The Template-directed Synthesis of Oligonucleotide. II.[1] The Interactive Properties of Nucleic Acid Base-binding Poly(4-vinylpyridine) and Its Related Compound with Nucleotides

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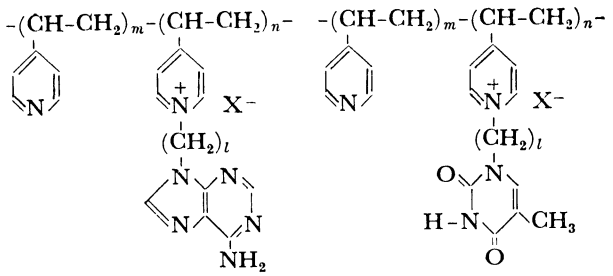
(Received June 11, 1977)

The interactive properties of positively charged polynucleotide analogs, such as nucleic acid base-binding poly(4-vinylpyridine)s and their derivatives, with nucleotides have been investigated. The interactions of the positively charged polynucleotide analogs such as *N*-alkylated poly(4-vinylpyridine)s with 9-(ω-chloroalkyl)adenine and of *N*-alkylated poly(4-vinylpyridine) with 1-(2-bromoethyl)thymine with nucleotides were attributed predominantly to a base-base stacking under reinforcement by the coulombic attractive force. The purine-purine base interaction was found to be stronger than the purine-pyrimidine and the pyrimidine-pyrimidine interactions. The interaction was reduced by an increase in the ionic strength. The higher the charge density of the nucleotide was, the stronger was the interaction observed. On the other hand, a cross-linked positively charged polynucleotide analog such as *N*-alkylated cross-linked poly(4-vinylpyridine) with 9-(2-chloroethyl)adenine showed considerable selective interaction with the complementary nucleotide, uridine-5′-phosphate (pU), in both aqueous and pyridine solutions.

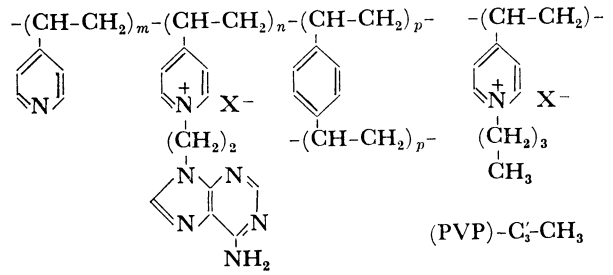
Various kinds of polynucleotide analogs have been prepared by the literature procedures.[2-12] Template-directed syntheses of oligonucleotide have also been attempted using neutral polynucleotide analogs such as poly(1-vinyluracil)[13] and oligonucleotide whose phosphoric acid moiety was blocked.[1] In general, interactions between the condensing nucleotide and the polynucleotide analogs are not very strong except for the interaction between the nucleotide and the polynucleotide analog with a positive charge. The polynucleotide analog with a positive charge is noteworthy for its coulombic interactive force with the condensing nucleotide. One of the polynucleotide analogs with a positive charge has been materialized by Okubo and Ise with poly(4-vinylpyridine) *N*-alkylated with a halogenoalkylated nucleic acid base;[14,15] they have also reported a noteworthy study of the interactions with polynucleotide and a nucleic acid base. The contribution of the coulombic force to the interaction is remarkable. However, the base-base stacking takes place predominantly in the interaction.

On the template-directed condensation of nucleotide, the most essential requirement for the template is a selective interaction between the template molecule and the condensing nucleotide. In this paper, we will describe our studies of the interactive properties of nucleotides with nucleic acid base binding poly(4-vinylpyridine)s and their derivatives.



