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# Functional Monomers and Polymers CLXXII† Synthesis and Interaction Studies on Water Soluble Nucleic Acid Analogs: Polyethyleneimine Derivatives Containing Thymine and Adenine

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ABSTRACT: Water soluble nucleic acid analogs were prepared by grafting adenine and thymine derivatives with homoserine onto polyethyleneimine. The analogs were found to form complementary polymer complexes with each other and with polynucleotides. The interactions were studied between these polymers, and with polynucleotides; polyuridylic acid (Poly U) and Polyadenylic acid (Poly A) in water.

KEY WORDS Nucleic Acid Analogs / Water Soluble / Thymine / Adenine / Polyethyleneimine / Homoserine / Poly A / Poly U / Polymer Complex / Specific Interaction /

Recently, a number of synthetic polynucleotide analogs have been prepared and their properties have been studied. These synthetic nucleic acid analogs were found to form polymer complexes by specific interaction between complementary nucleic acid bases.[1-13] Most of the nucleic acid analogs consist of hydrophobic polymer backbone and pendant nucleic acid bases, and are insoluble in neutral water. Therefore the interactions of these nucleic acid analogs were studied in organic solvents or in water-organic mixed solvents. On the other hand, polynucleotides are freely soluble in neutral water, and are insoluble in organic solvents. For the application of the nucleic acid analogs to biological fields, it is very important to study the interactions of the synthetic analogs with polynucleotides in neutral aqueous solution. For this purpose, in the previous paper, water soluble polyethyleneimine derivatives containing cytosine and hypoxanthine were prepared and the interactions of these polymers with polynucleotides were studied.[14]

In the present study, water soluble polyethyleneimine derivatives containing thymine and adenine are prepared, and their interactions with polyuridylic acid (Poly U) or polyadenylic acid (Poly A) in aqueous solution are investigated. The bioactivity of the polymer complex of the nucleic acid analogs and with polynucleotides will be shown elsewhere.[15]

## EXPERIMENTAL

### (±)-α-*N*-[3-(*thymin-1-yl*)*propionyl*]*amino*-γ-*butyrolactone* (*4T*; *Thy–Hse–L*)

To a solution of (±)-α-amino-γ-butyrolactone hydrobromide (910 mg; 5.0 mmol) in *N*,*N*-dimethylformamide (30 ml), pentachlorophenyl 3-(thymin-1-yl)propionate (3T)[16] (2.2 g; 5.0 mmol), triethylamine (0.7 ml; 5.0 mmol), and imidazole (340 mg; 5.0 mmol) were added in that order and stirred for one

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day at 50°C. After the reaction, the solvent was evaporated under reduced pressure. Acetone (150 ml) was added to the oily residue and stirred further for 4 h to give a light brown precipitate. The precipitate was washed with water (50 ml) and dried under reduced pressure. The yield was 1.4 g (97%); mp 274—276°C. IR (KBr, $cm^{-1}$): 1770 (lactone), 1650, 1530 (amide), 1700, and 1670 (thymine). Thin layer chromatography (TLC): *Rf* 0.48 (benzene–ethanol = 3 : 1). $^1H$ NMR (in dimethyl sulfoxide-$d_6$ at 25°C, ppm): 1.70 (s, 1H), 2.45 (t, 2H), 3.78 (t, 2H), 4.25 (m, 4H), 4.55 (m, 1H), 7.35 (s, 1H), 8.45 (d, 1H), and 11.05 (s, 1H).

*Anal.* Calcd for $C_{12}H_{15}N_3O_5$: C, 51.24%; H, 5.38%; N, 14.94%. Found: C, 50.95%; H, 5.37%; N, 14.90%.

(±)-α-*N*-[*3*-(*N*$^6$-*diphenylphosphinothioyladenin-9-yl*)*propionyl*]*amino-γ-butyrolactone* (*4PptA*; *PptAde–Hse–L*)

