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STUDIES ON
PHOTOCHEMICAL REACTIONS ON THE SYNTHETIC POLYMERS CONTAINING THYMINE BASES

YASUO KITA

1981

STUDIES ON PHOTOCHEMICAL REACTIONS ON THE SYNTHETIC POLYMERS CONTAINING THYMINE BASES

(チミンを側鎖に有する高分子の光反応に関する研究)

YASUO KITA

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January, 1981

Yasuo Kita

GENERAL INTRODUCTION

In recent years, a variety of functional polymers have been developed, such as polymer catalysts, biologically interesting polymers, photopolymers, polyelectrolytes and so on. And their applications have been also investigated actively. Especially, the photopolymers are useful as image and information processing materials and as photoresists which can be used for integrated circuit and large scale integration.

On the other hand, the studies on photochemistry and photobiology of nucleic acid have been developed extensively. In this connection, the photodimerization reaction of thymine bases has been a subject of intense interest to the area of photobiology for a number of years, primarily because this reaction is known to be involved in the photoinactivation of the deoxyribonucleic acid (DNA) in vivo and vitro. Exposure of nucleic acids to UV radiation in vivo and vitro leads to dimerization of the pyrimidine bases with the formation of cyclobutane derivatives [1]. This dimerization prevents the reduplication of DNA, and causes the UV mutation.

Detailed studies have been made on nucleic acids and the model systems involving photodimerization of thymine bases [2,3]. However, the systematic investigation for photoreaction of the synthetic polymers containing thymine bases as the model compound of DNA has not been reported. From a point of view described above, it is of interest to study the photochemical reactions on the synthetic polymers containing thymine bases.

The purpose of the present study is to synthesize the functional polymers containing pendant thymine bases and to see the relationship between intramolecular base-base interaction and photoreactivity of thymine bases in the polymers. Studies on photochemistry and molecular interactions of the synthetic polymers containing thymine bases would give useful informations on the design of functional polymers.

In Chapter I, photoreactions of N-2-isobutyloxyethyl thymine, bis[2-(2,4-dihydroxy-5-methyl pyrimidine-1-yl)ethyl]glutarate, poly(N-2-methacryloyloxyethyl thymine), and poly(N-2-acryloyloxyethyl thymine) were studied in dimethylformamide solution.

In Chapter II, photodimerization reactions of thymine bases present on the side chain of acryloyl and methacryloyl type polymers were studied in dimethyl sulfoxide, dimethylformamide, and dimethyl sulfoxide-ethylene glycol mixture. It was found that the photoreactivity was influenced by the intramolecular base-base interactions of polymers.

In Chapter III, photodimerization reactions of polyacrylic and polymethacrylic derivatives and dimeric model compound containing thymine bases were studied in the presence of adenine derivatives in dimethyl sulfoxide, dimethylformamide, and dimethyl sulfoxide-ethylene glycol mixture. The interactions of these complementary model compounds in the ground state were also studied by UV spectra in three kinds of solvents.

In Chapter IV, synthesis of polyacrylic and polymethacrylic derivatives containing different contents of pendant thymine unit by the polymer reaction using cyclic thymine derivative and photodimerization reactions of these copolymers were studied in dimethylformamide solution. It was found that the thymine contents of these polymers influenced the photoreactivity of the polymers.

In Chapter V, photodimerization reactions of the synthetic polymers containing thymine bases were studied in polymethyl methacrylate film and were discussed in comparison with the photodimerization reactions in solution.

References

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[3] A.D.McLaren and D.Shugar, *"Photochemistry of Proteins and Nucleic Acids"*, Pergamon Press, New York, 1964.

Chapter I. Photochemical reactions on the acrylic and methacrylic polymers containing thymine bases

I - 1. Introduction

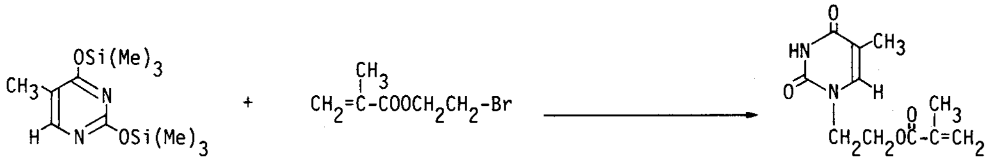
In our series of articles specific interaction due to base-base pairing between some synthetic polymers with pendant nucleic acid bases has been widely studied [1]. The complex formation of polymers that contain the nucleic acid base is influenced by two competitive interactions, that is, intramolecular self-association of the bases and intermolecular base pairing. If the self-association of the bases along the polymer chain is strong enough, the strength of the intermolecular interaction between the polymers will be negligible. Although the formation of the polymer complex by intermolecular base pairing was studied extensively by spectroscopic measurement [2], a similar study of the intramolecular self-association of bases along the polymer chain would be difficult because the interaction in question would be too weak. Such an intramolecular interaction was also studied by emission and UV spectroscopies. Although the intramolecular interaction of adenine bases in poly-9-vinyladenine was estimated by these spectroscopies [3], excimer emission of the methacrylate-type polymers could not be observed and the hypochromicity of the polymers was not so high as that of poly-9-vinyladenine because the distance between bases in methacrylate-type polymers is long enough. The other method of estimating the intramolecular interaction is a photochemical reaction of the bases.

In this chapter a study of the photodimerization of thymine bases along the polymer chain was made to estimate the degree of intramolecular self-association of the bases. The photodimerization reaction was also studied for monomeric and dimeric model compounds. Formation of thymine photodimers by UV irradiation onto nucleic acid is well known and the photodimerization of nucleic acid, its model compounds, and pyrimidine bases has been reported by many groups [4]. The photodimerization between pyrimidine bases of the vinyl-type polymers is unknown however.

I - 2. Results and Discussion

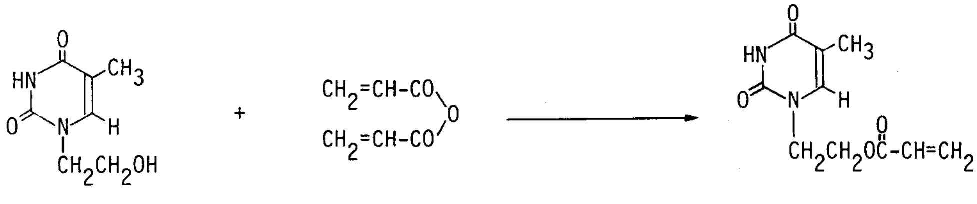
I - 2 - 1. Synthesis of Polymers and Model Compounds

Two kinds of thymine-containing polymer, that is, poly(N-2-methacryloyloxyethyl thymine) (polyMAOT) and poly(N-2-acryloyloxyethyl thymine) (polyAOT), were prepared by free radical polymerization of the corresponding monomers. A monomer, N-2-methacryloyloxyethyl thymine (2) (MAOT), was prepared from 5-methyl-bis(trimethylsiloxy)pyrimidine (1) and 2-bromoethyl methacrylate [5]:



(1) (2) MAOT

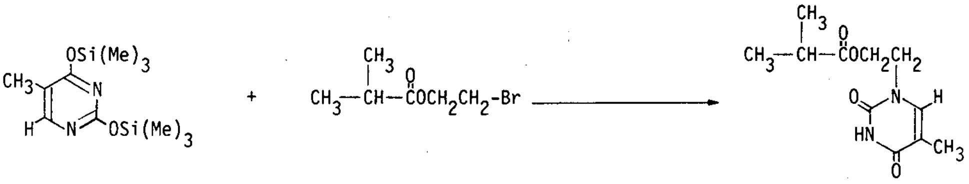
In the preparation of N-2-acryloyloxyethyl thymine (4) (AOT) the procedure described above is unfavorable because it yields a hydroxyethyl derivative of thymine. Neither does the reaction of 2-hydroxyethyl thymine (3) with acrylic chloride give acryl-type monomers. The monomer AOT, however, was successfully prepared by the reaction of (3) with acrylic anhydride:



(3) (4) AOT

Polymerization of these monomers was carried out in a dimethyl sulfoxide (DMSO) solution at 60 °C with azobisisobutyronitrile (AIBN) as an initiator. The polymers obtained were soluble in dimethylformamide (DMF), DMSO, and pyridine and insoluble in water and alcohols. The molecular weight of the polymers was determined by membrane osmometry to be 120,000 for polyMAOT and 31,000 for polyAOT.

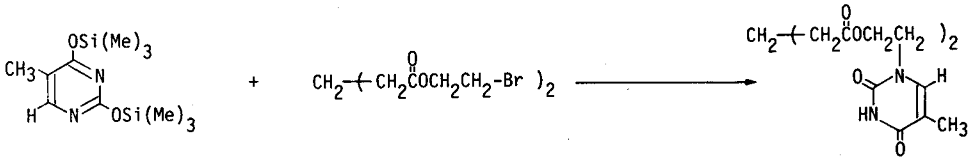
The monomeric model compound, N-2-isobutyroyloxyethyl thymine (5) (T-M), was prepared from (1) and 2-bromoethyl isobutyrate by using a similar method described for the methacrylate monomer synthesis:



(1)

(5) T-M

The dimeric model compound, bis[2-(2,4-dihydroxy-5-methyl pyrimidine-1-yl)ethyl]glutarate (6) (T-T), was also prepared from (1) and bis(2-bromoethyl)glutarate:



(1)

(6) T-T

I - 2 - 2. Spectral Properties of Polymers and Model Compounds

The UV absorption spectra of the polymers and the model compounds were measured in DMF solution at low concentrations (ca. 1×10^{-4} mol/l) to avoid intermolecular association so that interaction between thymine moieties within the identical molecule could be detected. From UV spectra the percentage of hypochromocity, H (%), was obtained. The UV spectral data and the H values are listed in Table I-1.

The λ_{max} values of the polymers and the model compounds were almost identical, whereas their ε_{max} values differed considerably.

Hypochromicities at λ_{max} of the polymers and dimeric model compound were obtained as 13.9, 11.8, and 7.0 %, respectively. From these data it was suggested that the thymine bases in dimeric model compound and polymers interact intramolecularly in the ground state and the order of this intramolecular interaction deduced from hypochromicity values was polyAOT > polyMAOT > T-T. The percentage of hypochromicity, as a partial means of assessing interaction, is assumed to relate to the relative ease of internal photodimerization in these compounds.

I - 2 - 3. Isolation and Identification of the Photodimer

Photolysis of the dimeric model compound which led to the formation of photodimers was carried out in DMF solution (2×10^{-3} mol/l) in a Pyrex reaction vessel with light from a 500-W high-pressure mercury lamp. After photolysis for 3 hr the solvent was distilled off under vacuum to yield an oily product. The product showed no absorption of thymine at 270 nm in UV spectral region and gave signals assigned to the photodimer by NMR spectroscopy (Table I-2).

For the photodimerization of thymine four isomeric products are assumed to be formed (Figure I-1) [6]. It is known that cis-syn and trans-syn isomers are formed by photolysis of 1,1'-trimethylene-bis-thymine [7], TpT [8], and denaturated DNA [9]. Examination of CPK models for T-T indicated that these thymine derivatives can form syn-type photodimers and the backbone prevents the formation of anti-types. The chemical shift of the 5-methyl proton at 1.12 to 1.18 in our case, as seen in Table I-2, suggests the formation of cis-syn and trans-syn isomers. It was found that when acetone was used as photosensitizer the products had photodimer distribution similar to that obtained without sensitizer.

The NMR spectra of the photochemical products of the polymers were almost the same as those of the dimeric model (Table I-2). This fact suggests that the isomeric photodimers in the polymers were the same ones, that is, the cis-syn and trans-syn isomers.

After the photolysis of the polymers viscosity decreased, which suggests that the photodimerization was an intramolecular reaction in

Table I-1. UV Spectral Data for the Polymers and Corresponding Model Compounds in DMF

Compound	λ max (nm)	ε [a]	Hypochromicity (%) [b]
T - M	271.0	9300	0
T - T	271.0	17300	7.0
polyMAOT	270.5	8200	11.8
polyAOT	270.5	8100	13.9

[a] Molar extinction coefficient.

[b] Hypochromicity based on T-M.

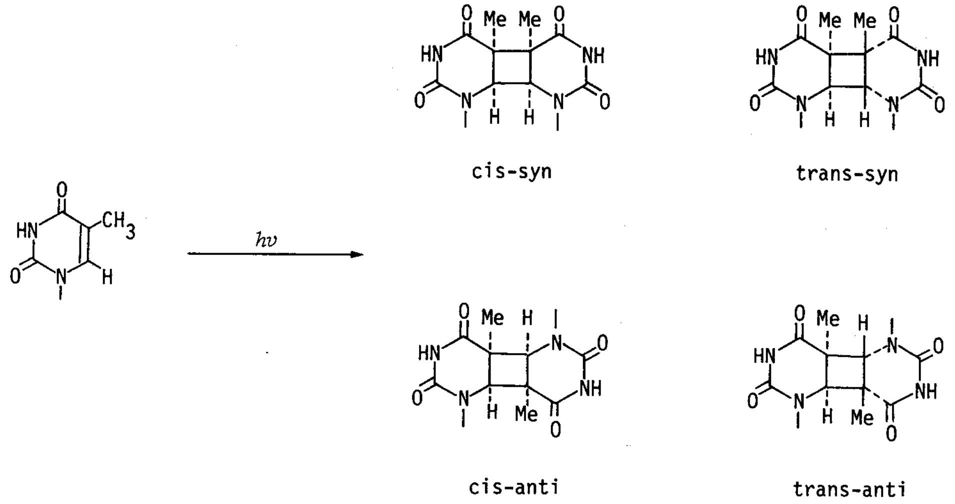


Figure I-1. Isomers of thymine dimer.

the identical polymer chain that caused contraction.

I - 2 - 4. The Rate of Photodimerization

The rate of photodimerization of the polymers and their dimeric and monomeric model compounds was measured. Formation of the photodimers in DMF solution was followed by an absorption change at 275 nm. It was found that the absorbance of monomeric model compound did not change with time, which suggests that neither intermolecular photodimerization nor other reactions, including decomposition of thymine rings, was occurring under the condition used. From this fact it was demonstrated for the photodimerization of the dimeric model compound and polymers that all the loss of absorbance at 275 nm could be accounted for by the intramolecular photodimerization. Figure I-2 shows how the photodimerization proceeds clearly in the case of the polymers and the dimeric model compound. The absorbance of the latter decreased linearly with time.

The dimerization reaction in a system in which only a photodimer is essentially formed should have a process related to thymine concentration by equation (1) for monochromic light:

$$-\frac{d[\mathrm{T}]}{dt} = \phi I_a = \phi I_0(1 - 10^{-D}) \qquad (1)$$

where D, ϕ, and I_0 denote optical density or absorbance, quantum efficiency, and intensity of light in mol/l·sec, respectively. At higher D values, equation (1) reduces to

$$-\frac{d[\mathrm{T}]}{dt} = \phi I_0 \qquad (2)$$

Our results of the photodimerization of the dimeric model compound could be explained by equation (2). When the concentration of the dimeric model compound was changed, the results also satisfied this equation.

In polymers, however, loss of thymine units by irradiation did not produce a straight line according to equation (2). The data for the polymers deviated from equation (1). Photodimerization of thymine

Table I-2. Chemical Shifts of 5-Methyl Protons of Photodimer in DMSO-d_6

Compound	δ in ppm (from TMS) $5\text{-}CH_3$ [a]	
T - T	1.12	1.18
T - T [b]	1.12	1.18
polyMAOT	1.11	1.18
polyAOT	1.12	1.20

[a] δ in ppm (from TMS) for T-T, polyMAOT, and polyAOT are 1.74, 1.84, and 1.80, respectively.

[b] Photosensitized irradiation photodimer.

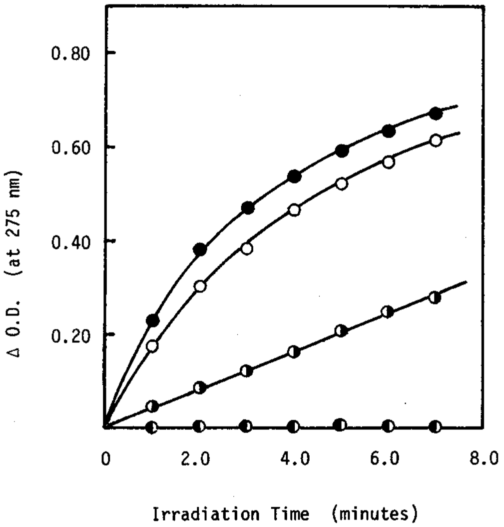


Figure I-2. Irradiation of thymine derivatives; (◐) T-M, (◑) T-T, (○) polyMAOT, (●) polyAOT.

in the polymers was not complete because some of the thymine units became isolated. When pairs of neighboring thymine units along a long polymer chain dimerize intramolecularly with one another, some of the substituents eventually fail to react because of isolation among neighbors which have reacted. The fraction of the substituents that becomes isolated at completion of the reaction has been calculated by statistical methods by Flory for removal of chlorine from vinyl chloride polymers with zinc [10]. His results show that if the polymer has all its chlorides in a 1-3 relationship the portion $1/e^2$ (or 13.53 %) of the original chlorides will become isolated, a result for which there is experimental support.

The rate of disappearance of thymine unit (a tangent in Figure I-2) was plotted against the conversion in Figure I-3. The plots in Figure I-3 produced a straight line which extrapolated to a rate of zero at the conversion $X = 0.86$ $(= 1 - e^{-2})$. This is almost identical with the fraction of the isolated substituents calculated by Flory. The rate of photodimerization was statistically calculated assuming that (1) the degree of polymerization, n, was high enough, (2) the effect of the end group was ignored, and (3) reaction occurred randomly.

At the beginning of the reaction the rate of reaction is given by

$$-\frac{d[\mathrm{T}]}{dt} = \phi I_0 P_0 = \phi I_0 \tag{3}$$

where the probability of reaction of the thymine unit (P) was unity. At time = t, x mole units of thymine are reacted and the polymer is divided into groups of thymine units. The probability of reaction in the end units of these groups is decreased from 1 to $(1 - a)$ (a is a parameter):

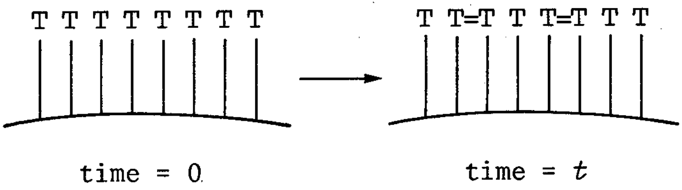


Table I-3. ϕI_0 Values

Compound	$\phi I_0 \times 10^7$ (mol/l·sec)
T - T	0.75
polyMAOT	4.3
polyAOT	5.4

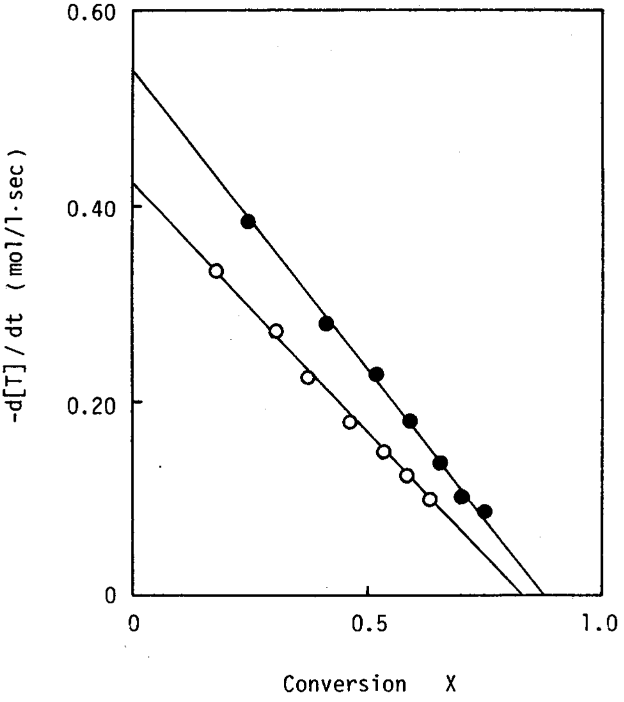


Figure I-3. Photodimerization of polymers as a function of conversion; (○) polyMAOT, (●) polyAOT.