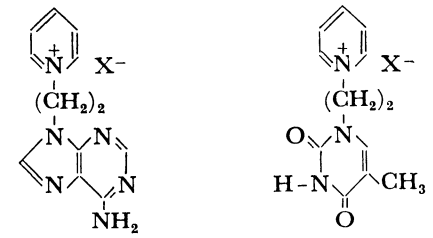
$l=2,3,4,6$
(PVP)-C$_l$′-Ade$_n$

$l=2$
(PVP)-C$_l$′-Thy$_n$



$p=2$
$n=61$
[PVP]$_p$-C$_2$′-Ade$_n$

(PVP)-C$_3$′-CH$_3$



Py-C$_2$′-Ade

Py-C$_2$′-Thy

Results and Discussion

Nucleic acid base-binding poly(4-vinylpyridine)s were synthesized by the alkylation of poly(4-vinylpyridine) with the halogenoalkyl derivatives of nucleic acid bases. The nucleic acid base-binding poly(4-vinylpyridine)s and their related compounds adopted in the present paper are shown on the right.

The polymers, nucleic acid base-binding poly(4-vinylpyridine)s (PVP)–C$_l$′–Ade$_n$ and (PVP)–C$_l$′–Thy$_n$ and their related compounds, were soluble in water and interacted with nucleotides. The solution of the nucleic acid base-binding poly(4-vinylpyridine) followed Beer's law in the range from 10^{-5} mol/dm^3 to 8×10^{-4} mol/dm^3. No conformational change which affects an intramolecular base-base interaction occurred in the present concentration range.

For example, the mixing curve of (PVP)–C$_2$′–Ade$_{48}$ and adenosine-5′-phosphate, pA, is shown in Fig. 1. The hypochromicity was calculated at the wavelength where the differences in the UV absorbances of the solu-

Table 1. Maximum hypochromicities in the mixing curves of (PVP)-C_l'-Ade_n, (PVP)-C_l'-Thy_n and nucleoside

Analog	Nucleoside	Hypochromicity %	Ratio[a]	Wavelength[b] nm	Concentration[c] mol/dm³
(PVP)-C_2'-Ade_{48}	Ado	1.7±0.2	1 : 2.1	260	4.8×10^{-4}
	Guo	1.0±0.1	1 : 5.5	255	4.8×10^{-4}
	Urd	1.8±0.2	1 : 0.5	261	4.8×10^{-4}
(PVP)-C_2'-Ade_{52}	Ado	1.6±0.2	1 : 2.0	260	5.0×10^{-4}
	Guo	1.0±0.2	1 : 3.0	255	5.0×10^{-4}
	Thy	2.2±0.3	1 : 0.7	263	5.0×10^{-4}
(PVP)-C_2'-Thy_{100}	Ado	5.7±0.3	1 : 0.7	260	6.7×10^{-4}
	Guo	6.2±0.3	1 : 0.4	258	6.7×10^{-4}
	Cyd	1.9±0.2	1 : 2.9	268	6.7×10^{-4}
	Urd	2.1±0.2	1 : 0.7	263	6.7×10^{-4}

a) The ratio of the polynucleotide analog and nucleoside where the maximum hypochromicity was given. b) The wavelength where the hypochromicity was calculated. c) Concentration shows the total concentration of nucleic acid bases in the mixture.

Table 2. Maximum hypochromicities in the mixing curves of (PVP)-C_l'-Ade_n, (PVP)-C_l'-Thy_n and nucleotide[a]

