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Polyethyleneimine Derivative for Nucleic Acid Model

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Abstract Water-soluble polyethyleneimine (PE) derivatives containing nucleic acid bases and hydrophilic amino acids such as homoserine (Hse) and serine were prepared by the activated ester method as nucleic acid models. From spectroscopic measurements, the polymers were found to interact with DNA accompanied by an induction of conformational change. Hypochromicity in UV spectra indicated that a stable polymer complex was formed between poly (A) with PEI-Hse-Ura by complementary hydrogen bonding with equimolar nucleic base units (adenine:uracil = 1:1). The induced conformation of DNA by the interaction with the polymer containing uracil and homoserine (PEI-Hse-Ura) was concluded to be a super triple helical structure. The formation of the polymer complex, DNA : PEI-Hse-Ura, was found to be affected by the presence of metal ions such as Ca^{2+} and Cu^{2+}.

Keywords: nucleic acid model, polyethyleneimine, homoserine, interaction, metal ions

INTRODUCTION

Recently, a number of investigation for sequence specific DNA-binding proteins, regulate gene expression and also serve structural role in other cellular processes, have been reported. From these studies, detailed surface structures of major and minor groove play an important role in specific recognition. DNA is known to form a double helical structure by specific hydrogen bonds between complementary nucleic acid bases. The effects of the chemical structure of DNA on specific polymer interactions have been studied using synthetic nucleic acid analogs [1-6]. Polyethyleneimine derivatives containing adenine and thymine have been prepared by polymer grafting using the activated ester method [7]. These polymers interact with each other to form polymer complexes by base pairing between adenine and thymine [8-12]. The polymers also interact with polynucleotides such as poly (A) or poly (U) [8]. The polyethyleneimine derivatives of the nucleic acid base in the previous study, however, were hardly soluble in water at a neutral pH. Therefore, their interaction studies were restricted to the water-ethylene glycol system.

Polymer derivatives of nucleic acid bases have been prepared for the application as a DNA chip probe [13,14]. Other applications of these nucleic acid base derivatives include HPLC resin for the separation of components of nucleic acids and polynucleotides using the specific binding characteristics, while ion-exchange and reverse-phase systems are non-specific separation systems [15-18].

In the present study, water-soluble nucleic acid models were prepared using hydrophilic amino acids such as serine and homoserine as spacers. These polymers made it possible for us to study the interactions with water soluble polynucleotides and nucleic acids in an aqueous solution [19-22]. This paper deals with the polyethyleneimine derivatives having uracil as a nucleic acid base and homoserine as the spacer (PEI-Hse-Ura).

The formation of polymer complexes with natural nucleic acids, DNA, RNA and polynucleotides, was investigated. Conformational changes of DNA by the interaction with polyethyleneimine were studied by CD spectra. The effects of additives such as metal ions, Cu^{2+} and Ca^{2+} on the formation of the polymer complex were also studied.

MATERIALS AND METHODS

Materials

Polyethyleneimine (MW = 800~1,200) was purchased from Nakarai (Japan). Polyethyleneimine derivatives containing uracil and homoserine (PEI-Hse-Ura) were prepared by the activated ester method [21] as shown in Fig. 1. DNA from a calf thymus origin, DNA from herring sperm, RNA from yeast, poly (A), and poly (U) were purchased from Yamasa Shoyu Co. Ltd (Japan).