The probability of reaction in the thymine units at time = t is given by

$$P_t = \frac{(n - x) - ax}{n} = 1 - (1 + a)X \qquad (4)$$

where $(n - x)$ is unreacted thymine units and $X = x/n$ is the conversion of the photodimerization. From equation (4) the reaction rate at time = t is given by

$$-\frac{d[\mathrm{T}]}{dt} = \phi I_0 P_t = \phi I_0[1 - (1 + a)X] \qquad (5)$$

At time = ∞ Flory's result gives

$$P_\infty = 0, \qquad X = 1 - e^{-2} \qquad (6)$$

Combination of equations (5) and (6) gives

$$-\frac{d[\mathrm{T}]}{dt} = \phi I_0 - \phi I_0(1 - e^{-2})^{-1}X \qquad (7)$$

The plots in Figure I-3 fit equation (7). From the intercept of the line in this figure the ϕI_0 value for the polymers at the beginning of the reaction was obtained and tabulated in Table I-3.

The ϕI_0 values obtained for two kinds of polymer were about 5.7-7.2 times higher than the value of the dimeric model compound. By statistical calculation, however, the values of the polymers are twice as high as the value of the dimeric model compound. This discrepancy in the polymers may be attributed to the difference in mechanism of photodimerization. As shown later, the reaction in the polymers occurred in both singlet and triplet states, whereas the reaction in the dimeric model compound occurred only in the triplet state. The difference in the values between two kinds of polymers may be attributed to the triplet/singlet ratios of the photodimerization mechanism, which was caused by the mobility of the thymine units in the polymers. In methacrylate polymer (polyMAOT) mobility of the main and side chains may be affected by the steric hindrance of the methyl group in the main chain. This fact was also supported by T_g values, that is, 127 and

132 °C for polyMAOT and polyAOT, respectively.

I - 2 - 5. Quantum Efficiency for Photodimerization Reaction

Quantum efficiency of the polymers and the dimeric model compound for the photodimer formation reaction was measured in DMF solution (1×10^{-4} mol/l of thymine unit) with a JASCO SS-25 monochromator coupled to a xenon arc lamp set at 280 nm, with the light intensity measured by potassium ferrioxalate actinometry. The data are given in Table I-4. The value of the polymers was found to be higher than that for the dimeric model compound. This tendency is in agreement with that of the rate of photodimerization.

I - 2 - 6. Quenching of the Photodimerization

The nature of the excited state responsible for the photodimerization reaction is of basic mechanistic interest. Degassed DMF solutions of T-T, polyMAOT, and polyAOT (1×10^{-4} mol/l of thymine unit), which contained various concentrations of isoprene, were irradiated. Temperature was controlled at 20 °C. Relative quantum efficiencies of thymine disappearance were determined by UV analysis.

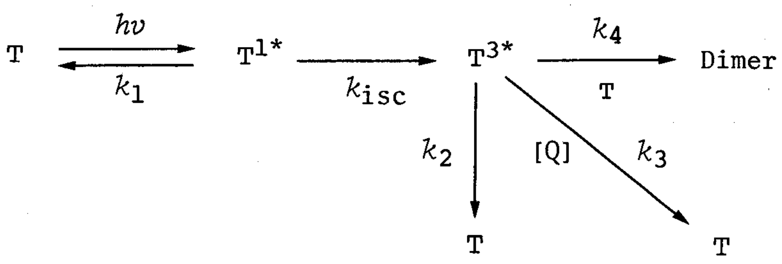
The amount of dimerization was plotted against isoprene concentration in the usual Stern-Volmer manner (i.e., $\phi_{DF}{}^{\circ}/\phi_{DF}$ vs. [Q], where $\phi_{DF}{}^{\circ}$ represents quantum efficiency at zero isoprene concentration). The plots are shown in Figure I-4.

In T-T partial and complete (0.02 mol/l isoprene) quenching of the photodimerization by the triplet quencher Q, that is, isoprene, was observed and a straight line was obtained. This fact indicates that the photodimerization of T-T occurs almost completely in the triplet state. On the other hand, the Stern-Volmer plots for the reaction of polymers does not produce straight lines, only curves (Figure I-4). This suggests that both singlet and triplet states are involved in the photodimerization in question.

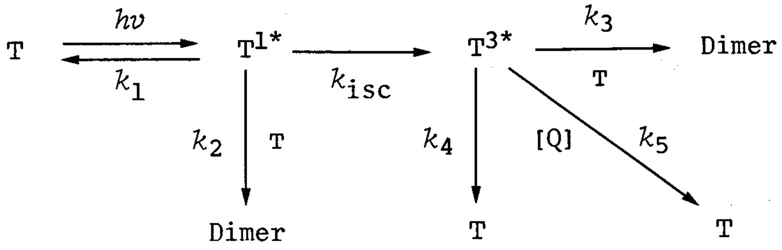
Mechanisms for the photodimerization of both dimeric model compound and polymers could, therefore, be shown in Schemes I-1 and I-2. In these schemes T represents the ground state of thymine units. T^{1*} and T^{3*} are the excited singlet and triplet states, respectively, and

k_1, k_2, k_3, k_4, k_5, and k_{isc} are rate constants.

Scheme I-1



Scheme I-2



For Scheme I-1 the dimerization quantum efficiency of T-T by using a steadystate treatment is then shown by

$$\phi_{DF}{}^{\circ} = \left(\frac{k_{isc}}{k_{isc} + k_1}\right)\left(\frac{k_4}{k_2 + k_4}\right) \tag{8}$$

$$\phi_{DF}{}^{\circ} = \phi_{isc}\frac{k_4}{k_2 + k_4} \tag{9}$$

In this treatment the reaction was assumed to be an intramolecular reaction and independent of the thymine unit concentration.

When the triplet quencher is present, equation (9) becomes

$$\phi_{DF} = \phi_{isc}\frac{k_4}{k_2 + k_4 + k_3[Q]} \tag{10}$$

Combination of equations (9) and (10) yields

$$\frac{\phi_{DF}{}^{\circ}}{\phi_{DF}} = 1 + \frac{k_3}{k_2 + k_4}[Q] \tag{11}$$

The photodimerization quantum efficiency of polymers (Scheme I-2) is described by

Table I-4. Quantum Efficiency for Photodimerization in DMF [a]

Compound	ϕ_{DF}°
T - T	0.0012
polyMAOT	0.0084
polyAOT	0.010

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

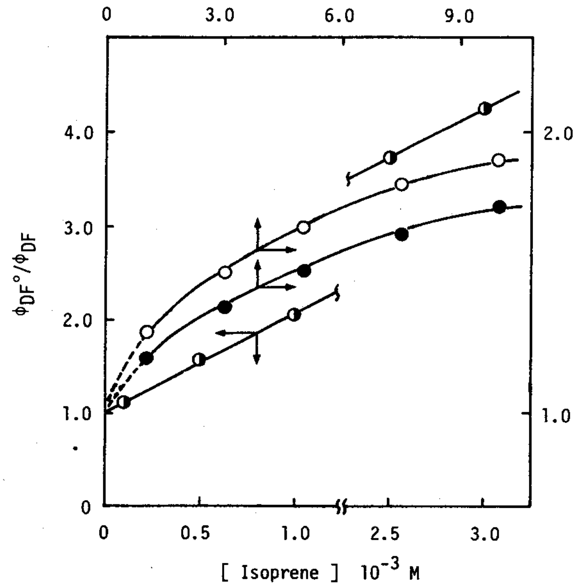


Figure I-4. Representative Stern-Volmer plots for quenching photodimerization of thymine derivatives by isoprene. The concentration of thymine derivatives was always 1×10^{-4} mol/l of thymine unit; (◑) T-T, (○) polyMAOT, (●) polyAOT.

$$\phi_{DF}{}^{\circ} = \left(\frac{1}{k_1 + k_2 + k_{isc}}\right)\left(k_2 + \frac{k_{isc}k_3}{k_3 + k_4}\right) \tag{12}$$

If the triplet quencher is present, equation (12) becomes

$$\phi_{DF} = \left(\frac{1}{k_1 + k_2 + k_{isc}}\right)\left(k_2 + \frac{k_{isc}k_3}{k_3 + k_4 + k_5[Q]}\right) \tag{13}$$

Treatment of equations (12) and (13) leads to

$$\frac{\phi_{DF}{}^{\circ}}{\phi_{DF}} = \left(\frac{k_2k_3 + k_2k_4 + k_{isc}k_3}{k_3 + k_4}\right)\left(\frac{k_3 + k_4 + k_5[Q]}{k_2k_3 + k_2k_4 + k_2k_5[Q] + k_{isc}k_3}\right) \tag{14}$$

Equation (14) is rewritten as

$$\frac{1}{\phi_{DF}{}^{\circ}/\phi_{DF} - 1} = \frac{(k_3 + k_4)k_2}{k_{isc}k_3} + \left(\frac{k_3 + k_4}{k_5}\right)\left(1 + \frac{(k_3 + k_4)k_2}{k_{isc}k_3}\right)\frac{1}{[Q]} \tag{15}$$

Equation (15) indicates that the plots of $1/(\phi_{DF}{}^{\circ}/\phi_{DF} - 1)$ vs. $1/[Q]$ should give a straight line. The data for the polymers (in Figure I-4) were plotted with equation (15) which gave straight lines satisfactorily, as shown in Figure I-5. From the intercepts of the lines in this figure the values of $\phi_{DF}{}^{\circ}/\phi_{DF}$ were obtained as 2.4 and 3.3 for polyAOT and polyMAOT, respectively. The quantum efficiencies for photodimerization that resulted from the singlet state were calculated as 41 and 32 %, respectively.

The data of hypochromicity gave evidence of appreciable ground-state aggregation. This singlet-derived photodimerization of polymers seems to be the dimerization from these aggregates by excimer intermediate. In fact, a number of experiments have provided support for the singlet excimer as an intermediate for the photodimerization of thymine in ice, T_pT, and DNA [11].

I - 3. Experimental

I - 3 - 1. Instrumentation

UV spectra were measured with a Union SM-401 spectrophotometer and NMR spectra were measured with a JNM-4H-100 spectrometer (JEOL),

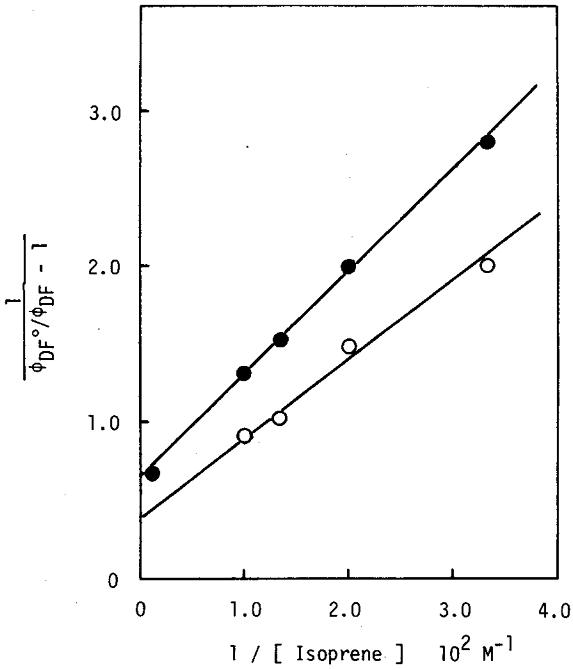


Figure I-5. The relationship between $1/(\phi_{DF}°/\phi_{DF} - 1)$ and 1/[isoprene]; (○) polyMAOT, (●) polyAOT.

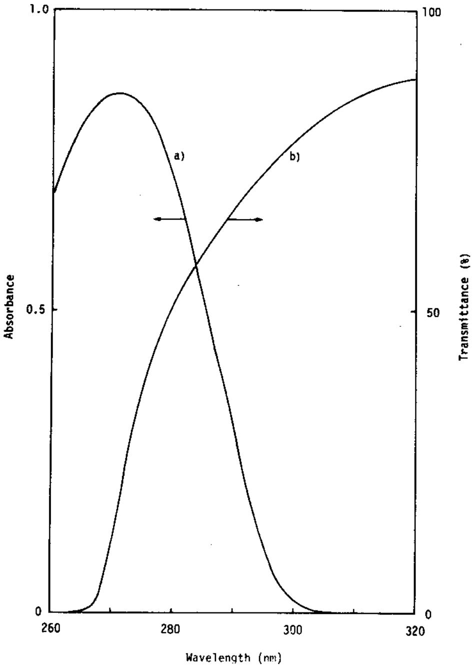


Figure I-6. UV Absorption of thymine derivative and 20 mm DMF solution filter; a) absorbance of T-T (1×10^{-4} mol/l of thymine unit in DMF solution), b) percent transmittance of the filter.

tetramethylsilane was used as an internal standard. Tg values of polyAOT and polyMAOT were obtained with a Perkin-Elmer differential scanning calorimeter (DSC-1). Molecular weight of the polymers was determined by means of osmometry using a Hewlett-Packard high speed membrane osmometer (Model 502).

I - 3 - 2. Photolysis

Photolysis was done in a 10-mm quartz cell as the reaction vessel filled with 4 ml of solution at 20 °C. Thymine derivatives concentration was always kept at 1×10^{-4} mol/l of thymine unit and degassed with purified nitrogen. The absorbance of 1×10^{-4} mol/l of thymine unit for dimeric model compound (T-T) is shown in Figure I-6. The same absorption spectra are obtained for other thymine derivatives. A 500-W high-pressure mercury lamp filtered through 20 mm of DMF was used as the light source. Transmittance of this filter is also shown in Figure I-6. This setup allowed 275.2, 283.0, and 289.4 nm lines on the mercury arc to be absorbed by thymine derivatives. Consequently, only thymine moieties, not those of photodimers and isoprene could absorb the radiation. Quenching studies were carried out in a similar manner with 0.01 - 0.001 mol/l isoprene. At a given period intervals, concentration of the thymine remaining was determined by UV analysis at 275 nm, at which neither the photodimers nor isoprene absorb significantly. Absorbance of thymine follows Beer's law.

Quantum efficiency data were obtained with a JASCO SS-25 monochromator coupled with a xenon arc lamp set at 280 nm.

Light intensities were determined by potassium ferrioxalate actinometry. The quantum yield for oxalic acid decomposition was 1.24 at 297/302 nm [12].

Preparative photolysis was carried out in a single Pyrex reaction vessel. The thymine derivatives were dissolved in DMF (2×10^{-3} mol/l) and irradiated with a 500-W high-pressure mercury lamp set inside the reaction vessel. The vessel was cooled by immersing it in ice water, and nitrogen gas was bubbled through the sample solution during the entire photochemical reaction. Photosensitized irradiation was carried out with acetone (2×10^{-2} mol/l) as photosensitizer. The irradiation time was 3 hr for ordinary and sensitizing reactions.

I - 3 - 3. Materials

N-2-Methacryloyloxyethyl thymine (MAOT) (2). This compound was prepared from 5-methyl-bis(trimethylsiloxy)pyrimidine and 2-bromoethyl methacrylate in a procedure reported earlier [5].

Poly(N-2-methacryloyloxyethyl thymine) (polyMAOT). Polymerization of MAOT (0.2 mol/l) was carried out at 60 °C for 8 hr in DMSO solution, with AIBN (4×10^{-3} mol/l) as initiator. Conversion attained was 97.8 %. The molecular weight of the polymers obtained was 120,000 by osmometry.

N-2-Hydroxyethyl thymine (3). To 5-methyl-bis(trimethylsiloxy) pyrimidine (2.7 g, 0.01 mol) was added 2-bromoethyl acetate (3.4 g, 0.02 mol), the mixture was then stirred at 60 °C for 10 days. After the reaction 100 ml of methanol and 10 ml of 6N HCl were added to the reaction mixture. After refluxing for 1 hr methanol was distilled off under vacuum to provide a crystalline product. Recrystallization from ethanol gave colorless cubes: yield, 2.7 g (80 %); mp 180-181 °C (lit. [13] 179-181 °C).

N-2-Acryloyloxyethyl thymine (AOT) (4). Acrylic anhydride (2.6 g, 0.02 mol) was added to N-2-hydroxyethyl thymine (1.8 g, 0.01 mol) and stirred for 3 hr at 70 °C to give a clear solution. The reaction mixture was cooled to 0 °C to precipitate the product. The product was filtered and washed thoroughly with diethyl ether and recrystallized from ethanol: yield, 1.9 g (87 %), colorless needles; mp 145-146 °C; UV (EtOH), λ_{max} 270 nm (ε 8800); NMR (in DMSO-d_6), δ 11.29 (br s, 1, 3-NH), 7.49 (s, 1, 6-H), 5.80-6.40 (m, 3, -CH=CH_2), 4.32 (t, 2, NCH_2C), 3.94 (t, 2, CCH_2O), and 1.75 (s, 3, 5-CH_3).

Anal. Calcd for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39; N, 12.50. Found: C, 53.09; H, 5.35; N, 12.56.

Poly(N-2-acryloyloxyethyl thymine) (polyAOT). Polymerization of AOT, like MAOT, was carried out at 60 °C for 8 hr in DMSO solution. Conversion attained was 48.5 %. Molecular weight of the polymer obtained was 31,000 by osmometry.

N-2-Isobutyloxyethyl thymine (T-M) (5). The monomeric model compound was prepared from 5-methyl-bis(trimethylsiloxy)pyrimidine (2.7 g, 0.01 mol) and 2-bromoethyl isobutyrate (2.0 g, 0.01 mol) as described for MAOT. Recrystallization from water produced colorless needles: mp 151-152 °C; yield, 1.8 g (76 %); NMR (in DMSO-d_6) δ 11.27 (br s, 1, 3-NH), 7.47 (s, 1, 6-H), 4.25 (t, 2, NCH_2C), 3.89 (t, 2, CCH_2O), 2.51 (m, 1, CH), 1.75 (s, 3, 5-CH_3), 1.05 (s, 3, CH_3), and 0.96 (s, 3, CH_3).

Anal. Calcd for $C_{11}H_{16}N_2O_4$: C, 55.20; H, 6.78; N, 11.83. Found: C, 54.99; H, 6.71; N, 11.66.

Bis[2-(2,4-dihydroxy-5-methyl pyrimidine-1-yl)ethyl]glutarate (T-T) (6). The dimeric model compound was prepared from 5-methyl-bis(trimethylsiloxy)pyrimidine (5.4 g, 0.02 mol) and bis(2-bromoethyl) glutarate (3.5 g, 0.01 mol)by the same procedure. Recrystallization from water gave colorless needles: mp 174-175 °C; yield, 2.7 g (61 %); NMR (in DMSO-d_6) δ 11.34 (br s, 2, 3-NH), 7.46 (s, 2, 6-H), 4.22 (t, 4, NCH_2C), 3.86 (t, 4, CCH_2O), 2.30 (t, 4, $COCH_2C$), 1.74 (s, 6, 5-CH_3), and 1.70 (m, 4, CCH_2C).

Anal. Calcd for $C_{19}H_{24}N_4O_8$: C, 52.29; H, 5.54; N, 12.84. Found: C, 51.93; H, 5.50; N, 12.85.

The reagents and solvents were obtained commercially and purified in the usual manner. Spectral quality solvents were used in all photochemical experiments.

I - 4. Summary

Photodimerization of N-2-isobutyloxyethyl thymine (T-M), bis[2-(2,4-dihydroxy-5-methyl pyrimidine-1-yl]glutarate (T-T), poly(N-2-methacryloyloxyethyl thymine) (polyMAOT), and poly(N-2-acryloyloxyethyl thymine) (polyAOT) was studied in dimethylformamide solution. The rate of intramolecular photodimerization of polyMAOT and polyAOT was 5.7 and 7.2 times faster, respectively, than that of T-T. In T-M, however, intermolecular photodimerization did not occur under the reaction condition used. Quenching studies with isoprene suggest that the photodimerization of T-T occurs predominantly in an excited triplet state, whereas that of polyMAOT and polyAOT occur in singlet and triplet states. It was concluded that the photodimers of T-T, polyMAOT, and polyAOT are two syn-fused cyclobutane-type dimers (cis-syn and trans-syn). Quantum efficiencies for photodimerization of thymine base were determined to be 0.0012 for T-T, 0.0084 for polyMAOT, and 0.010 for polyAOT.

