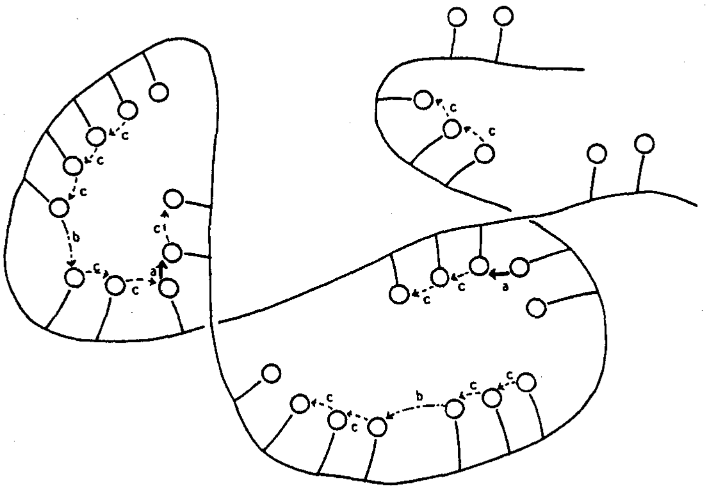


Fig.II-7. Singlet energy migration in a polymer: (a) a long range energy migration between remote units; (b) a short range energy migration along the polymer chain; (c) a energy migration in a sequence.

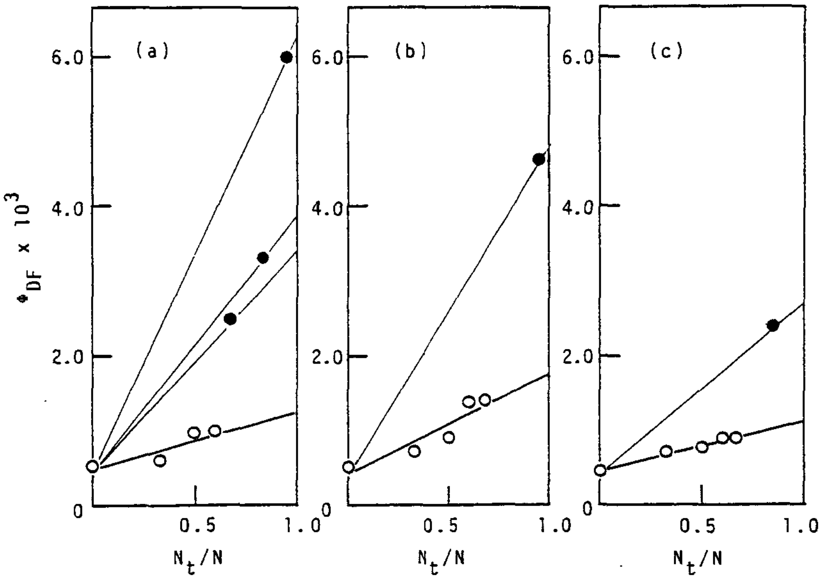


Fig.II-8. Relationships between quantum yields for the photodimerization of tymine bases (Φ_{DF}) and Nt/N values in aqueous solution at pH 11.3 for (a) EI-T-n and PEI-T-n; (b) EI-T-βAla-n and PEI-T-βAla; (c) EI-T-αAla-n and PEI-T-αAla: (○) Oligomers; (●) Copolymers.

This equation was also derived from the data of PEI-T-n in DMF solution (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 essentially 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 intramolecular photodimerization of thymine bases was influenced by the interaction of them in the ground state, particularly for the oligomeric 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 copolymer 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 migration 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.

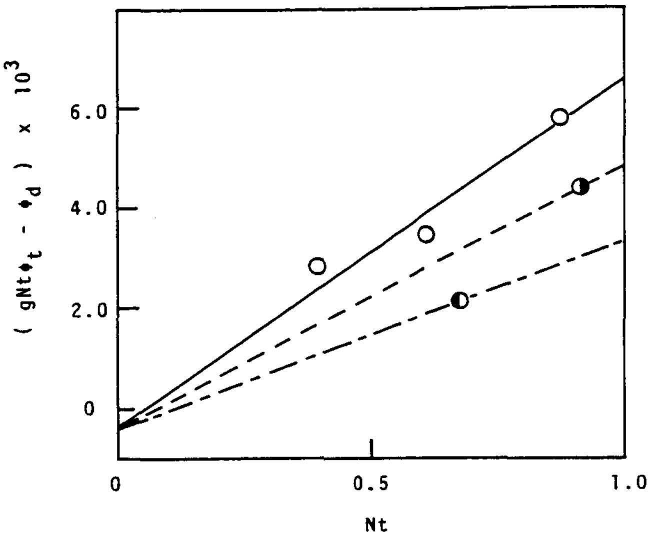


Fig.II-9. Relationships between (gNtϕ_t-ϕ_d) values and Nt values at pH 11.3: (○) PEI-T-n; (◑) PEI-T-βAla; (◐) PEI-T-αAla.

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, pentachlorophenyl 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. Recrystallization 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 (λ>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 1×10^{-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 molecular 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 thymine 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

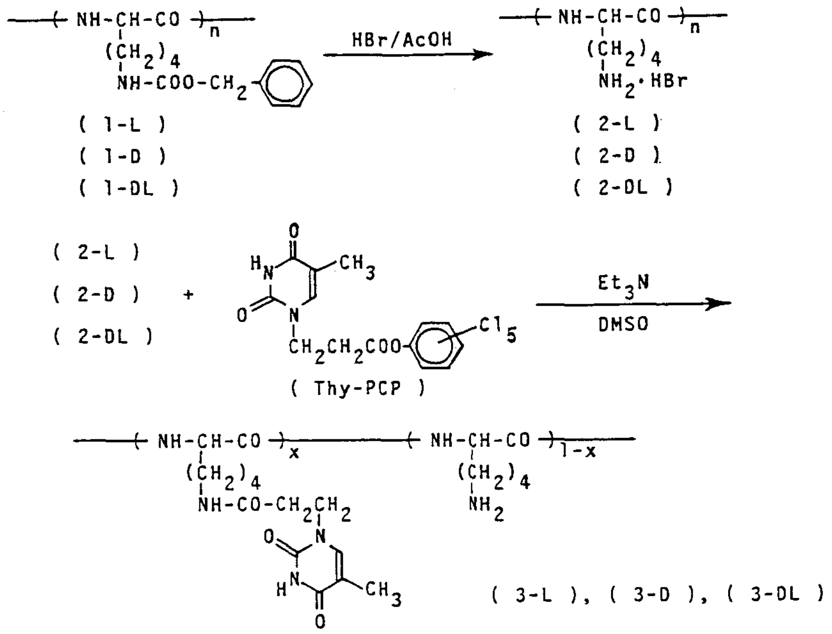
The polymers used here were poly-D-, poly-L-, and poly-DL-lysines that contained pendant thymine bases. These polymers were prepared by the grafting reaction of thymine base, Thy-PCP, according to Scheme III-1. Based on the absolute configuration of the asymmetric carbon atoms in lysine residue, these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