Polymer Complex Formation

Two kinds of polymer solutions in Kolthoff buffer at

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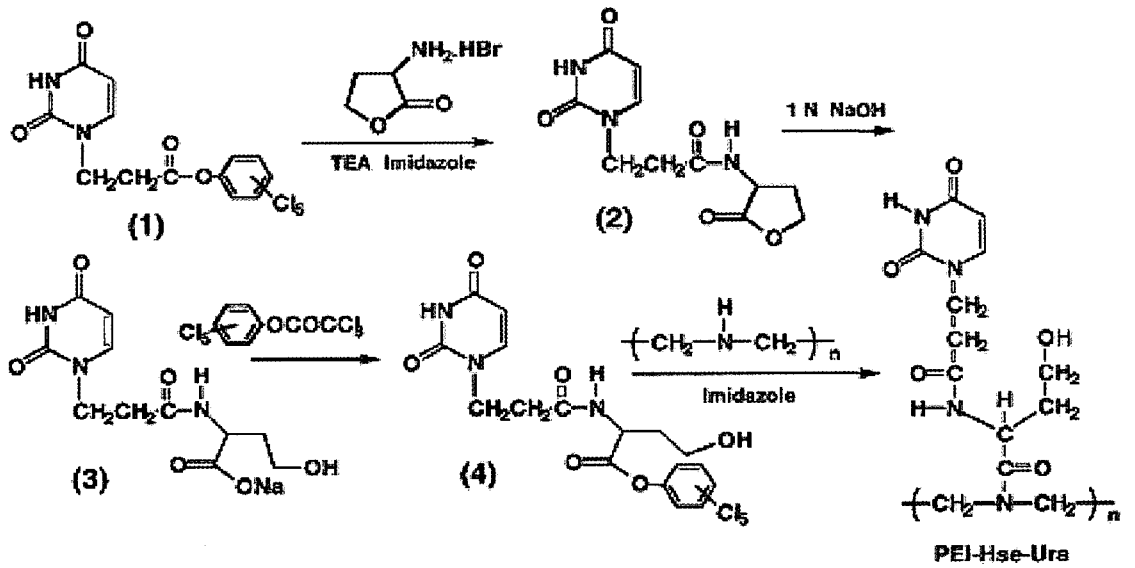

Fig. 1. Synthesis of a polyethyleneimine derivative containing uracil and homoserine (PEI-Hse-Ura).

pH 7 (1/10 M KH_2PO_4 and 1/20 M $Na_2B_4O_7 \cdot 10H_2O$) were mixed in various ratios to give a polymer mixture of 10^{-4} M total concentration of nucleic acid base units in a solution.

Spectroscopic Measurement

The hypochromicity was measured by UV spectroscopy. The UV spectra were measured with a JASCO UV-660 spectrometer (Japan) equipped with a temperature controller at 20°C. The circular dichromism (CD) spectra were measured under the same condition used for the UV spectra with a JASCO CD J-40 spectrometer (Japan) at room temperature.

RESULTS AND DISCUSSION

Polyethyleneimine Derivative Preparation

Polyethyleneimine derivatives containing uracil and homoserine (PEI-Hse-Ura) were prepared as follows. At first, the activated ester of carboxyethyluracil (1, in Fig. 1) was reacted with (±)-α-amino-γ-butyrolactone hydrobromide, followed by hydrolysis to give the uracil derivative of homoserine (3). The carboxyl group of the obtained compound was activated (4), and reacted with polyethyleneimine (the degree of polymerization was around 500) to give PEI-Hse-Ura. The degree of substitution of this polymer was obtained from UV spectra of the hydrolyzed sample to be 94%. The obtained polymer was freely soluble in an aqueous buffered solution.

Interaction with Poly (A)

Interaction studies of these polymers with DNA were carried out using UV spectra [19-22]. Fig. 2A shows the UV absorbance at 260 nm of the polyethyleneimine derivative (PEI-Hse-Ura) at various molar ratios of poly (A). The dotted line shows the absorbance for 3 h after mixing, and the solid line shows the absorbance overnight after mixing. From the curve in Fig. 2A, the maximum hypochromicity value obtained was 54.4% at a 0.5 mole fraction. Hypochromicity in UV spectra has been widely used to indicate the interaction of nucleic acid derivatives. Therefore, the present data indicated that a stable polymer complex was formed between poly (A) with PEI-Hse-Ura by complementary hydrogen bonding with equimolar nucleic base units (adenine:uracil = 1:1).

To determine whether the interaction between the bases was due to the complementary nucleic acid bases, the interaction of PEI-Hse-Ura with poly (U) was measured under the same condition. In Fig. 2B, the mixing curve for poly (U):PEI-Hse-Ura shows no hypochromicity after 3 h nor even after overnight. This result indicates that PEI-Hse-Ura did not interact with uracil bases or the phosphate units in poly (U). Therefore, the hypochromicity observed for the poly (A):PEI-Hse-Ura system may be concluded to be caused by the complementary adenine-uracil interaction.