Analog	Nucleotide	Hypochromicity %	Ratio[b]	Wavelength[c] nm	Concentration[d] mol/dm³
(PVP)-C_2'-Ade_{48}	pA	11.8±0.2	1 : 0.6	260	4.8×10^{-4}
	pG	7.5±0.2	1 : 0.5	255	4.8 ± 10^{-4}
	pC	6.7±0.2	1 : 2.2	265	4.8 ± 10^{-4}
	pU	5.4±0.2	1 : 2.5	260	4.8 ± 10^{-4}
	ATP	ppt.	—	—	4.8 ± 10^{-4}
(PVP)-C_2'-Ade_{52}	pA	10.9±0.3	1 : 0.8	260	5.7×10^{-4}
	pG	7.2±0.3	1 : 1.5	255	5.7×10^{-4}
	pC	7.0±0.3	1 : 0.9	265	5.7×10^{-4}
	pU	5.2±0.3	1 : 0.5	260	5.7×10^{-4}
(PVP)-C_3'-Ade_{56}	pA	9.8±0.3	1 : 1.1	260	5.7×10^{-4}
	pC	6.9±0.3	1 : 0.8	265	5.7×10^{-4}
	pU	6.0±0.3	1 : 0.6	260	5.7×10^{-4}
(PVP)-C_4'-Ade_{39}	pA	9.7±0.3	1 : 1.3	260	5.7×10^{-4}
	pC	7.0±0.3	1 : 0.6	265	5.7×10^{-4}
	pU	6.1±0.3	1 : 0.4	260	5.7×10^{-4}
(PVP)-C_6'-Ade_{47}	pA	10.0±0.3	1 : 1.1	260	5.7×10^{-4}
	pC	7.0±0.3	1 : 0.8	265	5.7×10^{-4}
	pU	5.9±0.3	1 : 0.4	260	5.7×10^{-4}
(PVP)-C_2'-Thy_{100}	pA	10.9±0.4	1 : 0.8	263	6.7×10^{-5}
	pG	9.0±0.4	1 : 4.0	259	6.7×10^{-5}
	pC	4.3±0.4	1 : 1.0	269	6.7×10^{-5}
	pU	5.3±0.4	1 : 0.4	264	6.7×10^{-5}
	ATP	ppt.	—	—	6.7×10^{-5}

a) Disodium salts of nucleotides were used. b) This shows the ratio of the polynucleotide analog and nucleotide where the maximum hypochromicity was given. c) This shows the wavelength where the hypochromicity was calculated. d) Concentration shows the total concentration of nucleic acid bases in the mixture.

tion mixtures were most extended. However, the magnitude of the hypochromicity was effectively the same as that observed at 260 nm. The hypochromicity is seen as resulting from interaction between the nucleic acid bases of two molecules. In the present paper, the maximum hypochromicities are listed in the Tables. The maximum hypochromicities were not given at a 1 : 1 molar ratio of those two molecules.

Tables 1 and 2 show the maximum hypochromicities in the mixing curves of (PVP)–C_l'–Ade_n and nucleoside, (PVP)–C_l'–Thy_n and nucleoside, (PVP)–C_l'–Ade_n and nucleotide, and (PVP)–C_l'–Thy_n and nucleotide. Polynucleotide analogs, such as (PVP)–C_l'–Ade_n (l=2, 3, 4, 6) and (PVP)–C_l'–Thy_n (l=2), had a stronger interaction with a purine base than with a pyrimidine base. This tendency is similar to the result which was obtained by Okubo *et al.*[15] in the cases of the interactions of the nucleic acid base-binding poly(4-vinylpyridine) both with polynucleotide and with a nucleic acid base. It was also observed that the interaction of the poly-

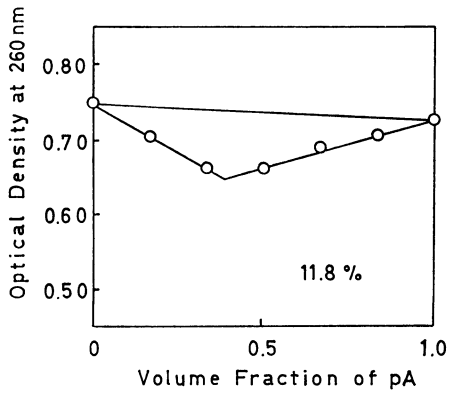


Fig. 1. Apparent hypochromicity in the mixing curve of (PVP)-C_2'-Ade_{48}-pA mixture.
Concentration, 4.8×10^{-4} mol/dm^3; temp, 15 °C.