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Chapter II. Solvent effect of the photochemical reactions on the acrylic and methacrylic polymers containing thymine bases

II - 1. Introduction

In a series of extensive studies of the interaction of polymers with pendant nucleic acid bases the effect of organic solvents was important [1]. Although no significant interaction was observed between polymers with complementary nucleic acid bases in dimethyl sulfoxide (DMSO), the addition of ethylene glycol (EG) to the DMSO solution produced a more favorable interaction between the polymers in question. This solvent effect has been explained by the competition between solvation by DMSO and base pairing with complementary bases [2,3].

In the previous chapter a study of the photodimerization of thymine bases along the polymer chain was discussed in an effort to estimate the degree of intramolecular self-association between the bases. The quantum efficiency for the intramolecular photodimerization of thymine bases on the polymers was larger than that of the dimer model compound [4].

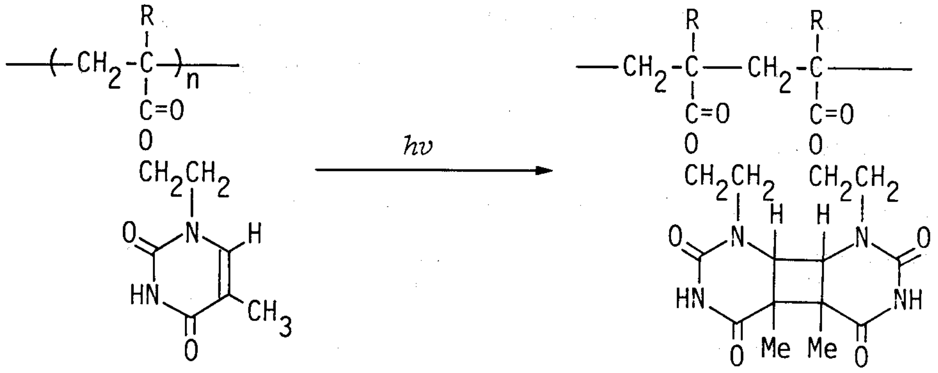
In this chapter photodimerization reactions of thymine bases in acrylic and methacrylic polymers were undertaken in different organic solvents to determine how the solvents affect the degree of intramolecular self-association of the bases along the polymer chain (Figure II-1). The photodimerization was measured in DMSO, N,N-dimethylformamide (DMF), and a mixture of DMSO and EG (3/2, v/v). Hypochromicities of the thymine derivatives as well as the intrinsic viscosities of the polymers were studied in these solvents. The results were related to those of the photodimerization.

II - 2. Results

II - 2 - 1. Quantum Efficiencies for Photodimerization

Quantum efficiencies for photodimer formation of thymine derivatives were measured in three kinds of solvents; DMSO, DMF, and DMSO-EG mixture (3/2, v/v). The data obtained are listed in Table II-1.

It was found that quantum efficiencies for photodimerization of the dimer model compound (T-T) produced almost identical values in



R: CH_3 polyMAOT
H polyAOT

Figure II-1. Photodimerization reaction of polyMAOT and polyAOT.

Table II-1. Quantum Efficiency for Photodimerization in Various Solvents [a]

Compound	Solvent	ϕ_{DF}°
T - T	DMSO	0.0011
	DMF	0.0012
	DMSO-EG [b]	0.0012
polyMAOT	DMSO	0.0074
	DMF	0.0084
	DMSO-EG [b]	0.011
polyAOT	DMSO	0.0077
	DMF	0.010
	DMSO-EG [b]	0.013

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

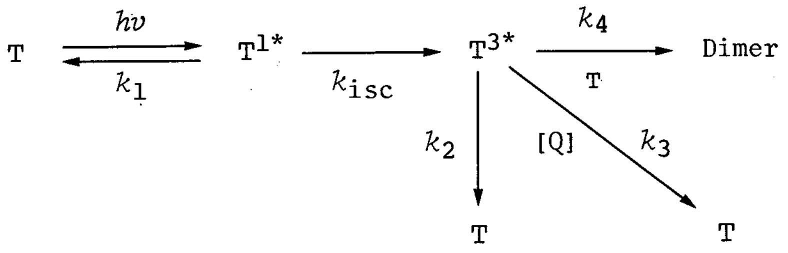
[b] DMSO/EG = 3/2 (v/v).

these solvents. The values of the polymers (polyMAOT and polyAOT) were higher than that of T-T and varied in these solvents. For both types of polymer the quantum efficiencies for photodimerization increased in the following order: DMSO < DMF < DMSO-EG mixture. The values of polyAOT were slightly higher than those of polyMAOT.

II - 2 - 2. Quenching of the Photodimerization

Photodimerization of the thymine derivatives in three kinds of solvents was studied in the presence of isoprene as the triplet quencher. The data obtained were plotted by using the Stern-Volmer equation (1) to give Figures II-2, II-3, and II-4. Equation (1) was derived from Scheme II-1 in which only the excited triplet state was involved in photodimerization [4]. Photodimerization of the thymine dimer model (T-T) was quenched by isoprene and the Stern-Volmer plot gave straight lines as shown in Figure II-2. This fact seems to indicate that the photodimerization of T-T occurs almost completely in the triplet state.

Scheme II-1



$$\frac{\phi_{DF}^{\circ}}{\phi_{DF}} = 1 + \frac{k_3}{k_2 + k_4}[Q] \qquad (1)$$

On the other hand, Stern-Volmer plots of the polymers did not show straight lines (Figures II-3 and II-4). In DMF and DMSO-EG mixture, in particular, only a small amount of quenching of the photodimerization was observed. For the photodimerization of the polymer it was assumed that the excited singlet and triplet states were involved as shown in Scheme II-2. Equation (2) was derived in the previous chapter on the basis of Scheme II-2 [4]. The data for the

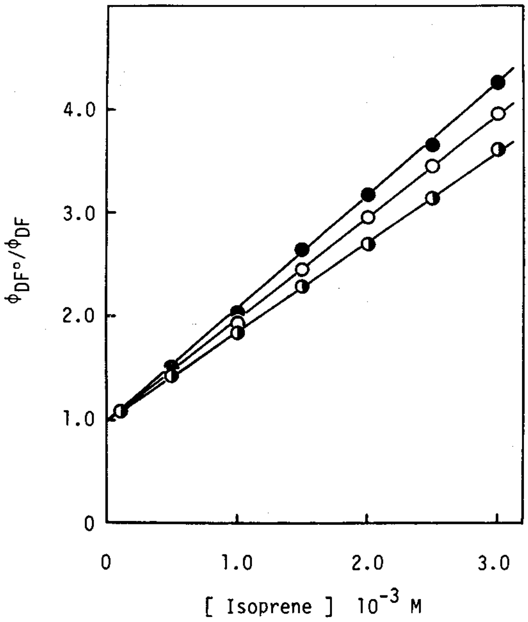


Figure II-2. Representative Stern-Volmer plots for the quenching of T-T by isoprene in various solvents. [T-T] = 1×10^{-4} mol/l of thymine unit; (○) in DMSO, (●) in DMF, (◑) in DMSO-EG mixture (3/2, v/v).

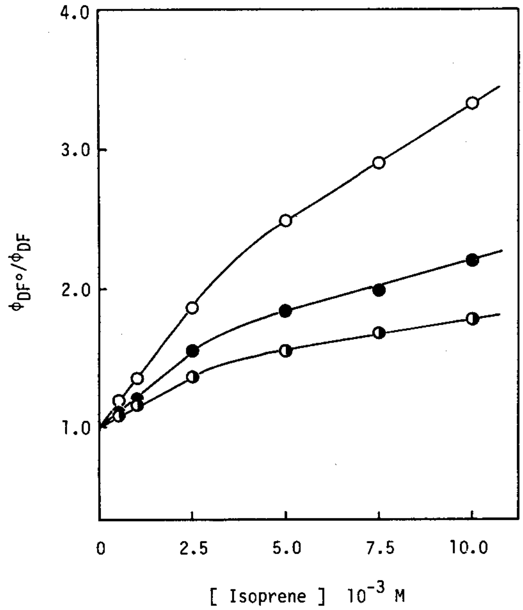
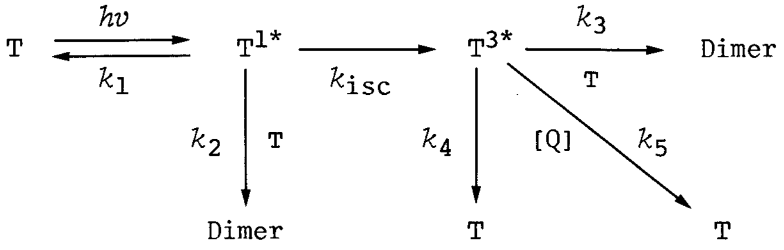


Figure II-3. Representative Stern-Volmer plots for the quenching of polyMAOT by isoprene in various solvents. [polyMAOT] = 1×10^{-4} mol/l of thymine unit; (○) in DMSO, (●) in DMF, (◑) in DMSO-EG mixture (3/2, v/v).

polymers were plotted with equation (2), which gave straight lines for polyMAOT (Figure II-5) and polyAOT (Figure II-6). These figures indicate that both singlet and triplet states were involved in the photodimerization of thymine bases in the polymers.

Scheme II-2



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

The values of $\phi_{DF}{}^{\circ}/\phi_{DF}$ at 1/[isoprene] = 0 were obtained from the intercepts of the lines in Figures II-5 and II-6. The quantum efficiency for the photodimerization that resulted in the singlet state was estimated with the values obtained above and tabulated in Table II-2. Photodimerization in the singlet state was a minor reaction in DMSO solution. In DMSO-EG, however, about one-half the quantum efficiency for photodimerization was derived from the singlet state.

II - 2 - 3. Spectral Properties of Thymine Derivatives

To determine the intramolecular interaction, that is, self-association of thymine bases in the thymine derivatives at the ground state, UV spectra of these compounds were measured in three kinds of solvents. From the spectra the percentage of hypochromicity at 275 nm was calculated, based on the value of the extinction coefficient of the monomeric model compound of thymine (T-M). The values obtained are tabulated in Table II-3.

It is to be noted that significant hypochromicity was observed even in the dimer model at values in the increasing order of DMSO < DMF < DMSO-EG. The hypochromicity values of polyMAOT and polyAOT were higher than those of T-T, although the variance was small. The hypo-

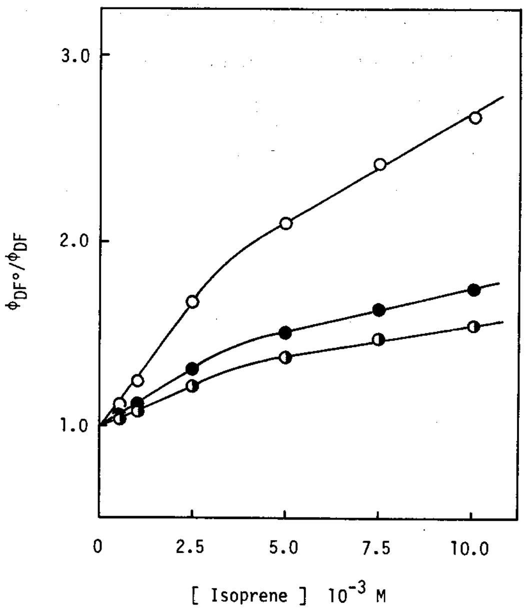


Figure II-4. Representative Stern-Volmer plots for the quenching of polyAOT by isoprene in various solvents. [polyAOT] = 1 × 10^{-4} mol/l of thymine unit; (○) in DMSO, (●) in DMF, (◑) in DMSO-EG mixture (3/3, v/v).

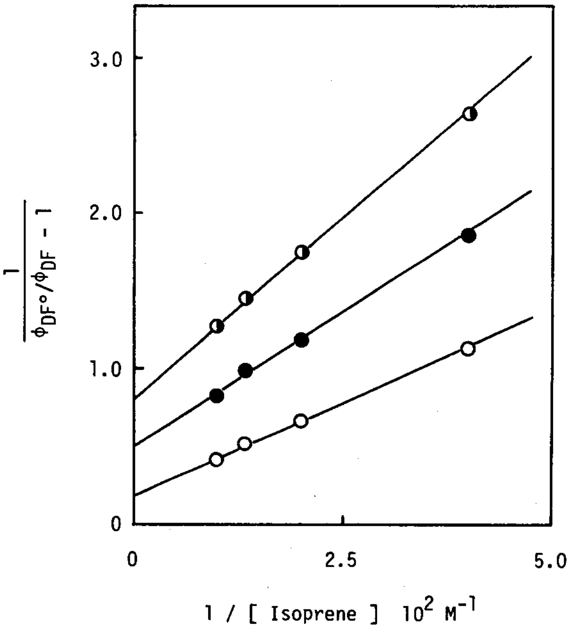


Figure II-5. Relationship between $1/(\phi_{DF}°/\phi_{DF} - 1)$ and 1/[isoprene] of polyMAOT in various solvents; (○) in DMSO, (●) in DMF, (◑) in DMSO-EG mixture (3/2, v/v).

chromicity values of the polymers also varied in different solvents as observed in T-T.

II - 2 - 4. Intrinsic Viscosity of the Polymers

The intrinsic viscosity of the polymers was measured at 25 °C in three kinds of solvents to determine the form of the polymer chain in the solvents in question. The values of the intrinsic viscosity as well as the Huggins' constants for polyMAOT and polyAOT are listed in Table II-4.

It was found that the intrinsic viscosity of polyMAOT was larger than that of polyAOT, probably because of the difference in molecular weight [4]. The intrinsic viscosity of the polymer tended to vary significantly with the solvent, as did the Huggins' constant.

II - 3. Discussion

II - 3 - 1. Self-association of Thymine Bases at the Ground State

From the UV spectral data shown in Table II-3 it is possible to estimate the intramolecular interaction, that is, self-association of the thymine bases in the dimer model and polymers. The self-association was affected by the kind of solvents and tended to increase in the following order: DMSO < DMF < DMSO-EG mixture. The solvent effect on the self-association observed here was closely related to the solvent effect on the formation of the polymer complexes by specific base pairing [1,2].

Data of the intrinsic viscosity in Table II-4 indicate that the conformation of the polymer chains is affected by the nature of the solvents. Because the polymer is soluble in DMSO but insoluble in EG, the addition of EG to DMSO may cause shrinkage of the polymer chain. A decrease in the intrinsic viscosity (Table II-4) appears to correspond to an increase in the hypochromicity (Table II-3).

In conclusion the self-association of thymine bases seems to be small in DMSO, the polymer chain keeps the expanded conformation in this solvent because of the interaction between DMSO and thymine bases. In DMSO-EG mixture the self-association of thymine bases is strong enough and the polymer chain is assumed to be in a shrunken confor-

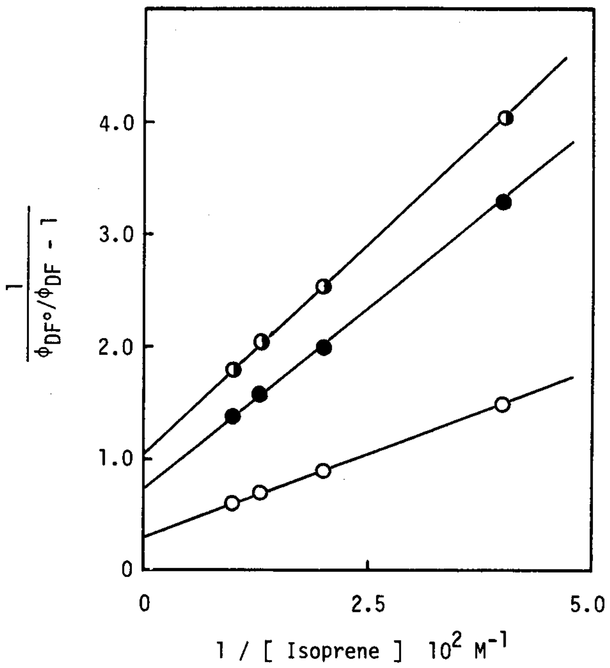


Figure II-6. Relationship between $1/(\phi_{DF}^\circ/\phi_{DF} - 1)$ and 1/[isoprene] of polyAOT in various solvents; (○) in DMSO, (●) in DMF, (◑) in DMSO-EG mixture (3/2, v/v).

Table II-2. Photodimerization of polyMAOT and polyAOT in Various Solvents

Compound	Solvent	Singlet (%)	Triplet (%)
polyMAOT	DMSO	17	83
	DMF	33	67
	DMSO-EG [a]	43	57
polyAOT	DMSO	23	77
	DMF	42	58
	DMSO-EG [a]	51	49

[a] DMSO/EG = 3/2 (v/v).

mation because DMSO molecules can interact with EG molecules. In DMF solution the salf-association of thymine base and the shrinkage of the polymer chain are assumed to be moderate because DMF molecules interact moderately with thymine bases.

II - 3 - 2. Photodimerization of Thymine Bases

It was found that the quantum efficiencies for photodimerization of the polymers were higher than those of T-T and were influenced by the solvents (Table II-1). From the result of the quenching by isoprene the photodimerization of thymine bases in the polymers occurred in both singlet and triplet states as shown in Scheme II-2. The ratio of singlet to triplet states involved in the photodimerization was estimated in Table II-2 and was clearly affected by the solvents. From the UV spectral data as well as the intrinsic viscosity the self-association of thymine bases in the polymers was confirmed and found to be affected by the solvents. By varying the solvents an increase in the self-association of thymine bases in the polymer tended to correspond to an increase in quantum efficiency and the ratio of singlet to triplet states.

From these facts it may be concluded that the addition of EG to DMSO induces the self-association of thymine bases in the polymer. Increase in the self-association is reflected in the increase in hypochromicity and shrinkage of the polymer chain. The self-association of thymine bases in the polymer chain may be sterically assisted by the neighboring groups on the polymer chain. This sterically assisted self-association of thymine bases on the polymer chain causes photodimerization in the singlet state and accelerates the photodimerization in the triplet state. The quantum efficiency would be higher if the singlet state was involved in the photodimerization because the quantum efficiency for photodimerization of the singlet state is known to be exceedingly high [5].

Quantum efficiency for photodimerization of the dimer model was low and unaffected by the solvents (Table II-1). On the other hand, the self-association of thymine bases in the T-T molecule at the ground state could be confirmed by the UV spectra and was influenced by the

Table II-3. UV Spectral Data for the Polymers and Corresponding Model Compounds in Various Solvents

Compound	Solvent	ε [a]	Hypochromicity (%)[b]
T - M	DMSO	9000	0
	DMF	8910	0
	DMSO-EG [c]	9120	0
T - T	DMSO	8430	6.33
	DMF	8250	7.41
	DMSO-EG [c]	8230	9.76
polyMAOT	DMSO	7990	11.2
	DMF	7840	12.0
	DMSO-EG [c]	7880	13.6
polyAOT	DMSO	7960	11.5
	DMF	7760	12.9
	DMSO-EG [c]	7850	13.9

[a] Molar extinction coefficient at 275 nm.

[b] Hypochromocity based on T-M.

[c] DMSO/EG = 3/2 (v/v).

Table II-4. Intrinsic Viscosity and Huggins' k' Constant of polyMAOT and polyAOT in Various Solvents at 25 °C

Compound	Solvent	$[\eta]$	k'
polyMAOT	DMSO	0.63	0.19
	DMF	0.50	0.24
	DMSO-EG [a]	0.31	0.37
polyAOT	DMSO	0.080	0.47
	DMF	0.076	0.52
	DMSO-EG [a]	0.065	0.71

[a] DMSO/EG = 3/2 (v/v).

solvents. The photodimerization of T-T occurred in the triplet state as shown in Scheme II-1 and the values $k_3/(k_2+k_4)$ were only slightly affected by the solvents (Figure II-2). This fact seems to explain that the lifetime of the self-association of thymine bases in T-T molecule observed in the UV spectra is too short to induce the singlet-derived photodimerization and too short to accelerate the triplet-derived photodimerization. Contrary to the case of the polymer, the self-association of the thymine bases in the T-T molecule may be unstable because the association does not appear to be sterically assisted by the neighboring groups.

