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Polynucleotides. XXX.[1] Synthesis and Properties of Oligonucleotides of Cyclouridine Phosphate. Hybridization with the Oligomer of *S*-Cycloadenosine Phosphate to Form Left-Handed Helical Complexes[2]

Seiichi Uesugi, Toru Tezuka, and Morio Ikehara*

Contribution from the Faculty of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan. *Received May 9, 1975*

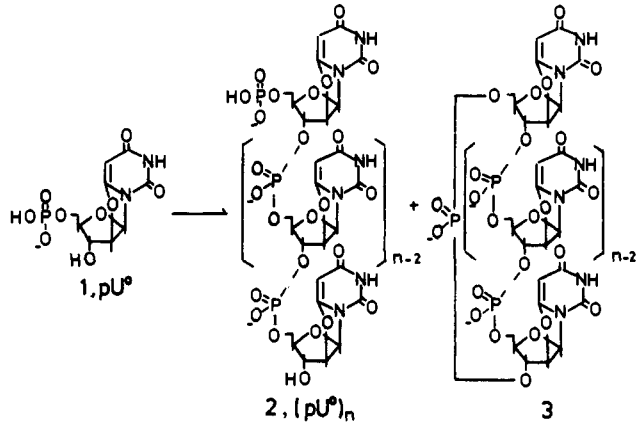
Abstract: Oligonucleotides containing 6,2′-anhydro-1-(β-D-arabinofuranosyl)-6-hydroxyuracil (U°) were synthesized by chemical polymerization of its 5′-phosphate with dicyclohexylcarbodiimide in pyridine. After separation by chromatography on a DEAE-cellulose column and purification by paper chromatography, a series of linear oligonucleotides and some cyclic oligonucleotides were obtained. Chain lengths of the linear oligomers were determined by phosphorus analyses of the oligomers and their corresponding dephosphorylated products. The linear oligomers of chain length up to 10, cyclic dinucleotide, and cyclic trinucleotide were characterized by paper chromatography and paper electrophoresis. Uv absorption and CD spectra for these oligomers are reported. From these data, it was concluded that these oligomers do not adopt stacked helical conformation in aqueous solution. Although the linear octamer did not form a complex with poly(A), it did hybridize with the octamer of 8,2′-*S*-cycloadenosine phosphate, which is assumed to have a left-handed helical conformation, forming a double- or a triple-stranded complex depending on the conditions. On the basis of their CD spectra, a left-handed multistranded helical structure was proposed for these complexes.

Introduction

As part of our continuing study on the effect of the torsion angle about the glycosidic linkage on the conformation of polynucleotides, various oligomers of cycloadenosine phosphates were synthesized and their properties examined.[2–6] The dinucleoside monophosphate (A^spA^s) of 8,2′-*S*-cycloadenosine was fully examined by uv absorption, CD and NMR spectroscopy and proved to have a highly stacked conformation with a left-handed screw axis.[3,4] The oligonucleotides, $(pA^s)_n$,[5,6] and the dinucleotide monophosphate (A^opA^o)[6,7] of 8,2′-*O*-cycloadenosine gave similar CD spectra to that of A^spA^s, suggesting that they have the same kind of left-handed helical conformation. None of these compounds hybridized with poly(U), which is assumed to form a right-handed helix, but $(pA^s)_8$ formed a complex with poly(formycin phosphate) in which the torsion angle about the glycosidic linkage is assumed to be easily rotatable.[8] It may be concluded that oligomers of cycloadenosine phosphate, in which the torsion angle, χ,[9] is fixed at around 120°, can form hydrogen-bonded complexes with oligo- and polynucleotides which can have similar torsion angles. In this respect, we were interested in the hybridization of $(pA^s)_n$ with oligomers of cyclouridine phosphate which is the complementary pyrimidine counterpart of cycloadenosine phosphate. The synthesis and properties of the dinucleoside monophosphate, U^opU^o, have been reported.[11] In this paper we report the synthesis and properties of the oligonucleotides of 6,2′-*O*-cyclouridine (U°), which is assumed to have nearly the same torsion angle as 8,2′-cycloadenosines.[12] From uv absorption and CD studies, these oligomers, $(pU^o)_n$, have almost no stacked or helical conformation in aqueous solution. The octamer, $(pU^o)_8$, did not form a complex with poly(A), but did hybridize with $(pA^s)_8$. The resulting complexes are assumed to have left-handed multistranded helical structures.