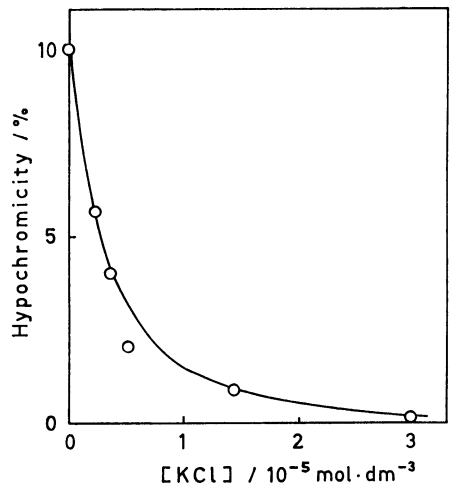


Fig. 2. Influence of salt on the apparent hypochromicity of (PVP)-C_2'-Thy_{100}-pA mixture.
Concentration, 6.7×10^{-5} mol/dm^3; temp, 15 °C; (PVP)-C_2'-Thy_{100}: pA=1 : 1.

nucleotide analog with nucleotide was stronger than that with nucleoside. On the interaction of the polynucleotide analog with adenosine triphosphate, ATP, a precipitation took place. Generally, a UV hypochromicity is not observed in mixtures of polynucleotide and nucleotide in the present concentration range. Such a stronger interaction suggests a dominant contribution of a coulombic force in the interaction between a polynucleotide analog with a positive charge and a nucleotide with a negative charge. Even in the case of the interaction of the polynucleotide analog with nucleoside, no repulsive coulombic force takes place. In neither cases was the quantitative ratio of the bases in the interaction, which was shown as the ratio at the maximum hypochromicity being given, always unity. This fact shows that the hydrogen-bond formation between complementary bases is not main force participating in the intermolecular interactions of the bases; rather, it suggests that a hydrophobic interaction is overwhelming. The influence of the side-chain length of the polynucleotide analog, (PVP)-C_l'-Ade_n, in the interaction between (PVP)-C_l'-Ade_n and nucleotide was not obvious where the length, l was below 6.

Table 3. pH dependence of apparent hypochromicities in the mixing curve of (PVP)-C_2'-Ade_{48} and pA[a)]

pH	4.0	5.0	5.5
Hypochromicity/%	1.8	2.4	11.8

(PVP)-C_2'-Ade_{48}: pA=1 : 0.6; Wavelength, 260 nm; concn, 4.8×10^{-4} mol/dm^3.
a) Disodium salt of pA was used.

Table 4. Apparent hypochromicities in the mixing curves of (PVP)-C_3'-CH_3 and nucleotide[a)]

Analog	pA	pU	ATP
(PVP)-C_3'-CH_3	1.5	0.6	15.8

Concn, 6.7×10^{-4} mol/dm^3.
a) Disodium salts of nucleotides were used.

Table 3 shows the pH dependence of the interaction of (PVP)-C_2'-Ade_{48} with adenosine-5′-phosphate, pA. A decrease of the pH of the solution made the maximum hypochromicity decrease. This result can be explained as follows; the lower pH makes the dissociation of the phosphate group in the nucleotide, pA, decrease and the amino group in the adenine moiety protonate, followed by a repulsion between the positively charged amino group and the positively charged (PVP)-C_2'-Ade_{48} upon the interaction.

In general, the addition of salt to the solution reduces the coulombic interaction of charged molecules. Figure 2 shows the influence of KCl added to the solution of the maximum hypochromicity of the (PVP)-C_2'-Thy_{100}-pA mixture. An increase in the ionic strength made the hypochromicity decrease. This shows that the interaction between the polynucleotide analog and nucleotide is governed by the coulombic attractive force.

