L-Adenylyl-(3′–5′)-L-adenosine and L-Adenylyl-(2′–5′)-L-adenosine*

Ichiro Tazawa, Setsuko Tazawa, Lloyd M. Stempel, and Paul O. P. Ts'o

ABSTRACT: L-Adenylyl-(3′–5′)-L-adenosine and L-adenylyl-(2′–5′)-L-adenosine were synthesized from L-adenosine. In comparative studies with their respective D isomers, both the D and L dimers have the same ultraviolet absorption spectra, the same proton magnetic resonance spectra, the same R_F values on silica thin-layer chromatography, and the same mobilities in paper electrophoresis. The circular dichroic spectra of the L-ApA(s) are opposite in sign but the same (or nearly the same within experimental error) in magnitude as those of the corresponding D-ApA(s). All these studies confirm the expectation that the conformation of L-ApA(s) is the mirror image of the corresponding D-ApA(s). Therefore, while the conformation of the D dimers is an *anti,anti*, right-handed stack (Kondo *et al.* (1970), *Biochemistry 9*, 3479), the conformation of the L dimers is an *anti,anti*, left-handed stack. The L-ApA(s) are either completely or extremely resistant to the hydrolytic attack of spleen and venom phosphodiesterase, but are not inhibitory to the action of these enzymes. L-ApA(s) form a complex with D-poly U having the same stoichiometry (1A:2U) as the complex of D-ApA(s) with D-poly U. In addition, the circular dichroism spectra of the four poly U–ApA complexes are very similar to each other, especially the circular dichroism spectra of D-$A_{3'}p_{5'}A\cdot 2$D-poly U and L-$A_{3'}p_{5'}A\cdot 2$D-poly U which are nearly identical. This observation suggests that the overall conformations of these helical complexes are very similar. In Tris (pH 7.5)–Mg^{2+} (0.01 M), these complexes have different T_m values: D-$A_{3'}p_{5'}A\cdot 2$D-poly U (13.7°), L-$A_{3'}p_{5'}A\cdot 2$D-poly U (5–5.5°), D-$A_{2'}p_{5'}A\cdot 2$D-poly U (11.2°), and L-$A_{2'}p_{5'}A\cdot 2$D-poly U (13.3°). The thermodynamic analyses of these melting data together with the symmetry consideration on the energy state of the enantiomers show that the D-2′–5′ dimer can form left-handed stacks more readily than the D-3′–5′ dimer. In other words, the selectivity in chirality of the screw axis of the stack of the 2′–5′ dimer is less than the selectivity of the 3′–5′ dimer.

In the preceding papers of this series, we have reported the general conformation of 25 dinucleoside mono- and diphosphates (Ts'o *et al.*, 1969) and the influence of the position of the phosphodiester linkage (2′–5′, 3′–5′, and 5′–5′) on the conformation of dinucleoside monophosphates (especially ApA) as studied concurrently by proton magnetic resonance, circular dichroism, and ultraviolet absorption spectroscopy (Kondo *et al.*, 1970). Conformational models have been constructed for these dimers. In general, the nucleosidyl units all have the *anti* conformation with respect to the sugar–base torsion angle, and the turn of the 3′–5′ screw axis of the stack is right handed. Yet, in the construction of these models, it was immediately recognized that the left-handed stack may also exist in the population, especially for the 2′–5′ dimers (Ts'o *et al.*, 1969). However, there is no simple method to study quantitatively this question about the chirality of the screw axis of the stack in solution. For this reason, we have synthesized L-adenylyl-(3′–5′)-L-adenosine (L-$A_{3'}p_{5'}A$) and L-adenylyl-(2′–5′)-L-adenosine (L-$A_{2'}p_{5'}A$) starting from L-adenosine. With these enantiomers of D-$A_{3'}p_{5'}A$ and D-$A_{2'}p_{5'}A$, we can study the effect of this unnatural sugar phosphate backbone on the properties and interaction of ApA(s), especially the question of chirality.