Synthesis of Oligonucleotides

Oligonucleotides (**2** and **3**) were synthesized by chemical



polymerization of pU° (**1**). As the pyridinium salt pU° was not soluble in pyridine, the polymerization reaction was carried out with dicyclohexylcarbodiimide in DMF in the presence of pyridinium Dowex 50 resin. The reaction mixture containing pU° (0.7 mmol) was kept at 30° for 2 weeks. After the reaction was stopped by addition of 50% aqueous pyridine, acetic anhydride–pyridine treatment was carried out to cleave any pyrophosphate linkages. After deacetylation with 9 N ammonium hydroxide, the products were sep-

Table I. Paper Chromatography, Electrophoresis,[a] and Identification of Polymerization Products

Peak no.[b]	Compd	Paper chromatography solvent A[c]	Paper chromatography solvent B[c]	Paper chromatography solvent B[d]	Paper chromatography solvent C[e]	TLC solvent E[c]	Paper electrophoresis[f]	Yield[g]
I	pU^o	1.00	1.04		0.19	1.00	1.05	6.0
III	$(pU^o)_2$	0.41	0.80		0.05	0.64	1.04	4.6
V	$(pU^o)_3$	0.17	0.59		0.02	0.40	1.02	5.4
VI	$(pU^o)_4$	0.14	0.32	1.00			1.01	6.5
VII	$(pU^o)_5$	0.07	0.18	0.65			1.03	6.2
VIII	$(pU^o)_6$	0.05	0.11	0.31			1.03	4.0
IX	$(pU^o)_7$	0.02	0.04	0.21			1.03	3.2
X	$(pU^o)_8$			0.16			0.96	2.8
XI	$(pU^o)_9$			0.09			0.99	2.0
XII	$(pU^o)_{10}$			0.04			0.92	1.3
II	Cyclic dinucleotide	0.69	0.94		0.07	0.45	0.86	7.3
IV	Cyclic trinucleotide	0.36	0.73		0.04	0.33	0.96	5.0

[a] Composition of solvent and buffer systems are given in the Experimental Section. [b] Peak numbers are shown in Figure 1. [c] Relative R_f to pU is given. [d] Relative R_f to $(pU^o)_4$ is given. [e] R_f is given. [f] Relative mobility to pU is given. [g] Overall yield from 0.7 mmol of pU^o. Total recovery of absorbance units was about 80%.

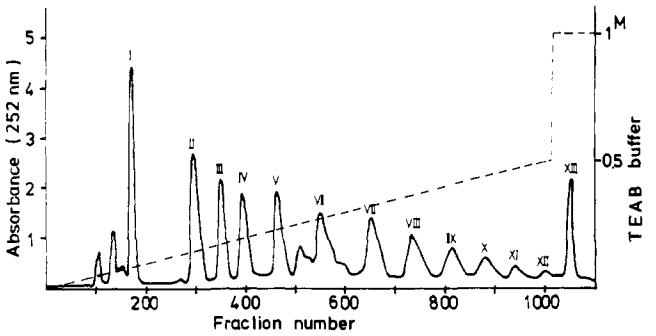


Figure 1. Chromatography of the products from the polymerization of pU^o on a column (3.5 × 4.0 cm) of DEAE-cellulose (bicarbonate). Elution was carried out with a linear gradient of triethylammonium bicarbonate (pH 7.5, 0–0.50 M, total 16 l.). Fractions of 16 ml were collected at 10-min intervals.

arated by chromatography on a DEAE-cellulose column. Elution was carried out with a linear gradient of triethylammonium bicarbonate (TEAB) buffer. The chromatogram is shown in Figure 1. Yields of oligonucleotides in the peaks and the results of identifications are shown in Table I. The pooled fractions from each peak were desalted by coevaporation with water. The oligonucleotides were further purified by paper chromatography on Whatman 3MM paper. The purified samples were used for identification. The octamer, $(pU^o)_8$, from peak X was purified by chromatography on a Sephadex G-50 column.