To a solution of (±)-α-amino-γ-butyrolactone hydrobromide (910 mg; 5.0 mmol) in *N*,*N*-dimethylformamide (20 ml), pentachlorophenyl 3-(*N*$^6$-diphenylphosphinothioyladenin-9-yl)propionate (3PptA)[17] (3.4 g; 5.0 mmol), triethylamine (0.7 ml; 5.0 mmol) and imidazole (340 mg; 5.0 mmol) were added. The mixture stirred at 60°C for 20 h to afford a light brown clear solution. After the reaction, the solvent was evaporated under reduced pressure. The residue was washed twice with 30 ml of diethyl ether and then with 40 ml of ethyl acetate. The oily residue stirred with 40 ml of diethyl ether at room temperature for 48 h. Resulting precipitate was collected and washed with 30 ml of water and dried to give light yellow powder (2.5 g; 97%). mp 115—117°C. IR (KBr, $cm^{-1}$): 1770 (lactone), 1650, 1530 (amide), 1660, 1600, and 1580 (Ppt–adenine). TLC: *Rf* 0.53 (benzene–ethanol = 3 : 1). $^1H$ NMR (in dimethyl sulfoxide-$d_6$ at 25°C, ppm): 2.78 (t, 2H), 3.53 (t, 2H), 4.40 (m, 4H), 4.68 (m, 1H), 7.50 (s, 10H), 8.32 (s, 1H), 8.51 (s, 1H), and 8.60 (s, 1H).

*Anal.* Calcd for $C_{24}H_{23}N_6O_3PS$: C, 56.92%; H, 4.56%, N, 16.59%. Found: C, 56.52%; H, 4.36%; N, 16.24%.

(±)-α-*N*-[*3*-(*adenin-9-yl*)*propionyl*]*amino-γ-butyrolactone hydrobromide* (*4A; Ade–Hse–L*)

PptAd–Hse–L (4PptA) (2.0 g; 4.0 mmol) was added to 10 ml of trifluoroacetic acid. The mixture stirred at room temperature for 2 h to afford a light brown clear solution. Then 30 ml of 25% hydrogen bromide acetic acid solution was added to this solution. The reaction was carried out at 0°C for 2 h and then at room temperature for 12 h. After the reaction, the mixture was evaporated under reduced pressure. The resulting residue was washed several times with diethyl ether. The precipitate was collected and washed with 20 ml of acetone to yield 1.2 g (78%). mp 130—133°C. *Rf* 0.05 (benzene–ethanol = 3 : 1).

*Anal.* Calcd for $C_{12}H_{14}N_6O_3$ HBr: C, 38.83%; H, 4.07%; N, 22.63%. Found: C, 38.15%; H, 3.81%; N, 22.65%.

*Poly-N*-[{*2*-[*3*-(*thymin-1-yl*)*propionyl*]*amino-4-hydroxy*}*butanoyl*]*ethyleneimine* (*5T*; *PEI–Hse–Thy*)

To a suspension of Thy–Hse–L (4T) (1.0 g; 3.6 mmol) in water (5.0 ml), 1 *N* NaOH aqueous solution (3.6 ml; 3.6 mmol) was added. The mixture stirred at 40°C for 1 h to afford a light brown clear solution. After the reaction, the solvent was evaporated under reduced pressure. Resulting residue was washed with acetone (20 ml), then with diethyl ether (30 ml) to give a sodium salt of thymine derivative (Thy–Hse–Na) in 96% yield (1.1 g, powder). TLC: *Rf* 0.00 (benzene–ethanol = 3 : 1), (ethanol), (*n*-butanol–acetic acid–water = 4 : 5 : 3).

To a suspension of Thy–Hse–Na (1.1 g; 3.2 mmol) in 10 ml of dry *N*,*N*-dimethylformamide, pentachlorophenyl trichloroacetate (1.8 g; 4.5 mmol) and catalytic amount of triethylamine were added. The mixture stirred at 0°C for 2 h and then at 50°C until gas evolu-

tion was ceased to give a light brown clear solution. After the reaction, the solvent was evaporated under reduced pressure. The residue was washed with diethyl ether (30 ml) to give the pentachlorophenyl ester of thymine derivative (Thy–Hse–PCP) (yield 92%; 1.8 g). TLC: *Rf* 0.82 (benzene–ethanol = 3 : 1).