L-Ribose has not been found in nature, though its enantiomer D-ribose has a wide occurrence in the biological world. Among the L sugars, L-arabinose, L-lyxose, L-galactose, L-sorbose, and L-xylulose have been found in nature (Staněk *et al.*, 1963). The synthesis of L-adenosine was accomplished by Acton *et al.* (1964) and Shimizu *et al.* (1965) independently. The latter authors also synthesized L-inosine, L-5′-AMP, L-5′-IMP (Shimizu *et al.*, 1965; Asai *et al.*, 1967), and both α and β anomers of L-5′-UMP, L-5′-CMP, L-5′-ribosyl-TMP (Shimizu *et al.*, 1967). They studied the optical rotatory dispersion of these L-nucleotides and found that these L-nucleotides, as expected, have the opposite optical rotatory dispersion spectra to their naturally occurring D enantiomers (Nishimura *et al.*, 1968). Synthesis of L-uridine has been reported by Wu and Chargaff (1969). Recently, Holý and Šorm (1969) reported the synthesis of many L-nucleosides and L-nucleotides including L-adenosine, L-guanosine, L-cytidine, L-uridine, L-ribosylthymidine, as well as the 2′,3′-cyclic phosphates and 2′(3′)-phosphates of these nucleosides, L-6-azauridine and L-inosine 2′,3′-cyclic phosphate. They also synthesized D-$G_{3'}p_{5'}$L-A and D-$G_{3'}p_{5'}$L-C, dinucleoside monophosphates with both D- and L-nucleoside units. In the deoxyriboside series, L-thymidine (Šmejkal and Šorm, 1964) and both α and β anomers of L-deoxyadenosine and L-deoxyguanosine (Robins *et al.*, 1970) have been synthesized.

In this paper, the synthesis and properties of L-$A_{3'}p_{5'}A$ and L-$A_{2'}p_{5'}A$ are reported. These L-ApA(s) share many properties with their D isomers, *i.e.*, ultraviolet spectra, proton magnetic resonance spectra, R_F values on silica thin-layer

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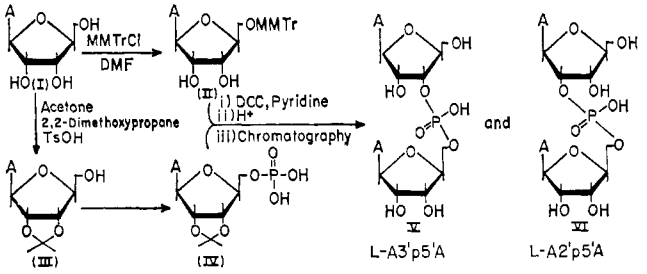

FIGURE 1: The synthetic scheme for L-$A_{3'}p_{5'}A$ and L-$A_{2'}p_{5'}A$.

chromatography, and electrophoretic mobilities. Their circular dichroism, however, are opposite to those of D isomers. The L-ApA(s) are extremely resistant to spleen and venom phosphodiesterase in contrast to their D isomers, but are not inhibitory to these enzymes. The L-ApA(s) form a complex with D-poly U with 1A:2U stoichiometry as do their D isomers. It appears that the overall conformation of these L-ApA·2D-poly U complexes closely resembles those of D-ApA·2D-poly U complexes based on the similarity of circular dichroism spectra of all these complexes. From the thermodynamic analyses on the T_m values of these complexes, aided by the symmetry consideration on the energy state of the enantiomers, the question about the chirality of the screw axis of the ApA dimers can be studied in a quantitative manner. In view of the fact that the L-ApA(s) are resistant to the action of certain nucleolytic enzymes and form complexes with D-poly U, polynucleotides containing L-pentose in their backbone should be of biological interest.

Materials

Enzymes, Polynucleotides, and D-*Ribosyladenine Dinucleoside Monophosphates.* Spleen phosphodiesterase and venom phosphodiesterase (EC 3.1.4.1) were purchased from Worthington Biochemical Co., Freehold, N. J. Poly U was obtained from Miles Laboratories, Elkhart, Ind., and used without further purification. The maximum molar extinction coefficient of 9.2×10^3 was used for poly U. D-$A_{2'}p_{5'}A$ and D-$A_{3'}p_{5'}A$ were purchased from Zellstofffabrik Waldhof, Mannheim, Germany. Both compounds were found tc be chromatographically pure and to be free from each other as examined by spleen phosphodiesterase.

Synthesis of L-$A_{2'}p_{5'}A$ *and* L-$A_{3'}p_{5'}A$. L-$A_{2'}p_{5'}A$ and L-$A_{3'}p_{5'}A$ were synthesized from L-adenosine by the procedure shown in Figure 1. The L-adenosine (Acton *et al.*, 1964) was obtained through the courtesy of Dr. Leon Goodman, Stanford Research Institute, Menlo Park, Calif., and of Dr. Harry B. Wood, Cancer Chemotherapy, National Service Center, National Cancer Institute, Bethesda, Md. We are most grateful for their assistance in acquiring this starting material.