II - 4. Experimental

II - 4 - 1. Instrumentation

UV spectra were measured with a Shimazu UV-180 spectrophotometer. Intrinsic viscosities were measured with a Ubbelohde-type capillary viscometer at 25 °C.

II - 4 - 2. Photolysis

Photochemical reactions were carried out in a 10-mm quartz cell filled with 4 ml of solution at 20 °C. A 1-kw super-high pressure mercury lamp filtered through a Pyrex filter was used as the light souce. The filter showed 0 % transmittance below 265 nm and 50 % transmittance at 297 nm. Light intensities were determined by potassium ferrioxalate actinometry. The quantum yield for oxalic acid decomposition was 1.24 at 297/302 nm [6]. Concentration of the thymine derivatives was kept at 1×10^{-4} mol/l of thymine unit and a stream of purified nitrogen gas was passed through the solution to displace the air.

Quenching studies were performed in a similar manner, as mentioned above for photochemical reaction, with isoprene as a triplet quencher.

II - 4 - 3. Materials

Polymers and the model compounds used here were prepared according to the method reported in previous chapter [4]. The reagents were obtained commercially and purified in the usual manner. Spectral quality solvents were used in all photochemical experiments.

II - 5. Summary

Photodimerization reaction of thymine bases present on the side chain of acryloyl and methacryloyl type polymers was studied in dimethyl sulfoxide, dimethylformamide, and dimethyl sulfoxide-ethylene glycol mixture. Quantum efficiencies for photodimerization and quenching with isoprene were influenced by the solvents. The self-association of thymine bases estimated from their ultraviolet spectra and intrinsic viscosity were related to the effect of solvent on the photodimerization reaction.

II - 6. References

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Chapter III. Photochemical reactions on the acrylic and methacrylic polymers containing thymine bases in the presence of adenine derivatives

III - 1. Introduction

The photodimerization of thymine bases has received much attention in the nucleic acid chemistry [1-3]. The formation of such dimer structure has been a subject of interest to the area of photobiology, because the reaction in question is known to be involved in photoinactivation of deoxyribonucleic acid (DNA) [4-6]. As the mechanism of the photodimerization of DNA is complicated, the reaction was studied rather on the simple model compounds such as thymine derivatives [7,8], thymine [9-11], and polythymidylic acid (poly T) [12,13]. From these results, it was concluded that the thymine bases form photodimers with the nearest neighboring ones which exist in stacking interaction with each other along the polymer chain [1].

In a series of our extensive studies on the photodimerization of thymine bases present in the side chain of synthetic polymers, the quantum efficiency for photodimerization was found to depend on the intramolecular interaction of the thymine bases along the polymer chain. For the thymine bases which lie on the polymer chain, the hypochromicity and quantum efficiency for photodimerization were found to be greater than those for low molecular weight model compounds [14-16].

In DNA, thymine bases are known to interact with neighboring ones along the polymer chain (stacking interaction), and also with adenine bases in another polymer chain (hydrogen bonding interaction) to form a double-stranded helical structure. Such a specific base pairing between adenine and thymine bases was reported to lower the quantum efficiency for photodimerization of thymine bases [1].

This chapter deals with the photodimerization of thymine bases both in the polymers and in the dimer model compound, which was studied in the presence of adenine derivatives, in order to see the effect of such specific base pairing between adenine and thymine bases on the photodimerization.

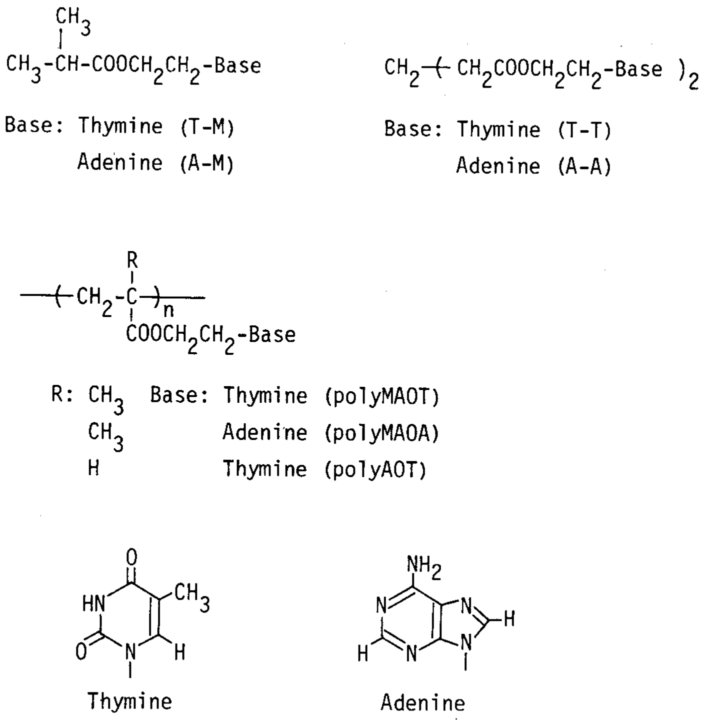


Figure III-1. Thymine and adenine derivatives.

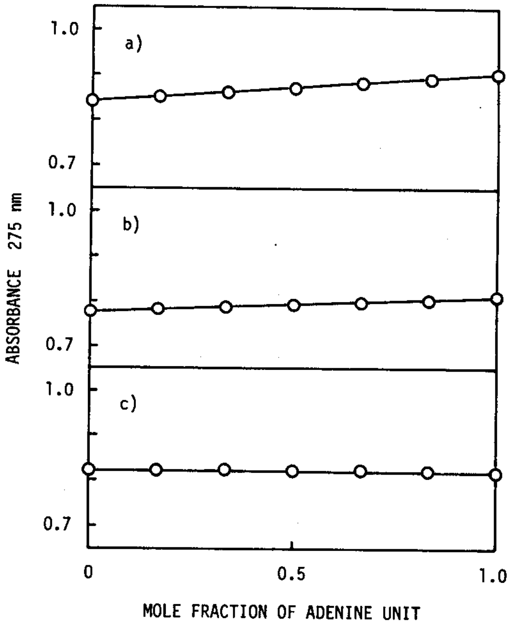


Figure III-2. Mixing curves between T-T and A-M in various solvents. Absorbance at 275 nm was obtained in 10 mm cell at 25 °C. A series of mixed solutions was allowed to stand at 50 °C for 24 hr; a) in DMSO, b) in DMF, c) in DMSO-EG mixture (3/2, v/v).

III - 2. Results and Discussion

III - 2 - 1. Interaction between Adenine and Thymine Bases in Ground State

The intramolecular interaction of thymine bases (stacking interaction) in homopolymers, copolymers, and oligomers was studied previously [14,16,17]. These interaction can be estimated from the hypochromicity in UV spectra. The hypochromicities of the thymine derivatives were found to be affected by the nearest neighboring thymine bases and also affected by solvent [15]. It was also found that the hypochromicity for the homopolymer was the highest value among these derivatives.

When the polymer containing pendant thymine bases forms a polymer complex with the polymer containing pendant adenine bases, additional hypochromicity can be observed, as well as the case of polynucleotide or the synthetic polymers containing nucleic acid bases. The double-stranded structure is more rigidly stacked than the single-stranded one, so that additional hypochromicity of the former over the latter structure is seen in these polymer systems [18,19].

The interaction between adenine and thymine derivatives in ground state was studied by UV spectroscopy in dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and DMSO-ethylene glycol (EG) mixture (3/2, v/v). The thymine and adenine derivatives used here were summarized in Figure III-1. Figures III-2 to III-10 show the mixing curves for these systems. As seen in these figures, the formation of the complex was observed only for the polyMAOA with polyMAOT, or with polyAOT system. The complex formation in question may depend on the degree of polymerization of the polymers used. For the polynucleotide system, the critical length for forming the double-stranded structure has been estimated from the CD data to be six base pairs [20].

The complex formation was also found to be affected by the sort of solvents. In DMSO, which is a good solvent for both polyMAOA and polyMAOT, no significant interaction was observed because of the strong solvation of DMSO with thymine and adenine bases. On the other hand, in DMF, which is a good solvent for polyMAOT but a poor solvent for

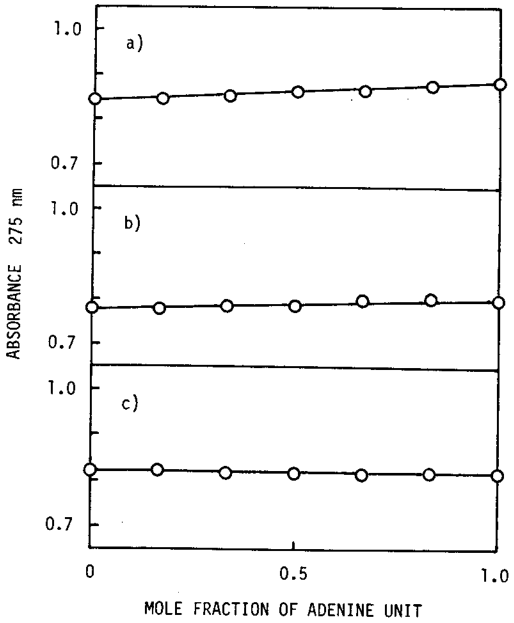


Figure III-3. Mixing curves between T-T and A-A in various solvents. Absorbance at 275 nm was obtained in 10 mm cell at 25 °C. A series of mixed solutions was allowed to stand at 50 °C for 24 hr; a) in DMSO, b) in DMF, c) in DMSO-EG mixture (3/2, v/v).

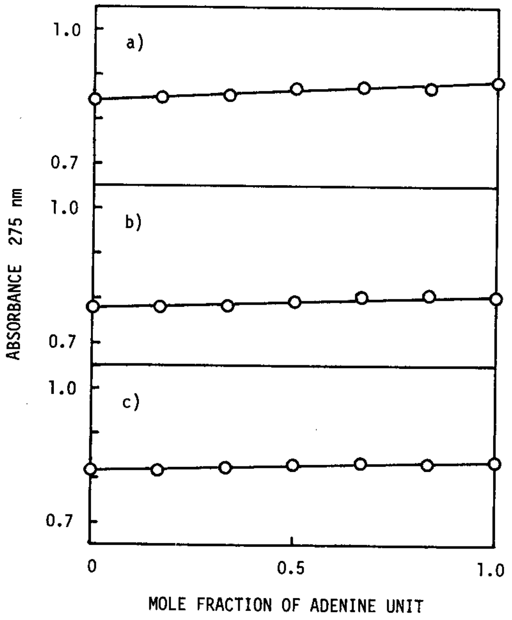


Figure III-4. Mixing curves between T-T and polyMAOA in various solvents. Absorbance at 275 nm was obtained in 10 mm cell at 25 °C. A series of mixed solutions was allowed to stand at 50 °C for 24 hr; a) in DMSO, b) in DMF, c) in DMSO-EG mixture (3/2, v/v).

polyMAOA, the complex formation between polyMAOA and polyMAOT was observed. In DMSO-EG mixture, in which EG is a good solvent for polyMAOA but a poor solvent for polyMAOT, the most stable polymer complex was formed. Similar solvent effect on the formation of the polymer complex was also reported [21].

From these facts it should be concluded that the intermolecular interaction between adenine and thymine bases in ground state depends on the degree of polymerization of the polymers and on the solvent used.

III - 2 - 2. Photodimerization of Thymine Bases

Photolysis of both the polymers (polyMAOT and polyAOT) and the dimer model compound (T-T) containing thymine moieties was then carried out in DMSO, DMF, and DMSO-EG mixture. In all of these systems, the formation of the photodimer between adjacent thymine bases was ascertained as reported in Chapter II [15]. The quantum efficiencies for photodimerization of these systems were summarized in Table III-1. This table indicated that the quantum efficiency depends on the adenine derivatives added, and on the solvent used.

It was known that the photodimer of thymine bases is formed from the excited triplet state and/or from the excited singlet state [1]. Addition of adenine derivatives to the thymine system causes decrease in the quantum efficiency for photodimerization. The photodimerization of thymine in freezed aqueous solution was depressed by the addition of adenine [22,23]. The rate of photodimerization of polydeoxythymidylic acid (poly dT) was faster than that of poly dA · poly dT (double-stranded polymer complex) [24]. The photodimerization of 1-cyclohexylthymine in acetonitrile was quenched by 9-ethyladenine, and was regained by further addition of triplet sensitizer [25].

As the triplet and singlet energies of adenine derivatives are higher than those of thymine derivatives, energy transfer from thymine base to adenine base is impossible [26]. Therefore, the photodimerization of thymine bases is considered to be quenched by the hydrogen bonding interaction between adenine and thymine bases. And the quenching by hydrogen bonding is caused in the singlet state by

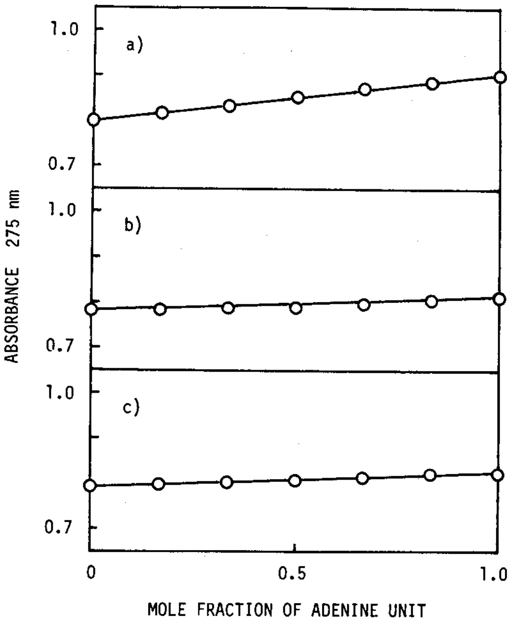


Figure III-5. Mixing curves between polyMAOT and A-M in various solvents. Absorbance at 275 nm was obtained in 10 mm cell at 25 °C. A series of mixed solutions was allowed to stand at 50 °C for 24 hr; a) in DMSO, b) in DMF, c) in DMSO-EG mixture (3/2, v/v).

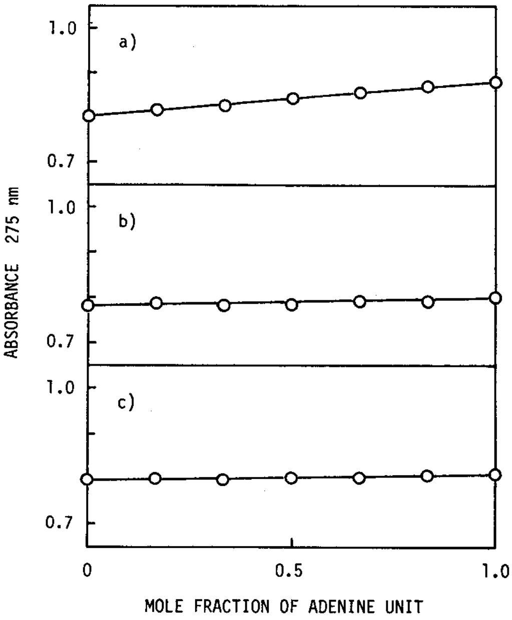


Figure III-6. Mixing curves between polyMAOT and A-A in various solvents. Absorbance at 275 nm was obtained in 10 mm cell at 25 °C. A series of mixed solutions was allowed to stand at 50 °C for 24 hr; a) in DMSO, b) in DMF, c) in DMSO-EG mixture (3/2, v/v).

geometry modification, and not by proton transfer [26].

It may then be expected that the extent of the quenching of the photodimerization of thymine bases by adenine bases indicates the extent of the interaction between adenine and thymine bases in the excited singlet state.

Dimer Model Compound (T-T)

In the previous study on the photodimerization of thymine bases of the dimer model compound (T-T), it was found that the triplet state was the precursor of all the photodimer formed [14]. Although addition of equimolar isoprene (triplet quencher) to the T-T system caused slight decrease in the quantum efficiency, addition of equimolar adenine units caused significant decrease in the quantum efficiency as shown in Table III-1. As the adenine derivatives added may quench the singlet state of the thymine bases as mentioned above, the mechanism of photodimer formation for T-T in the presence of adenine derivatives may be given as Scheme III-1. In this scheme, the quenching of the singlet state of thymine base by the adenine base may cause decrease in the concentration of the triplet state of the thymine bases. Such quenching results in the inhibition of the photodimerization from the triplet state.

Scheme III-1

$$\begin{tikzcd}
T \arrow[r, shift left, "h\nu"] & T^{1*} \arrow[l, shift left, "k_1"] \arrow[r, "k_{isc}"'] \arrow[d, "k_3"', "{[A]}"] & T^{3*} \arrow[r, "k_4", "T"'] \arrow[d, "k_2"'] & \text{Dimer} \\
 & T & T &
\end{tikzcd}$$

In Scheme III-1 T, T^{1*}, T^{3*}, and A denote the ground state, excited singlet and triplet states of thymine and adenine bases, respectively. k_1, k_2, k_3, k_4, And k_{isc} are rate constants. For the Scheme III-1, the quantum efficiency of T-T for photodimerization by using a steady-state treatment is given by

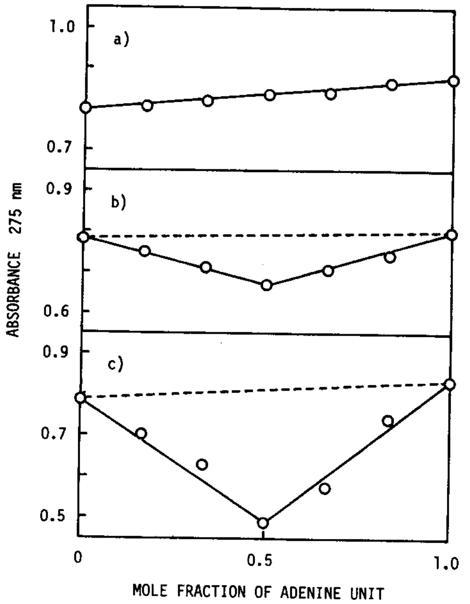


Figure III-7. Mixing curves between polyMAOT and polyMAOA in various solvents. Absorbance at 275 nm was obtained in 10 mm cell at 25 °C. A series of mixed solutions was allowed to stand at 50 °C for 24 hr; a) in DMSO, b) in DMF, c) in DMSO-EG mixture (3/2, v/v).

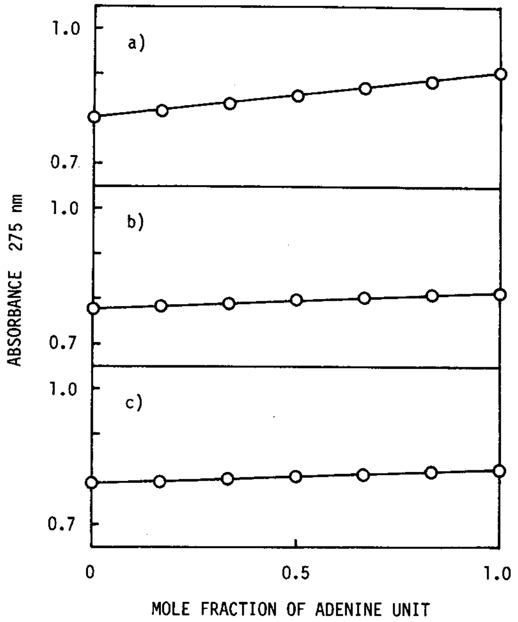


Figure III-8. Mixing curves between polyAOT and A-M in various solvents. Absorbance at 275 nm was obtained in 10 mm cell at 25 °C. A series of mixed solutions was allowed to stand at 50 °C for 24 hr; a) in DMSO, b) in DMF, c) in DMSO-EG mixture (3/2, v/v).

$$\phi_{DF}{}^{\circ} = \left(\frac{k_{isc}}{k_{isc} + k_1}\right)\left(\frac{k_4}{k_2 + k_4}\right) \qquad (1)$$

If the adenine derivatives are present in the system, the equation (1) becomes

$$\phi_{DF} = \left(\frac{k_{isc}}{k_{isc} + k_1 + k_3[A]}\right)\left(\frac{k_4}{k_2 + k_4}\right) \qquad (2)$$

Combination of equations (1) and (2) gives

$$\frac{\phi_{DF}{}^{\circ}}{\phi_{DF}} = 1 + \left(\frac{k_3}{k_{isc} + k_1}\right)[A] \qquad (3)$$

From the data shown in Table III-1, the value of $\phi_{DF}{}^{\circ}/\phi_{DF} - 1$ was calculated and tabulated in Table III-2. Assuming the equation (3), the values in Table III-2 give the quantum efficiency quenched by the adenine derivatives, and therefore give the extent of interaction between adenine and thymine bases in the excited state. In the systems of T-T with A-M, A-A, and polyMAOA, additional hypochromicity caused by hydrogen bonding interaction between T-T and the adenine derivatives could not be observed from UV spectra. Table III-2, however, shows clearly the interaction of T-T with the adenine derivatives by hydrogen bonding in excited state.