As a control experiment, the interaction between poly (A) and poly (U) was studied under the same condition (Fig. 2C). The polymer complex formation was observed immediately after mixing of the polymer solutions, and the maximum hypochromicity value was obtained as 40.3%, which is smaller than the value obtained for the poly (A):PEI-Hse-Ura system. Compared with the poly (A):poly (U) system, the formation of the complex was slow for the poly (A):PEI-Hse-Ura system. This may be due to the intramolecular interaction of uracil bases in PEI-Hse-Ura, which was reported for the polymethacrylate derivative of uracil [23].

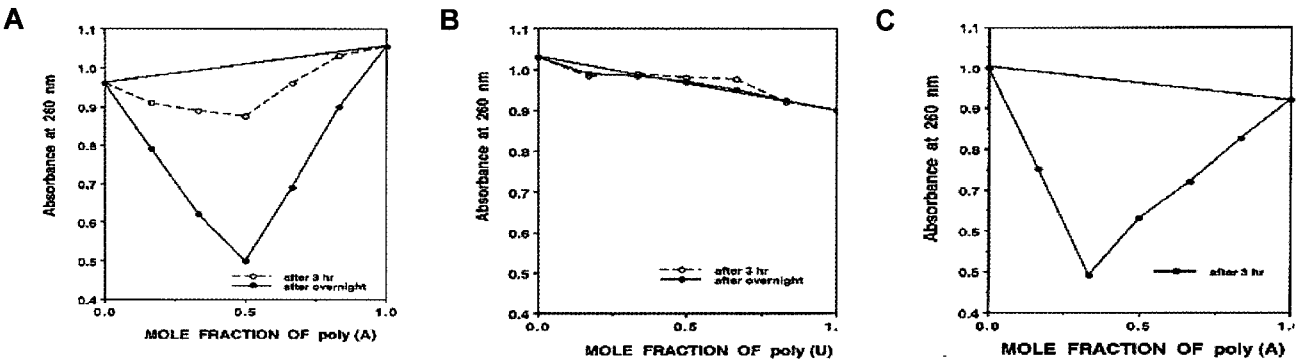

Fig. 2. UV absorbance curves for A: the mixture of poly (A) and PEI-Hse-Ura in Kolthoff buffer at pH 7, B: the mixture of poly (U) and PEI-Hse-Ura in Kolthoff buffer at pH 7 and C: the mixture of poly (A) and poly (U). The straight lines represent theoretically predicted data without an interaction.

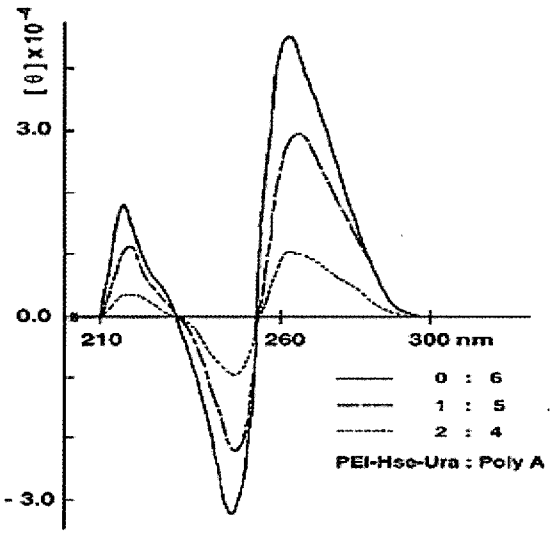

Fig. 3. CD spectra of poly (A) with PEI-Hse-Ura.