5'-*O*-MONOMETHOXYTRITYL-L-ADENOSINE (II). L-Adenosine (I) (107 mg, 0.4 mmole) was converted into 5'-*O*-monomethoxytrityl-L-adenosine (II) with monomethoxytrityl chloride in dimethylformamide according to the published procedure (Lohrmann and Khorana, 1964). After recrystallization, a white powder (105 mg, yield 48.5%) was obtained, which gave a single spot on thin-layer chromatography (silica gel, solvent $CHCl_3$–EtOH, 7:1, v/v). The R_F value (0.54) was the same as that of the 5'-*O*-monomethoxytrityl-D-adenosine which was synthesized by the same procedure from D-adenosine.

2',3'-*O*-ISOPROPYLIDENE-L-ADENOSINE (III). L-Adenosine (I) (107 mg, 0.4 mmole) was converted into 2',3'-*O*-isopropylidene-L-adenosine by a reaction which was carried out in acetone in the presence of 2,2-dimethoxypropane and *p*-toluenesulfonic acid (Fromageot *et al.*, 1967). The reaction product was isolated as described by Hampton (1961). Recrystallization from water gave crystalline III (60.8 mg, yield 50.4%) which had the same R_F value (0.69) on thin-layer chromatography (silica gel, solvent $CHCl_3$–EtOH, 7:1, v/v) as that of 2',3'-*O*-isopropylidene-D-adenosine prepared by the same procedure from D-adenosine. An additional 25.5 mg (yield 20.8%) of III, which was essentially homogeneous on thin-layer chromatography, was obtained from the mother liquor.

2',3'-*O*-ISOPROPYLIDENE-L-ADENOSINE 5'-PHOSPHATE (IV). Crystalline 2',3'-*O*-isopropylidene-L-adenosine (58.3 mg, 0.19 mmole) was phosphorylated with phosphorus oxychloride in triethyl phosphate according to a published procedure (Yoshikawa *et al.*, 1967; Yamazaki *et al.*, 1968). The reaction mixture was poured into ice water which contained 6 mole equiv of NaOH. This solution was acidified with acetic acid to pH 4 and treated with charcoal. The charcoal was then filtered and washed with water. Materials adsorbed to the charcoal were eluted with 2% NH_4OH in 50% EtOH. The eluate was concentrated to dryness and dissolved in a small volume of water, filtered, and the filtrate applied to a DEAE-cellulose column (bicarbonate form). Linear gradient elution with 0–0.2 M triethylammonium bicarbonate buffer (pH 7.5) gave 2',3'-*O*-isopropylidene-L-adenosine 5'-phosphate (IV) as the major product ($OD_{T,260\,m\mu}$ = 2200, yield 75.3%). The R_F values of IV in paper chromatography (0.26 in solvent A, 0.42 in solvent C) were the same as those of 2',3'-*O*-isopropylidene-D-adenosine 5'-phosphate which was prepared by the same procedure from 2',3'-*O*-isopropylidene-D-adenosine. The electrophoretic mobility (0.95 relative to 5'-AMP) and $\lambda_{max}^{H_2O}$ (259 mμ) of IV were also the same as those of the D isomer.

L-ADENYLYL-(3'–5')-L-ADENOSINE (V) AND L-ADENYLYL-(2'–5')-L-ADENOSINE (VI). An aqueous solution of triethylammonium 2',3'-*O*-isopropylidene 5'-phosphate (IV) ($OD_{T,260\,m\mu}$ = 3210, 0.208 mmole) was passed through a column of Dowex 50-X8 (pyridinium) ion-exchange resin. Elution with 10% aqueous pyridine gave the pyridinium salt of IV, which was concentrated to dryness and rendered anhydrous by repeated evaporation of anhydrous pyridine. 5'-*O*-Monomethoxytrityl-L-adenosine (II) (58 mg, 0.107 mmole) and dry pyridinium Dowex 50-X8 ion-exchange resin (500 mg) were added, and the mixture was rendered anhydrous by repeated evaporation of anhydrous pyridine. Anhydrous pyridine (3 ml) and dicyclohexylcarbodiimide (408 mg, 2 mmoles) were added and the mixture was stirred for 8 days in the dark. After this period, water (3 ml) was added and the mixture was extracted three times with pentane. After standing overnight the aqueous layer was diluted with 50% aqueous pyridine and filtered from insoluble material. The filtrate was evaporated to dryness and residual pyridine was removed by repeated addition and evaporation of water. The residue was dissolved in 80% acetic acid and kept for 4 hr at room temperature. Acetic acid was removed