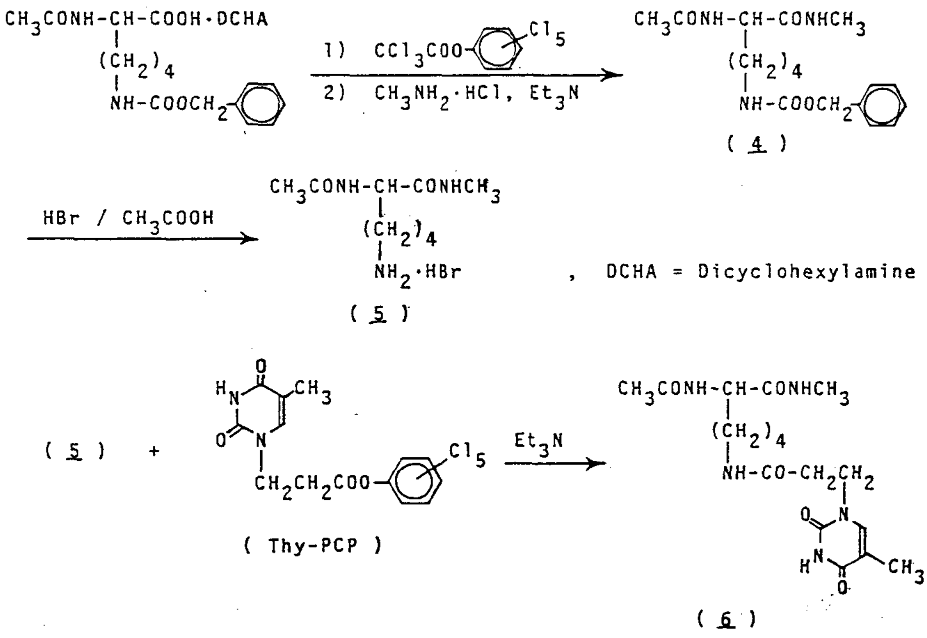
Nuclear magnetic resonance spectra of these compounds were measured in DMSO-d_6 at 90°C (Table III-3). The NMR data for poly(Thy-L-lys)s and oligo(Thy-L-lys) have been reported[8,9] and were exactly same as those for poly(Thy-D-lys)s. It appears that no marked difference in NMR spectra could be detected 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)



(Scheme III-2)



DCHA = Dicyclohexylamine

were measured in aqueous solution. The thymine unit concentration was low (1 x 10^{-4}mol/L) to avoid intermolecular interaction between 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):

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

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 insolubility 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.

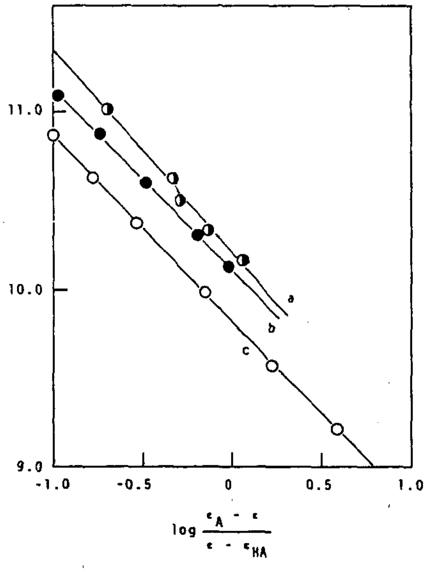
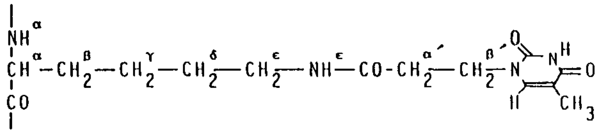


Fig.III-1. Relationship between pH and log($\varepsilon_A - \varepsilon$)/($\varepsilon - \varepsilon_{HA}$) of thymine derivatives: (a) poly(Thy-D-lys)-88; (b) poly(Thy-D-lys)-72; (c) T-M.

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.



b) T-M: 1.84ppm ($\underline{CH_3}CONH$-); 7.50ppm ($CH_3\underline{NH}CO$-); 2.56ppm ($\underline{CH_3}NHCO$-).

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]		
	0.5*N* NaOH	6*N* HCl	0.5*N* NaOH	6*N* HCl	p*K*$_a$
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.

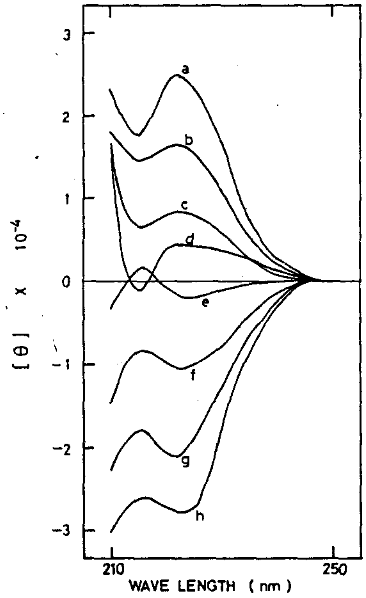


Fig.III-2. CD Spectra of poly(Thy-D-lys)-88 at pH: (a) 10.18; (b) 10.63; (c) 11.02; (d) 11.35; CD spectra of poly(Thy-L-lys)-93 at pH: (e) 11.16; (f) 10.80; (g) 10.61; (h) 10.28.

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.

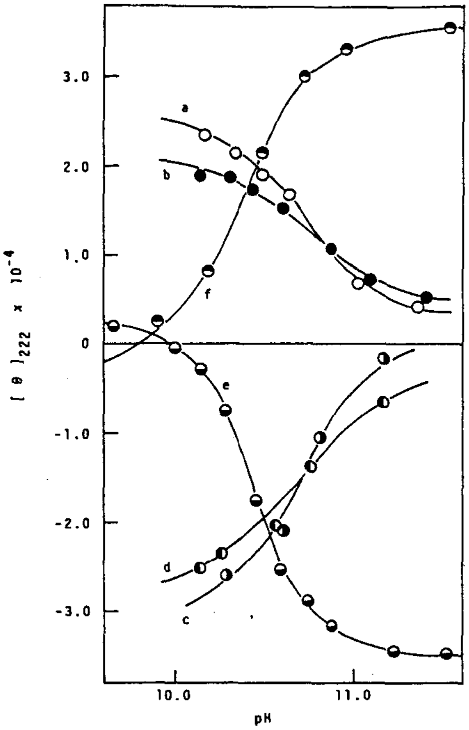


Fig.III-3. Dependence of molar ellipticity at 222 nm ($[\theta]_{222}$) on pH: (a) poly(Thy-D-lys)-88; (b) poly(Thy-D-lys)-72; (c) poly(Thy-L-lys)-93; (d) poly(Thy-L-lys)-79; (e) poly(L-lysine); (f) poly(D-lysine).

