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Syntheses of Cationic Mononucleotide Analogs and Their Interaction with Polynucleotide and Polynucleotide Analogs

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Cationic mononucleotide analogs were synthesized through a quaternization reaction of *N*-halogenoethylated derivatives of nucleic acid bases or 5′-halogeno-5′-deoxynucleoside. When they interacted with polynucleotide and polynucleotide analogs with negative charges, base-base interactions were observed. The interactions between complementary bases were stronger than the others. It is suggested that an anionic field originating from the polynucleotide or polynucleotide analog contributes to the interaction of complementary bases.

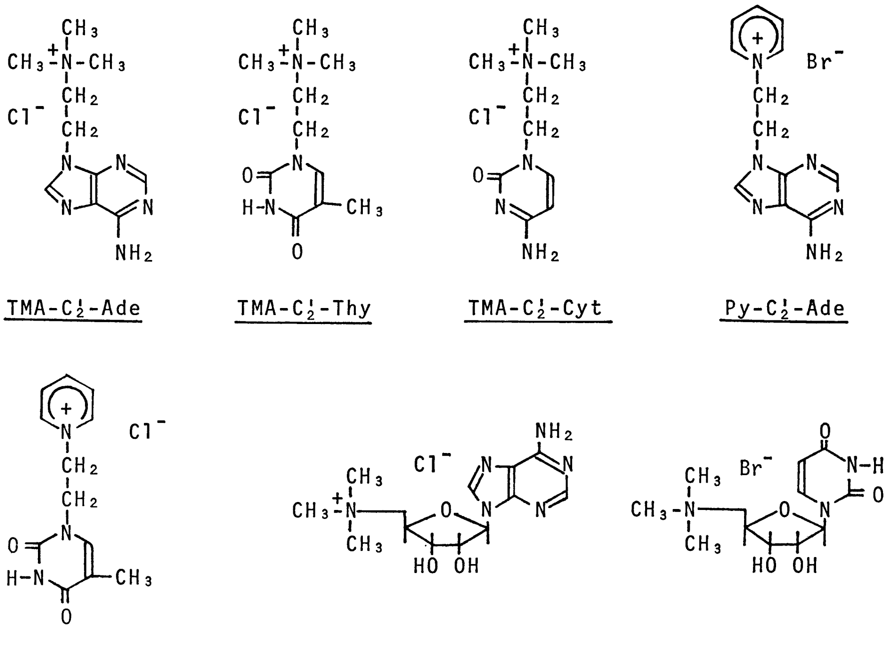
Several polynucleotide analogs with positive charges have been synthesized.[1,2] Their interactive properties with nucleic acid bases,[3] nucleotides,[2,4] polynucleotides,[3] and nucleotide analogs with negative charges[5,6] have also been investigated. In these studies, the mode of interactions was predominantly the base-base stacking. This evidence for the contribution of stacking interaction has also been found in the interaction of nucleotides or nucleotide analogs with negative charges in the cationic polyelectrolyte.[5] The mode of the interaction was also affected by the hydrophobic polymer domain. The cross-linked cationic polynucleotide analogs have a tendency to interact with the complementary nucleotide,[4] and so template-directed syntheses of oligonucleotides were carried out.[7]

In comparison with those phenomena, it is significant to elucidate the effect of the ionic field originating from the polynucleotide. For this purpose, some nucleotide analogs with the charge opposite to that of the nucleotide were synthesized. The present paper will give the details of the syntheses of the cationic mononucleotide analogs and the interactive properties with polynucleotides and their analogs with negative charges. Some consideration regarding the contribution of the ionic field to the base-base interaction will also be paid.

Results and Discussion

The cationic mononucleotide analogs shown in Fig. 1 were synthesized in the following manner. Nucleic acid bases were first converted to the corresponding *N*-halogenoethylated derivatives by the literature method.[8] 5′-Halogeno-5′-deoxynucleoside was synthesized by the literature procedure.[9] To *N*-halogenoethylated derivatives and 5′-halogeno-5′-deoxynucleosides, pyridine and trimethylamine were added; cationic mononucleotide analogs were then obtained through a quaternization reaction. In general, it is recommended that the reactions are carried out without any solvent. In the case of trimethylammonium derivatives, though, a good result was also yielded by using ethanol as a solvent. The precipitates during the reaction were almost purely the desired substances. These derivatives were hygroscopic.