Table 4 shows the maximum hypochromicity in the mixing curve of (PVP)-C_3'-CH_3 and nucleotide. In the case of (PVP)-C_3'-CH_3-monoculeotide, the magnitude of the hypochromicity was low. This result indicates that the interaction among the nucleotides which are concentrated in the vicinity of the positively charged polynucleotide analog can scarecely be evaluated. The result also shows that it is not necessary to take the interaction between the pyridinium moiety and the nucleotide into account in considering the hypochromicity. On the other hand, the certain magnitude of the hypochromicity for (PVP)-C_3'-CH_3 and ATP suggests that there exists a stacking among the ATPs concentrated at the cationic (PVP)-C_3'-CH_3. The distinction between the nucleotides and the ATP may be due to the charge density following its concentration.

The monomeric analogs of such polynucleotide analogs as Py-C_2'-Ade and Py-C_2'-Thy all have a single cationic charge. Table 5 shows the muximum hypochromicity in the mixing curve of the monomeric analogs and the nucleotides. The hypochromicity was not observed in the single-single charge system, although the two species were oppositely charged. On the other hand, a definite interaction between the monomeric

Table 5. Apparent hypochromicities in the mixing curves of Py-C_2'-Ade, Py-C_2'-Thy and nucleotide[a)]

Mono. analog	pA	pU	ATP
Py-C_2'-Ade	0	0	1.0[b)]
Py-C_2'-Thy	0	0	1.0[b)]

Concn, 6.7×10^{-4} mol/dm^3.
a) Disodium salts of nucleotides were used. b) [Py-C_2'-Ade]/[ATP] and [Py-C_2'-Thy]/[ATP] were 4.2 and 5.2, respectively.

Table 6. Selective adsorption of nucleotide on [PVP]$_2$-C_2'-Ade$_{61}$ in aqueous solution

Nucleotide mmol	H_2O cm^3	Adsorbed nucleotide mmol
pA (0.50)	1.0	pA (0.19)
pU (0.50)	1.0	pU (0.17)
pA (0.50), pU (0.50)	1.0	pA (0.07), pU (0.12)
pA (0.50), pU (0.50), pC (0.50)	2.0	pA (0.03), pU (0.12), pC (0.05)

[PVP]$_2$-C_2'-Ade$_{61}$, 2.5×10^{-4} mol equiv; Column, 2 mm id; Eluent (H_2O), 20 cm^3.

Table 7. Selective adsorption of nucleotide on [PVP]$_2$-C_2'-Ade$_{61}$ in pyridine

Nucleotide mmol	Pyridine cm^3	Adsorbed nucleotide mmol
pA (0.50)	1.0	pA (0.20)
pU (0.50)	1.0	pU (0.20)
pA (0.50), pU (0.50)	1.0	pA (0.08), pU (0.16)
pA (0.50), pU (0.50), pC (0.50)	1.0	pA (0.04), pU (0.15), pC (0.05)

Eluent (pyridine), 20 cm^3: The other conditions are the same as Table 6.

analogs and ATP, which has a multi-charge, was observed. This result suggests that an effective interaction requires the charge of one interactive species to be more than unity, at least.

The nucleic acid base-binding cross-linked poly(4-vinylpyridine) such as [PVP]$_2$-C_2'-Ade$_{61}$ was synthesized by the reaction of 9-(2-chloroethyl)adenine and poly(4-vinylpyridine), which had been cross-linked by divinylbenzene. Tables 6 and 7 show the adsorptions of nucleotides on [PVP]$_2$-C_2'-Ade$_{61}$ in water and in pyridine, respectively. Compared with the above-described water-soluble polynucleotide analogs such as (PVP)-C_i'-Ade$_n$ and (PVP)-C_i'-Thy$_n$, the [PVP]$_2$-C_2'-Ade$_{61}$ had a considerable tendency to interact with the complementary nucleotide. In both solvent systems, the total amount of the adsorbed nucleotides on the [PVP]$_2$-C_i'-Ade$_{61}$ was constant, independent of the kind of nucleotide. That is, the total amount of the nucleotide adsorbed depended on the total number of charges in the [PVP]$_2$-C_2'-Ade$_{61}$. The selectivity in the adsorption as the complementary interaction rose and appeared to be marginal when more than one nucleotides were used. The order of the addition of the nucleotide did not affect the selectivity in the adsorption. This means that the adsorption is reversible. The occurrence of the considerable complementary base-base interaction in this case is important in connection with the template molecule in the template-directed synthesis of oligonucleotide. The formation of the cross-linked structure is preferable to the complementary base-base interaction, though it gives a heterogeneous reaction system. It is though that a matrix which is formed by the cross-linked structure is advantageous for the stability of the hydrogen-bond formation. The adsorbed nucleotide can be eluted with a concentrated salt solution. These properties suggest that the nucleic acid base-binding cross-linked poly(4-vinylpyridine) is useful for the template-directed synthesis of oligonucleotide and for affinity chromatography.