Identification of the Oligonucleotides. The compound in peak I was identical with authentic pU^o on paper chromatography and paper electrophoresis and gave U^o after dephosphorylation with *E. coli* alkaline phosphatase. The compound from peak III was dephosphorylated with phosphatase to give U^opU^o,[7] which was identical with the authentic sample on paper chromatography and paper electrophoresis. The compound was thus proved to be $(pU^o)_2$. The chain lengths of higher linear oligonucleotides, which were major components in peaks V–IX and resistant to enzymatic digestion,[3] were determined by the technique used previously in the case of cycloadenosine oligonucleotides.[5] Each oligomer was dephosphorylated by *E. coli* alkaline phosphatase to give a product possessing a higher R_f value in paper chromatography as shown in Table II. $\epsilon(p)1$ and $\epsilon(p)2$ were determined for the compounds before and after dephosphorylation, respectively, by uv absorption and phosphorus analysis. The ratio of $\epsilon(p)1$ to $\epsilon(p)2$ was compared with the calculated value (Table II). Thus, the trimer, tetramer, pentamer, hexamer, and heptamer were identified. As far as the compounds from peak X–XII are concerned,

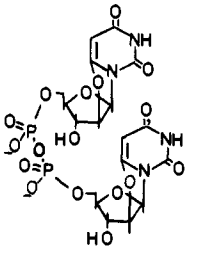
Table II. Chain Length Analysis by Phosphorus Determination of Oligomers before and after Dephosphorylation[a]

Compound	Paper chromatography R_f in solvent B $(pU^o)_n$	Paper chromatography R_f in solvent B $U^o(pU^o)_{n-1}$	$(P)_2/(P)_1$[b] Calcd	$(P)_2/(P)_1$[b] Found
$(pU^o)_3$	0.59	0.76	1.50	1.55
$(pU^o)_4$	0.32	0.48	1.33	1.34
$(pU^o)_5$	0.18	0.23	1.25	1.26
$(pU^o)_6$	0.11	0.12	1.20	1.19
$(pU^o)_7$	0.04	0.06	1.16	1.18

[a] Experimental details are described in the Experimental Section. [b] $\epsilon(P)_1$ stands for $\epsilon(P)$ of the oligomer before dephosphorylation and $\epsilon(P)_2$ for the oligomer after dephosphorylation.

their chain lengths were estimated to be 8, 9, and 10, respectively, from their elution positions on column chromatography and their mobilities on paper chromatography.

Compounds from peak II and IV were resistant to phosphatase which suggested that they were cyclic oligonucleotides (**3**). The compound from peak II ran faster than $(pU^o)_2$ on paper chromatography in solvents A and B and was different from the pyrophosphate derivative (**4**) (which



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was synthesized separately by a standard procedure) on paper chromatography in solvent C (peak II: R_f 0.07; (**4**): R_f, 0.13). The compound from peak IV ran faster than $(pU^o)_3$ on paper chromatography in solvents A and B and moved slightly slower than $(pU^o)_3$ on paper electrophoresis. Both compounds showed similar CD curves which differed from those of $(pU^o)_n$. It was concluded that the compound from peak II was the cyclic dinucleotide (n = 2 in **3**) and that the compound from peak IV was the cyclic trinucleotide (n = 3 in **3**).

Physical Properties of the Oligonucleotides

Uv absorption and CD data for the linear oligonucleotide, $(pU^o)_n$, are shown in Table III. The uv absorption spectrum of $(pU^o)_n$ is almost the same as that of pU^o. The ϵ per base residue is between 1.4 and 1.5 × 10^4, with very little hypochromicity. The shape and $[\theta]_{min}$ of the CD bands

Table III. Ultraviolet Absorption and Circular Dichroic Data of Cyclonucleotides Oligomers[a]

$(pU^o)_n$	Ultraviolet absorption		Circular dichroism	
n	λ_{max}, nm	$\epsilon_{max} \times 10^{-4}$	λ_{min}, nm	$[\theta]_{min} \times 10^{-4}$
1	252.5	1.53	252	−4.1
2	252.5	1.45	251	−4.1
3	252.5	1.46	251	−3.9
4	253	1.45		
5	252.5	1.48		
6	253	1.41		
7	253	1.42		
8	253	1.45	251	−3.8

[a] Obtained at room temperature in 0.1 M KF, 0.01 M phosphate buffer (pH 7.3).

