Syntheses and Characterization of Polymers

Containing Nucleic Acid Bases

by

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SYNOPSIS

Two different types of the polymers containing nucleic acid bases in backbone or in pendant groups were prepared. The polymers of the first type were polyureas obtained by the polyaddition reaction of uracil and adenine with hexamethylene diisocyanate (HMDI). The second type, that is cationic polyurethanes containing nucleic acid bases in pendant groups, were obtained by Menschutkin reaction of halogenated derivatives of uracil and adenine with a linear polyurethane containing tertiary nitrogen atoms which was based on HMDI and N-methyldiethanolamine. Base-base interactions were studied for the polymers by UV and NMR spectra. A relatively high value of hypochromicity, ca. 19%, was observed for the mixture of the ionic polyurethane with uracil pendant and herring sperm DNA. Complementary hydrogen bonding interaction was detected for the mixture of the ionic polyurethane with adenine pendant and that with uracil pendant. Nonthrombogenic character of the polymers was examined according to the modified Lee-White method. The ionic polyurethanes with adenine and uracil pendant exhibited fairly good anti-clotting property.

INTRODUCTION

It is expected that the synthetic polymers containing nucleic acid bases in the structure are highly functional materials because of their ability for complementary base pairing. They may exhibit template characteristics through this specific interaction which governs the process of replication and the genetic code transcription *in vivo*. Specific catalytic activity is also expected in these polymers. Furthermore, if they obtain helical and double strand structure similar to DNA through their selective hydrogen bonding and specific interaction between bases, the possible conforma-

tional response caused by the chemical and physical excitation may offer a fundamental mechanism for the design of new mechanochemical system. Thus the polymers will work as a novel functional material and will serve as a useful model for the elucidation of delicate mechanism realized in biopolymers. From this viewpoint a number of studies have been carried out on the syntheses and characterization of the polymers containing nucleic acid bases.

Jones et al.[1] synthesized 5'-O-acryloyluridine and 5'-O-acryloylthymidine and found that the polymers of these compounds have inter-

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actions with denatured DNA. Takemoto et al.[2] prepared a variety of similar compounds, which were mainly vinyl compounds, and observed hypochromic effect in UV spectra owing to selective interactions between the polymers. Kaye[3] and Pitha et al.[4] studied the interactions between poly(9-vinyladenine) and poly U by CD and concluded the two-dimensional network structure for the complex of the two polymers. Seita et al.[5] synthesized the polymers containing nucleic acid bases and acid esters, and found strong interactions between their polymers and DNA. Okubo et al.[6] prepared and studied the cationic polymers from poly(4-pyridylethylene) by quaternization with chloroethyl derivatives of nucleic acid bases.

In this paper the authors attempted to prepare the novel polymers in which nucleic acid bases are introduced in backbone or side chain, and determined hydrogen bonding and stacking interaction between bases. The potentiality as biomaterials was also examined for the prepared polymers.

EXPERIMENTAL

1. Reagents

Uracil, 5-bromouracil, adenine, and herring sperm DNA were commercial grade and used without further purification. Other starting materials and solvents were purified by conventional methods.

2. Synthesis of poly [2, 4-pyrimidinedione-1,3-diyl) carbonyliminohexamethyleneiminocarbonyl] (Ura-HMDI).

A mixture of uracil (1.12 g; 0.01 mol) and HMDI (1.68 g; 0.01 mol) in 30 ml of N, N-dimethylformamide (DMF) was stirred at 80°C for 1 hr. Then the reaction mixture was poured into a large amount of water. A white powder precipitated was filtered off, washed with water, and dried at 80°C *in vacuo* until a constant weight was reached. This compound will be abbreviated as Ura-HMDI. Yield 50%.