To a solution of polyethyleneimine[14] (160 mg; 3.5 mmol) in *N,N*-dimethylformamide (10 ml), Thy–Hse–PCP (2.1 g, 3.5 mmol) and imidazole (210 mg; 3.5 mmol) were added and stirred at 60°C for 48 h. After the reaction, the solution was concentrated under reduced pressure and was poured into excess acetone to precipitate the polymer. The obtained polymer was purified by reprecipitation from *N,N*-dimethylformamide into excess acetone. The polymer was dissolved in a small amount of water and freeze-dried to give light brown powder (860 mg, 78%); mp 116—120°C. IR (KBr, $cm^{-1}$): 1650 and 1550 (amide). TLC: *Rf* 0.0 (benzene–ethanol = 3 : 1), (ethanol). $^1H$ NMR (in dimethyl sulfoxide-$d_6$ at 25°C, ppm): 1.75 (s, 3H), 2.70 (t, 2H), 3.40 (m, 8H), 3.80 (m, 4H), 7.20 (s, 1H), 7.50 (d, 1H), and 8.40 (s, 1H).

*Poly-N-[{2-[3-(adenin-9-yl)propionyl]amino-4-hydroxy}butanoyl]ethyleneimine (5A; PEI–Hse–Ade)*

The polymer was prepared by two methods.

I) The polymer was prepared from 4A (1.4 g; 3.6 mmol) and polyethyleneimine (160 mg; 3.6 mmol) according to a similar procedure described for PEI–Hse–Thy (5T) (yield 68%; 820 mg), mp 95—98°C. IR (KBr, $cm^{-1}$): 1650 and 1550 (amide). $^1H$ NMR (in dimethyl sulfoxide-$d_6$ at 25°C, ppm): 2.60 (t, 2H), 3.70 (m, 8H), 4.30 (m, 3H), 7.40 (s, 1H), 7.60 (s, 1H), and 8.60 (s, 1H).

II) Poly-*N*-[{2-[3-($N^6$-diphenylphosphinothioyl-adenin-9-yl)propionyl]amino-4-hydroxy}butanoyl]ethyleneimine (5PptA; PEI–Hse–PptAde) was prepared from 4PptA (2.0 g; 4.0 mmol) and polyethyleneimine (180 mg; 4.0 mmol) according to the similar procedure described for PEI–Hse–Thy (5T). Resulting PEI–Hse–PptAde (5PptA, 1.6 g; 2.9 mmol; 73%) was dissolved in 20 ml of trifluoroacetic acid and 10 ml of 25% hydrogen bromide acetic acid solution was added. The reaction was carried out at 0°C for 5 h and then at room temperature overnight. After the reaction, the mixture was concentrated under reduced pressure and poured into excess acetone to give PEI–Hse–AdeHBr. The obtained polymer was dissolved in 5 ml of water and the pH of the solution was adjusted to 7.0 by NaOH aqueous solution. After purification of the polymer from water–acetone, the polymer was dissolved in a small amount of water and freeze-dried to give 770 mg of PEI–Hse–Ade (80%); mp 93—97°C. IR (KBr, $cm^{-1}$): 1650 and 1550 (amide). $^1H$ NMR (in dimethyl sulfoxide-$d_6$ at 25°C, ppm): 2.60 (t, 2H), 3.70 (m, 8H), 4.30 (m, 3H), 7.40 (s, 1H), 7.60 (s, 1H), and 8.60 (s, 1H).

**Table I.** Contents of PEI–Hse–Thy and PEI–Hse–Ade

| | PEI–Hse–Thy | PEI–Hse–Ade |
|---|---|---|
| Contents (unit, mol%) | 97 | 92 |

*Hydrolysis of the Polymer*

The polyethyleneimine derivatives were hydrolyzed in 6 *N* hydrochloric acid at 80°C for 48 h, into polyethyleneimine hydrochloride and carboxyethyl derivatives of nucleic acid bases. The quantitative calculation was made by using the corresponding carboxyethyl derivatives as standard samples. The nucleic acid base contents of the polymers are tabulated in Table I.

*Interaction between the Polymers*

Interaction of the polymers was estimated by hypochromicity values in UV spectra as reported previously.[18]

The UV spectra were measured with a JASCO UV-660 spectrometer equipped with a temperature controller at 20°C. Polyuridylic

acid (Poly U) (sodium salt) ($s^0_{20,w}$: 6—12) and polyadenylic acid (Poly A) (sodium salt) ($s^0_{20,w}$: 6—12) were obtained from Yamasa Shoyu Co., Ltd. PEI–Hse–Thy (5T), PEI–Hse–Ade (5A) and polynucleotides were dissolved in Kolthoff buffer (pH 7.0) (1/10 M $KH_2PO_4$–1/20 M $Na_2B_4O_7 \cdot 10H_2O$). These solutions stocked for 2 days at 20°C were mixed to give a polymer mixture of $10^{-4}$ mol $l^{-1}$ total concentration of nucleic acid base units in aqueous solution.