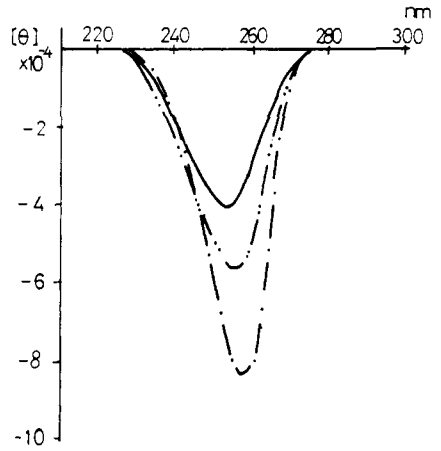

Figure 2. CD spectra of cyclic dinucleotide (—·—), cyclic trinucleotide (—··—), and linear $(pU^o)_n$ (—) in 0.1 M KF, 0.01 M phosphate buffer (pH 7.3) at room temperature.

of each oligomer are almost identical with those of the monomer. No temperature dependence of uv absorption and CD spectra is observed over the temperature range 0 to 60° even for $(pU^o)_8$. All the above evidence suggests that $(pU^o)_n$ does not have a base-stacked conformation and may exist in a random coil state in aqueous solution. In the case of uridylic acid oligonucleotides, even UpU shows a different CD spectrum from that of the monomer, and this CD spectrum exhibits temperature dependence.[13] On the other hand, A^opA^o exhibits even greater hypochromicity and the same magnitude of CD bands when compared with ApA. The pyrimidine cyclonucleoside residue, with a smaller base ring system and less stacking ability, may have some difficulty in adopting a left-handed helical conformation because of the space-taking cyclo bonds between the base and the sugar.

The cyclic oligonucleotides give essentially the same uv absorption spectra as those of the corresponding $(pU^o)_n$ but possess slightly higher ϵ values: $(pU^o)_2$, 1.45×10^4; cyclic dinucleotide, 1.59×10^4; $(pU^o)_3$, 1.46×10^4; cyclic trinucleotide, 1.56×10^4. However, their CD spectra are significantly different from those of $(pU^o)_n$ as shown in Figure 2. Both cyclic nucleotides give a negative Cotton band, which is similar to those of $(pU^o)_n$ in shape in the 250–260 nm region, but they are larger in magnitude and have $[\theta]_{min}$ shifted to longer wavelength. The difference is greater in the cyclic dinucleotide, and hence the cause may be a distortion of the sugar moiety and/or the chromophore caused by cyclization of the sugar–phosphate backbone, rather than a stacking interaction between the base. In the case of cyclic oligonucleotides of thymidylic acid[14] and adenylic acid,[15] their CD spectra are totally different from those of the corresponding monomer and linear oligomers and some kind of

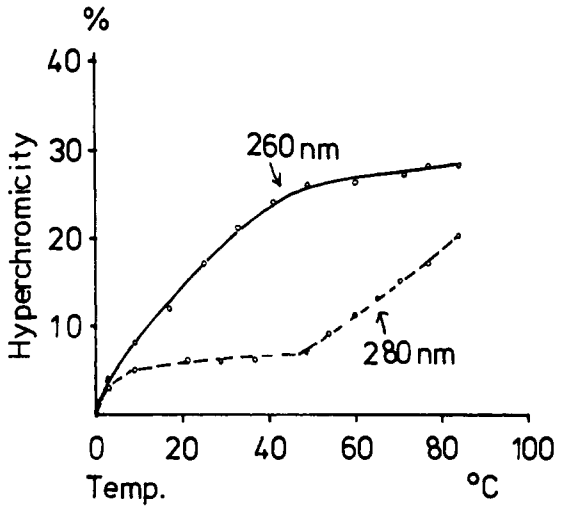

Figure 3. Uv absorption temperature profiles of the 1:1 complex between $(pU^o)_8$ and $(pA^s)_8$ in 0.1 M KF, 0.01 M phosphate buffer (pH 7.3). The total base concentration is 0.05 mM.

base–base interaction has been suggested.[14] It may be noted that the torsion angle about the glycosidic bond is variable in these cyclic oligonucleotides.