Experimental

UV Measurements. Standardized solutions of the polynucleotide analogs, nucleotides, their analogs, and nucleosides were mixed in varying proportions. The solutions were not buffered. The mixtures were stored for periods of up to 6 h at 5 °C, until no further optical density change occurred. UV measurements were then carried out on Hitachi EPS-3T spectrophotometer at 15 °C. The hypochromicity was calculated according to the following equation:[18)]

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

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

Alkylating Reagents. 9-(2-Chloroethyl)adenine was synthesized by a literature procedure.[16)]

All the 9-(ω-chloroalkyl)adenines except 9-(2-chloroethyl)-adenine were synthesized by a literature procedure.[17)]

1-(2-Bromoethyl)thymine was synthesized by a modification of the procedure of Ueda *et al.*[16)] A mixture of 31.6 g (0.19 mol) of 1-(2-hydroxyethyl)thymine and 126 g (0.61 mol) of thionyl bromide, plus a few drops of pyridine, was heated under reflux for 45 min. Then the excess thionyl bromide was removed under reduced pressure, and the remainder was washed with water. The residual solid was extracted with chloroform in a Soxleht extractor. A white, powdery 1-(2-bromoethyl)thymine was obtained from the chloroform solution (18.1 g, 41 %); IR(KBr): 3000, 2330, 1710, 1663, 1360, 1280, 900, 515 cm^{-1}; R_f (2-propanol : concn NH_4OH : H_2O = 7 : 1 : 2, Whatman 3MM) : 0.72; Found: C, 35.57; H, 3.79; N, 11.94; Br, 32.13%. Calcd for $C_7H_9N_2O_2Br$: C, 36.07; H, 3.89; N, 12.02; Br, 34.29%.

Polynucleotide Analogs, (PVP)-C_i'-Ade$_n$ and (PVP)-C_i'-Thy$_n$. The alkylations of poly(4-vinylpyridine) were carried out using the corresponding ω-halogenoalkylated nucleic acid bases mentioned above, according to a literature procedure.[14)] The degree of alkylation was estimated by means of elementary analysis. The results are tabulated in Table 8.

Cross-linked Polynucleotide Analog, [PVP]$_2$-C_2'-Ade$_{61}$. Freshly distilled 4-vinylpyridine and 1.5 mol% of divinylbenzene (55% solution of ethylbenzene) were placed in a flask with a few seeds of cross-linked polystyrene. After the reaction mixture has then been kept at 60 °C for 1 day, a faint coral-reddish, porous popcorn polymer was obtained. The polymer was ground and thoroughly washed with ethanol. The degree of the cross-linkage was calculated to be 2 % by means of the C/N ratio in the elementary analysis (Found: C, 79.81; H, 7.27; N, 12.92%).