Found   C: 50.19   H: 6.84   N: 19.68
Calcd   C: 51.41   H: 5.76   N: 22.83
$[\eta] = 0.1$ (m-cresol, 30°C)

3. Synthesis of poly [imino(6, 9-purindiyl) carbonyliminohexamethyleneiminocarbonyl] (Ade-HMDI)

A mixture of adenine (1.35 g; 0.01 mol) and HMDI (1.68 g; 0.01 mol) in 40 ml of N, N-dimethylacetamide (DMA) was stirred at 60°C. After 30 min the reaction mixture that was inhomogeneous at the beginning of the reaction became transparent, and then a white powdery precipitate started to separate out. During the course of the reaction the precipitate increased in its amount. The extent of reaction was followed by titrating unreacted NCO group according to amine equivalent method. After 72 hr no residual NCO group was detected. The product was filtered off, washed with DMA, and dried at 60°C *in vacuo*. This compound will be abbreviated as Ade-HMDI. Yield 40%.

Found   C: 50.21   H: 5.61   N: 33.96
Calcd   C: 51.46   H: 5.66   N: 32.33

4. Synthesis of poly [oxyethylene(methyl-imino)ethyleneoxycarbonyliminohexamethyleneiminocarbonyl]   (N-MePU)

A mixture of N-methyldiethanolamine (17.90 g; 0.15 mol) and HMDI (25.22 g; 0.15 mol) in 200 ml of DMA was stirred at 80°C for 3hr. The reaction mixture was poured into a large amount of water. A white powder precipitated was filtered off, washed with water, and dried at 80°C *in vacuo*. This compound will be abbreviated as N-MePU. Yield 80%.

Found   C: 54.36   H: 8.77   N: 14.98
Calcd   C: 54.44   H: 8.71   N: 14.63

5. Synthesis of poly [oxyethylene [methyl-5-(2,4(1H,3H)-pyrimidinedionyl)imino]ethyleneoxycarbonyliminohexamethyleneiminocarbonyl bromide]   (PU-Ura)

A mixture of N-MePU (2.51 g; 8.7 mmol) and 5-bromouracil (2.0 g; 10.4 mmol) in DMA

was stirred at 80°C for 160 hr. By pouring the reaction mixture into a large amount of water, a white powder was precipitated. It was filtered off, washed with water, and dried at 80°C *in vacuo*. This compound will be abbreviated as PU-Ura. Yield 30%.

Found   C: 41.38   H: 5.78   N: 14.98
Calcd   C: 42.68   H: 5.85   N: 14.63

6. Synthesis of poly[oxyethylene[methyl-2-hydroxy-2-(1*H*-purin-6-amino-9-yl)ethyl-imino]ethyleneoxycarbonyliminohexamethyl-eneiminocarbonyl bromide] (PU-AdeEBH)

A mixture of adenine (2.00 g; 14.7 mmol), epibromohydrin (2.08 g; 15.2 mmol), and a trace amount of anhydrous potassium carbonate in 40 ml of DMF was stirred at 60°C. After 24 hr N-MePU (0.96 g; 3.3 mmol) was added to the reaction mixture. After 100 hr, the small amount of the unreacted adenine was discarded by filtration. The filtrate was poured into a large amount of acetone, and then a brown solid was precipitated. It was filtered off, washed with acetone, and dried at 60°C *in vacuo*. This will be abbreviated as PU-AdeEBH. Yield 30%.

Found   C: 41.41   H: 6.42   N: 17.79
Calcd   C: 45.07   H: 6.32   N: 20.03

7. Synthesis of 9-(2'-bromoethyl)adenine (AdeEtBr)

A mixture of adenine (2.70 g; 0.02 mol), ethylene carbonate (1.76 g; 0.02 mol), and a trace amount of sodium hydroxide in 40 ml of DMF was stirred at 110°C for 1 hr[7]. After cooling, the solvent was removed to complete dryness under reduced pressure. Recrystallization of the residue from ethanol gave 9-(2'-hydroxyethyl)adenine (AdeEtOH) as colorless plates.

In a three-necked round-bottomed flask equipped with a stirrer and a dropping funnel was placed 25 ml of pyridine containing AdeEtOH (0.01 mol; 1.8 g). The flask was cooled externally with crushed ice and salt to −10°C. PBr(1.08 g; 4mmol)was added slowly to the flask during agitation. After 30 min the temperature of the reaction mixture was raised to room temperature and was allowed to stand overnight. Then the solvent was removed under reduced pressure. Recrystallization of the residue from ethanol gave 9-(2'-bromoethyl) adenine (AdeEtBr) as white needles. It was filtered off and dried at 60°C *in vacuo*. Yield 20%.