## RESULTS AND DISCUSSION

Various kinds of synthetic nucleic acid analogs have been prepared by polymerization of the corresponding monomers or by grafting of the nucleic acid base derivatives on polymers.[1] Most of these analogs, however, are insoluble in water at neutral pH, while polynucleotides are freely soluble in water. Therefore the interaction studies of the nucleic acid analogs were limited in organic solvents or in the mixture of water and organic solvents. A water soluble nucleic acid analog makes it possible to study the interactions of the polymers with polynucleotides in aqueous solution and biological activity of the analogs.

The most convenient method of preparing the nucleic acid analog is the grafting of the nucleic acid base derivatives to functional polymers. By this method, the nucleic acid analogs have been prepared such as polyacrylate, polylysine, and polyethyleneimine derivatives containing nucleic acid bases. In order to prepare the water soluble polymers containing high content of nucleic acid bases, hydrophilic units should be introduced to the side chains. Overberger and Inaki prepared polyethyleneimine derivatives containing nucleic acid bases, in which amino acids were used as the spacer.[19] One of these derivatives was the polyethyleneimine having nucleic acid base and serine as a spacer, which was soluble in water. For the preparation of the serine derivative, however, protections of the functional groups were necessary.

In the previous study, (±)-α-amino-γ-butyrolactone hydrobromide was used as a starting compound.[14] This lactone has a stable lactone ring where hydroxyl and carbonyl groups are protected, and gave homoserine unit by ring opening reaction. By using this lactone, polyethyleneimines containing nucleic acid bases and homoserine unit were successfully prepared in high yield. Schemes 1 and 2 show the preparation of the water soluble nucleic acid analogs containing thymine and adenine. The butyrolactone derivatives of thymine and adenine were obtained as stable solid in high yield.

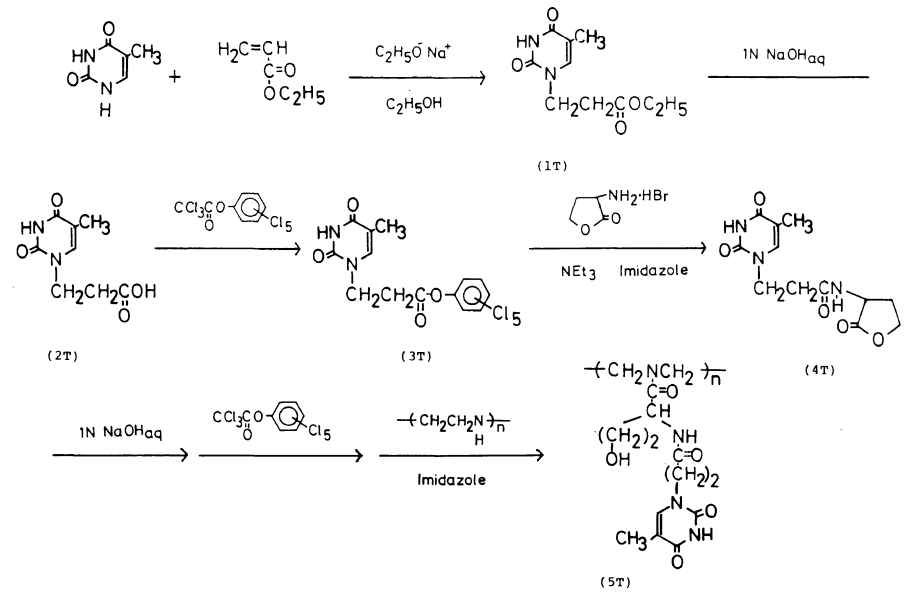
### *Homoserine Derivatives of Nucleic Acid Bases*

Starting with thymine or adenine, the carboxyethyl derivatives of the nucleic acid bases (1T and 1A) were prepared by Michael type addition reaction of ethyl acrylate. The $N^6$ amino group of the adenine base was protected by diphenylphosphinothioyl group in order to suppress the intramolecular catalytic activity of the adenine base for the activated ester derivative.