Hybridization Experiments of $(pU^o)_8$ with Poly(A)

Mixing experiments between $(pU^o)_8$ and poly(A) were carried out under two conditions, in 0.1 M KF, 0.01 M potassium phosphate buffer (pH 7.3) at 1.5° and in 0.1 M NaCl, 0.01 M $MgCl_2$, 0.01 M potassium cacodylate buffer (pH 7.0) at 0°. No hybridization was observed even in the presence of $MgCl_2$. In this condition, octauridylic acid should hybridize with poly(A) giving T_m around room temperature.[16] It is previously reported that oligomers of *S*-cycloadenosine phosphate, in which the torsion angle, χ, is nearly the same as that of U^o, do not form a complex with poly(U) either.[4,5] So it seems that these oligomers of cyclonucleotides cannot form a right-handed double helix with ordinary homopolynucleotides in which the torsion angle, χ, is considered to be around 0°.

Hybridization between $(pU^o)_8$ and $(pA^s)_8$

It has been shown previously that oligomers containing 8,2′-*S*-cycloadenosine, A^s, form a left-handed helix, contrary to ordinary polynucleotides. We can, therefore, expect that oligomers of 6,2′-*O*-cyclouridine phosphate, $(pU^o)_n$, have the ability to form a left-handed helix, though they do not associate with a right-handed helix of poly(A). This possibility was proved by a mixing experiment between $(pU^o)_8$ and $(pA^s)_8$ in 0.1 M KF, 0.01 M potassium phosphate buffer (pH 7.3) at 4°. The result has been shown in a preliminary report[2] and $(pU^o)_8$ did form a 1:1 complex with $(pA^s)_8$ at low temperature. A uv absorption–temperature profile of the 1:1 mixture is shown in Figure 3. The curve at 260 nm reaches a plateau around 50° and the curve at 280 nm reaches a plateau around 15°. Absorbance at both wavelengths is still changing at 1° and so at least the first T_m should be below 8°. As shown in Figure 4, the CD spectrum of the 1:1 mixture is clearly different from the summation curve from both components at 4°, suggesting that a complex is really formed. At 60°, the observed curve for the 1:1 mixture is identical with the summation curve, suggesting absence of interaction between the two components. A pair of CD bands, the negative one around 280 nm and the positive one around 260 nm, which are the characteristics of $(pA^s)_n$ having a left-handed helical conformation, are still retained in the CD spectrum of the complex at low temper-

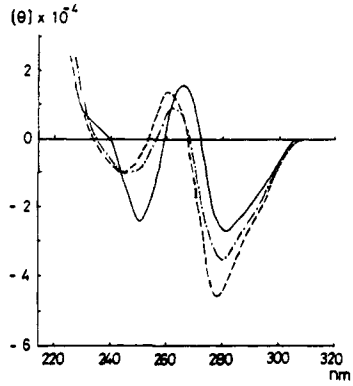

Figure 4. CD spectra of $(pU^o)_8$·$(pA^s)_8$ at 4° (—) and at 60° (—·—) and the summation curve from both components at 4° (— —), in 0.1 M phosphate buffer (pH 7.3). The summation curve at 60° is identical with the CD spectrum of the complex at 60°. The same sample as in Figure 3 was used.

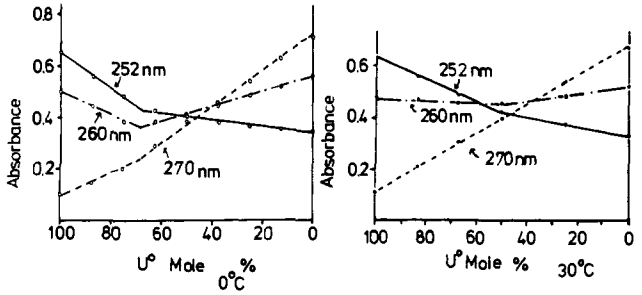

Figure 5. Mixing curve between $(pU^o)_8$ and $(pA^s)_8$ at 0° (shown on the left) and 30° (shown on the right) in 0.1 M NaCl, 0.01 M $MgCl_2$, 0.01 M sodium cacodylate buffer (pH 7.0). The total base concentration is 0.04 mM.