Found   C: 32.19   H: 3.90   N: 26.78
Calcd   C: 34.73   H: 3.34   N: 28.93

8. Synthesis of poly[oxyetylene[methyl-2-(purin-6-amino-9-yl)ethylimino]ethyleneoxy-carbonyliminohexamethyleneiminocarbonyl bromide] (PU-AdeEtBr)

A mixture of N-MePU (0.38 g; 1.3 mmol) and AdeEtBr (0.32 g; 1.3 mmol) in 40 ml of DNA was stirred at 60°C for 100 hr. By pouring the reaction mixture into acetone, a brown precipitate appeared. It was filtered off, washed with acetone, and dried at 60°C *in vacuo*. This compound will be abbreviated as PU-AdeEtBr.

9. Ultraviolet absorption spectra

Ultraviolet absorption spectra of the obtained polymers, DNA, and the mixtures of the polymers and DNA were measured in dilute solutions of Walpole buffer solution (CH₃COOH-CH₃COONa pH 4.5) by using a Hitachi UV-VS spectrometer model 323 and fused quartz cells of approximately 1.0 cm path. All spectra were recorded at room temperature. In the case of the mixtures of the prepared polymers and DNA, the separate solutions dissolving each component were mixed and allowed to stand 2 days before spectroscopic measurement in order to make sure inter-polymer interactions. An apparent hypochromicity was calculated according to the following equation,[8] where m and n are the volume fractions of the solution of polymer a and that of polymer b, and I_a,I_b, and I_{a+b} the absorbances of the solutions of polymer a, polymer b, and the mixture of the two solutions, respective-

ly.

$$\% \text{ Hypochromicity} = 100 \left(1 - \frac{I_{a+b}}{m\,I_a + n\,I_b}\right)$$

10. Nuclear magnetic resonance spectra

Nuclear magnetic resonance spectra were recorded on a Nihon Denshi NMR spectrometer model JNM-MH-100 operating at 100Mc. TMS and DSS internal standards were used for DMSO-d_6-benzene-d_6 mixed solution and for D_2O-D_2SO_4 solution, respectively, The measurements were made on 0.05, 0.1, and 0.15 mol solution at room temperature, 50°, and 75°C.

11. Examination of anti-clotting property of the polymers

Modified Lee-White method[9] was used for the examination of nonthrombogenic character of the polymers. Two test tubes of 10 mm diameter were prepared for each polymer. The inside walls of test tubes were coated with the obtained polymers. For the control, two tubes were not treated by polymer. An appropriate amount of whole human blood was withdrawn from an arm vein using a small syringe. One milliliter of blood just withdrawn was transfered into cach tube. The one of the two tubes for each polymer was rotated endwise every thirty seconds. The other tube was left without any disturbance. At the point at which the blood no longer flows from its position but maintains its surface contour when inverted, the other tube was started to rotate. The point at which the blood in the second tube no longer flowed as described above was taken as the end point. The time from withdrawing of the blood from vein to final clotting was recorded as clotting time.

RESULTS AND DISCUSSION

1. Preparation

The preparation of the polymers containing nucleic acid bases in backbone is illustrated in Fig. 1. The reactions proceeded smoothly. The obtained polymers, Ura-HMDI and Ade-HMDI, were identified with those expected from the reaction scheme. The polymers were white
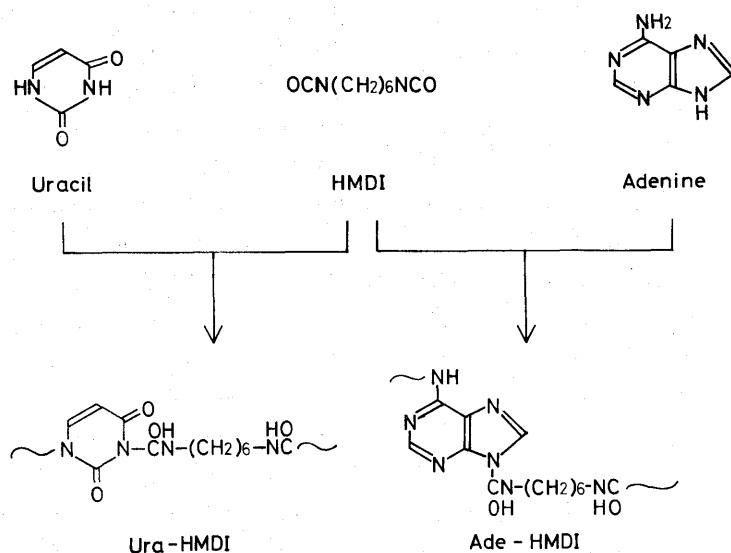