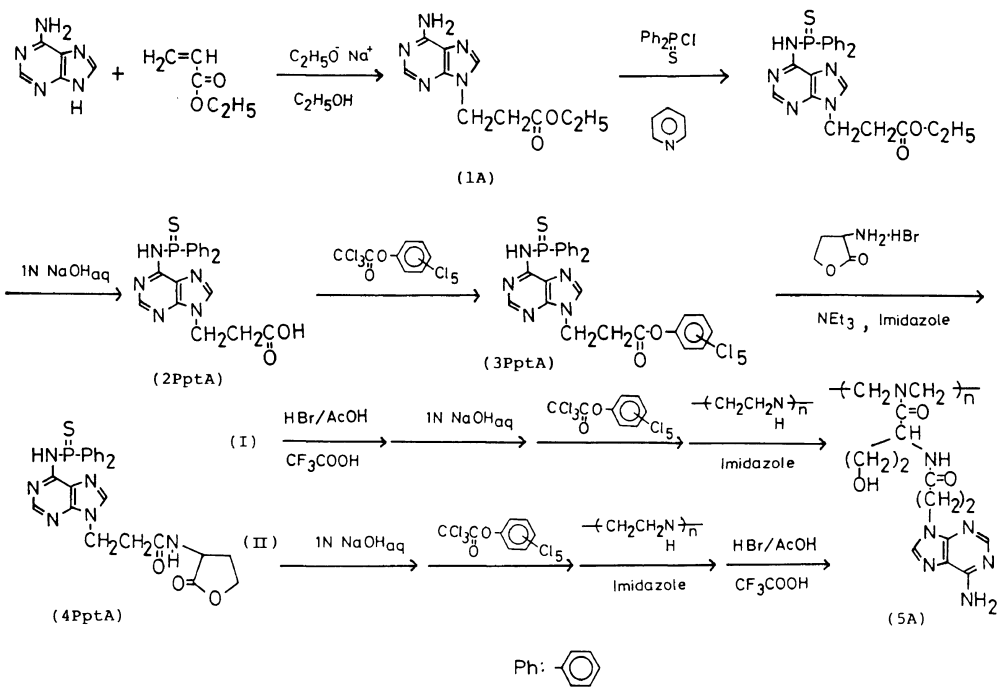
The carboxyethyl derivative of thymine (2T) or adenine (2PptA) was reacted with (±)-α-amino-γ-butyrolactone hydrobromide to give γ-butyrolactone derivative of thymine (4T) or adenine (4PptA). For the reactions, pentachlorophenyl ester derivatives were used with imidazole as a catalyst.[16]

### *Grafting onto Polyethyleneimine*

Since the reactivity of the γ-lactone is low, the direct reaction of the lactone derivative with polyethyleneimine was hardly occurred. Therefore the activated ester method was used. The lactone derivatives were hydrolyzed to the 3-hydroxybutyric acid derivatives, followed by the condensation with polyethyleneimine using the activated ester method. The grafting reaction was carried out in *N*,*N*-dimethylformamide, where a small amount of 4-pyrrolidinopyridine was used as an effective catalyst.



Scheme 1.



Scheme 2.

The polymers containing thymine (PEI–Hse–Thy; 5T) was obtained in high yield. For the preparation of the polymers containing adenine (PEI–Hse–Ade; 5A), it is necessary to remove the protection group of adenine base. In the route (I) of Scheme 2, the protecting

group of the lactone derivative (4PptA) was removed before the grafting reaction. On the other hand, the protecting group was removed after the grafting reaction in route (II). The yield of the polymer in route (II) was higher than that of in route (I).

Nucleic acid base content of the polymer was determined by UV spectroscopy on their hydrolyzed samples. The quantitative calculation was made by using the corresponding carboxyethyl derivatives as standard samples, and nucleic acid base contents in the polymer (unit mol%) were tabulated in Table I.

*Interaction of the Polymers*

Natural and synthetic polynucleotides are known to form a polymer complex by specific base-base interaction between nucleic acid bases. The synthetic nucleic acid analogs such as polyethyleneimine and polylysine derivatives containing nucleic acid bases were also found to form polymer complexes with polynucleotides by the specific base-base interactions.[2,3] As the solubilities of these nucleic acid analogs in water were low, the specific interactions were studied in organic solvents or water-organic mixed solvents, such as dimethyl sulfoxide, ethylene glycol, and water–propylene glycol.[20-22]

On the contrary, the polyethyleneimine derivatives of thymine or adenine having homoserine spacer unit were soluble in water over the entire range of pH values. This fact made it possible for the nucleic acid analogs to interact with polynucleotides in neutral aqueous solution. The complex formation of the nucleic acid analogs with polynucleotides in water is very important for the investigation of bioactivity of the polymers.[23] The interactions in water were thus studied between the polyethyleneimine derivatives, between the polymers and polynucleotides such as Poly U and Poly A.