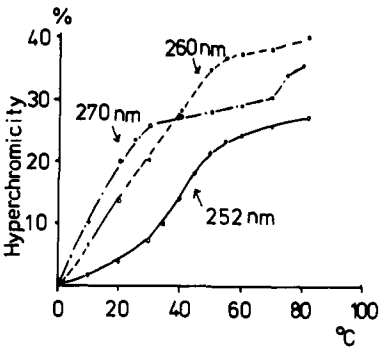

Figure 6. Uv absorption temperature profiles of $2(pU^o)_8$·$(pA^s)_8$ in 0.1 M NaCl, 0.01 M $MgCl_2$, 0.01 M sodium cacodylate buffer (pH 7.0). The total base concentration is 0.04 mM.

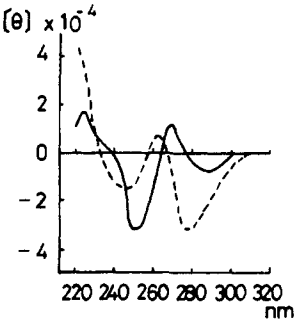

Figure 7. CD spectrum of $(pU^o)_8$·$(pA^s)_8$ (solid line) and the summation curve (broken line) from both components in 0.1 M NaCl, 0.01 M $MgCl_2$, 0.01 M sodium cacodylate buffer (pH 7.0) at 3°. The total base concentration is 0.04 mM.

ature. These two bands are considered to originate from splitting of the monomer's transition around 270 nm. The pattern of splitting, a negative band at longer wavelength and a positive band at shorter wavelength, suggests left-handed stacking of the bases.[17] So the double-stranded complex between $(pU^o)_8$ and $(pA^s)_8$ may have a left-handed helical turn.

A mixing experiment was carried out next in the presence of $MgCl_2$, namely in 0.1 M NaCl, 0.01 M $MgCl_2$, 0.01 M sodium cacodylate buffer (pH 7.0) at 0° (Figure 5, on the left). Under this condition, a triple-stranded complex, $2(pU^o)_8$·$(pA^s)_8$, is formed. A uv absorption temperature profile of a 2:1 mixture of $(pU^o)_8$ and $(pA^s)_8$ is shown in Figure 6. From the curve at 270 nm, there seems to be a T_m below 15°, and the second T_m of 38° is suggested from the curve at 252 nm. A mixing experiment in the same solvent at 30° exhibits the existence of the 1:1 complex at this temperature. It may be concluded that the 2:1 complex, $2(pU^o)_8$·$(pA^s)_8$, is stable at low temperature and melts with T_m below 15° to give the 1:1 complex which dissociates with T_m at 38° to give free components. The same phenomenon, transition from a triple-stranded complex to a double-stranded complex by increasing temperature, is observed in poly(A)–poly(U) interaction at low salt concentration[18] and in poly(A)–decathymidylic acid at high salt concentration (1 M LiCl).[19]