Fig. 1   Synthetic route of the polymers containing nucleic acid bases in backbone.

powder.

Since the polymerization mechanism is essentially polyaddition of isocyanate with amine, pyrimidine and purin bases were polymerized by the formation of substituted urea linkage. As a result of it, N-H groups of nucleic acid bases are used up after polymerization. This consumption of N-H groups will cause the decrease in basic character of the nucleic acid bases and the lack of specific interactions between bases. Though the polymers obtained consist of nucleic acid base structure, it seems appropriate to regard them as novel polyureas with specific heterocyclic ring structure in backbone.

In order to keep the character of nucleic acid bases, it is better to incorporate bases as pendant groups to some polymeric stems. This is in line with the structure of polynucleotides.

In the case of uracil and adenine, the base is connected to the ribose at N_1 and N_9 position, respectively. In Fig. 2 are shown the synthetic route of the polymers containing nucleic acid bases as pendant groups studied in this work. The stem polymer is a linear polyurethane containing tertiary nitrogen atom to which halogenated derivatives of nucleic acid bases add by Menschutkin reaction, resulting in the quaternization of nitrogen atom. Thus, the resultant polymer obtain cationic character in addition to the heterocyclic ring structure of nucleic acid base. In the case of uracil, C_5 position was used for the connecting site because 5-bromuracil was obtained commercially. As for adenine, it was necessary to prepare halogenated intermediates. The N_9 position was available for this purpose in most cases except for AdeEBH. In the latter case the
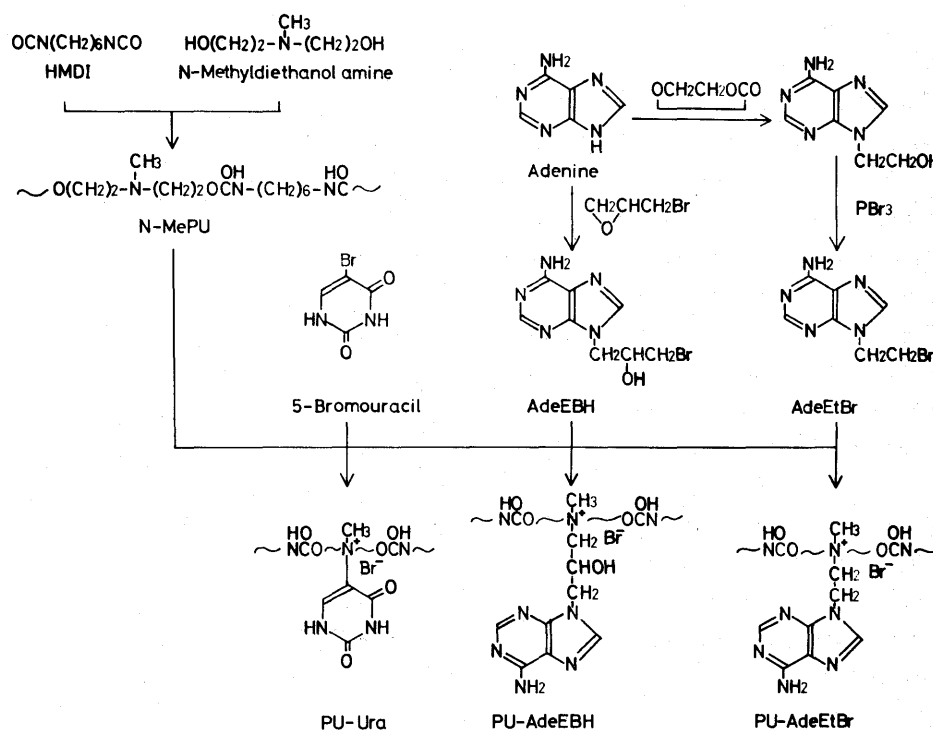