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:

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

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

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

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:

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

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

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

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):

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

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; $k^1 \gg 1$. In this case eq.(III-8) can be rewritten as eq.(III-9):

$$h \fallingdotseq [\mathrm{NH_2}] \qquad \text{(III-9)}$$

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

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

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

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

where $[\theta]_{222}^{\mathrm{NH_3^+}}$ and $[\theta]_{222}^{\mathrm{NH_2}}$, 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):

$$\mathrm{T} \rightleftharpoons \mathrm{T^-} + \mathrm{H^+} \qquad \text{(III-12)}$$

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

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

$$[\mathrm{NH_3^+}] + [\mathrm{NH_2}] + N = 1 \qquad (\text{III-15})$$

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):

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

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

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

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, $k^T >> 1$ [eq.(III-19)]:

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

The helix content of the copolymer can then be written as

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

$$A = \frac{K^1}{1 + K^1[\mathrm{H}^+]} \qquad B = \frac{[\mathrm{H}^+]}{K^{\mathrm{T}} + [\mathrm{H}^+]} \qquad (\text{III-21})$$

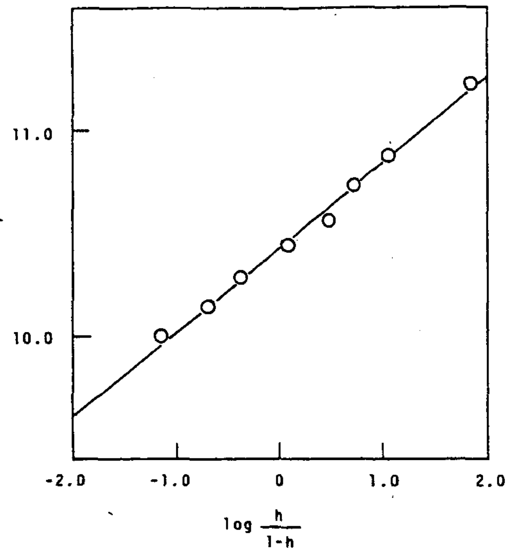


Fig.III-4. Relationship between pH and log(h/1-h) of poly(L-lysine)

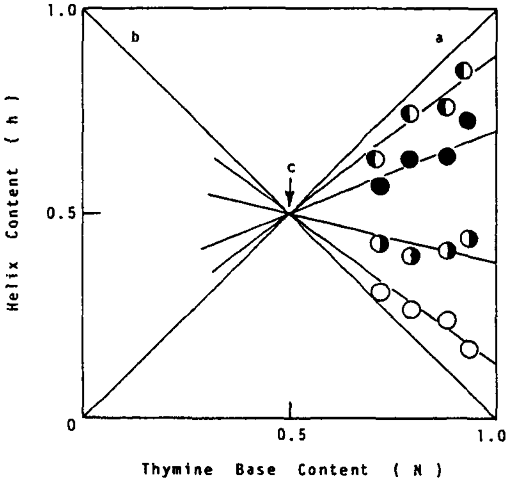


Fig.III-5. Relationship between hlix content (**h**) and thymine base content (N) of poly-lysine derivatives: pH = 10.4 (●); pH = 10.8 (◑); pH = 11.2 (○); pH = 10.0 (◐).

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 ($pH \ll pK^{1}, pK^{T}$) and line **b** is at high pH ($pH \gg pK^{1}, pK^{T}$). 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.

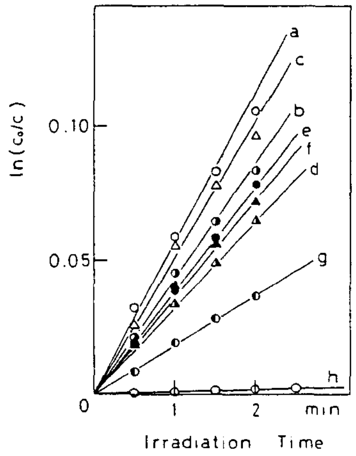


Fig.III-6. Photolysis of thymine base derivatives at pH 10.8: (a) poly(Thy-D-lys)-88; (b) poly(Thy-D-lys)-72; (c) poly(Thy-L-lys)-93; (d) poly(Thy-L-lys)-79; (e) poly-(Thy-DL-lys)-85; (f) poly(Thy-DL-lys)-79; (g) oligo(Thy-L-lys); (h) T-M.

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 substantially low (1×10^{-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 quantum 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)]

	Φ_0		
Compound	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

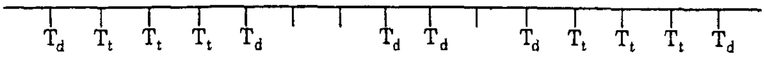
	Φ_{DF}		
Compound	pH 10.4	pH 10.8	pH 11.2
Poly(Thy-D-lys)-88	0.0077	0.0048	0.0037
Poly(Thy-D-lys)-72	0.0055	0.0033	0.0028
Poly(Thy-L-lys)-93	0.0084	0.0060	0.0042
Poly(Thy-L-lys)-79	0.0063	0.0043	0.0027
Poly(Thy-DL-lys)-85	0.0053	0.0032	0.0029
Poly(Thy-DL-lys)-79	0.0046	0.0030	0.0024
Oligo(Thy-L-lys)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

(Scheme III-3)



$$\sum T_d = N_d\ , \quad \sum T_t = N_t$$

$$N = N_d + N_t$$

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

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 photodimerization[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 derivatives (Chapter I). This fact indicates that the molecular weight 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 present in a helical structure has higher reactivity to the photodimerization than the thymine bases present in an unordered structure of poly(Thy-DL-lys)s. The straight lines in Fig.III-8 were extrapolated to Nt = 1 to give quantum yields of the photodimerization 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 photodimerization 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 region, about 30% of the quantum yield may be derived from the helical 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)

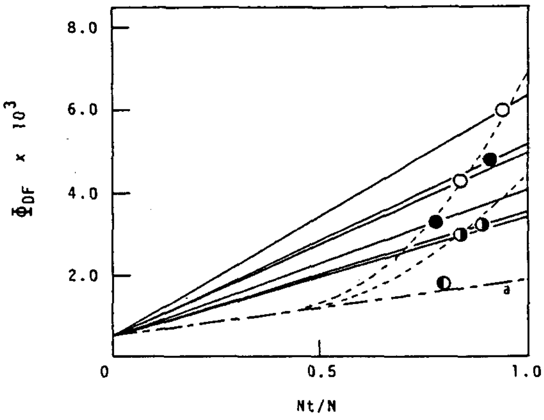


Fig.III-7. Relationship between quantum yield for the photodimerization (Φ_{DF}) and Nt/N value at pH 10.8: poly(Thy-L-lys)s (○); poly(Thy-D-lys)s (●); poly(Thy-DL-lys)s (◑); oligo(Thy-L-lys) (◐). The line a was obtained from the data of the oligomers of ethyleneimine derivatives (ref. 1).

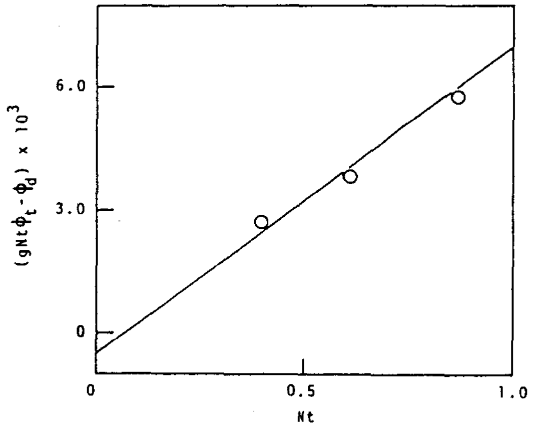


Fig.III-9. Relationship between Φ_{DF} and helix content (*h*) for poly-lysine derivatives with 100% content of thymine bases.