The values of T-T in Table III-2 tend to increase in the order: A-M < A-A < polyMAOA. The order is in agreement with that of the interaction between complementary oligonucleotides [27]. The values in Table III-2 suggest the solvent effect of quenching, and it increased in the order: DMSO < DMF < DMSO-EG mixture. The solvent effect observed here gave similar tendency with those of hypochromicity [15], formation of polymer complex [28], and photodimerization reaction [15]. This fact suggests that the values in the Table III-2 represent the extent of the interaction between adenine and thymine bases.

The results on the quenching experiment in the presence of adenine derivatives can be explained by (1) the excited singlet state of

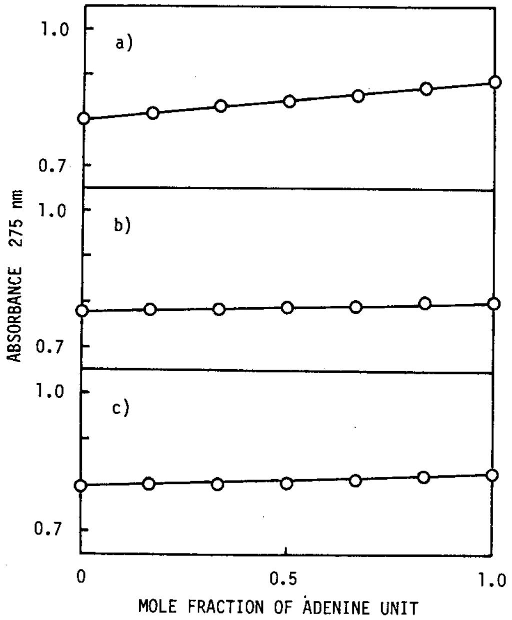


Figure III-9. Mixing curves between polyAOT and A-A in various solvents. Absorbance at 275 nm was obtained in 10 mm cell at 25 °C. A series of mixed solutions was allowed to stand at 50 °C for 24 hr; a) in DMSO, b) in DMF, c) in DMSO-EG mixture (3/2, v/v).

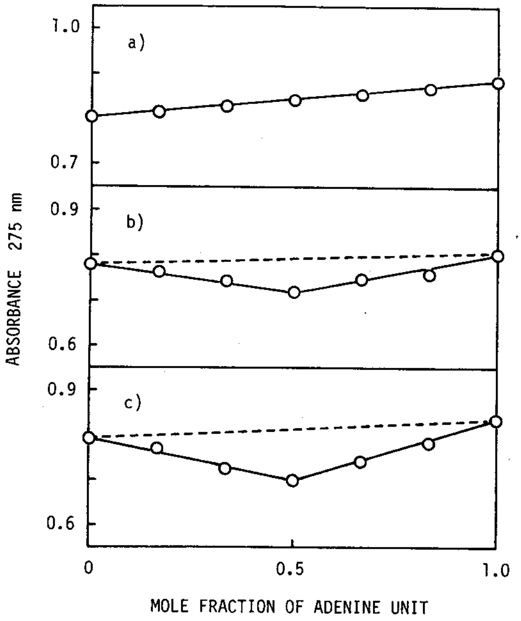


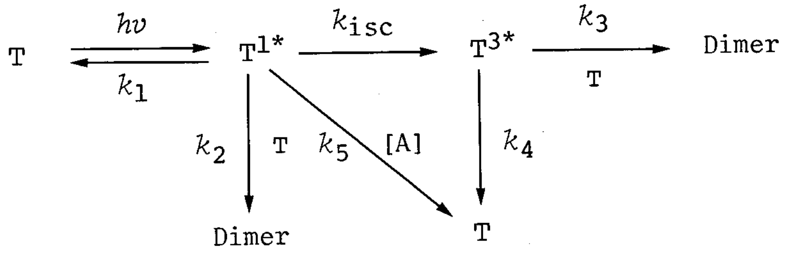
Figure III-10. Mixing curves between polyAOT and polyMAOA in various solvents. Absorbance at 275 nm was obtained in 10 mm cell at 25 °C. A series of mixed solutions was allowed to stand at 50 °C for 24 hr; a) in DMSO, b) in DMF, c) in DMSO-EG mixture (3/2, v/v).

thymine bases was quenched by the adenine-thymine interaction which was too weak to be detected in the ground state, or (2) the excited singlet state was quenched by the interaction between the excited singlet state of thymine base and ground state of adenine base, and the bases in the excited state have much stronger interactions than they do in the ground state.

Polymers (polyMAOT and polyAOT)

For the synthetic polymers containing thymine bases, the photodimerization of the bases along the polymer chain was studied in previous chapter [14]. According to this, both singlet and triplet state were found to be the precursors of the photodimer formation. The extent of the singlet derived photodimerization was affected by the nearest neighboring thymine units as seen for the hypochromicity. It was also found that the energy of the singlet state of thymine bases in the polymer migrates intramolecularly to another thymine bases in the identical polymer chain, so that the energy absorbed could be effectively used for the photodimerization reaction [17].

Scheme III-2



As shown in Table III-1, the photodimerization of thymine bases in the polymer chain was quenched by adding the adenine derivatives. As for the polymer system as well as T-T system, it may also be assumed that the singlet state of thymine bases in the polymer chain was quenched by the adenine bases forming hydrogen bonding interaction. Therefore, the mechanism of the photodimer formation in cases of polyMAOT and polyAOT in the presence of adenine derivatives may be shown as in Scheme III-2. In this scheme, decrease in the concentra-

Table III-1. Quantum Efficiency for Photodimerization in Various Solvents[a]

Compound	Solvent	Additives[b]				
		none[d]	A - M	A - A	polyMAOA	Isoprene
T - T	DMSO	0.0011	0.00080	0.00080	0.00075	0.00090
	DMF	0.0012	0.00084	0.00078	0.00064	0.00088
	DMSO-EG[c]	0.0012	0.00076	0.00071	0.00059	0.0011
polyMAOT	DMSO	0.0074	0.0038	0.0045	0.0049	0.0062
	DMF	0.0084	0.0040	0.0044	0.0049	0.0074
	DMSO-EG[c]	0.011	0.0074	0.0059	0.0041	0.010
polyAOT	DMSO	0.0077	0.0049	0.0053	0.0056	0.0066
	DMF	0.010	0.0054	0.0058	0.0060	0.0089
	DMSO-EG[c]	0.013	0.0079	0.0079	0.0048	0.012

[a] Concentration of thymine unit was always 1 × 10^{-4} mol/1.

[b] Concentration of isoprene and adenine unit were always 1 × 10^{-4} mol/1.

[c] DMSO/EG = 3/2 (v/v).

[d] Reference 15.

tion of thymine base in the singlet state quenched by the adenine-thymine interaction results in the depression of the photodimerization reaction derived from both singlet and triplet states.

For the mechanism shown in Scheme III-2, the quantum efficiency for photodimerization can be described by

$$\phi_{DF}{}^{\circ} = \left(\frac{1}{k_{isc} + k_1 + k_2}\right)\left(k_2 + \frac{k_{isc}k_3}{k_3 + k_4}\right) \tag{4}$$

If the adenine bases are present, the equation (4) becomes to

$$\phi_{DF} = \left(\frac{1}{k_{isc} + k_1 + k_2 + k_5[A]}\right)\left(k_2 + \frac{k_{isc}k_3}{k_3 + k_4}\right) \tag{5}$$

Treatment of equations (4) and (5) leads to

$$\frac{\phi_{DF}{}^{\circ}}{\phi_{DF}} = 1 + \left(\frac{k_5}{k_{isc} + k_1 + k_2}\right)[A] \tag{6}$$

Assuming the equation (6), values for polyMAOT and polyAOT in Table III-2 should indicate the quantum efficiency quenched by the adenine bases, and such values may show the extent of the interaction between adenine and thymine bases.

In DMSO-EG mixture, the values shown in Table III-2 tend to increase in the order: A-M < A-A < polyMAOA. In the ground state, additional hypochromicity was observed for the polyMAOA-polyMAOT and polyMAOA-polyAOT systems, suggesting the formation of stable polymer complex by specific base-pairing between the complementary bases. From these facts, the order of the values in Table III-2 for polyMAOT and polyAOT in DMSO-EG mixture can be correlated to the hydrogen bonding interaction between adenine and thymine bases of each polymers.

In DMSO, the complex formation in the ground state could not be observed because of strong solvation of DMSO molecules to the nucleic acid bases. As can be seen from Table III-2, however, interaction between adenine and thymine bases in the excited state was observed for polyMAOT and polyAOT systems. The reason for these results ap-

Table III-2. $\phi_{DF}^{\circ}/\phi_{DF} - 1$ Values

Compound	Solvent	Additives				
		none	A - M	A - A	polyMAOA	Isoprene
T - T	DMSO	0	0.38	0.38	0.47	0.22
	DMF	0	0.43	0.54	0.88	0.36
	DMSO-EG [a]	0	0.58	0.69	1.03	0.091
polyMAOT	DMSO	0	0.95	0.64	0.51	0.19
	DMF	0	1.10	0.91	0.71	0.14
	DMSO-EG [a]	0	0.49	0.86	1.68	0.078
polyAOT	DMSO	0	0.57	0.45	0.38	0.17
	DMF	0	0.85	0.72	0.67	0.12
	DMSO-EG [a]	0	0.64	0.64	1.71	0.066

[a] DMSO/EG = 3/2 (v/v).

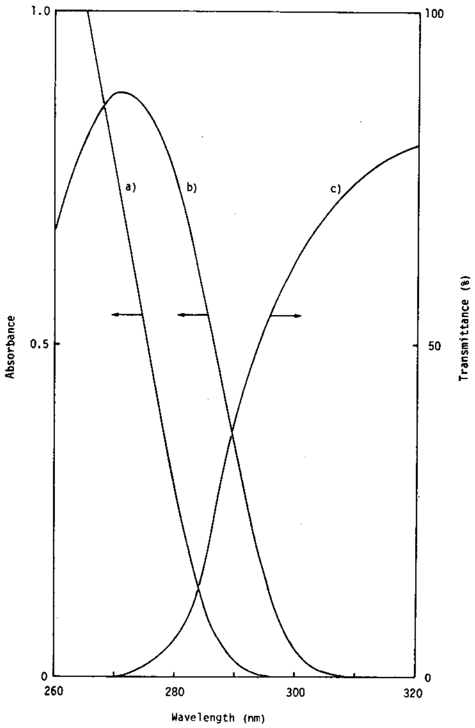


Figure III-11. UV Absorption of thymine derivative, adenine derivative, and the combination of colored glass and 10 mm DMF solution filter;
a) absorbance of A-M (1×10^{-4} mol/l of adenine unit in DMF solution),
b) absorbance of T-T (1×10^{-4} mol/l of thymine unit in DMF solution),
c) percent transmittance of the filter.

pears to be similar to that mentioned for T-T. The bases in excited state have much stronger interaction than they do in the ground state. Table III-2 shows that the tendency of quenching in DMSO solution is different from that in DMSO-EG mixture. The order of quenching in DMSO is polyMAOA < A-A < A-M. This fact may be caused by the difference in the penetration ability of the adenine derivatives, A-M can easily penetrate into the polyMAOT and polyAOT chains, while polyMAOA can hardly penetrate into the polyMAOT and polyAOT chains.

In DMF solution, the interaction between adenine and thymine bases in the ground state was observed for polyMAOA-polyMAOT and polyMAOA-polyAOT systems. The interaction in the excited state, however, tended to increase in the order as observed in DMSO; polyMAOA < A-A < A-M (Table III-2). The result shown here in DMF solution may be explained as follows, that is, as the interaction in the ground state was less stable in DMF solution as compared with that in DMSO-EG mixture, the penetration ability of the adenine derivatives became the main factor for the quenching of the singlet state of thymine bases.

In conclusion, the interaction between adenine and thymine bases in the ground state could be observed when the stable polymer complex was formed, that is, polyMAOA-polyMAOT and polyMAOA-polyAOT in DMF and DMSO-EG systems. On the other hand, the interaction between adenine and thymine bases in the excited singlet state was observed even for T-T and A-M systems where the interaction was weak enough in the ground state. The study of the interaction by quenching of the photodimerization may give an useful information to a weak interaction between adenine and thymine bases.

III - 3. Experimental

III - 3 - 1. Photolysis

Photochemical reactions were carried out using 10-mm quartz cell filled with 4 ml of solution at 20 °C. A 1-kw super-high pressure mercury lamp filtered through a combination of colored glass and 10 mm DMF solution filters 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 297/302 nm [29].

Concentration of the thymine derivatives was kept constant at 1×10^{-4} mol/l of thymine unit and a stream of purified nitrogen gas was passed through the solution to displace air.

Quenching studies were made in a similar manner, as mentioned above for the photochemical reactions, with isoprene (1×10^{-4} mol/l) and adenine derivatives (1×10^{-4} mol/l of adenine unit).

The absorbance of 1×10^{-4} mol/l of base unit for T-T and A-M in DMF solution are shown in Figure III-11. The same absorption spectra were obtained in DMSO and DMSO-EG mixture for other thymine and adenine derivatives. In order to avoid ambiguities in interpreting the results obtained from the photochemical reactions, light with wavelength between 280 and 300 nm, where thymine bases absorb but adenine bases do not, should be used. Therefore, a 1-kw super-high pressure mercury lamp with the combination of colored glass and 10 mm DMF solution filters was used as the light source. Transmittance of this filter is also shown in Figure III-11. Light with $\lambda < 270$ nm emitted by the super-high pressure mercury lamp was cut off by this filter. The effect of adenine derivatives on the photodimerization of thymine derivatives thus cannot be attributed to the reduction of light energy sufficient for the reaction due to the absorption by adenine derivatives.

At a given period intervals, the concentration of thymine bases remained in solution was determined by UV analysis at 275 nm (A Shimazu UV-180 spectrophotometer was used), at which neither the photodimers nor isoprene absorb significantly.

III - 3 - 2. Materials

The thymine derivatives used here were prepared according to the method described in the previous chapter [14]. The adenine derivatives were prepared according to the method reported earlier [21,28]. The reagents were obtained commercially and purified in the usual manner. Spectral quality solvents were used in all photochemical experiments.

III - 4. Summary

Photodimerization reactions of polyacrylic and polymethacrylic derivatives and the dimeric model compound containing thymine bases were studied in the presence of adenine derivatives in dimethyl sulfoxide, dimethylformamide, and dimethyl sulfoxide-ethylene glycol mixture. The photodimerization of thymine bases both in the polymers and in the dimeric model compound was found to be quenched by the addition of adenine derivatives. Base-base interaction in ground state was also studied by UV spectroscopy in three kinds of solvents. The quenching of the photodimerization of thymine bases in the presence of adenine derivatives was discussed in terms of the specific interaction between adenine and thymine bases both in ground and excited states.

III - 5. References

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Chapter IV. Photochemical reactions on the acrylic and methacrylic copolymers containing thymine bases

IV - 1. Introduction

Photodimerization of thymine bases seems to have been a subject of growing interest to the area of photobiology for a number of years. Photolysis of thymine [1], dimethylthymine [2,3], and tetramethyluracil [4] have been studied in aqueous and organic media, and it was suggested that these aggregates in ground state could play an important role in the photodimerization.

The chemistry of nucleic acid analogs has recently received much attention and a number of such synthetic polymers has been hitherto designed, and the specific interaction due to base-base pairing between the polymers having pendant nucleic acid bases has been extensively studied [5-7].

Studies on the photodimerization of thymine bases along the polymer chain were previously done in order to estimate the degree of intramolecular self-association of the thymine bases in the ground state. It was concluded in these cases that the intramolecular self-association of thymine bases plays an important role in the photodimerization reaction [8,9]. The quantum efficiency as well as the mechanism of photodimerization were extremely affected by the extent of intramolecular self-association of thymine bases.

In this chapter, photodimerization reaction of polyacrylic and polymethacrylic derivatives containing different pendant thymine content was investigated. As previously reported [8], photodimerization of thymine bases in the homopolymers seems to have been incomplete because of the isolation of some thymine bases by the photodimerization processes. In order to see the effect of thymine base isolation during the reaction, the copolymers were chosen in this chapter, and the data obtained were compared with those for homopolymers reported in Chapter I.

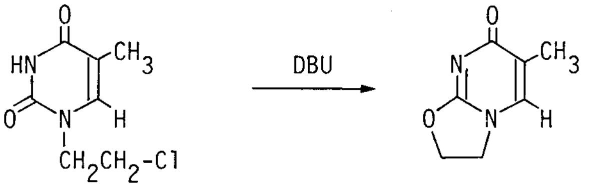
IV - 2. Results

IV - 2 - 1. Synthesis of Polyacrylic and Polymethacrylic Derivatives Containing Different Contents of Pendant Thymine Units

Cyclonucleosides are valuable intermediate compounds for synthesizing various nucleoside derivatives [10]. As a model of the cyclonucleosides, cyclic compound of thymine (2) was prepared [11]. This cyclic derivative showed similar reactivity as the cyclonucleosides against a number of reagents, and was substantially useful as intermediate compounds for the preparation of thymine derivatives [12].

A series of polyacrylic and polymethacrylic derivatives having thymine units was prepared by the polymeranalogous reaction of the cyclic compound of thymine (2) with polyacrylic and polymethacrylic acids.

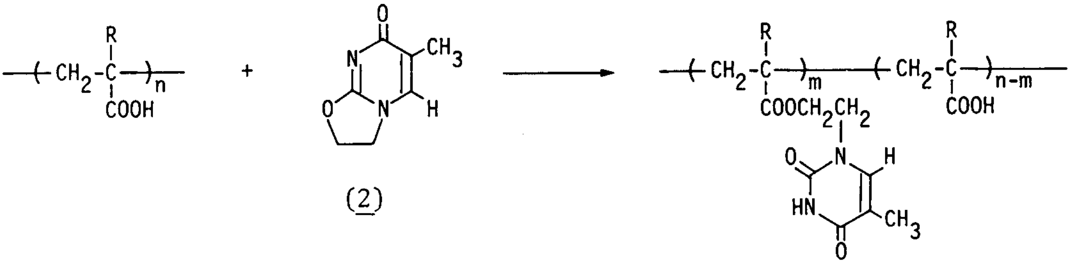
Cyclic derivative of thymine (2) was obtained in high yield from the chloroethylthymine (1) by dehydrochlorination with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in dimethylformamide (DMF) solution. Structure of (2) was identified by NMR, IR, UV, and Mass spectra, as well as elemental analysis [11].



(1) (2)

Reactions of the cyclic compound with different kinds of reagents gave the thymine derivatives conveniently at room temperature without catalyst. One of the typical reaction is the esterification by acetic acid in bulk, which affords the corresponding acetate. When this reaction was applied to polymeric acids, that is, polyacrylic and polymethacrylic acids, polyacrylate and polymethacrylate having pendant thymine bases were obtained in high yield.