Two grams of the resulting polymer, 4.0 g of 9-(2-chloro-

Table 8. Results of the alkylation of poly(4-vinylpyridine) with halogenoalkylated nucleic acid bases

Alkylating reagent	g	DMF cm³	Time h	Degree of alkylation %	λ_{max} nm
9-(2-Chloroethyl)adenine	1.0	1.5	73	48	261
9-(2-Chloroethyl)adenine	1.5	2.0	72	52	261
1-(2-Bromoethyl)thymine	11.8	10.8	72	100	267
9-(3-Chloropropyl)adenine	1.7	2.0	72	56	261
9-(4-Chlorobutyl)adenine	2.0	2.5	70	39	261
9-(6-Chlorohexyl)adenine	2.0	2.5	85	47	261

Poly(4-vinylpyridine) 0.5 g was used. React. temp, 95—100 °C. Products were washed with alcohol and ether, until no ultraviolet absorption was observed in the washings.

ethyl)adenine, and 7 cm³ of DMF were placed in a sealed bottle, and the mixture was heated at 80 °C with stirring for a week. The resulting material was thoroughly washed with hot ethanol and water. The degree of alkylation was calculated to be 63 % (*cf.* the pyridine moiety in the polymer) by the change in the C/N ratio in the elementary analysis from that of the starting polymer (Found: C, 60.50; H, 5.62; N, 24.59, Cl, 9.29%).

Poly(1-butyl-4-vinylpyridinium bromide), (PVP)–C_3'–CH_3.

Twenty-six cm³ of 4-vinylpyridine and 32 cm³ of butyl bromide were dissolved in 100 cm³ of benzene, and the mixture was heated at 50 °C with stirring for 115 h. A reddish violet solution resulted. The benzene was then removed, and the residue was dissolved in water, followed by dialysis. The resulting solution was lyophilized. A hygroscopic, faint yellow, powdery product was thus obtained (11.2 g).

1-[2-(Adenin-9-yl)ethyl]pyridinium Chloride, Py–C_2'–Ade.

In 2 cm³ of a freshly distilled pyridine, 0.11 g of 9-(2-chloroethyl)adenine was dissolved. The mixture was sealed under nitrogen and then heated in boiling water for 68 h. A white precipitate was filtered, washed with ethyl acetate, and then dried (46 mg); mp 290—291 °C, $\lambda_{max}^{H_2O}$, 260 nm; $\varepsilon_{max}^{H_2O}$, 1.63×10^4; Found: C, 52.16; H, 4.90; N, 30.30; Cl, 12.69%. Calcd for $C_{12}H_{13}N_6Cl$: C,52.08; H,4.74; N,30.37; Cl,12.81%.

1-[2-(Thymin-1-yl)ethyl]pyridinium Chloride, Py–C_2'–Thy.

In 5 cm³ of freshly distilled pyridine, 1.0 g of 1-(2-chloroethyl)thymine was dissolved. The mixture was then refluxed on a steam bath for 5 h. A white needle crystal was obtained after cooling. It was washed with ethanol and then dried (230 mg): $\lambda_{max}^{H_2O}$, 266 nm; $\varepsilon_{max}^{H_2O}$, 1.3×10^4; 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%.

Adosorption. One half mmol of nucleotide was dissolved in a certain amount of water and then placed on 100 mg (2.5×10^{-4} mol equiv) of the [PVP]$_2$–C_2'–Ade_{61}. The adsorption of the nucleotide on the [PVP]$_2$–C_2'–Ade_{61} was completed within 1 h, with stirring. The whole mixture was then placed in a column whose inner diameter was 2 mm. Then the column was washed by the addition, drop by drop, of 20 cm³ of water for a 10 min period. The content of the nucleotide in the effluent was analyzed by paper chromatography and by liquid chromatography (Shimadzu-Dupont 830, Permaphase AAX). From the composition of the nucleotides in the effluents, the amounts of nucleotides adsorbed on the [PVP]$_2$–C_2'–Ade_{61} were calculated. For the experiment in the pyridine-solution system, the pyridinium form of the nucleotides was used, and pyridine was used as the effluent in lieu of water. Before analysis, the pyridine was evaporated; the residue was dissolved in water and then analyzed.

The authors are indebted to Professor K. Fukui for his encouragement and to Mr. H. Azuma and Mr. Y. Yonehara for their technical assistances. The present work was supported by a Grant-in-Aid from the Ministry of Education.

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