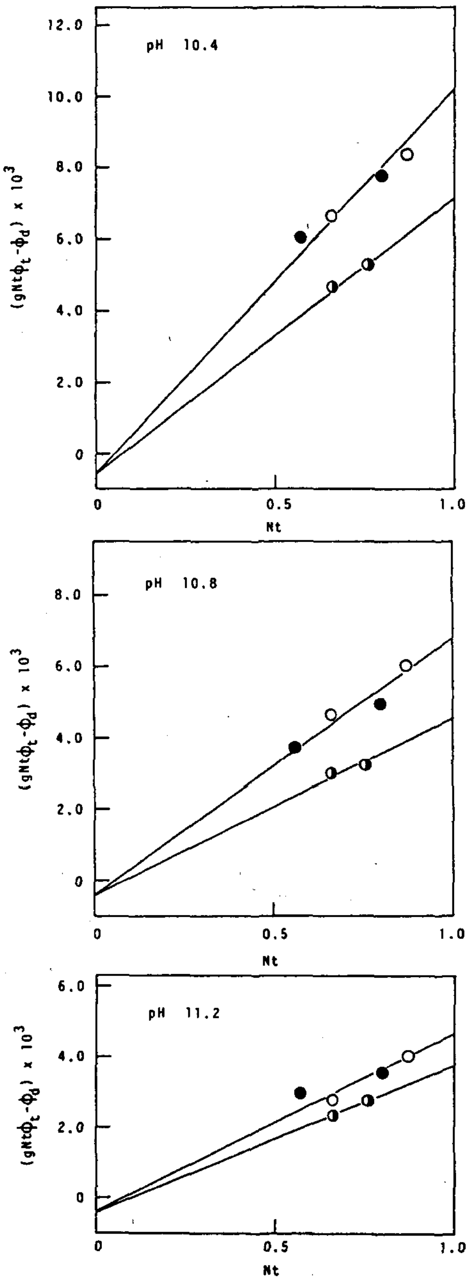


Fig.III-8. Relationships between ($gNt\Phi_t$ - Φ_d) and Nt values at each pH poly(Thy-L-lys)s (○); poly(Thy-D-lys)s (●); poly(Thy-DL-lys)s (◐).

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 dissolved 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 stirred 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^{ε}-Carboxyethylthymin-1-yl-L-lysine), Oligo(Thy-L-lys)

Oligo(Thy-L-lys) was obtained by the polymerization of N^{α}-carboxyanhydride of N^{ε}-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^{α}-Acetyl-N^{ε}-Carboxyethylthymin-1-yl-L-Lysine Methylamide (T-M)(6)

N^{α}-Acetyl-N^{ε}-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 (4): yield 31%, mp 163°C; IR (KBr) 3320, 1690, 1640, 1550, and 1280 cm^{-1}.

The compound (4) (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 (5) was precipitated, washed thoroughly with diethyl and petroleum ethers, and dried in vacuo.

To a solution of the compound (5) 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 1×10^{-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 containing poly-lysine derivatives

IV-1. Introduction

In a series of our studies on the synthetic nucleic analogues, both intermolecular and intramolecular interactions have been extensively 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 between a base grafted poly-L-lysine and a vinyl type polymer containing 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 descrived previous chapters. In chapter III, the effect of the conformation 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-

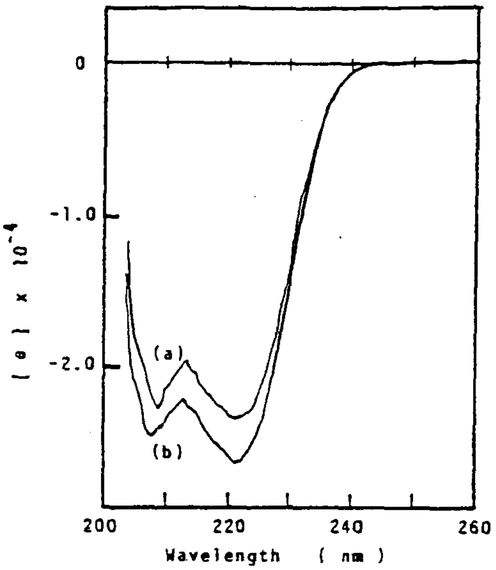
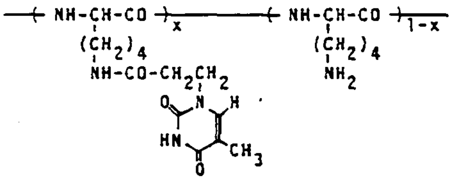


Fig.IV-1. CD Spectra of poly(Thy-L-lys)-93 at pH 10.2. Photolysis with a glass filter: (a) before (b) after irradiation.



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.

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, $|[\theta]_{222}|$, increased during UV irradiation.

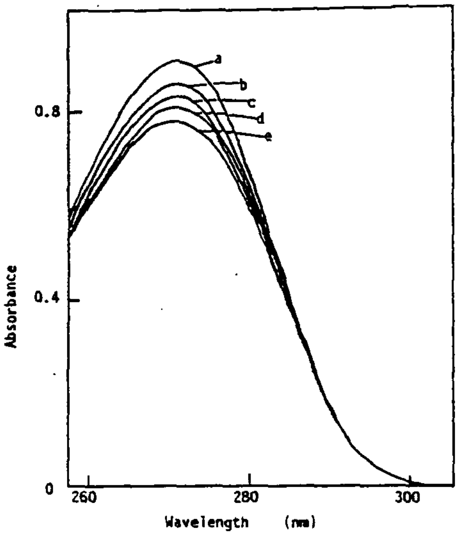


Fig.IV-3. UV Spectra of poly(Thy-L-lys)-93 at pH 11.0. Photolysis with a glass filter: (a) 0 min, (b) 3.0 min, (c) 6.0 min, (d) 10.0 min, (e) 15.0 min.

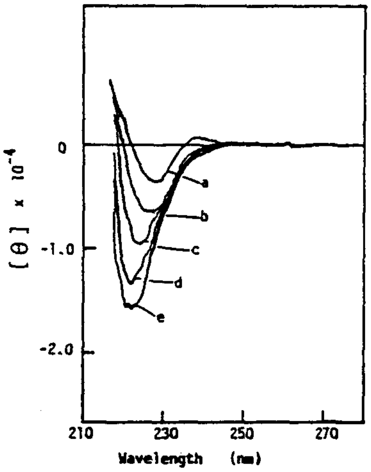


Fig.IV-4. CD Spectra of poly(Thy-L-lys)-93 at pH 11.0. Photolysis with a glass filter: (a) 0 min, (b) 3.0 min, (c) 6.0 min, (d) 10.0 min, (e) 15.0 min.