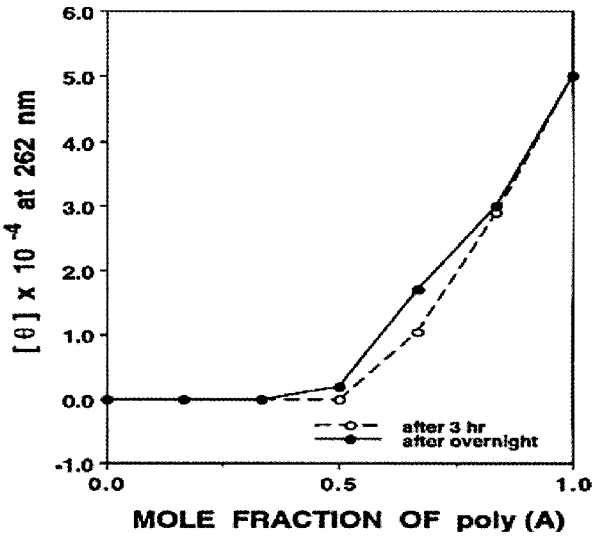

Fig. 4. Molecular ellipticity [θ] at 262 nm *vs.* mole fraction of poly (A).

Conformation of the Poly (A):PEI-Hse-Ura Complex

Poly (A) is known to form a right handed single stranded structure caused by stacking of adenine bases in a neutral aqueous solution, while the strand is unstable compared with DNA [24]. The conformation of poly (A) was changed by the formation of the polymer complex with PEI-Hse-Ura as shown in the CD spectra (Fig. 3) where the bands were assigned to poly (A) because PEI-Hse-Ura was optically inactive. The molar ellipticity ([θ]) at 262 nm was plotted according to the mixing ratio of the two polymers in Fig. 4. As mentioned above, the hypochromicity in UV spectra indicated that the formation of the complex was slow for the poly (A):PEI-Hse-Ura system (Fig. 2A). However, the hypochromicity in CD spectra caused by the conformational change of poly (A) was observed immediately after mixing of the two polymer solutions (Fig. 4). From the UV and CD spectra, the formation of the polymer complex between poly (A) and PEI-Hse-Ura was considered as follows (Fig. 5).

(1) Before mixing, poly (A) forms a single stranded structure, and PEI-Hse-Ura exists in a random coiled conformation with an intramolecular interaction of uracil bases.

(2) After mixing (3 h), base pairing between adenine and uracil was partly formed (UV spectra), and the single stranded structure of poly (A) changed to a random coiled conformation (CD spectra).

(3) During one night, the intramolecular interaction in PEI-Hse-Ura dissociated slowly to form the intermolecular base pairing (UV spectra). The conformation of poly (A), however, scarcely changed overnight (CD spectra).

Interaction with DNA

DNA is known to form a double helical structure by specific hydrogen bonds between complementary nucleic acid bases. Although various structures are known for DNA, the most stable structure of DNA from calf thymus origins is a B-type double stranded conformation [25,26].