The UV characteristics, R_f values, and electropho-



TMA-C_2'-Ade TMA-C_2'-Thy TMA-C_2'-Cyt Py-C_2'-Ade

Py-C_2'-Thy TMA-Ado TMA-Urd

Fig. 1. Cationic mononucleotide analogs.

Table 1. $\lambda_{\max}$, R_f values, and electrophoretical mobilities of cationic mononucleotide analogs

Analog	$\lambda_{\max}$ (H_2O)[a]	Paper chromatography[b] Solvent A	Paper chromatography[b] Solvent B	Paper electrophoresis[c] Relative mobility
TMA-C_2'-Ade	261	0.35	0.46	−2.0
TMA-C_2'-Thy	266	0.65	0.35	−2.0
TMA-C_2'-Cyt	272	0.57	0.55	−1.9
Py-C_2'-Ade	260	0.10	0.44	−2.5
Py-C_2'-Thy	266	0.28	0.66	−2.2
TMA-Ado	261	0.53		−2.4
TMA-Urd	262	0.24	0.62	−1.6

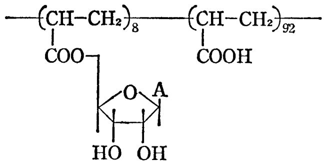
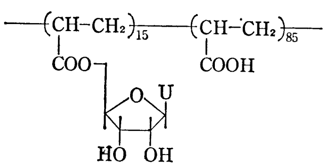
a) The UV measurements were carried out in water. The pH of the unbuffered solutions were 5.4—5.6. b) The solvent systems have been shown in the text. c) Electrophoresis was carried out in a 0.1 M phosphate buffer(pH 7.0). The relative mobility was based on the corresponding nucleotides.

Table 2. Apparent hypochromicities in the mixing curves of cationic mononucleotide analogs and polynucleotides[a]

Polynucleotide	Analog	Hypochromicity[b] %
Poly A	TMA-C_2'-Ade	2.8
	TMA-C_2'-Thy	4.5
	TMA-C_2'-Cyt	3.3
	Py-C_2'-Ade	3.3
	Py-C_2'-Thy	5.0
	TMA-Ado	4.9
	TMA-Urd	5.6
Poly U	TMA-C_2'-Ade	2.6
	TMA-C_2'-Thy	1.5
	TMA-C_2'-Cyt	0.0
	Py-C_2'-Ade	4.3
	Py-C_2'-Thy	3.0
	TMA-Ado	2.1
	TMA-Urd	2.5

The total concentration of nucleic acid bases in the mixture was 5.4×10^{-5} mol dm^{-3}.
a) Potassium salts of polynucleotides were used. b) The hypochromicities were calculated at 260 nm.

Table 3. Apparent hypochromicities in the mixing curves of cationic mononucleotide analogs and anionic polynucleotide analogs[a]

Anionic polynucleotide analog	Cationic mono-nucleotide analog	Hypochromicity[b] %
PAA-Ado$_8$ 	TMA-C_2'-Ade	1.0
	TMA-C_2'-Thy	5.8
	TMA-C_2'-Cyt	2.5
	Py-C_2'-Ade	0.0
	Py-C_2'-Thy	1.5
	TMA-Ado	1.0
	TMA-Urd	2.3
PAA-Urd$_{15}$ 	TMA-C_2'-Ade	2.5
	TMA-C_2'-Thy	1.0
	TMA-C_2'-Cyt	0.0
	Py-C_2'-Ade	1.6
	Py-C_2'-Thy	0.0
	TMA-Ado	2.4
	TMA-Urd	0.0

The total concentration of nucleic acid bases in the mixture was 5.4×10^{-5} mol dm^{-3}.
a) Anionic polynucleotide analogs were synthesized by the condensation reaction of poly(acrylic acid) with nucleoside. The content of adenine or uridine was determined by a UV measurement. b) The hypochromicities were calculated at 260 nm.

retical mobilities of cationic mononucleotide analogs are shown in Table 1. Their mobilities were almost twofold in the opposite direction, based on the corresponding nucleotides. This result indicates that they have a cationic charge even in neutral aqueous media.