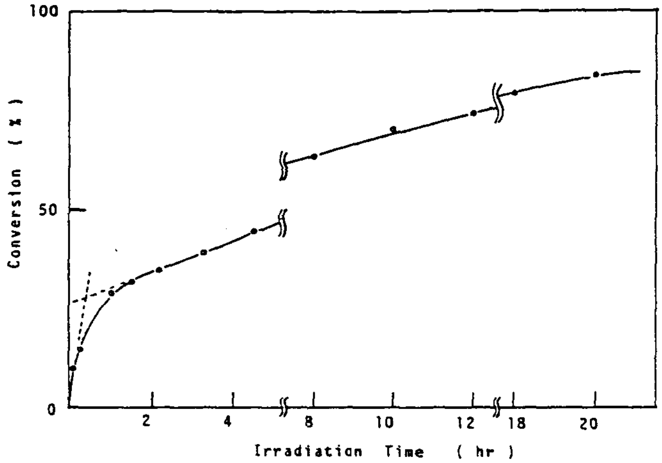


Fig.IV-5. A time-conversion curve for poly(Thy-D-lys)-88 at pH 11.2. Photolysis with a glass filter.

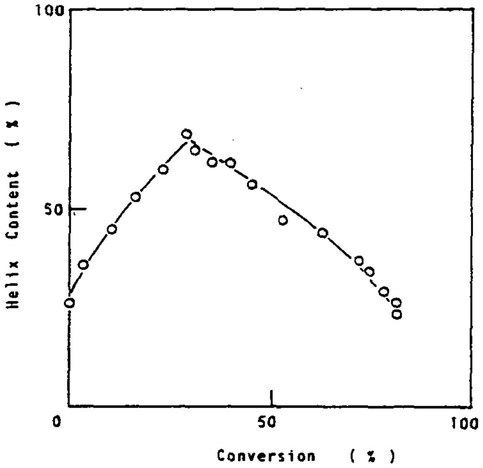


Fig.IV-6. The relationship between the conversion of thymine bases and the helix content of the polymer for poly(Thy-D-lys)-88 at pH 11.2. Photolysis with a glass filter

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 $|[\theta]_{222}|$ tended to decrease during UV irradiation. When thymine bases are present in poly(L-lysine) solution, the rate of decrease in the value of $|[\theta]_{222}|$ is slower than when thymine bases are present. These facts suggest that the photoracemization

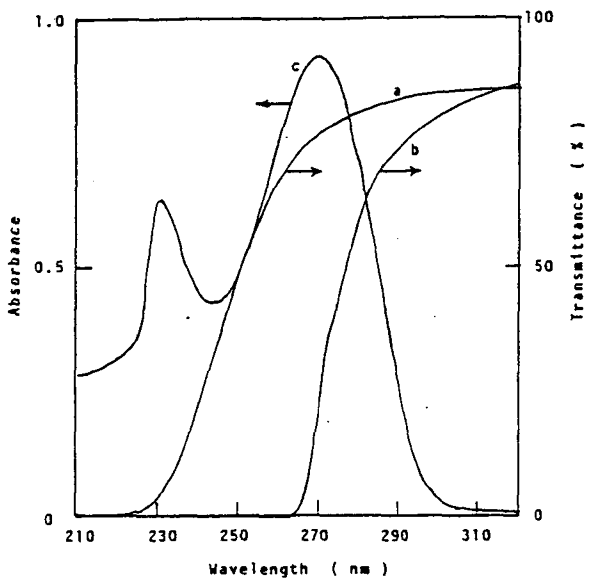


Fig.IV-7. Percent transmittances of the filter: (a) a Corning 9-30 glass filter; (b) a 10-mm pathlength DMF solution filter; (c) UV absorbance of poly(Thy-L-lys)-93 at pH 11.2.

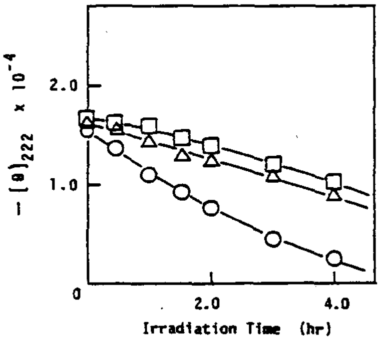


Fig.IV-8. Photolysis of poly(L-lysine) with a glass filter in the presence of thymine bases: [thymine]/[lysine unit] = 0 (○), 1.0 (△), 5.0 (□).

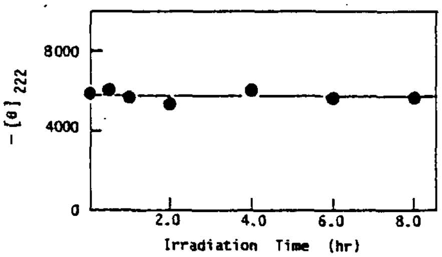


Fig.IV-9. Photolysis of poly(L-lysine) with a DMF filter.

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 $|[\theta]_{222}|$ 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 photoracemization occurred. From these aspects, it can be considered that the photoracemization may be regarded as a factor in the decrease 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 photodimerization; 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-

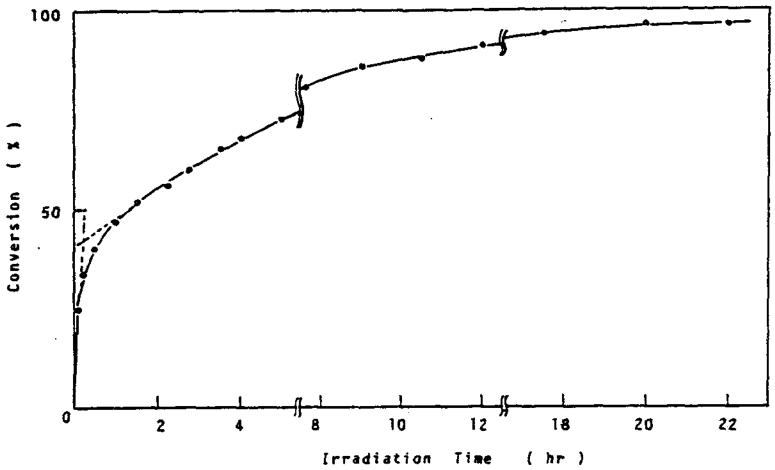


Fig.IV-10. A time-conversion curve for poly(Thy-L-lys)-93 at pH 10.5. Photolysis with a DMF filter.

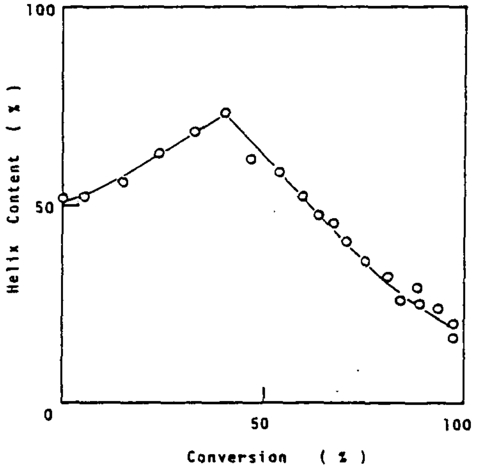


Fig.IV-11. The relationship between the conversion of thymine bases and the helix content of the polymer for poly(Thy-L-lys)-93 at pH 10.5. Photolysis with a DMF filter.