By using this reaction, copolymers of acrylic acid and N-2-acryloyloxyethyl derivative of thymine, and also those of methacrylic acid and the methacrylate of the thymine were successfully prepared, which had the same degree of polymerization as the original polyacrylic and polymethacrylic acids. A series of the copolymers was obtained by varying the molar ratio of the cyclic compound to the polymeric acid. Molecular weight of polyacrylic and polymethacrylic acids used here was about 15,000.



(2)

R: CH_3 copolyMAOT (3b)
H copolyAOT (3a)

The content of thymine base in the copolymer was determined by measuring UV absorbance of the oxyethyl derivative of thymine which was obtained by hydrolysis of the copolymer. The analytical data are listed in Table IV-1. This table shows that the cyclic compound reacted almost quantitatively with polyacrylic and polymethacrylic acid. From NMR, IR, and UV spectra, the copolymer having the highest content of the thymine base was found to be identical to the authentic homopolymer which was prepared by free radical polymerization of the corresponding vinyl monomer.

IV - 2 - 2. Spectral Properties of Copolymers

In order to get information about the intramolecular interaction of thymine bases in copolymers, the UV spectra of the copolymers were measured in DMF solution. The concentration of these copolymers were low enough (1×10^{-4} mol/l of thymine unit) to avoid intermolecular association. The UV spectral data were listed in Table IV-2. From the data, percent hypochromicity of the copolymers was estimated and listed also in the same table. This table contains further the data of the dimer (T-T), monomer (T-M), and polymers (polyMAOT and polyAOT) reported in Chapter I. The λ_{max} values of the copolymers afforded

Table IV-1. Synthesis of the Copolymers

Compound	Base Content	
	Calcd. (%) [a]	Found (%) [b]
copolyAOT-100	100	87
copolyAOT-75	75	65
copolyAOT-50	50	36
copolyAOT-25	25	19
copolyAOT-10	10	7.4
copolyAOT-5	5	4.0
copolyMAOT-80	80	69
copolyMAOT-60	60	44
copolyMAOT-40	40	37
copolyMAOT-20	20	19
copolyMAOT-10	10	7.9
copolyMAOT-5	5	3.9

[a] Mole percent of the cyclic compound (2) used for the polymer reaction to the total concentration of carboxylic acid groups.

[b] Thymine base contents in the copolymer after the polymer reaction (unit mol %).

Table IV-2. UV Spectral Data of the Copolymers

Compound	ε [a]	Hypochromicity (%) [b]
polyAOT	7740	12.7
copolyAOT-100	7800	12.1
copolyAOT-75	7890	11.0
copolyAOT-50	8050	9.2
copolyAOT-25	8160	8.0
copolyAOT-10	8200	7.6
copolyAOT-5	8240	7.1
polyMAOT	7820	11.8
copolyMAOT-80	7890	11.0
copolyMAOT-60	8060	9.1
copolyMAOT-40	8090	8.8
copolyMAOT-20	8160	8.0
copolyMAOT-10	8250	7.0
copolyMAOT-5	8290	6.5
T - T [c]	16500	7.0
T - M [c]	8870	0

[a] Molar extinction coefficient at 275 nm in DMF, $\lambda_{max} = 270.5$ nm.

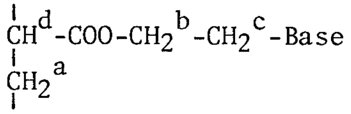
[b] Hypochromicity based on T-M.

[c] Reference 8.

Table IV-3. NMR Spectral Data of the Copolymers [a]

Compound	$-CH_2-$			$-CH$	Base	
	a	b	c	d	$5-CH_3$	6-H
polyAOT	1.70	3.94	4.28	2.32	1.80	7.32
copolyAOT-100	1.68	3.94	4.28	2.36	1.80	7.34
copolyAOT-75	1.63	3.92	4.26	2.34	1.80	7.34
copolyAOT-50	1.64	3.92	4.26	2.36	1.80	7.34
copolyAOT-25	1.60	3.92	4.26	2.36	1.79	7.36
copolyAOT-10	1.62	3.92	4.26	2.32	1.79	7.36
copolyAOT-5	1.60	3.92	4.26	2.30	1.79	7.36

[a] In DMSO-d6 at 150 °C, δ in ppm from TMS.

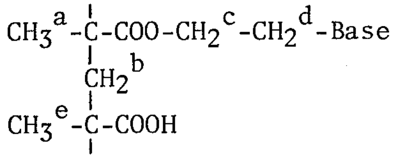


$$-CH^{d}-COO-CH_2{}^{b}-CH_2{}^{c}-Base$$
$$-CH_2{}^{a}-$$

Table IV-4. NMR Spectral Data of the Copolymers [a]

Compound	$-CH_3$				$-CH_2-$			Base	
	a		e		b	c	d	$5-CH_3$	6-H
polyMAOT	0.84	0.95			1.92	4.00	4.16	1.84	7.40
copolyMAOT-80	0.84	0.96	1.06	1.14	1.92	4.00	4.20	1.84	7.40
copolyMAOT-60	0.84	0.96	1.06	1.14	1.92	4.00	4.20	1.84	7.39
copolyMAOT-40	0.84	0.96	1.06	1.14	1.92	4.00	4.20	1.83	7.38
copolyMAOT-20	0.84	0.96	1.06	1.14	1.93	4.00	4.20	1.83	7.38
copolyMAOT-10			1.06	1.14	1.92	3.96	4.16	1.82	7.39
copolyMAOT-5			1.06	1.14	1.93	3.96	4.16	1.81	7.38

[a] In DMSO-d6 at 150 °C, δ in ppm from TMS.



$$\begin{array}{l} CH_3{}^{a}-\overset{|}{\underset{|}{C}}-COO-CH_2{}^{c}-CH_2{}^{d}-Base \\ \qquad\ \ CH_2{}^{b} \\ CH_3{}^{e}-\overset{|}{\underset{|}{C}}-COOH \end{array}$$

Table IV-5. Quantum Efficiency for Photodimerization of the Copolymers [a]

Compound	$\phi_{DF}°$	Nd/N Value
polyAOT	0.010	0
copolyAOT-100	0.0093	0.10
copolyAOT-75	0.0072	0.28
copolyAOT-50	0.0053	0.56
copolyAOT-25	0.0038	0.74
copolyAOT-10	0.0029	0.86
copolyAOT-5	0.0021	1.0
polyMAOT	0.0084	0
copolyMAOT-80	0.0064	0.24
copolyMAOT-60	0.0050	0.48
copolyMAOT-40	0.0045	0.54
copolyMAOT-20	0.0033	0.74
copolyMAOT-10	0.0025	0.86
copolyMAOT-5	0.0019	1.0

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

the almost identical wave length, while the molar extinction coefficient (ε) of these copolymers differed considerably. In both copolyAOT and copolyMAOT series, the value of hypochromocity decrease with decreasing the thymine content, and becomes to be closely near the value of the dimeric model compound (T-T).

The NMR spectral data of the copolymers in DMSO-d_6 at 150 °C were shown in Tables IV-3 and IV-4. The peak position of the copolymers afforded the almost identical and coincided with those of the authentic homopolymers (polyMAOT and polyAOT). The chemical shift of the protons of thymine base due to the carbonyl groups in the same molecule was not observed. The NMR spectra of the copolymers did not seem to depend on the content of thymine units in the copolymers.

IV - 2 - 3. Quantum Efficiency for Photodimerization

Quantum efficiencies of thymine bases in the copolymers for photodimer formation were measured in DMF solution. The photochemical reactions were carried out in 10 mm quartz cell filled with 4 ml of a solution at 20 °C, and the concentration of the thymine unit was kept always at 1×10^{-4} mol/l. The quantum efficiencies obtained are given in Table IV-5. It was found that the quantum efficiency of the copolymers decreased with decreasing the thymine content in the copolymers. This feature was in agreement with the result obtained for the photodimerization of the homopolymer [8]. The rate of photodimerization reaction of thymine bases in the homopolymer decreased during the intramolecular photodimerization and with decreasing thymine units along the polymer chain.

IV - 2 - 4. Quenching of the Photodimerization

The photodimerization of thymine bases in the copolymers was quenched by isoprene as the triplet quencher. The data obtained were plotted using Stern-Volmer equation (Figures IV-1 and IV-2). The Stern-Volmer plots for the copolymers did not give straight lines, because the excited singlet and triplet states were involved in the photodimerization reaction of the polymers. For the photodimerization reaction in question, it was assumed that both excited singlet

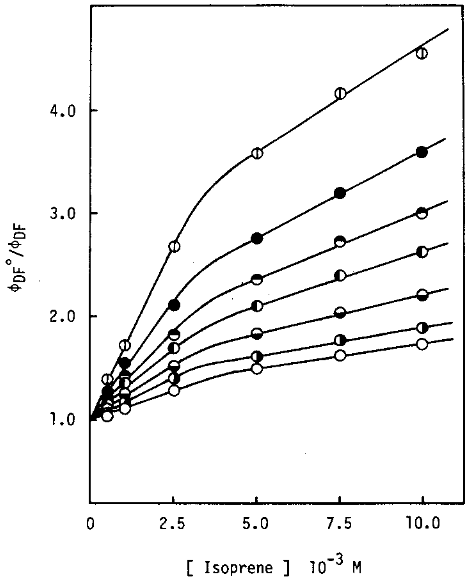


Figure IV-1. Representative Stern-Volmer plots for quenching photodimerization of copolymers by isoprene. The concentration of thymine unit was kept to 1 × 10^{-4} mol/l; (○) polyAOT, (◑) copolyAOT-100, (◒) copolyAOT-75, (◐) copolyAOT-50, (◓) copolyAOT-25, (●) copolyAOT-10, (⦶) copolyAOT-5.

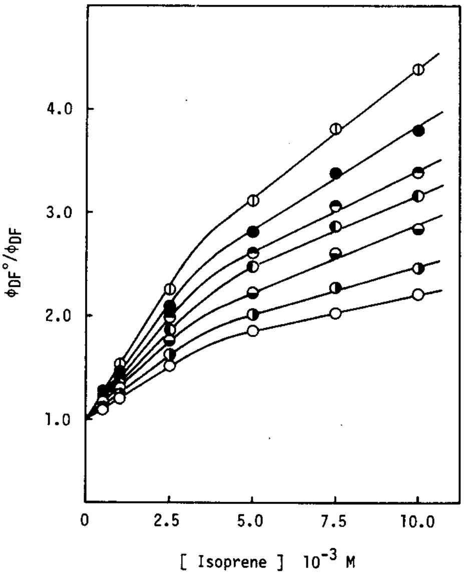


Figure IV-2. Representative Stern-Volmer plots for quenching photodimerization of copolymers by isoprene. The concentration of thymine unit was kept to 1 × 10^{-4} mol/l; (○) polyMAOT, (◑) copolyMAOT-80, (◒) copolyMAOT-60, (◐) copolyMAOT-40, (◓) copolyMAOT-20, (●) copolyMAOT-10, (⦶) copolyMAOT-5.

and triplet states were involved, and the equation (1) was derived [8].

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

The data for the copolymers were plotted using the equation (1) to give straight lines, as shown in Figures IV-3 and IV-4. From the intercepts of the lines, the portion of the quantum efficiency for photodimerization resulted only from the singlet state was estimated (Table IV-6). It was found from this table that the ratio of the singlet to triplet state tends to decrease with decreasing thymine base content in the copolymers.

IV - 3. Discussion

IV - 3 - 1. Photodimerization Reaction

In the previous chapter, the rate of photodimer formation was plotted against the conversion of the photodimerization [8]. The plots gave straight lines which were extrapolated to zero value at the conversion of 86 % for both polyMAOT and polyAOT. As the intramolecular photodimerization reaction occurred randomly along the polymer chains, some of thymine units, that is, 14 %, seems to have remained isolated. From the result it should be expected that the photodimerization reaction could not take place for the copolymers containing thymine units below about 14 %.

In the present study, however, the photodimerization reaction took place even for the copolymers containing only 4 % thymine content (Table IV-5). Figures IV-5 and IV-6 show the plots of the relative quantum efficiencies in Table IV-5 against the thymine content of the copolymers. In these figures, the data for the homopolymers, that is, polyMAOT and polyAOT, were also plotted. The relative quantum efficiencies for photodimerization reaction of the copolymers were found to decrease with decreasing the thymine content in the copolymers. The straight line was extrapolated to the value of dimeric model compound at zero thymine content, but could not be extrapolated to zero at 14 % thymine content. The following reasons can be

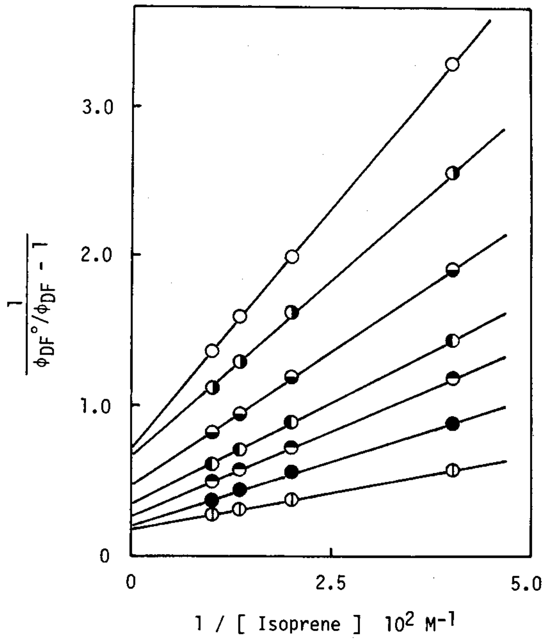


Figure IV-3. Relationship between $1/(\phi_{DF}^{\circ}/\phi_{DF} - 1)$ and 1/[Isoprene] for copolyAOT series; (○) polyAOT, (◑) copolyAOT-100, (◒) copolyAOT-75, (◐) copolyAOT-50, (◓) copolyAOT-25, (●) copolyAOT-10, (⦶) copolyAOT-5.

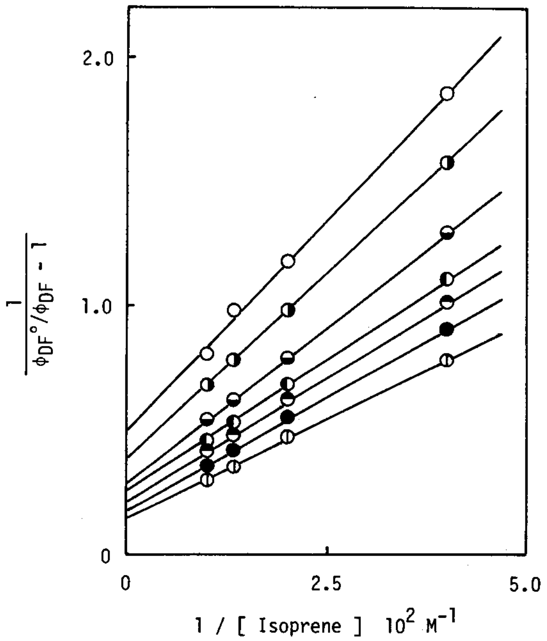
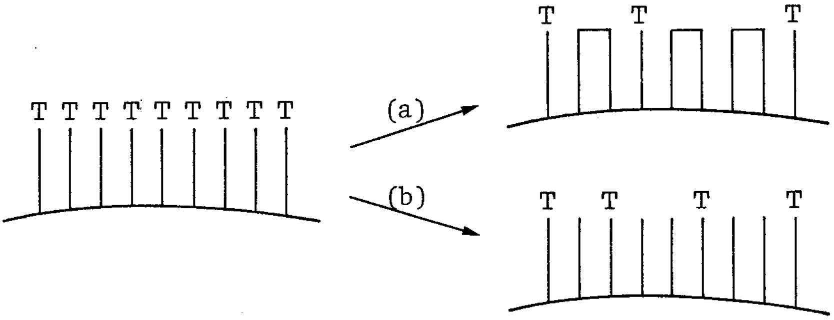


Figure IV-4. Relationship between $1/(\phi_{DF}^{\circ}/\phi_{DF} - 1)$ and 1/[Isoprene] for copolyMAOT series; (○) polyMAOT, (◑) copolyMAOT-80, (◒) copolyMAOT-60, (◐) copolyMAOT-40, (◓) copolyMAOT-20, (●) copolyMAOT-10, (⦶) copolyMAOT-5.

considered for the result: (1) The extent of thymine units isolated during the photodimerization reaction is different in each other for the case of homopolymers and that of copolymers, and (2) the copolymers prepared by the polymer reaction are not the random ones, but block in structure.

The thymine units isolated along the polymer chain were statistically calculated using the Monte Carlo procedure for the two systems shown in Scheme IV-1: (a) Pairs of thymine units in the polymers were deleted owing to the isolation of thymine units during the photodimerization reaction, and (b) the thymine units in the polymers were deleted one by one owing to the isolation by copolymerization. In this Monte Carlo procedure, the simulation can be made for the photodimerization and polymeranalogous reaction positions along the polymer chains. From the results of the calculation, the non-isolated thymine units were plotted against thymine content to afford two lines as shown in Figures IV-5 and IV-6. As the probability of the photodimerization may correspond to the amount of the non-isolated thymine units present, it should become zero at 14 % thymine content for (a) system, and 0 % thymine content for (b) system.

Scheme IV-1



If the thymine units in the copolymers were incorporated randomly by polymeranalogous reaction, the $\phi_{DF}°$ values for the copolymers should become zero at 0 % thymine content in the copolymers. In our results for the copolymers as shown in Figures IV-5 and IV-6, however, the $\phi_{DF}°$ values were not extrapolated to zero at 0 % thymine content. The results indicated that the copolymers used here should be block ones,

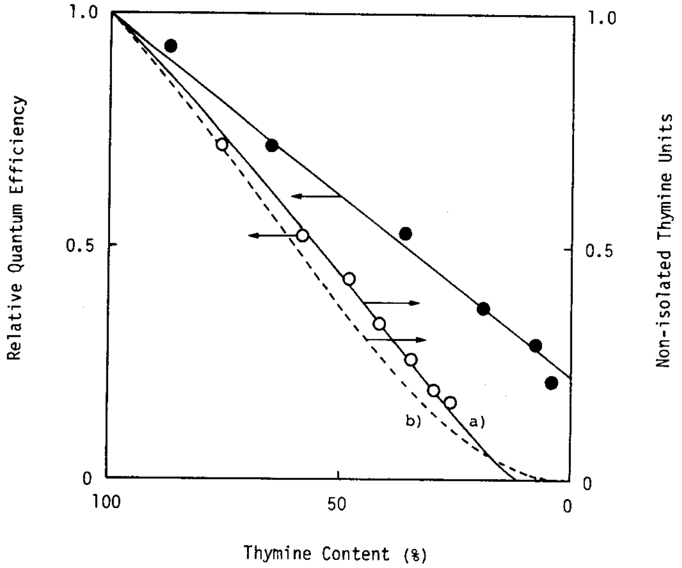


Figure IV-5. Relationship between relative quantum efficiency for photodimerization and thymine content; (○) polyAOT, (●) copolyAOT series. Relationship between non-isolated thymine units and thymine content; a) isolated by the photodimerization reaction, b) isolation by the polymeranalogous reaction.