The interactive properties of the present cationic mononucleotide analogs have also been investigated. Evidence for interaction between cationic mononucleotide analogs and polynucleotides came from the apparent hypochromicities in mixtures of them. When solutions of polynucleotides were mixed with solutions of cationic mononucleotide analogs, apparent hypochromicities down to a low percentage were observed. The results are shown in Table 2. Generally, a UV hypochromicity is not observed in a mixture of polynucleotide and nucleotide at the present concentration range. Such an interaction suggests a dominant contribution of Coulombic forces in the interaction between the cationic mononucleotide analog and the polynucleotide.

The observed hypochromicities of the complementary base's sets (Poly A-T derivative, Poly A-U derivative, and Poly U-A derivative) were larger than those of the incomplementary base's sets (Poly A-A derivative, Poly A-C derivative, and Poly U-C derivative, Poly U-T derivative, and Poly U-U derivative). This fact indicates that both a base stacking and a hydrogen bonding contribute to the mode of intermolecular base-base interaction.

The apparent hypochromicities in the mixing curves of the cationic mononucleotide analogs and the anionic polynucleotide analogs are shown in Table 3. A mode of interaction similar to that of polynucleotide was observed. This shows that hydrogen bonding between complementary bases takes place in the intermolecular interaction of the bases. As the contrary, previous studies of the interaction between nucleotides and cationic polynucleotide analogs showed that the stacking interaction was overwhelming.[2,4]

Now we can deduce that the anionic field originating from polynucleotides and polynucleotide analogs makes a large contribution to the base-base interaction; the anionic field contributes to the hydrogen bonding in the complementary base-base interaction.

Experimental

General Method. Paper chromatographies were carried out by the descending technique using Whatman 3 MM paper in the following solvent systems: A, 2-propanol–concentrated ammonia–water (7:1:2, v/v); B, ethanol–1 M ammonium acetate (pH 7.5) (7:3, v/v). Paper electrophoresis was carried out on the same paper at 35 V cm^{-1} in a 0.1 M phosphate buffer (pH 7.0). The UV absorption spectra were obtained on a Hitachi EPS-3T spectrophotometer. The NMR spectra were recorded with a Varian HR-220 spectrometer.

Reagents. The pyridine was distilled by a general method. A 30% trimethylamine–ethanol solution was obtained by dissolving a dry trimethylamine gas in ice-cooled ethanol.

Materials. The 9-(2-bromoethyl)adenine, 9-(2-chloroethyl)adenine, and 1-(2-chloroethyl)thymine were synthesized by the literature procedure.[8] The 1-(2-chloroethyl)cytosine was synthesized by the modification of the literature method:[8] 15 cm^3 of thionyl chloride was added to 1 g of 1-(2-hydroxyethyl)cytosine, and the mixture was stirred for 90 min at 35 °C and then was refluxed for 30 min. After the removal of the excess thiony chloride under reduced pressure, the residue was dissolved in water and treated with 5% of a sodium carbonate solution, and the precipitate thus formed was filtered off and dried. Recrystallization from ethanol gave 1-(2-chloroethyl)cytosine as pale yellow needles (48% yield). Found: C, 41.38; H, 4.69; N, 23.81; Cl, 19.63%. Calcd for $C_6H_8N_3OCl$: C, 41.51; H, 4.65; N, 24.20; Cl, 20.42%. The 5′-bromo-5′-deoxyuridine was synthesized by the literature method.[9] The 5′-chloro-5′-deoxyadenosine was kindly given by the Tanabe Pharmacy Company, Ltd.