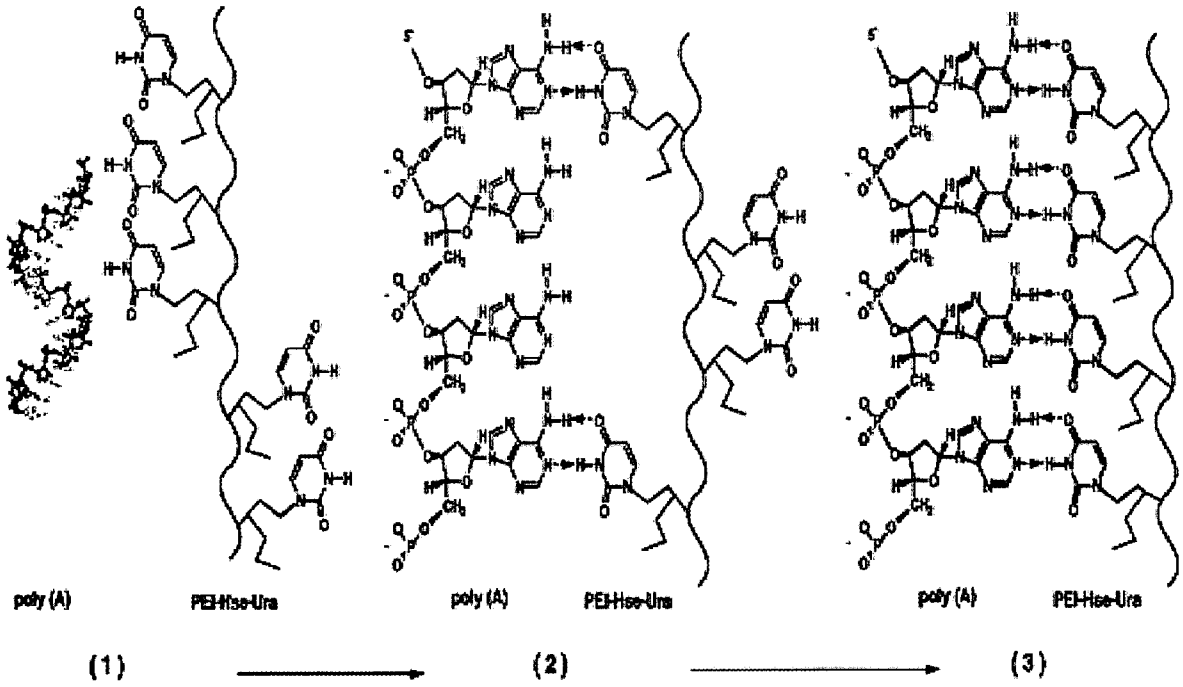

Fig. 5. Formation of the polymer complex between poly (A) and PEI-Hse-Ura.

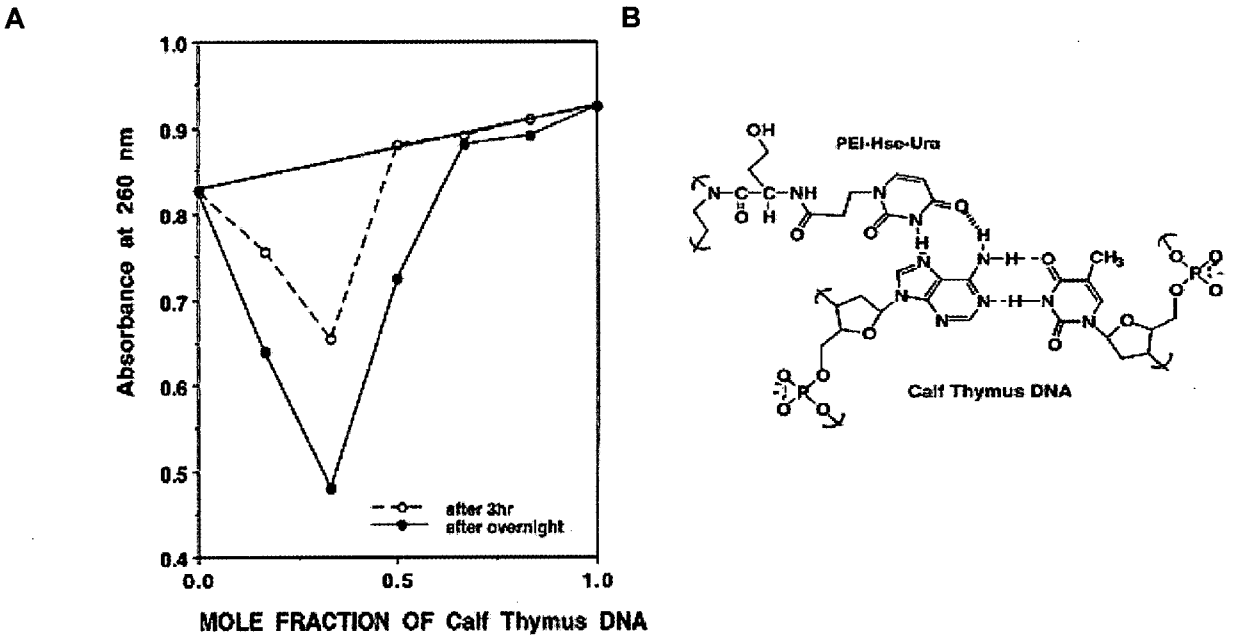

Fig. 6. A: UV absorbance curve for the mixture of calf thymus DNA and PEI-Hse-Ura B: DNA-PEI-Hse-Ura triple strand.

The polyethyleneimine derivative of uracil (PEI-Hse-Ura) was found to form a polymer complex with DNA.