Fig. 2 Synthetic route of the polymers containing nucleic acid bases in pendant groups.

selectivity of the reaction of epibromohydrin with adenine was low, giving a mixture of the two isomers, 9-(2-hydroxy-3-bromopropyl) adenine and 3-(2-hydroxy-3-bromopropyl) adenine. Because of the similar properties of the two isomers, the further elaborative separation was avoided and the mixture was used for the next step. The use of ethylene carbonate instead of epibromohydrin, yielded the highly selective addition and therefore led the single halogenated derivative (AdeEtBr). The reaction of halogenated intermediates with N-MePU proceeded almost quantitatively by sufficiently prolonged reaction.

Thus the five different polymers, Ura-HMDI, Ade-HMDI, PU-Ura, PU-AdeEBH, and PU-AdeEtBr were obtained.

2. Base-base interaction

It is a well known phenomenon that polynucleotides show hypochromism, which is the decrease in absorbance per chromophore in the polymer compared to that of the monomer. The origin of hypochromism has been explained in different ways. Tinoco and Rhodes[10,11] attributed hypochromism to dipole-dipole interaction between transition moments in neighboring oscillators. This has been termed an off-resonance interaction because the interacting dipoles originate from different transitions. When the bases are arranged in vertical stacking arrangement, that is, base planes are arranged parallel and one above another, this model predicts hypochromism. Bolton and Weiss[12] have attributed hypochromism to a local field effect, which originates from the induced electric dipole moments in neighboring oscillators. Since self-interaction of single transition of the monomer is considered, this has been termed a resonance interaction. This resonance effect can lead to hypochromism even in the absence of secondary structure or vertical stacking of the bases. Later, Thomas and Kyogoku[13] showed that the

dimeric hydrogen-bonded complexes between bases (complementary base pairing) lead to hypochromism, and therefore concluded that parallel stacking of the bases is not the only condition for hypochromism.

By consulting these studies, it seems safe to consider that the existence of hypochromism suggests base-base interactions which may be hydrogen bonding or vertical stacking interaction.

Fig. 3 shows example of hypochromic effect of PU-Ura mixed with DNA in Walpole buffer at room temperature. The absorbance at 260 nm, when plotted against the volume fraction of PU-Ura, show a negative deviation from Beer's low, reaching the relatively high value of 14% hypochromicity at volume fraction of 0.9. Table 1 summarizes the results of hypochromicity between the prepared polymers and DNA. Though the accurate molar relationships are not known because of uncertainty of the structure of DNA, it is concluded that the synthetic polymers in this study can interact with DNA,
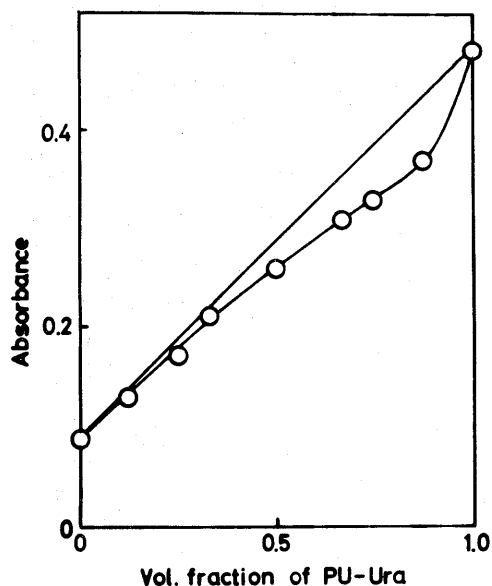

Fig. 3   Comparison of observed and Beer's law absorbances at 275 nm for the mixture of PU-Ura and DNA in Walpole buffer.

Table 1. Apparent hypochromicity of the mixture of the polymers containing nucleic acid bases and herring sperm DNA.