*Interaction of PEI–Hse–Thy and PEI–Hse–Ade*

The interactions between water soluble polyethyleneimine derivatives containing thymine (PEI–Hse–Thy) and adenine (PEI–Hse–Ade) at pH 7.0 were studied with continuous variation techniques (Job plot[17]). As shown in Figure 1b, the stoichiometry of the complex based on nucleic acid base units was determined to be 1 : 1 (thymine : adenine) from the base ratio at the maximum hypochromicity value (10.4%). Hypochromicity has been widely used to indicate the interaction of nucleic acid base derivatives. The hypochromicity values for the PEI–Hse–Thy and PEI–Hse–Ade system (1 : 1 base unit) were determined at pH 2.2 and 5.5. At pH 2.2 the hypochromicity was negligible even after 3 days. On the other hand, the hypochromicity value at pH 5.5 was 7.1%, which was comparable to the value at pH 7.0. Adenine base has a pKa value at 4.15, and exists in a protonated form at pH 2.2. The protonated adenine base can not form a complex with the complementary thymine base.[21] This may be the reason for the negligible hypochromicity values at pH 2.2 and the high hypochromicity value at pH 5.5. From these facts, the polymer complex between PEI–Hse–Thy and PEI–Hse–Ade at pH 7.0 was concluded to be formed by the complementary nucleic acid base interaction.

In this system, time dependence of hypochromicity value was observed as shown in Figure 1. The hypochromicity was not observed in 3 h after mixing of the polymer solutions (Figure 1a). The absorbance decreased (the hypochromicity increased) and then became constant after 3 days (Figure 1b). Therefore, the conformational change of the synthetic polymers should be important for the formation of a stable polymer complex.[24]

It has been known that formation of the polymer complexes between the nucleic acid analogs is influenced by several essential factors: nature of the polymers such as flexibility, stereoregularity, and electric charge, effect of

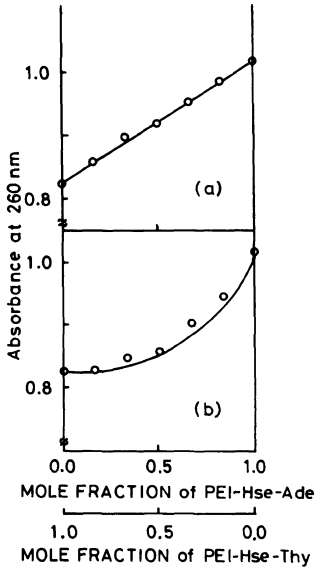



**Figure 1.** Continuous variation curve of PEI–Hse–Thy and PEI–Hse–Ade. Absorbance at 260 nm after (a) 3 h and (b) 3 days in 0.05 M Kolthoff buffer solution (pH 7.0) at 20°C. [PEI–Hse–Thy] = $1.0 \times 10^{-4}$ mol l$^{-1}$; [PEI–Hse–Ade] = $8.3 \times 10^{-5}$ mol l$^{-1}$.

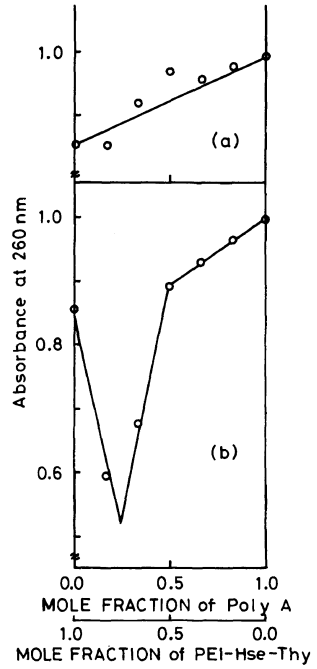



**Figure 2.** Continuous variation curve of PEI–Hse–Thy and Poly A. Absorbance at 260 nm after (a) 3 h and (b) 3 days in 0.05 M Kolthoff buffer solution (pH 7.0) at 20°C. [PEI–Hse–Thy] = $1.1 \times 10^{-4}$ mol l$^{-1}$; [Poly A] = $1.1 \times 10^{-4}$ mol l$^{-1}$.