Fig. 6A shows the UV absorbance curve for the mixture of the DNA:PEI-Hse-Ura system, which suggests the highest hypochromicity value of 47%. The overall stoichiometry of the complex based on the nucleic acid base units was 1:2 (DNA:PEI-Hse-Ura), which is different from 1:1 for the system poly (A):PEI-Hse-Ura. From this result, the polymer complex of DNA:PEI-Hse-Ura was concluded as a triple strand (Fig. 6B). The uracil bases in PEI-Hse-Ura can interact with adenine bases in DNA that already form a base pair with thymine in DNA.

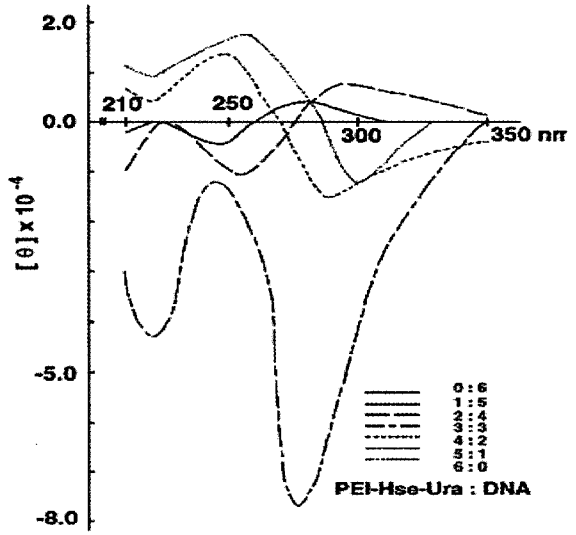

Fig. 7. CD spectra of calf thymus DNA with PEI-Hse-Ura.

One adenine base in poly (A) is known to interact with two uracil bases in poly (U) forming the poly (A):(poly (U))2 triple complex [8].

PEI-Hse-Ura also formed a polymer complex with DNA from another origin:herring sperm.

Conformation of the DNA:PEI-Hse-Ura Complex

A Drastic change of CD spectra was observed for DNA by the addition of the polyethyleneimine derivative as shown in Fig. 7. The spectra of DNA (0:6=PEI-Hse-Ura: DNA) show a typical B type conformation [27]. With an increase of PEI-Hse-Ura, the positive band at 280 nm shifted to red (2:4=PEI-Hse-Ura:DNA), and a drastic change of the spectrum was observed at the 3:3 molar ratio. The negative band at 280 nm with high intensity indicated a highly condensed form of DNA (Ψ(-) DNA) [28-30]. This type of conformation may be a super helical structure. Furthermore, with excess PEI-Hse-Ura (5:1 =PEI-Hse-Ura:DNA), the spectrum showed a negative band at 290 nm and a positive band at 245 nm, which should be assigned to a Z-type conformation of DNA [31].

Interaction in the Presence of Metal Ions

An anomalous CD spectrum for the 1:1 mixture of DNA with PEI-Hse-Ura was investigated after the additions of sodium chloride and metal ions. Fig. 8 shows the spectral change of the 1:1 mixture with various sodium chloride concentrations. With an increase of the NaCl concentration, the intensity of the negative band at 280 nm decreased (up to 100 mM), and became a normal spectrum of DNA with 200 mM of NaCl. The triple strand of DNA:PEI-Hse-Ura probably dissociated to a double strand of DNA, because a double strand of DNA is known to be stabilized in the presence of neutral salt [32].

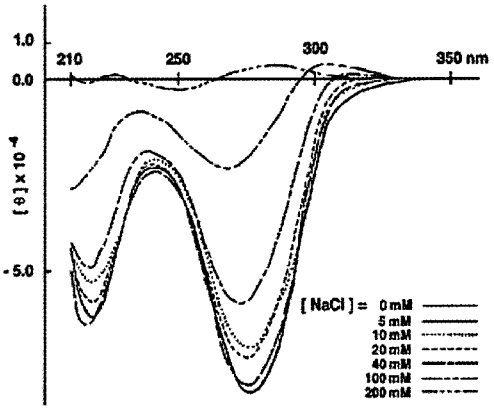

Fig. 8. Effect of NaCl on the CD spectra of calf thymus DNA with PEI-Hse-Ura.