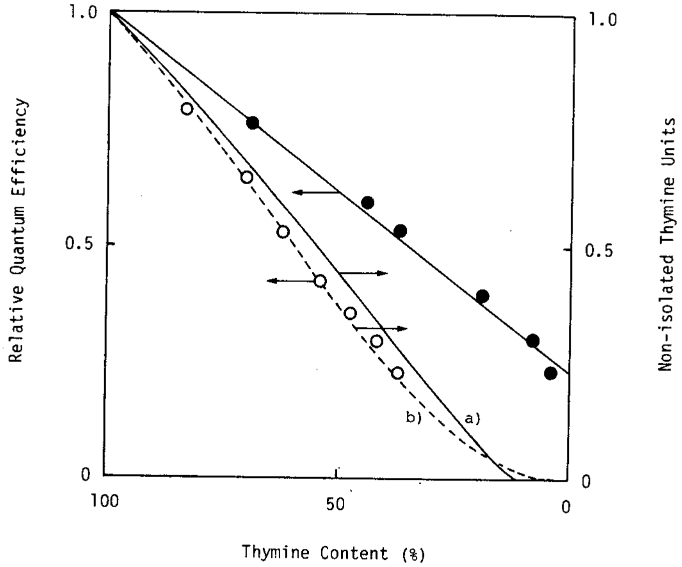
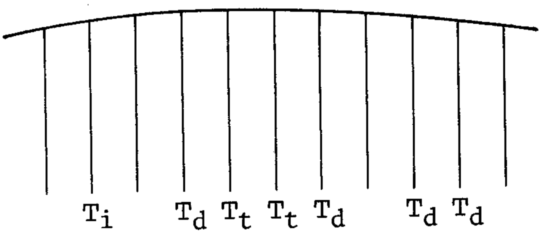


Figure IV-6. Relationship between relative quantum efficiency for photodimerization and thymine content; (○) polyMAOT, (●) copolyMAOT series. Relationship between non-isolated thymine units and thymine content; a) isolated by the photodimerization reaction, b) isolation by the polymeranalogous reaction.

and the thymine units in the copolymers have not been incorporated in a random way by the polymeranalogous reaction. The NMR spectra of the copolymers also suggested the block type structure for the copolymers; the signals of 6-H in thymine bases did not shift even for the copolymer having 4 % thymine content (Tables IV-3 and IV-4).

As shown in Figures IV-5 and IV-6, the $\phi_{DF}°$ values obtained did not fit to the calculated line (b). This fact may be explained by the effect of the neighboring thymine units along the polymer chain. In a copolymer as shown in Scheme IV-2, thymine unit T_i is isolated, thymine unit T_d has neighboring thymine unit only on one side, and the thymine unit T_t has neighboring thymine units on both sides.

Scheme IV-2



N, N_i, N_d, And N_t are the number of total, T_i, T_d, and T_t thymine units, respectively. The total number of thymine unit is then given by equation (2):

$$N = N_i + N_d + N_t \tag{2}$$

For the copolymer in question, the quantum efficiency of the thymine bases for photodimerization may be given by equation (3):

$$\phi_{DF}° = (N_i\phi_i + N_d\phi_d + N_t\phi_t) / N \tag{3}$$

where ϕ_i, ϕ_d, and ϕ_t denote quantum efficiency of T_i, T_d, and T_t, respectively. When the copolymer is a block one, the equation (3) can be rewritten as equation (4), using the equation (2):

$$\phi_{DF}° = \frac{N_d}{N}\phi_d + \frac{N_t}{N}\phi_t$$

Table IV-6. Photodimerization Reaction of the Copolymers

Compound	Singlet (%)	Triplet (%)
polyAOT	42	58
copolyAOT-100	40	60
copolyAOT-75	32	68
copolyAOT-50	26	74
copolyAOT-25	21	79
copolyAOT-10	18	82
copolyAOT-5	16	84
polyMAOT	33	67
copolyMAOT-80	28	72
copolyMAOT-60	23	77
copolyMAOT-40	21	79
copolyMAOT-20	18	82
copolyMAOT-10	16	84
copolyMAOT-5	14	86

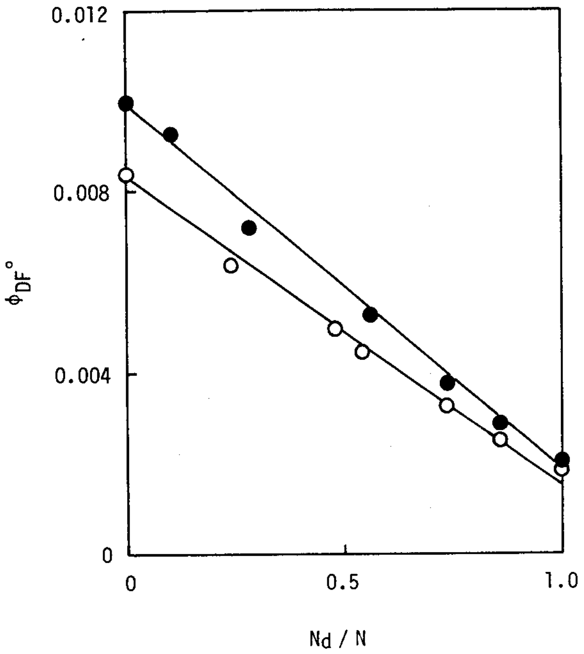


Figure IV-7. Relationship between quantum efficiency for photodimerization and Nd / N; (●) copolyAOT series, (○) copolyMAOT series.

$$= \phi_t - (\phi_t - \phi_d)\frac{N_d}{N} \qquad (4)$$

For the data obtained in this work, the equation (4) can be applied, because the copolymers in question are block ones as already mentioned. The value N_d was obtained by statistical calculation for the block copolymer ($N_i = 0$) using the Monte Carlo procedure and was listed in Table IV-5. By using the equation (4), the values in Table IV-5 were plotted against N_d / N to give straight lines as shown in Figure IV-7. From the intercept and the slope of the straight lines in this figure, ϕ_d and ϕ_t were obtained. The value of ϕ_t was found to agree with the value of $\phi_{DF}°$ of the homopolymers.

This finding indicates that the photodimerization of thymine bases in the copolymers was affected by the nearest neighboring thymine units, and was not affected by the remote ones. It was also suggested that the assumption of block type structure for the copolymer used here was right. It is to be noted throughout figures and tables that two sorts of copolymers, that is, copolyMAOT and copolyAOT series differ in each other in the mobility of the main and side chains which appears to be caused by the steric requirement of methyl groups present in the former copolymers.

IV - 3 - 2. Quenching of the Photodimerization

It was reported in a previous chapter that the photodimerization reaction occurs from both excited singlet and triplet states for polyMAOT and polyAOT [8]. For the copolymer system, the ratio of singlet to triplet states was found to decrease with decreasing the thymine content in the copolymers (Table IV-6).

For the singlet derived photodimerization, equation (5) may be given similar to equation (4):

$$S = S_t - (S_t - S_d)\frac{N_d}{N} \qquad (5)$$

where S, S_d, and S_t denote percentages of the singlet derived photodimerization reaction of thymine bases in the copolymer, T_d, and T_t, respectively. The data in Table IV-6 were plotted using equation (5) to give straight lines as shown in Figure IV-8. This fact suggests that the singlet derived photodimerization of thymine bases in the copolymer was affected by the nearest neighboring thymine units along the copolymer chain, and the extent of the singlet derived photodimerization determined the extent of quantum efficiency of the copolymer.

IV - 3 - 3. Self-association of Thymine Bases at the Ground State

From the UV spectral data shown in Table IV-2, it is evident that the thymine bases along the copolymer chain can interact intramolecularly at the ground state. It was found that the hypochromicity depends on the thymine content in the copolymer. The value of hypochromicity tended to decrease with decreasing the thymine content in the copolymer.

Its value is generally used as a measure of interaction between nucleic acid bases. The hypochromicity value, H (%), is defined as equation (6)

$$H = \frac{\varepsilon_1 - \varepsilon}{\varepsilon_1} \times 100 \qquad (6)$$

where ε_1 and ε are molar extinction coefficient of the monomeric model compound (T-M) and copolymer, respectively. As polyacrylic and polymethacrylic acid have no absorption around 275 nm, the molar extinction coefficient of the copolymer can be given as equation (7)

$$\varepsilon = (N_i\varepsilon_i + N_d\varepsilon_d + N_t\varepsilon_t) / N \qquad (7)$$

where ε_i, ε_d, and ε_t are molar extinction coefficients of T_i, T_d, and T_t, respectively. For the block copolymer ($N_i = 0$), the hypochromicity is given from equations (6) and (7) by

$$H = H_t - (H_t - H_d)\frac{N_d}{N} \qquad (8)$$

where $H_t = (\varepsilon_1 - \varepsilon_t)/\varepsilon_1 \times 100$, $H_d = (\varepsilon_1 - \varepsilon_d)/\varepsilon_1 \times 100$, and H_t and H_d are hypochromicity values of T_t and T_d, respectively.

Data of H (%) in Table IV-2 were plotted using the equation (8) to give straight lines as shown in Figure IV-9. From this figure, it was found that the hypochromicity values was also affected by the nearest neighboring thymine units along the copolymer chain.

It was thus concluded that the quantum efficiency for photodimerization (ϕ), singlet derived photodimerization (S), and hypochromicity (H) were found to depend on the thymine content in the copolymers, and to relate to the effect of the nearest neighboring thymine units along the copolymer chain.

The hypochromicity of thymine bases in the copolymer is affected by the nearest neighboring thymine units along the polymer chain, and the amounts of T_t (N_t) in Scheme IV-2 decreases with decreasing thymine content in the copolymer. Thus, the self-association of thymine units in the copolymer decreases with decrease of thymine cont content in the copolymer.

Singlet derived photodimerization of thymine bases in the polymer may relate to the self-association of the bases. Thus, the singlet derived photodimerization of the copolymer system tends to decrease with decreasing thymine content in the copolymer.

Quantum efficiency of thymine bases in the copolymer for photodimerization depends on the extent of the singlet derived photodimerization, because the quantum efficiency of the singlet derived photodimerization is known to be greater than that of the triplet derived one [13]. Thus, the quantum efficiency of the thymine bases in the copolymer for photodimerization decreases with decrease of the thymine content in the copolymer.

As the quantum efficiency of the thymine bases in the copolymers is closely related to the self-association of thymine bases along the polymer chain, photodimerization reaction of the polymer containing

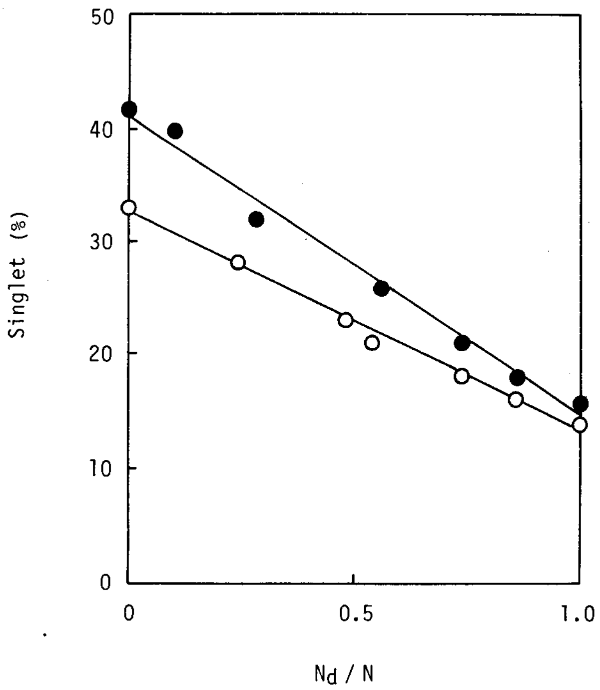


Figure IV-8. Relationship between percentage of singlet-derived photodimerization and Nd / N; (●) copolyAOTseries, (○) copolyMAOT series.

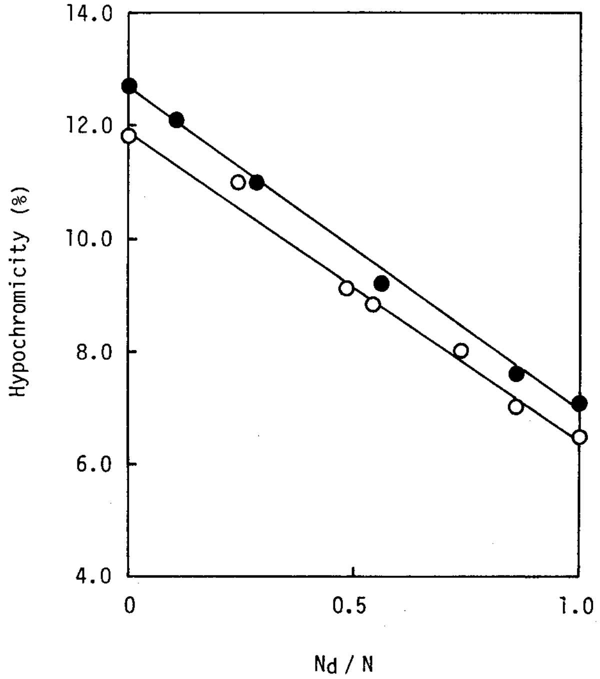


Figure IV-9. Relationship between percent hypochromicity and Nd / N; (●) copolyAOT series, (○) copolyMAOT series.

thymine moieties may be applied as a measure of self-association of thymine bases in the polymers.

IV - 4. Experimental

IV - 4 - 1. Instrumentation

UV Spectra were measured with a Shimazu UV-180 spectrophotometer and NMR spectra were measured with a JNM-PS-100 (JEOL) spectrometer using tetramethylsilane as an internal standard.

IV - 4 - 2. Photolysis

Photochemical reactions and the quenching studies were performed according to the method reported in Chapter II.

IV - 4 - 3. Monte Carlo Procedure

The Monte Carlo procedure is employed on a digital computer (NEC PC-8000 system) in order to generate non-overlapping randam reaction position on the polymer chain simulating the intramolecular photodimerization reaction and the polymeranalogous reaction for preparing the copolymers. In intramolecular photodimerization reaction, we assume that a group of polymer molecules each contains n structural units (and n substituents) and the substituents (thymine bases) are all in 1 - 3 relationship and all pairs of thymine bases which are 1 - 3 relationship to each other will be photodimerized. If a thymine base has as its nearest neighbor an inert substituent, that thymine base is isolated and hence incapable of being photodimerized.

As an example, the program of this Monte Calro method was shown in following scheme:

```
10  DIM X(1000)
20  DIM Y(1000)
30  INPUT N
40  INPUT Z
50  PRINT "CALCULATION OF AV.P. INTER"
60  PRINT "BRANCH NUMBER N"
70  PRINT "SAMPLE SIZE Z"
80  J=0
90  X(0)=1
100 X(N+1)=1
```

```
110 FOR I=1 TO N
120 X(I)=1
130 NEXT I
140 J=J+1
150 M=0
160 R=INT(RND(1)*N+1)
170 IF X(R)=0 THEN 160
180 X(R)=0
190 M=M+1
200 I=1
210 P=0
220 IF X(I)=0 THEN 250
230 IF X(I-1)=0 AND X(I+1)=0 THEN P=P
240 P=P+1
250 I=I+1
260 IF I=N+1 THEN 270 ELSE 160
270 Y(M)=Y(M)+P/N
280 IF P=0 THEN 290 ELSE 160
290 IF J=Z THEN 300 ELSE 90
300 FOR K=1 TO N
310 PRINT K, Y(K)/Z
320 NEXT K
330 STOP
340 END
```

IV - 4 - 4. Hydrolysis of Copolymers

The contents of the thymine base in the copolymers obtained were determined by measuring UV absorbance of the product after hydrolysis. Hydrolysis of the copolymer was carried out by 10N-hydrochloric acid at room temperature for 2 days, which gave oxyethyl derivative of thymine as well as polyacrylic or polymethacrylic acid. The authentic polymethacryloyloxyethylthymine was confirmed to be hydrolyzed to give oxyethylthymine with 98 % yield. For the quantitative calculation, the authentic oxyethylthymine was used.

IV - 4 - 5. Materials

Cyclic derivative of thymine (2). The cyclic derivative, that is, 1,2-O-ethanothymine was prepared from chloroethylthymine (1) by dehydrochlorination with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in a procedure reported earlier [11].

Grafting of cyclic thymine onto polyacrylic acid (copolyAOT) (3a). To a suspension of polyacrylic acid (1.44 g, 0.02 mol) in DMF (30 ml), 2 (3.04 g, 0.02 mol) was added at 80 °C with stirring. The suspension became a clear solution after few days. After stirring further 10 days, the solvent was distilled off under reduced pressure. Excess acetone was then added to the oily residue obtained to give precipitate of the polymer. Reprecipitation from DMF-acetone gave the polymer (3a) in a quantitative yield. Polyacrylic derivatives having different contents of thymine were also prepared using a similar procedure described above.

Grafting of cyclic thymine onto polymethacrylic acid (copolyMAOT) (3b). Polymethacrylic derivatives having different contents of thymine were prepared using the similar procedure described for preparing 3a.

The reagents and solvents were obtained commercially and purified in the usual manner. Spectral quality solvents were used in all photochemical experiments.

IV - 5. Summary

Photodimerization reaction of polyacrylic and polymethacrylic derivatives having different pendant thymine contents was studied in dimethylformamide solution. The quantum efficiency of thymine base for photodimerization was found to increase with increasing thymine contents in the copolymers. The quenching study using isoprene as the quencher revealed that the photodimerization resulted from excited singlet state increases with increasing the thymine content. The self-association of thymine bases in the polymer chain also estimated from their UV spectra. The photochemical results were discussed in terms of self-association of thymine bases in the polymer chain.

IV - 6. References

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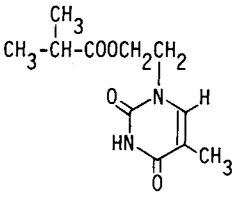
Chapter V. Photochemical reactions on the synthetic polymers containing thymine bases in polymethyl methacrylate film

V - 1. Introduction

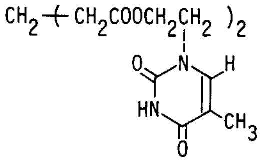
Thymine base is known to form easily a photodimer in which two bases are covalently bonded to give cyclobutane structure [1]. Thus, four isomeric cyclobutane-type photodimers can be formed as shown in Chapter I (Figure I-1) [2]. It has been reported that the quantum efficiency for photodimerization of thymine bases in aqueous solution is low, while that in an aggregated solution is very high [3]. The photodimerization of thymine bases in polythymidylic acid (poly T) is known to be faster than that of oligomer or monomer [4]. In these case, a stacking interaction of thymine bases is closely related to the photodimerization of the bases.

The photodimerization of thymine bases in solid states, such as in ice [5], dry film [6], and KBr pellet [7] has been also reported. The quantum efficiency for photodimerization in the solid state is very high as compared with that in solution. In solution, yields of the photodimers as well as the distribution of their isomers are determined largely by the properties of electronic excited states of parent compounds. On the other hand, the yields and the distribution of the isomers obtained depend primarily on the spacing and orientation of the monomers in their ground state, in a solid matrix such as a dry film or a microcrystalline ice.

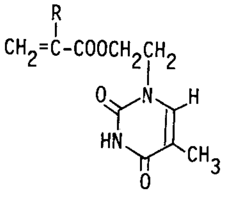
In a series of our extensive studies on the photodimerization reaction of thymine bases present on the side chain of the synthetic polymers, the quantum efficiency for photodimerization was found to depend on the intramolecular interaction between thymine bases (stacking interaction) along the polymer chain. In the polymer system in which the stacking interaction of thymine bases is strong enough, the photodimerization of thymine bases was caused from the excited singlet state as well as the excited triplet one to give high quantum efficiency [8]. The photodimerization of thymine bases on the polymer side chain was also found to be affected by the solvent used [9]. In a poor solvent for the polymer, in which the stacking interaction of the



T - M

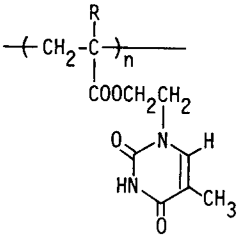


T - T



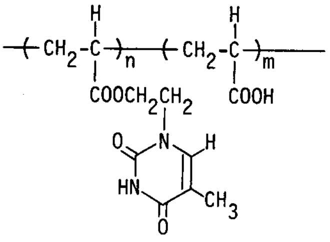
R: CH_3 MAOT

H AOT



R: CH_3 polyMAOT

H polyAOT



copolyAOT series

Figure V-1. Thymine derivatives.

thymine bases are strong because of weak solvation to the thymine bases, the quantum efficiency for photodimerization was high. In a solid state matrix, thymine bases can be expected to form photodimers with high quantum efficiency, because of inability of solvation to the thymine bases.