solvent, and temperature. In studies of the interaction of the synthetic nucleic acid analogs, it was revealed that these factors closely related to the intramolecular and the intermolecular interactions of the nucleic acid bases in the polymer.[16,17,24] The nucleic acid bases in the synthetic nucleic acid analogs are self-associated intramolecularly in a solution. If the self-association of the bases is weak, the polymer forms a intermolecular polymer complex with the complementary polymer immediately after mixing, which is accompanied by a conformational change. However, for strong self-association of the nucleic acid bases, it is necessary to break the association by heating, addition of good solvent, or change of pH value.

The time dependency in Figure 1 suggests that the self-association of the nucleic acid bases in the polyethyleneimine derivatives dissociated slowly accompanying change of conformation, and formed the intermolecular polymer complex by the interaction between adenine and thymine.

*Interaction of PEI–Hse–Thy with Poly A*

The polyethyleneimine derivatives containing both the nucleic acid bases and the homoserine units are soluble in water, therefore it is possible to study the interactions of the polymers with nucleic acids and polynucleotides in aqueous solution. Figure 2 shows the mixing curves for the PEI–Hse–Thy with Poly A at pH 7.0. The maximum hypochromicity value was obtained as 38.4% at the base unit ratio of 2 : 1 (thymine : adenine) (Figure 2b). The overall stoichiometry of the complex based on nucleic acid base units, therefore, was 2 : 1 (thymine : adenine).

However, Figure 2b indicates that the polymer complex contains both 2 : 1 and 1 : 1 complexes. The maximum hypochromicity value was higher than that of the PEI–Hse–Thy:

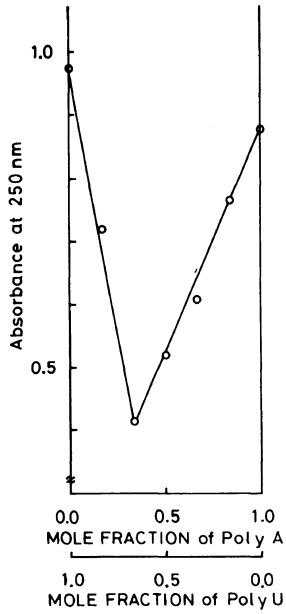



**Figure 3.** Continuous variation curve of Poly U and Poly A. Absorbance at 250 nm in 0.05 M Kolthoff buffer solution (pH 7.0) at 20°C. [Poly U] = $1.0 \times 10^{-4}$ mol l$^{-1}$; [Poly A] = $9.9 \times 10^{-5}$ mol l$^{-1}$.

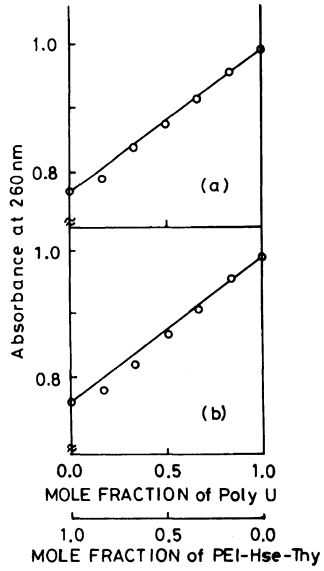



**Figure 4.** Continuous variation curve of PEI–Hse–Thy and Poly U. Absorbance at 260 nm after (a) 3 h and (b) 3 days in 0.05 M Kolthoff buffer solution (pH 7.0) at 20°C. [PEI–Hse–Thy] = $9.8 \times 10^{-5}$ mol l$^{-1}$; [Poly U] = $1.0 \times 10^{-4}$ mol l$^{-1}$.