The CD spectrum of 2:1 mixture of $(pU^o)_8$ and $(pA^s)_8$ in the presence of $MgCl_2$ at 3° is shown in Figure 7. It is quite different from the summation curve from both components, and the splitting bands, which is characteristic of $(pA^s)_n$, is also retained, suggesting a left-handed helical conformation. Thus, on association with $(pA^s)_8$, $(pU^o)_8$ may be able to form a left-handed helix along the rigid left-handed helix of $(pA^s)_8$. The resulting multistranded complexes between the two oligomers seem to be very stable, probably more stable than the corresponding $(pA)_8$–$(pU)_8$ complexes. It is reported that the 1:1 complex between the octamer, $(Up)_7U$, and poly(A) has a T_m of 20°,[16] whereas $(pU^o)_8$·$(pA^s)_8$ has a T_m of 38° under similar conditions. On complex formation between $(pU^o)_8$ and $(pA^s)_8$, the CD difference spectrum ($[\theta]_{complex}-[\theta]_{summation}$) has a positive band at longer wavelengths and a negative band at shorter wavelengths region with a crossing-over point around 263–265 nm (see Figures 4 and 7). Poly(U)–poly(A) complexes also give a pair of bands (different in sign) and the pattern of their appearance is opposite to those of $(pU^o)_8$–$(pA^s)_8$, a negative band at longer wavelengths and a positive one at shorter wavelength with a crossing-over point around 255–260 nm.[20] These phenomena are difficult to explain, but perhaps the base-pairings involved in $(pU^o)_8$–$p(A^s)_8$ complexes are similar to those in poly(U)–poly(A) complexes.

Experimental Section

Paper chromatography was carried out by the descending technique using Toyo filter paper No. 51A in the following solvent systems: A, 2-propanol–concentrated ammonia–water (7:1:2, v/v); B, 1-propanol–concentrated ammonia–water (55:10:35, v/v); C, 1-butanol–acetic acid–water (5:2:3, v/v); D, ethanol–1 M ammonium acetate (7:3, v/v). Cellulose thin-layer chromatography was performed on a plate (10 × 10 cm) prepared with Avicel SF (purchased from Funakoshi Co. Ltd.) in solvent E, 2-propanol–0.5 N ammonium hydroxide (5:3, v/v). Paper electrophoresis was carried out on Toyo filter paper No. 51A for characterization or Whatman 3MM paper for preparative purpose at 35 v/cm in 0.05 M triethylammonium bicarbonate buffer (pH 7.5). Ultraviolet absorption spectra were obtained on a Hitachi EPS-3T or Hitachi 124 spectrophotometer, and circular dichroic spectra were taken with a JASCO ORD/UV-5 spectropolarimeter equipped with a CD attachment. For obtaining absorption–temperature profiles and mixing curves, a Hitachi 124 spectrophotometer equipped with a Ko-

matsu Solidate SPD-H-124 thermostated cell was used. CD spectra at low temperature were measured using JASCO low-temperature device. The temperature within the cell was measured by a Cu-constantan thermocouple. Molecular extinction coefficient (ϵ) and molecular ellipticity ([θ]) are presented as per residue values.

Polymerization of 6,2′-Anhydro-1-(β-D-arabinofuranosyl)-6-hydroxyuracil 5′-Monophosphate (1, pU°). The mixture of pyridinium salt of 6,2′-anhydro-1-(β-D-arabinofuranosyl)-6-hydroxyuracil 5′-monophosphate[11] (pU°, 0.7 mmol, 11 000 AU_{252}) and pyridinium Dowex 50 ion-exchange resin (700 mg) was rendered anhydrous by repeated evaporation of its solution in a mixture of dimethylformamide (1 ml) and pyridine (1 ml). Evaporation was done in a pear-shaped flask equipped with a cocked joint. Admitting air into the flask and addition of the solvent were carried out in a drybox. Finally, the residue except for the resin was dissolved in dimethylformamide (1 ml), and dicyclohexylcarbodiimide (370 mg, 1.35 mmol) was added. As the mixture solidified immediately, dimethylformamide (1 ml) was added to dissolve the solid. Resulting solution was concentrated to a half volume. The reaction mixture was kept in a thermostated box at 30° for 2 weeks. Then, 50% aqueous pyridine (5 ml) was added and the precipitate of dicyclohexylurea was filtered off. The precipitate was washed with 50% aqueous pyridine (60 ml). The combined solution of the filtrate and washings was extracted with pentane (10 ml × 3). The water layer was evaporated to dryness in vacuo. To the residue, pyridine (8 ml), triethylamine (0.097 ml), and acetic anhydride (1.7 ml) were added, and the resulting solution was kept at room temperature for 2 days. The reaction mixture was evaporated to dryness in vacuo. The residue was dissolved in 50% aqueous pyridine (20 ml) and kept at room temperature for 1 day. The solution was evaporated to dryness in vacuo. To the residue, a mixture of dimethylformamide and methanolic ammonia saturated at 0° (1:1, v/v) was added, but a clear solution was not obtained. Addition of 9 N ammonium hydroxide (30 ml) gave a solution, and it was kept at room temperature for 1 h, then evaporated to dryness in vacuo. Examination of the reaction mixture by paper chromatography in solvent D gave no acetylated derivatives of pU° showing that deacylation reaction was complete. The residue was largely dissolved in water (200 ml), and the remaining solid was dissolved in 50% aqueous pyridine (30 ml). Both solutions were combined, and the pH of the resulting solution was adjusted to 8 with concentrated ammonia. Then the solution was applied to a column (3.5 × 4.0 cm) of DEAE-cellulose (bicarbonate form). After washing with water (1.6 l.), elution was carried out using a linear gradient of triethylammonium bicarbonate buffer pH 7.5 (0–0.25 M, total 8 l.). Further elution was carried out with a linear gradient of the same buffer (0.25–0.5 M, total 8 l.) and finally with 1 M buffer. In each case, fractions of 16 ml were collected at 10-min intervals. The combined fractions in each peak were desalted by repeated evaporation with water under reduced pressure. An aqueous solution of the final residue had a yellowish color. The elution pattern is shown in Figure 1, and the distribution of nucleotidic materials in different peaks is shown in Table I. The properties of the products in paper chromatography and electrophoresis are shown in Table II.