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):

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

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:

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

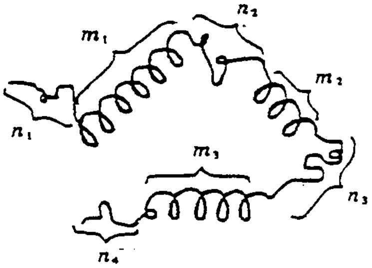
$$\frac{n_0}{m_0} = K^0 \qquad (\text{IV-6})$$

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

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 2·f units of residues may be fixed, where f is a parameter. The structure of f·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·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 photodimer of thymine bases on the side chain of the polymer.

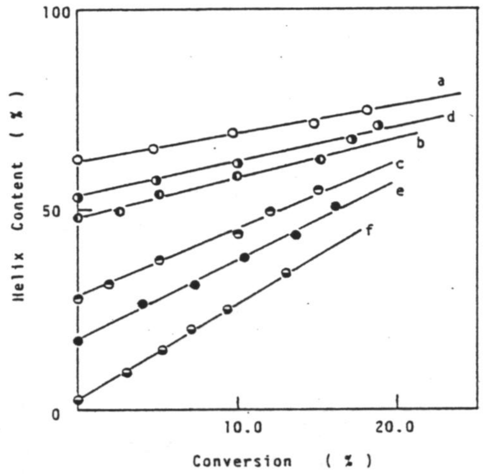


Fig.IV-12. The relationship between the conversion of thymine bases and the helix content of the polymer for poly(Thy-D-lys)-88 at pH: (a) 10.5, (b) 10.9, (c) 11.2. For poly(Thy-L-lys)-93 at pH: (d) 10.6, (e) 11.0, (f) 11.4. Photolysis with a glass filter.

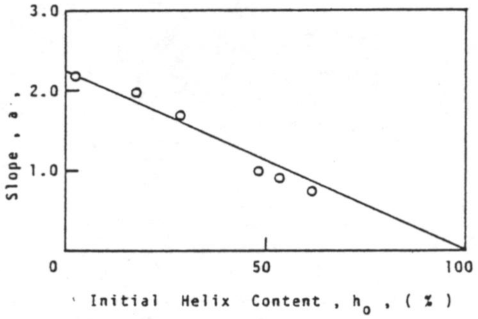


Fig.IV-13. The relationship between slope a and the initial helix content h_0.

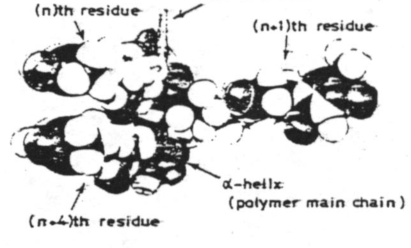


Fig.IV-14. A CPK model for poly(Thy-D-lys)-88 and/or poly(Thy-L-lys)-93.

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 photodimerization 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

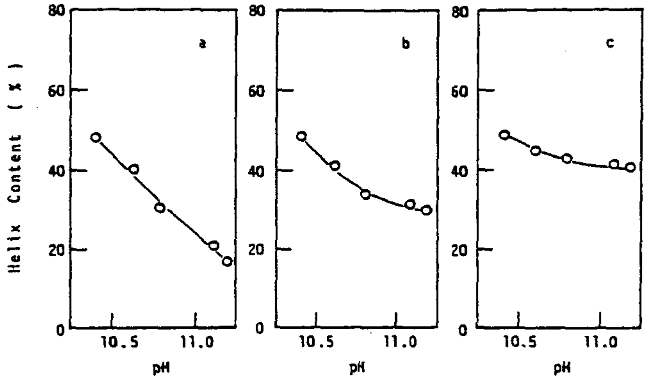


Fig.IV-15. Helix-coil equilibria for poly(Thy-D-lys)-88 irradiated at low helix content (pH 11.2). The conversion of thymine bases for photodimerization: (a) 0%, (b) 12%, (c) 42%.

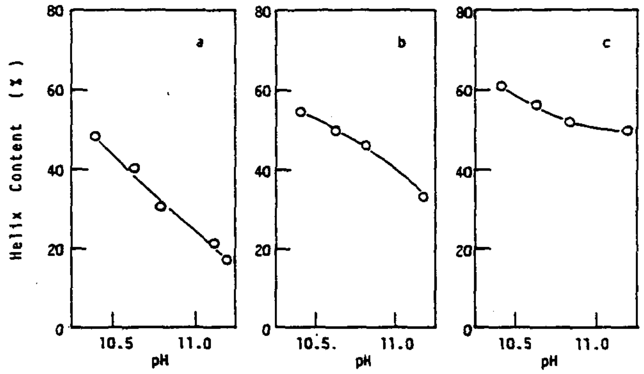


Fig.IV-16. Helix-coil equilibria for poly(Thy-D-lys)-88 irradiated at high helix content (pH 10.4). The conversion of thymine bases for photodimerization: (a) 0%, (b) 16%, (c) 27%.

(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 conversion; 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 photodimerization 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 hydrobromide) according to the method reported earlier[5]. Thymine-base content in the polymers was determined by measuring the UV absorbance of the product after hydrolysis[10]. From the absolute configuration of the asymmetric units in the polymer these polymers were abbreviated as poly(Thy-D-lys)-88 and poly(Thy-L-lys)-93, respectively; 88 and 93 represent the thymine-base content (mol%) of the polymers. The degree of polymerization was determined by viscometry 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 concentration of the polymer kept at 1×10^{-4} mol/L of the monomer unit.

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 bicarbonate 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 alkaline, aqueous, buffered solution. The helix content of the polymer was found to be changed by photodimerization. And it was proved 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 distortion 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 structure, 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].

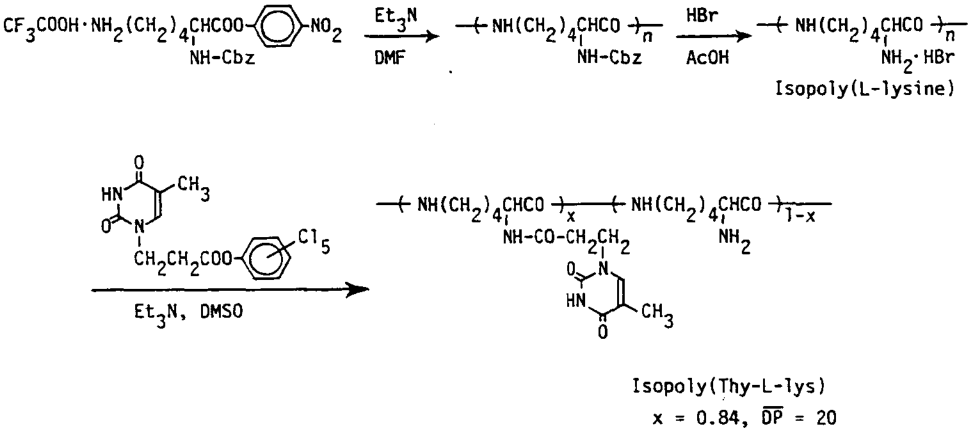
In this chapter, the effect of the β-sheet conformation of the polymer main chain on the photodimerization of the grafted thymine bases was mentioned by use of isopoly-L-lysine derivatives, which was able to be existed as β-sheet structure.