PEI–Hse–Ade system. The value was also higher than the value both for the PEI–Hse–Hyp : Poly C system (26%), and for the PEI–Hse–Cyt : Poly I (7%) system in the previous paper.[14]

The stoichiometry of the polymer complex PEI–Hse–Thy : Poly A, however, was different from that of the PEI–Hse–Thy : PEI–Hse–Ade complex. As a control experiment, the interaction between Poly A and Poly U was studied under the condition used here. As shown in Figure 3, the complex formation was observed immediately after mixing of the polymer solutions, and the maximum hypochromicity (40.3%) was observed at the base ratio of 2 : 1 (uracil : adenine). The reason of the formation of the 2 : 1 complex is known to be ability of single strand formation for Poly A. The 2 : 1 polymer complex of PEI–Hse–Thy : Poly A, therefore, may be formed by the same reason for the Poly A : Poly U complex.

To make sure that such complex formation is due to the complementary nucleic acid base interaction, the interaction of PEI–Hse–Thy with Poly U was measured under the same condition. Figure 4 shows the mixing curves for PEI–Hse–Thy : Poly U at pH 7.0. The hypochromicity, however, could not be observed after 3 h (Figure 4a) and even after 3 days (Figure 4b). From these facts, the formation of the PEI–Hse–Thy : Poly A complex was concluded to be caused by the complementary interaction between adenine and thymine.

Time dependence of absorbance was also observed in this case as shown in Figures 2a and 2b. The hypochromicity was scarcely observed in 3 h after mixing of the polymer solutions (Figure 2a), but the absorbance decreased and became constant in 3 days (Figure 2b). The fact may be caused by self association of the nucleic acid bases in the polyethyleneimine derivatives, which dissociated slowly to form an intermolecular polymer complex. On the other hand, the polymer complex formations were immediately observ-

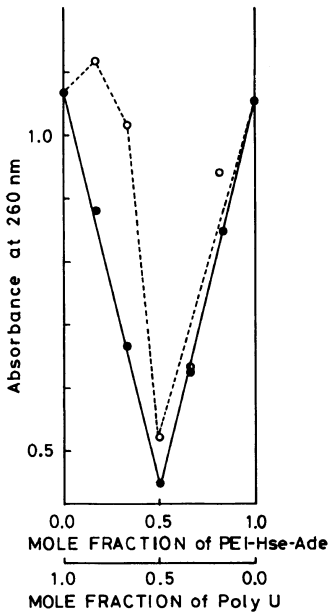



**Figure 5.** Continuous variation curve of PEI–Hse–Ade and Poly U. Absorbance at 260 nm (a) (---○---) 3 h and (b) (●) 3 days in 0.05 M Kolthoff buffer solution (pH 7.0) at 20°C. [Poly U] = $1.1 \times 10^{-4}$ mol $l^{-1}$; [PEI–Hse–Ade] = $8.3 \times 10^{-5}$ mol $l^{-1}$.

ed for the systems of Poly A : Poly U and PEI–Hse–Ade : Poly U after mixing of the polymer solutions. These facts suggested that the dissociation of the self-association of thymine bases accompanied by conformational change in PEI–Hse–Thy should be necessary to form the polymer complex in aqueous solution. A similar result was reported for the polymer complex formation between the polymethacrylate derivatives of uracil and adenine. In this case, the self association of the uracil bases in the polymer inhibited the polymer complex formation.[24]

*Interaction of PEI–Hse–Ade and Poly U*

Figure 5 shows the mixing curve between PEI–Hse–Ade (5A) and Poly U in 3 h (a) and 3 days (b) after mixing of the polymer solution. In this case, the formation of the polymer complex was observed even after 3 h, as shown in Figure 5a. The stoichiometry of the complex was 1 : 1 (thymine : adenine) and the maximum hypochromicity value was 49.6% at this base ratio. This value was high as compared with the values of PEI–Hse–Thy : PEI–Hse–Ade and Poly A : Poly U systems. The formation of the polymer complex, however, was negligible at pH 2.2, where the adenine base existed in a protonated form. From these facts, it should be concluded that the polymer complex between PEI–Hse–Ade and Poly U was caused by the specific interaction between adenine and thymine, and the self association of PEI–Hse–Ade in aqueous solution was negligible.

## CONCLUSION

Water soluble polyethyleneimine derivatives containing both the nucleic acid bases, thymine or adenine, and the homoserine unit were prepared. The polyethyleneimine derivatives, PEI–Hse–Thy and PEI–Hse–Ade formed the 1 : 1 polymer complex in aqueous solution. These polymers also formed the polymer complexes with polynucleotides, Poly A or Poly U, by the complementary interaction of the nucleic acid bases.

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