System	λmax nm	Hypochromicity %
PU-Ura/DNA	275	14
PU-AdeEBH/DNA	267	−5
PU-AdeEtBr/DNA	262	−39
PU-AdeEBH/PU-Ura	273	−17
PU-AdeEtBr/PU-Ura	275	−24

In Walpole buffer solution at room temperature.

which may be vertical stacking interactions.

Alternatively, interaction between bases can be studied by NMR spectra[14]. Table 2 shows the results of NMR spectra in $D_2O\text{-}D_2SO_4$ solution. The addition of D_2SO_4 was necessary for solubility problem. It is seen that the dilution shift and temperature shift of C_6-H proton of PU-Ura are trivial to draw any conclusion on base-base interactions. Also, the trivial downfield shift observed for the mixture of PU-Ura and PU-AdeEtBr does not give any evidence for the complex formation.

Table 2 Shifts in the resonant frequency of the C_6 proton of PU-Ura upon the addition of PU-AdeEtBr.

System	Conc. M/l	Temp. °C	$\delta(C_6-H)$ ppm	$\Delta\delta$ ppm
PU-Ura	0.05	50	8.55	
				+0.08
PU-Ura	0.05	75	8.47	
				−0.01
PU-Ura	0.10	75	8.48	
PU-Ura/ PU-AdeEtBr	0.05− 0.05	75	8.48	−0.01 (compared with 0.05 M sol)

In D_2O containing a trace of D_2SO_4 solvent.
Chemical shifts are given as δ values(ppm) relative to internal DSS.

In nonaqueous solvents (DMSO and DMF), the proton resonances are essentially independent of concentration. DMSO and DMF are hydrogen bonding acceptors and interact with bases with the result that solute molecules are interrupted to associate with each other. However, it has been observed that the use an equal volume mixture of benzene and DMSO is effective to detect a downfield hydrogen bonding shift of N-H proton in the base pairing interactions.[15] According to their observation an equal volume mixture of benzene and DMSO was used in this study. The results are shown in Table 3. As seen from the table, the resonance peak at around 7.3-7.7 ppm, which is assigned to N_3-H proton signal of pyrimidine ring of PU-Ura, showed a downfield shift (−0.1 ppm) upon mixing with PU-AdeEtBr. By elevating temperature from 25 to 50 to 75°C a marked upfield shifts are observed. Thus it is concluded that considerable self-association occurs between uracil and adenine moiety due to hydrogen bonding interaction.

Table 3. Chemical shifts of the hydrogen bonding protons of PU-Ura.

System	Temp. °C	$\delta(N_3-H)$ ppm	$\Delta\delta$ ppm
PU-Ura	25	7.59	
			−0.10
PU-Ura/PU-AdeEtBr	25	7.69	
			+0.19
PU-Ura/PU-AdeEtBr	50	7.50	
			+0.18
PU-Ura/PU-AdeEtBr	75	7.32	

In DMSO-d_6/benzene-d_6 (1:1 in vol.) mixed solvent. Concentration 0.05 mol/l. Chemical shifts are given as δ values (ppm) relative to internal TMS.

3. Anti-clotting property

It can be expected that the polymers in this study are of practical importance in the field of biomedical polymers, since nucleic acid base moieties are parts of biopolymers and naturally be expected blood compatible. Furthermore,

polyurethane backbone structure is known to be one of the most promising smooth, flexible, and blood and tissue compatible materials. Ammonium groups are known to impart pharmacological and bacteriocidal properties.

The clotting times of blood in contact with the prepared polymers measured by modified Lee-White method are shown in Table 4.

Table 4. Comparison of nonthrombogenecity of the polymers containing nucleic acid bases.

System	Total clotting time min
Control	10
Ura-HMDI	11
Ade-HMDI	12
PU-Ura	19
PU-AdeEtBr	20

PU-Ura and PU-AdeEtBr exhibited clotting time as long as twice that of the control. Though this clotting time is much less than that of Avcothane 51 (a block copolymer of polyurethane and silicone), this is comparable with those of silicone polymers. Further modification of the structure or heparinization will improve anti-clotting property.

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