2-(Adenin-9-yl)ethyltrimethylammonium Chloride, TMA-C$_2'$-Ade: In 25 cm^3 of a 30% trimethylamine–ethanol solution, 0.6 g of 9-(2-chloroethyl)adenine was dissolved. The mixture was sealed in a thick glass tube and then heated at 80 °C for 48 h. A white precipitate was filtered, washed with ethanol, then dried (63% yield). Found: C, 44.24; H, 6.86; N, 31.43; Cl, 13.48%. Calcd for $C_{10}H_{17}N_6Cl$: C, 46.78; H, 6.62; N, 32.74; Cl, 13.84%. NMR (D_2O) δ 8.45 (1H, s, 2 position of adenine), 8.43 (1H, s, 8 position of adenine), 4.93 (2H, t, 2 position of ethylene), 4.23 (2H, t, 1 position of ethylene), 3.23 (9H, s, trimethylammonium).

2-(Thymin-1-yl)ethyltrimethylammonium Chloride, TMA-C$_2'$-Thy: In 10 cm^3 of a 30% trimethylamine–ethanol solution, 0.5 g of 1-(2-chloroethyl)thymine was dissolved. The mixture was sealed in a thick glass tube and then heated at 80 °C for 70 h. A white precipitate was obtained after cooling. It was washed with ethanol and then dried (26% yield). Found: C, 43.26; H, 7.44; N, 15.29; Cl, 12.70%. Calcd for $C_{10}H_{18}N_3O_2Cl$: C, 47.74; H, 7.16, N, 16.70; Cl, 14.12%. NMR (D_2O) δ 7.92 (1H, s, 6 position of thymine), 4.74 (2H, t, 2 position of ethylene), 4.12 (2H, t, 1 position of ethylene), 3.69 (9H, s, trimethylammonium).

2-(Cytosin-1-yl)ethyltrimethylammonium Chloride, TMA-C$_2'$-Cyt: In 15 cm^3 of a 30% trimethylamine–ethanol solution, 0.2 g of 1-(2-chloroethyl)cytosine was dissolved. The mixture was sealed in a thick glass tube and then heated at 80 °C for 72 h. Pale yellow needles were filtered out, washed with ethanol, and then dried (52% yield). Found: C, 45.45; H, 7.37; N, 23.89; Cl, 14.96%. Calcd for $C_9H_{17}N_4OCl$: C, 46.45; H, 7.31; N, 24.09; Cl, 15.27%. NMR (D_2O) δ 7.98 (1H, d, 6 position of cytosine), 6.30 (1H, d, 5 position of cytosine), 4.49 (2H, t, 2 position of ethylene), 3.86 (2H, t, 1 position of ethylene), 3.41 (9H, s, trimethylammonium).

1-[2-(Adenin-9-yl)ethyl]pyridinium Bromide, Py-C$_2'$-Ade: In 2 cm^3 of a freshly distilled pyridine, 0.11 g of 9-(2-bromoethyl)adenine was dissolved. The mixture was sealed under nitrogen and then heated in boiling water for 78 h. A white precipitate was filtered out, washed with ethyl acetate, and then dried (28% yield). Found: C, 44.80; H, 3.97; N, 26.02; Br, 25.03%. Calcd for $C_{12}H_{13}N_6Br$: C, 44.87; H, 4.08; N, 26.17; Br, 24.88%. NMR (D_2O) δ 8.86 (2H, d, α proton of pyridinium), 8.80 (1H, dd, γ proton of pyridinium), 8.29 (1H, s, 8 position of adenine), 8.18 (2H, dd, β proton of pyridinium), 8.10 (1H, s, 2 position of adenine), 5.29 (2H, t, 2 position of ethylene), 5.02 (2H, t, 1 position of ethylene).