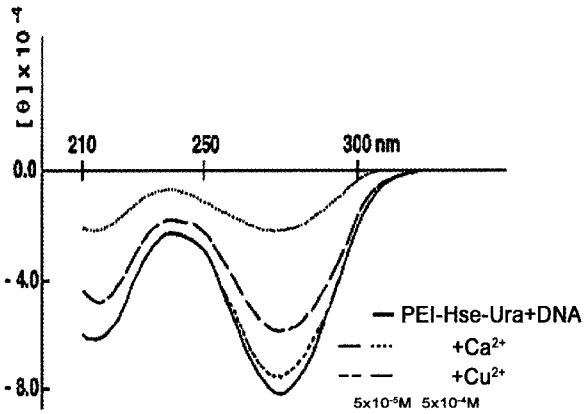

Fig. 9. Effect of metal ions on the CD spectra of calf thymus DNA with PEI-Hse-Ura.

A change of the spectra for the 1:1 mixture of DNA with PEI-Hse-Ura was also observed in the presence of metal salt. In the presence of Cu^{2+} (5×10^{-5} and 5×10^{-4} M), the intensity of the negative band at 280 nm decreased as shown in Fig. 9. In the presence of a calcium ion (Ca^{2+}), however, a conformational change was only slightly observed. Metal ions are also known to interact with the double strand of DNA [33-35], thus the triple strand of DNA:PEI-Hse-Ura probably dissociated to a copper (II) complex of DNA.

Formation of the polymer complex between DNA from herring sperm and PEI-Hse-Ura was also observed from the UV spectra (data not shown). A conformational change by PEI-Hse-Ura, however, was not observed for DNA from herring sperm. The double strand of DNA from herring sperm may be more stable than that of DNA from calf thymus because the guanine-cytosine (G-C) contents were different.

Interaction with RNA

The conformation of RNA is different from that of DNA.

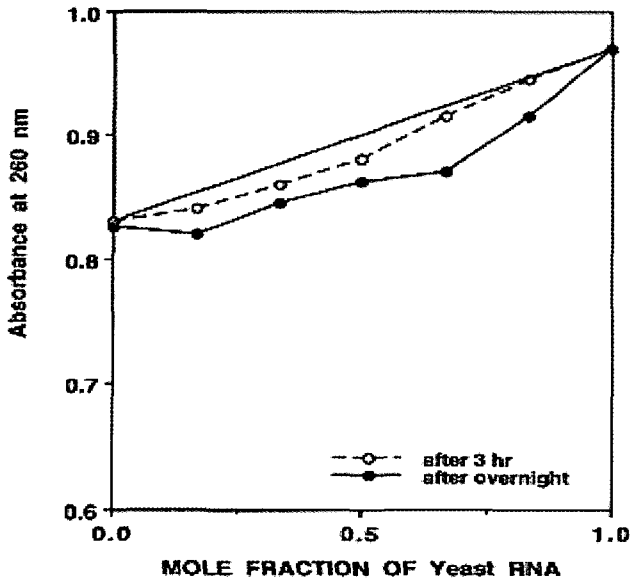

Fig. 10. UV absorbance curves for the mixture of yeast RNA and PEI-Hse-Ura in Kolthoff buffer at pH 7.

RNA from yeast has been reported to form an A-type double stranded conformation [36]. Fig. 10 shows the UV mixing curve of RNA with PEI-Hse-Ura. Hypochromicity was observed for this system, but the value was small compared with the system of DNA. The reason for this observation may be caused by the difference of the structure between DNA and RNA. DNA has a wide and shallow groove, but RNA has a narrow and deep groove where the base pairs are shielded. Uracil bases of PEI-Hse-Ura may penetrate into the major groove of DNA, and form a base pair with adenine of DNA. On the other hand, uracil bases of PEI-Hse-Ura hardly penetrate into the groove of RNA due to the shielded structure of RNA.

CONCLUSION

The polyethylenimine derivatives of uracil containing homoserine as a spacer (PEI-Hse-Ura) were soluble in water, and formed a polymer complex with poly (A) and DNA by complementary base pairing. PEI-Hse-Ura, however, hardly formed the polymer complex with RNA due the conformational change of poly (A) and DNA by formation of a polymer complex. The induced conformation of DNA with equimolar PEI-Hse-Ura was concluded.

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