V-2. Results and Discussion

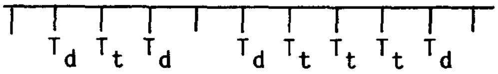
V-2-1. Thymine Derivative

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)



(Scheme V-2)



mined as 84 mol% from its NMR spectra.

V-2-2. Photochemical Reaction

Photochemical reaction of the polymer was carried out in aqueous buffered solution. The UV absorbance of the monomeric model compound of poly-L-lysine derivative[1] (Chapter III) did not decrease 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]:

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

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)

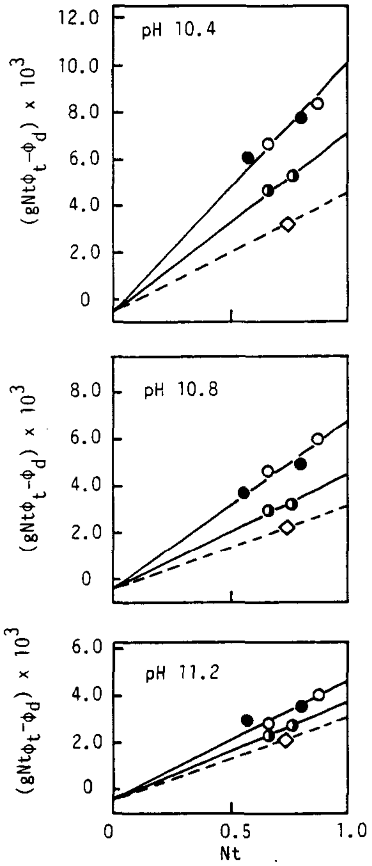


Fig.V-1. Relationships between ($gNt\phi_t-\phi_d$) values and Nt values at each pH: isopoly(Thy-L-lys) (◇); poly(Thy-DL-lys)s (◑); poly(Thy-L-lys)s (○); poly(Thy-D-lys)s (●).

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 ethyleneglycol-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-

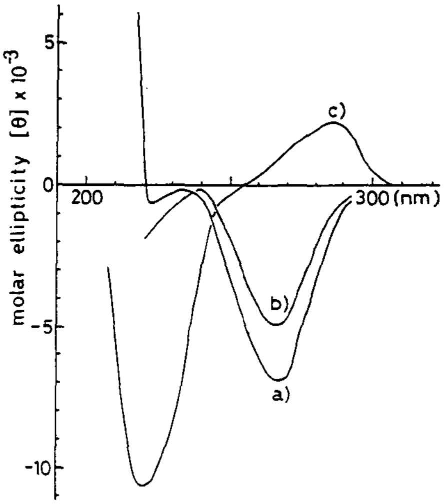


Fig.V-2. CD Spectra of isopoly(Thy-L-lys)-95 (ref. 3) in ethylene-glycol-water (1/1, v/v) mixed solvent at pH (a) 11.83; (b) 10.86; (c) 10.31.

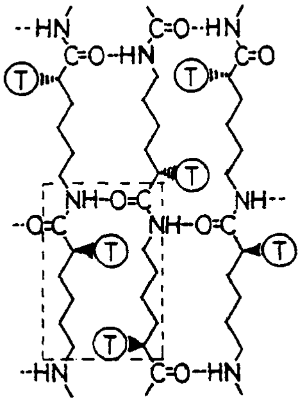


Fig.V-3. Anti-parallel β-sheet structure of isopoly(Thy-L-lys): Ⓣ = Thymine Base.

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 photodimerization 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 manner.

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 concentration 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 1×10^{-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 Polymethacrylate 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 photoresists 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 photodimerization 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-withdrawing 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 polymethacrylate derivatives as well as its monomeric model compound.

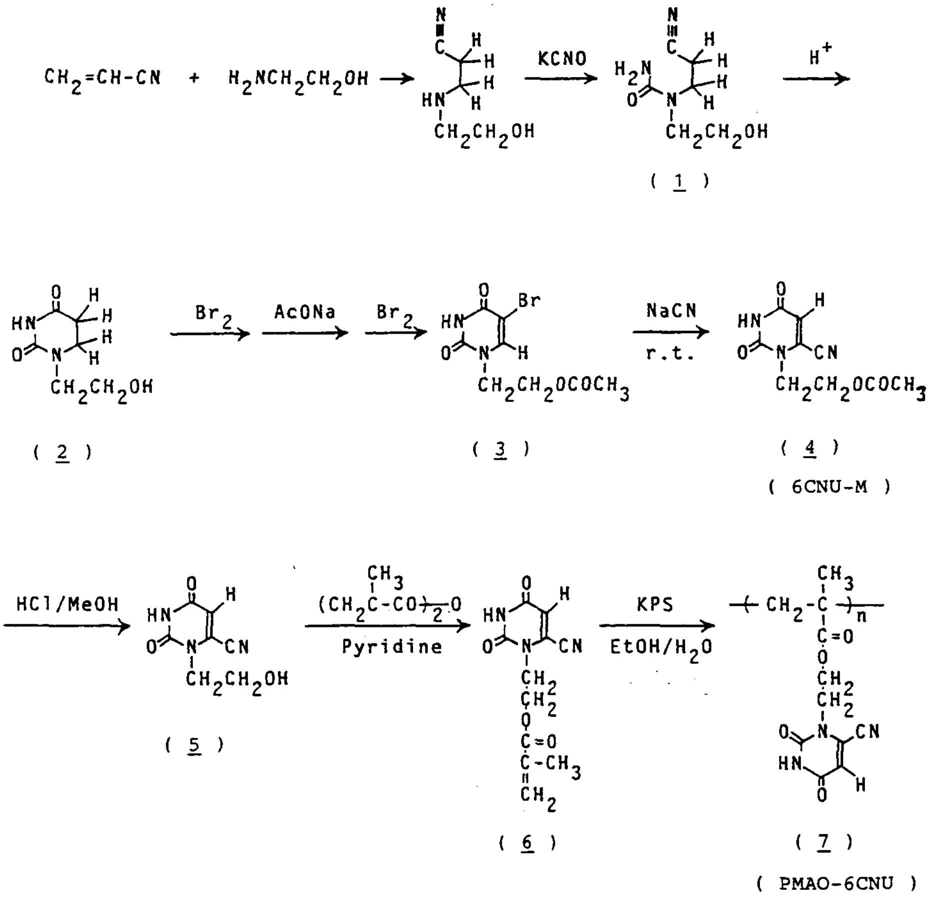
VI-2. Results and Discussion

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.