1-[2-(Thymin-1-yl)ethyl]pyridinium Chloride, Py-C$_2'$-Thy: In 5 cm^3 of a freshly distilled pyridine, 0.1 g of 1-(2-chloroethyl)thymine was dissolved. The mixture was then refluxed on a steam bath for 5 h. White needle crystals were obtained after cooling. They were washed with ethanol and then dried (17% yield). Found: C, 52.33; H, 5.34; N, 15.14; Cl, 13.15%. Calcd for $C_{12}H_{14}N_3O_2Cl$: C, 53.84; H, 5.27, N, 15.70; Cl, 13.24%. NMR (D_2O) δ 9.09 (2H, d, α proton of pyridinium), 8.82 (1H, dd, γ proton of pyridinium), 8.30 (2H, dd, β proton of pyridinium), 7.57 (1H, s, 6 position of thymine), 5.09 (2H, t, 2 position of ethylene), 4.05 (2H, t, 1 position of ethylene).

(Adenosin-5′-yl)trimethylammonium Chloride, TMA-Ado: In 15 cm^3 of a 30% trimethylamine–ethanol solution, 0.2 g of 5′-chloro-5′-deoxyadenosine was dissolved. The mixture was sealed in a thick glass tube and then heated at 55 °C for 70 h. A white precipitate was filtered, washed with ethanol, and then dried (38% yield). Found: C, 45.63; H, 6.35; N, 23.96; Cl, 10.43%. Calcd for $C_{13}H_{21}N_6O_3Cl$: C, 45.28; H, 6.10; N, 24.38; Cl, 10.30%. NMR (D_2O) δ 8.16 (1H, s, 8 position of adenine), 8.14 (1H, s, 2 position of adenine), 3.18 (9H, s, trimethylammonium).

(Uridin-5′-yl)trimethylammonium Bromide, TMA-Urd: In 20 cm^3 of a 30% trimethylamine–ethanol solution, 0.7 g of 5′-bromo-5′-deoxyuridine was dissolved. The mixture was sealed in a thick glass tube and then heated at 55 °C for 70 h. A white precipitate was filtered, washed with ethanol, and then dried (12% yield). Found: C, 38.74; H, 5.65; N, 10.96; Br, 22.36%. Calcd for $C_{12}H_{20}N_3O_5Br$: C, 39.36; H, 5.74; N, 11.48; Br, 21.84%. NMR (D_2O) δ 7.50 (1H, d, 6 position of uracil), 6.82 (1H, d, 5 position of uracil), 3.19 (9H, s, trimethylammonium).

UV Measurements. The measurements were carried out in water at 15 °C. The pH of the unbuffered solutions was 5.4—5.6. Absorbances were obtained in a 10-mm quartz cell. The apparent hypochromicity was calculated according to the following equation:[10]

$$\%\ \text{Hypochromicity} = \left(1 - \frac{I_{a+b}}{mI_a + nI_b}\right) \times 100$$

where m and n are the volume fractions of the solutions of Compounds a and b, and where I_a, I_b, and I_{a+b} are the absorbances of the solutions of Compound a, Compound b, and the mixture of Compounds a and b respectively.

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References

1) T. Okubo and N. Ise, *Polym. J.*, **4**, 674 (1974).

2) T. Shimidzu, Y. Konishi, and A. Murakami, *Makromol. Chem.*, **178**, 2581 (1977).

3) T. Okubo, K. Ban, and N. Ise, *Makromol. Chem.*, **175**, 49 (1974).

4) T. Shimidzu, A. Murakami, Y. Konishi, and M. Minami, *Bull. Chem. Soc. Jpn.*, **51**, 821 (1978).

5) T. Shimidzu, Y. Konishi, and A. Murakami, *Nucleic Acids Res.*, Sp. **2**, 35 (1976).

6) T. Shimidzu, A. Murakami, and K. Yamana, *Nucleic Acids Res.*, Sp. **5**, 379 (1978).

7) T. Shimidzu, A. Murakami, and Y. Konishi, *J. Chem. Soc., Perkin Trans. 1*, **1979**, 20.

8) N. Ueda, K. Kondo, M. Kono, K. Takemoto, and M. Imoto, *Makromol. Chem.*, **120**, 13 (1968).

9) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **106**, 113 (1934).

10) M. N. Lipsett, L. A. Happel, and D. F. Bradley, *J. Biol. Chem.*, **236**, 857 (1961).