In this chapter, the study on the photodimerization reaction of thymine bases present in the side chains of synthetic polymers in a solid state matrix, that is, in a polymethyl methacrylate film was reported in some detail.

V - 2. Results and Discussion

V - 2 - 1. Preparation of the Films Containing Thymine Derivatives

Polymethyl methacrylate films containing thymine derivatives were prepared by the thermal polymerization of the mixed solution of methyl methacrylate monomer and methanol (or dimethyl sulfoxide) which dissolves thymine derivatives, with azobisisobutyronitrile (AIBN) as an initiator. The thymine derivatives used here were shown in Figure V-1. The T-M and T-T were low molecular weight model compounds. The MAOT and AOT were vinyl type monomers having a pendant thymine unit and were able to be copolymerized with methyl methacrylate monomer [10]. The polyMAOT, polyAOT, and copolyAOT were the polymeric model compounds containing pendant thymine bases along the polymer chain. The copolyAOT series were polyacrylic derivatives containing different content of thymine units [11]. These copolymers were prepared by the polymer reaction [12].

Figure V-2 indicates the UV spectra of the extracts from the films containing T-M, T-T, MAOT, and AOT into methanol. The extraction was carried out on 1.4 g films with 40 ml methanol by a Soxhlet extractor for 7 hr. The films containing T-M and T-T exhibit the absorption peak due to thymine bases at 270 nm, suggesting that the thymine derivatives incorporated into films were extracted with methanol. On the other hand, the extracts from the films containing MAOT and AOT do not exhibit absorption peak at 270 nm. It can be seen in Figure

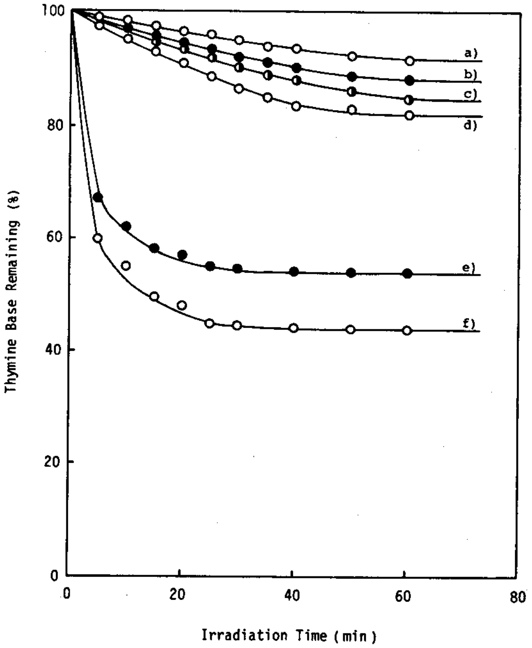


Figure V-3. Photodimerization reaction of thymine derivatives in polymethyl methacrylate film; a) T-M, b) MAOT, c) AOT, d) T-T, e) polyMAOT, f) polyAOT.

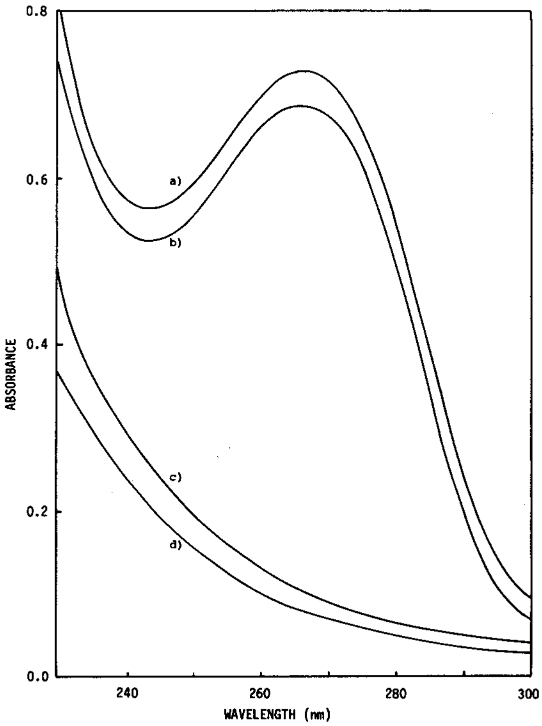


Figure V-2. UV Spectra of the extracts from the film into methanol; a) T-M, b) T-T, c) MAOT, d) AOT.

V-2 that the MAOT and AOT copolymerized with methyl methacrylate monomer and thymine bases of these films were incorporated into films as the copolymer.

The glass transition temperatures, Tg, of the films were measured by differential scanning calorimetry (DSC) at a scanning rate of 10 °C /min. The T_g values in every films were almost identical and revealed between 101 to 105 °C. These T_g values were in good agreement with that of polymethyl methacrylate [13].

V - 2 - 2. Photodimerization Reaction

By irradiating UV light onto the films, the absorption of thymine bases tended to decrease rapidly as shown in Figures V-3 and V-4. The quantum efficiency for the photodimer formation of thymine bases in polymethyl methacrylate film was measured from the initial rates and was listed in Table V-1.

Monomeric model compound (T-M)

In solution (1×10^{-4} mol/l of thymine unit), the monomeric model compound of thymine (T-M) did not form a photodimer [8]. In the polymethyl methacrylate film, however, formation of the photodimer from T-M was observed (Table V-1), in which the concentration of thymine unit was about 2×10^{-3} mol/l. As thymine at the concentration above 1×10^{-3} mol/l in aqueous solution is known to give photodimers with high quantum efficiency because of forming aggregation [3], the photodimerization observed in T-M in polymethyl methacrylate film may be caused by the aggregation of T-M. The maximum conversion of the photodimerization reaction in this system was substantially low (Figure V-3).

Dimeric model compound (T-T)

In solution, the thymine dimer model (T-T) gave photodimers with low quantum efficiency. It was found that the triplet state was the precursor of all the photodimers formed [8]. Although the hypochromicity of T-T depended on the sort of the solvents, the photodimerization reaction was never influenced by it [9]. The life time

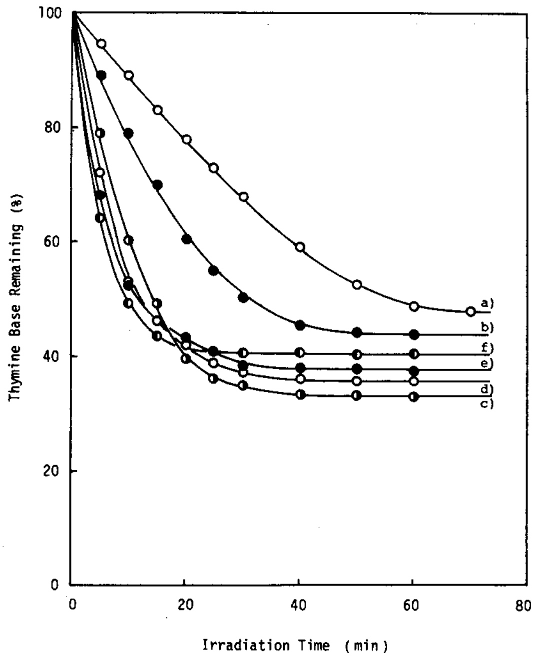


Figure V-4. Photodimerization reaction of thymine derivatives in polymethyl methacrylate film; a) copolyAOT-5, b) copolyAOT-10, c) copolyAOT-25, d) copolyAOT-50, e) copolyAOT-75, f) copolyAOT-100.

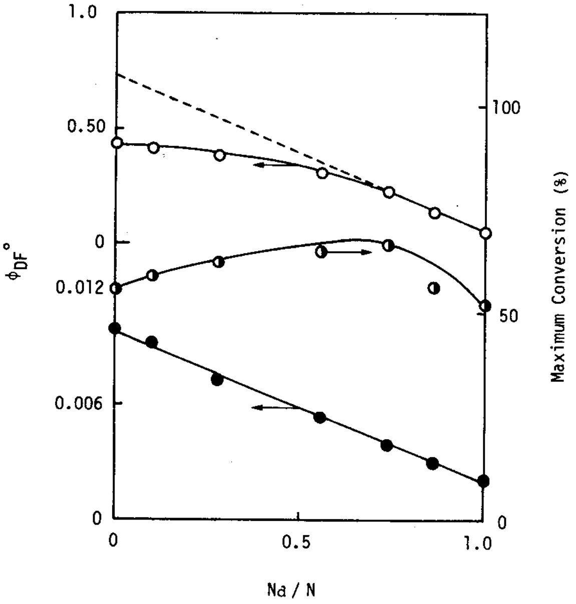


Figure V-5. Relationship between quantum efficiency for the photodimerization and Nd / N; (○) in polymethyl methacrylate film, (●) in DMF solution. Relationship between maximum conversion of photodimerization and Nd / N is shown with (◑).

of the singlet state of the thymine bases is known to be about 1 psec at room temperature [14], so that the conformational change of T-T in solution should be so fast that the reaction could not be occured from the singlet excited state.

In a solid matrix of polymethyl methacrylate film, however, the quantum efficiency of T-T for photodimerization was 20 times greater than the value obtained in solution (Table V-1). If T-T was tried to fix in the film, some of T-T may be fixed in a stacked conformation which could not be changed in solid, so that the quantum efficiency may attain the high value.

Vinyl monomers (MAOT and AOT)

When the vinyl monomers, MAOT and AOT were incorporated into a polymethyl methacrylate film, copolymers of MAOT and methyl methacrylate monomer, or AOT and methyl methacrylate monomer were formed. As MAOT and AOT are monomeric derivatives like T-M, distribution of them into the film should be similar to the case of T-M. The quantum efficiency of MAOT and AOT for photodimerization in the film, however, was about 1.5 times higher than that of T-M (Table V-1). As for the aggregation of T-M in the film, four kinds of aggregation which correspond to four isomers of the photodimers as shown in Figure I-1 can be expected. On the other hand, for MAOT and AOT which are incorporated as copolymers into the film, two kinds of aggregation which correspond to cis-syn and trans-syn photodimers are possible, like T-T and the polymers [8]. Therefore, the thymine base in MAOT and AOT in the film are more rigidly stacked than T-M in the film, and the quantum efficiency of MAOT and AOT systems is higher than that of T-M.

Acrylic and methacrylic polymers (polyAOT and polyMAOT)

The quantum efficiency of the polymers having thymine bases in polymethyl methacrylate film was about 40 times higher than the values obtained in dimethylformamide (DMF) solution (Table V-1). From the quenching study in the solution, it was found that both excited singlet and triplet states were the precursors of the photodimer [8]. The ratio of the singlet to triplet state of the precursor increased with

Table V-1. Photodimerization Reaction of Thymine Derivatives in Polymethyl Methacrylate Film

Compound	Base Content (%) [a]	Hypochromicity in DMF (%) [a]	$\phi_{DF}°$ in Film	$\phi_{DF}°$ in Solution [b]	Maximum Conversion (%) [c]
T - M		0	0.014		8.7
T - T		7.0	0.030	0.0012	18.0
MAOT			0.019		11.9
AOT			0.022		15.3
copolyAOT-5	4.0	7.1	0.057	0.0021	52.2
copolyAOT-10	7.4	7.6	0.13	0.0029	56.1
copolyAOT-25	19	8.0	0.23	0.0038	66.9
copolyAOT-50	36	9.2	0.31	0.0053	63.5
copolyAOT-75	65	11.0	0.38	0.0072	62.1
copolyAOT-100	87	12.1	0.42	0.0093	58.9
polyAOT	100	12.7	0.43	0.010	55.5
polyMAOT	100	11.8	0.34	0.0084	45.7

[a] Reference 11.

[b] Reference 16.

[c] Percentage of thymine bases converted to the photodimer in film.

increasing the stacking interaction of thymine bases [9].

In polymethyl methacrylate film, the stacking interaction of thymine bases along the polymer chain should be strong enough because of no solvation to thymine bases. The strong stacking interaction may reflect to the singlet derived photodimerization to result in high quantum efficiency. For the other possible reason of high quantum efficiency in the film, it appears that the thymine bases in the stacked conformation which is favorable for the photodimerization are fixed in the film at below T_g of the film. Gerdil showed that thymine crystallizes in ice with adjacent thymine molecules stacked in a position which is a suitable precursor for the cis-syn dimer [15].

For the photodimerization reaction of polyMAOT and polyAOT in the polymethyl methacrylate film, intermolecular photodimerization to form crosslinked structure can be excluded by considering the following evidence, that is, polyMAOT and polyAOT in the polymethyl methacrylate film did not give insoluble part in dimethyl sulfoxide before and after the photodimerization reaction.

Acrylic copolymers (copolyAOT series)

About the photodimerization of copolymers (Table V-1), the quantum efficiency of them was found to decrease with decreasing in the thymine units in the copolymers. This tendency was in agreement with the result obtained in DMF solution.

In the previous chapter, it was reported that the quantum efficiency of the copolymers in DMF solution was related to the amounts of thymine units which were in the nearest-neighboring situation [16]. Here a linear relationship was observed between the quantum efficiency and the value of N_d / N, where N and N_d denote the number of total thymine units and those having nearest-neighboring thymine units only on one side, as shown in Figure V-5. The data in Table V-1 obtained from the experiment in film were plotted against N_d / N in this figure. At the low content of thymine units in the copolymers, the quantum efficiencies tended to increase linearly with N_d / N. On the other hand, at higher content of thymine units in the copolymers, the value did not increase with N_d / N as shown in Figure V-5.

Maximum conversion of the photodimerization for polyMAOT and polyAOT in DMF solution was found to be about 86 %, because of the effect of isolating the thymine units still unreacted [8]. As shown in Figure V-5 and Table V-1, on the other hand, the maximum conversion of photodimerization for the polymers containing thymine bases was about 60 %. For copolyAOT-25 containing 19 unit mole % of thymine units, the highest maximum conversion was observed. Both the increase and decrease in the thymine units in the copolymers from 19 unit mole % caused the decrease of maximum conversion. The maximum conversion may relate to the amount of stacked thymine units and the flexibility of the side chain where the thymine bases present. With increasing thymine units in the copolymers, the amounts of the stacked thymine bases as well as the rigidity of side chains can be increased to give slow increase of the maximum conversion at higher thymine units.

Assuming the fact of the maximum conversion, it may be assumed that the copolymers having high thymine units are rigid, so the quantum efficiency cannot be increased linearly with increasing N_d / N value in the copolymers.

The AOT system is the homogeneously dispersed ones, but the copolyAOT-5 system is the mixture of copolymer and polymethyl methacrylate. Difference of the system caused the difference in the quantum efficiency (Table V-1). The copolyAOT-5 system may have a part containing high local concentration of thymine units. It was found in the previous chapter that the copolymer which was prepared by the polymeranalogous reaction on polyacrylic acid had characters of a block copolymer [16]. This fact also causes the high quantum efficiency for the copolymer system.

V - 3. Experimental

V - 3 - 1. Instrumentation

UV Spectra were measured with a Shimazu UV-180 spectrophotometer. T_g Values of the films containing thymine derivatives were obtained with a Perkin-Elmer differential scanning calorimeter (DSC-1).

V - 3 - 2. Photolysis

The films were irradiated by UV light for a given period with a 1-kw super-high pressure mercury lamp filtered through a Pyrex filter at room temperature, in the presence of air at atmospheric pressure. The filter showed no transmittance below 265 nm and 50 % transmittance at 297 nm. Light intensity was determined by potassium ferrioxalate actinometry. The quantum yield for oxalic acid decomposition was 1.24 at 297/302 nm [17]. Concentration of the thymine derivatives in film was kept at about 2×10^{-3} mol/l of thymine unit. At a given time intervals, concentration of the thymine bases remaining in the film was determined by UV analysis at 275 nm, at which neither photodimers nor polymethyl methacrylate absorb significantly.

V - 3 - 3. Materials

Thymine derivatives used here (Figure V-1) were prepared according to the method described in the previous chapter [8,11]. All the reagents and solvents were obtained commercially and purified in the usual manner.

agents and solvents were obtained commercially and purified in the usual manner.

V - 3 - 4. Preparation of the Films

The monomers (T-M, MAOT, and AOT) and dimer (T-T) were dissolved in a mixed solution of 2 ml of methanol and 10 g (0.1 mole) of methyl methacrylate monomer. The polymers (polyMAOT and polyAOT) and the copolymers (copolyAOT series) were dissolved in a mixed solution of 2 ml of dimethyl sulfoxide and 10 g (0.1 mole) of methyl methacrylate monomer.

The mixed solution was added azobisisobutyronitrile (AIBN) (1.2×10^{-4} mole) and a stream of purified nitrogen gas was passed through the mixed solution to flush off the air present. The polymerization of the mixed solution was carried out at 80 °C for 20 min. The prepolymer solution resulted was casted on a flat glass plate and the polymerization was further continued at 80 °C for 24 hr. The films thus formed were dried at 80 °C for 24 hr in vacuum to remove the solvent. Thickness of the films was about 5×10^{-2} cm. The films were colorless and transparent.

V - 4. Summary

Photodimerization reaction of the synthetic polymers and corresponding low molecular weight model compounds containing thymine bases was studied in polymethyl methacrylate film. The quantum efficiency for photodimerization of the thymine bases of these thymine derivatives which were fixed in the polymethyl methacrylate film was measured. The value in the film was found to be higher than that measured in solution.

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CONCLUSION

The purpose of the present study was to investigate the relationship between the intramolecular base-base interaction and the photoreactivity of the synthetic functional polymers containing thymine bases.

In Chapter I, the synthesis of polyacrylic, polymethacrylic derivatives, and corresponding low molecular weight model compounds containing pendant thymine bases was described. The photochemical reactions of these model compounds were investigated, and it was found that the quantum efficiency for the photodimerization of thymine bases on the polymers was larger than that of the low molecular weight model compounds. This result was explained by the degree of intramolecular self-association between the thymine bases.

In Chapter II, the photochemical reactions of polymers and dimer model compound containing thymine bases were investigated in three kinds of organic solvents. It was found that the quantum efficiency for the photodimerization of thymine bases was affected by the kind of solvent used and increased with increasing the degree of intramolecular self-association of thymine bases. This fact was explained by the interaction between solvent molecules and thymine bases along the polymer chain.

In Chapter III, the photodimerization reactions of polymers and dimer model compound were studied in the presence of adenine derivatives in three kinds of organic solvents. The photodimerization of thymine bases of these model compounds was found to be quenched by the addition of adenine derivatives. This finding was explained by in terms of the specific interaction between adenine and thymine bases both in ground and excited states.

In Chapter IV, the synthesis of polyacrylic and polymethacrylic derivatives containing different contents of pendant thymine unit and the photodimerization reactions of these copolymers were investigated. It was found that the quantum efficiency for the photodimerization of thymine bases and the intramolecular self-association of thymine bases depended on the thymine contents in the copolymers and related to the effect of the nearest neighboring thymine units along the copolymer chain. It was concluded that the photodimerization reactions of the polymers containing thymine bases may be applied as a measure of self-association of thymine bases in the polymers.

In Chapter V, photochemical reactions of the synthetic polymers and low molecular weight model compounds containing thymine bases were studied in polymethyl methacrylate film. It was found that the quantum efficiency for the photodimerization of thymine bases in film was extremely larger than that in solution. However, the percentage of thymine bases converted to photodimer in film was usualy less than that in solution. This fact was explained by the strong stacking interaction of thymine bases in film because of no solvation to thymine bases. It was concluded that the thymine bases were fixed in the film taking the stacked conformation which was favorable for the photodimerization. And the maximum conversion to photodimer related to the amount of stacked thymine units and the flexibility of the side chain where the thymine bases present.

SUPPLEMENTARY TREATISES

The supplementary treatises are as follows:

(1) A convenient synthesis of N-(2-methacryloyloxy)ethyl derivatives of nucleic acid bases
M.Akashi, Y.Kita, Y.Inaki, and K.Takemoto,
Makromol.Chem., 178, 1211(1977).

(2) A copolymerization study of methacryloyl type monomers containing nucleic acid bases in chloroform solution
M.Akashi, Y.Kita, Y.Inaki, and K.Takemoto,
J.Polymer Sci.Polymer Chem.Ed., 17, 301(1979).