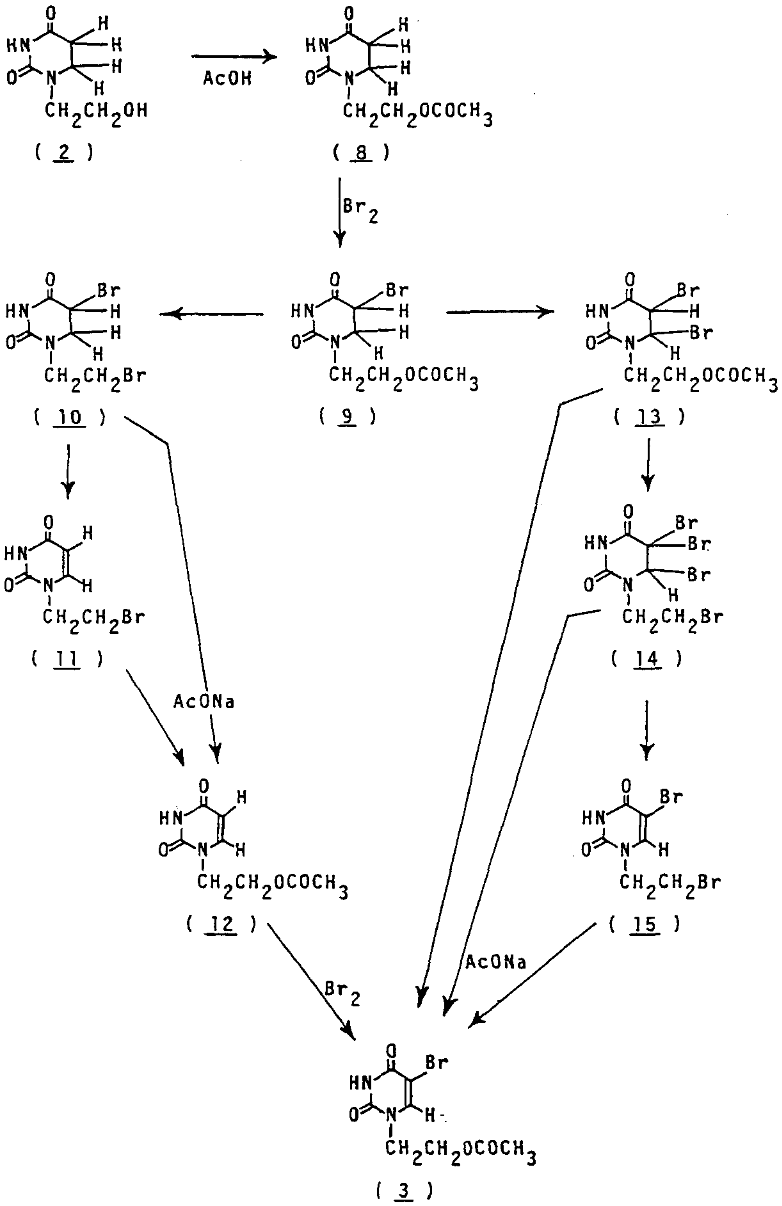
It was confirmed that the reaction from 1-(2-hydroxyethyl)-5,6-dihydrouracil (2) to compound (3) in Scheme VI-1 was occurred according to Scheme VI-2. In acetic acid, 2-hydroxyethyl group of

(Scheme VI-1)



$CH_2=CH-CN$ + $H_2NCH_2CH_2OH$
KCNO
H^+
(1)
(2)
Br_2
AcONa
Br_2
(3)
NaCN
r.t.
(4)
(6CNU-M)
HCl/MeOH
(5)
$(CH_2C(CH_3)-CO)_2O$
Pyridine
(6)
KPS
$EtOH/H_2O$
(7)
(PMAO-6CNU)

(Scheme VI-2)


AcOH
Br2
AcONa
AcONa
Br2
(2)
(8)
(9)
(10)
(11)
(12)
(13)
(14)
(15)
(3)
CH2CH2OH
CH2CH2OCOCH3
CH2CH2Br

(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 derivatives was hardly reacted[3]. To confirm the photoproduct of 6-cyanouracil derivatives, the acetone sensitized photolysis was carried out for 1-(2-acetoxyethyl)-6-cyanouracil (6CNU-M). The photolysis was carried out in acetone solution (9.0×10^{-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 accompanying 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 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 predominantly 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 photodimerization 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 temperature, 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

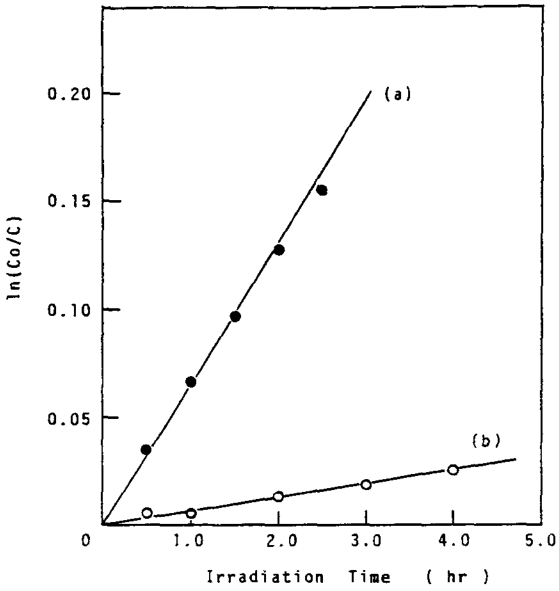


Fig.VI-1. Photolysis of 6-cyanouracil derivatives in DMSO solution: (a) PMAO-6CNU; (b) 6CNU-M.

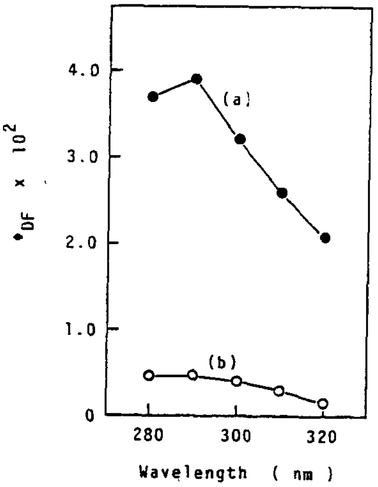


Fig.VI-2. Relationships between the quantum yield for the photodimerization of 6-cyanouracil and the wavelength of the irradiating monochromatic light in DMSO solution: (a) PMAO-6CNU; (b) 6CNU-M.

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 photodimerization 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 derivatives 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 containing polymer. This fact suggests that the 6-cyanouracil containing polymer is more desirable than the thymine containing polymers for the photoresist.

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

	λ max (nm)		ε max		Hypochromicity (%)[a)]	
Compound	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 photodimerization of 6-cyanouracil in the compounds[a)]

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

a) All data for solution 1 x 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 x 10^{-2}
PEI-T-βAla[d)]	210 - 310	275	6.49 x 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 (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 (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 (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 (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 $C_{11}H_{11}O_4N_3$: 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 (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 reaction 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 $C_{18}H_{18}O_8N_6$: 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 determined by potassium ferrioxalate actinometry. The quantum yield for oxalic acid decomposition was 1.24 at 313 nm. The concentration of 6-cyanouracil derivatives was kept constant at 1 x 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 dissolve 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.

The author is grateful to Japan Synthetic Rubber Co. Ltd. for the lithographic evaluation of the polymers.

VI-5. References

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CONCLUSION

The purpose of the present study was to be cleared the relationships between the properties of the polymers such as molecular weight, molecular structure and the conformation and the photodimerization 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 photodimerization 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 investigated for thymine-containing poly-lysine derivatives. It was stated that the photodimerization was greatly affected by the conformation of the polymer main chain and that its helical structure 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., 20, 1917 (1982).

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