Further Purification of Cyclonucleotide Oligomers. A part of the material in each peak was further purified by paper chromatography in solvent A or solvent B. The main band was extracted with water to give a pure oligomer. As to peak X which contained $(pU°)_8$, it was rechromatographed on a Sephadex G-50 column (1 × 90 cm). Elution was carried out with water. Fractions of 3 ml were at 1-h intervals. Appropriate fractions (tube no. 11-22) were combined and concentrated under reduced pressure. Examination by paper chromatography in solvent B showed only one spot. The purified oligomers thus obtained were used for analysis of the chain lengths.

Chain Length Analysis of the Linear Cyclonucleotide Oligomers. Each linear oligonucleotide, $(pU°)_n$, was dephosphorylated by *E. coli* alkaline phosphatase to give the corresponding oligomer, $U°(pU°)_{n-1}$, with no terminal phosphate group. For dephosphorylation, a mixture of substrate (5 AU_{252}/100 μl), ammonium bicarbonate (0.1 M), and phosphatase (0.04 mg/ml) was incubated at 37° for 4 h. The product was isolated by paper chromatography in solvent B. Phosphorus analysis of $(pU°)_n$ and $U°(pU°)_{n-1}$ was carried out by Allen's[22] method to give molecular absorption coefficients per phosphate residue, $\epsilon(P)_1$ and $\epsilon(P)_2$, respectively. The chain length of each oligomer was determined from the $\epsilon(P)_1/\epsilon(P)_2$ value as shown in Table II.

***P*$_1$,*P*$_2$-Bis[2′,6-anhydro-1-(β-D-arabinofuranosyl)-6-hydroxyuracil] 5′-Pyrophosphate (4).** The pyridinium salt of pU° (260 AU_{252}, 0.016 mmol) was passed through a Dowex 50 (H^+ form) column to give the free acid of pU°. It was converted into mono-4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt and rendered anhydrous by repeated evaporation with pyridine. Finally, it was dissolved in pyridine (3 ml), and DCC (8.3 mg) was added. The mixture was kept at 60° for 3 h, and water (3 ml) was added. After keeping it for 1 h at room temperature, the mixture was concentrated to a one-third volume in vacuo and filtered. The filtrate was extracted with petroleum ether (1 ml × 3), and the water layer was evaporated to dryness in vacuo. The residue was applied on Whatman 3 MM paper strips and the product was isolated by paper electrophoresis. Uv absorption: λ_{max} (H^+) 252.5, λ_{max} (H_2O) 252.5, λ_{max} (OH^-) 255 nm. Paper electrophoresis: $R_{pU°}$ 0.84. Paper chromatography: R_f 0.13 (pU°, 0.17) in solvent C, 0.42 (pU°, 0.47) in solvent B.

References and Notes

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