by lyophilization and the residue was dissolved in water. The pH of the solution was adjusted to about 8 with NH_4OH and the solution was applied on a column of DEAE-cellulose (bicarbonate). After linear gradient elution with 0–0.2 M triethylammonium bicarbonate buffer (pH 7.5), two fractions were obtained which contained mainly L-adenylyl-(3′–5′)-2′,3′-*O*-isopropyridene-L-adenosine and its 2′–5′ isomer. Each of these fractions was freed from the buffer by repeated addition and evaporation of water and then separately dissolved in 90% formic acid. After standing for 100 min at room temperature, the formic acid was removed by lyophilization. Each residue was dissolved in water and applied on a column of Dowex 1-X8 (formate). Linear gradient elution with 0–0.05 N formic acid gave two peaks in each column, the first peak in each case being L-$A_{2'}p_{5'}A$ (VI) and the second peak being L-$A_{3'}p_{5'}A$ (V). The two L-$A_{3'}p_{5'}A$ (V) peaks from the separate columns were combined as were the two L-$A_{2'}p_{5'}A$ (VI) peaks and evaporated to about 100 ml at 25°. Formic acid was removed by lyophilization. Both V and VI were purified further by preparative paper chromatography in solvent A. Finally, the chromatographically pure V and VI were put on separate columns of DEAE-cellulose (bicarbonate), which were then washed thoroughly with water. Compounds V and VI were eluted from the columns with 0.2 M ammonium bicarbonate solution. Compounds V and VI were obtained as ammonium salts after removal of ammonium bicarbonate by repeated evaporation of water. The yields of V and VI were 7.6 and 6.9%, respectively. These low yields were the result of the formation of many side products during the condensation reaction. It seems that the high nucleotide:nucleoside ratio (about 2:1) and relatively long reaction time (8 days) enhanced these side reactions. When the same condensation reaction was conducted with 2′,3′-*O*-isopropylidene-D-adenosine 5′-phosphate and 5′-*O*-monomethonytrityl-D-adenosine with nucleotide:nucleoside ratio 1:2 for 5 days, yields of D-(2′–5′)-ApA and D-(3′–5′)-ApA were 26.4 and 13.9%, respectively. The preferential formation of 2′–5′ isomer suggests the substantial steric hindrance on 3′-OH group by a 5′-*O*-monomethoxytrityl group. Aliquots of V and VI were hydrolyzed with 0.3 N KOH for 20 hr at 37°. Paper chromatography of the hydrolysates in solvent A gave two spots in both cases which corresponded to L-adenosine and L-adenosine 2′(3′)-phosphate, respectively. From the comparison of TOD at 259 mμ ($\lambda_{max}^{H_2O}$), the nucleoside:nucleotide ratio was determined to be 0.94 for V and 0.89 for VI. Chromatographic properties, electrophoretic mobilities, nuclear magnetic resonance spectra, ultraviolet spectra, and circular dichroism spectra of V and VI were all consistent with the assigned structure and are discussed in the Results section.

Methods

Chromatography and Electrophoresis. Descending paper chromatography was performed using Whatman No. 1 paper. The solvent systems used were solvent A, 2-propanol–concentrated ammonia–water (7:1:2, v/v); solvent B, 1-propanol–concentrated ammonia–water (55:10:35, v/v); solvent C, ethanol–1 M ammonium acetate (pH 7.5) (7:3, v/v). Ascending thin-layer chromatography was carried out on Eastman chromatographic silica gel sheets No. 6060. Solvent systems used were the same as those used in paper chromatography. Paper electrophoresis was performed using Whatman No. 1 paper and 0.05 M ammonium bicarbonate buffer (pH 7.5) for about 1 hr at a potential of about 40 V/cm.

Proton Magnetic Resonance Spectra, Circular Dichroic Spectra, and Ultraviolet Absorption Spectra. A Varian HA-100 spectrometer was used for the proton magnetic resonance spectra as described in the preceding paper (Kondo *et al.*, 1970). A Cary 60 recording spectropolarimeter equipped with the circular dichroism attachment was used for the circular dichroism spectra as described in the preceding paper (Kondo *et al.*, 1970). A Cary 15 spectrophotometer was used for the ultraviolet absorption spectra and for the mixing curve experiments. A thermostatically controlled cell compartment was used for the experiments with temperature variation. The actual temperature of the cell was measured by Model 42 SC thermometer manufactured by Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio. Condensation of moisture at low temperature was prevented by passing dry nitrogen through the sample compartment.

Enzyme Digestion. Incubations of ApA's with spleen and venom phosphodiesterase were carried out under the following conditions. Spleen phosphodiesterase: incubation mixture (0.1 ml) contained ammonium acetate buffer (pH 6.5) (10 μmoles), ApA (0.15 μmole), and the enzyme (*ca.* 0.16 unit) and was incubated at 37°. Venom phosphodiesterase: incubation mixture (0.1 ml) contained ammonium bicarbonate buffer (pH 9.0) (10 μmoles), ApA (0.15 μmole), and the enzyme (40 μg) and was incubated at 37°.

Results

Properties of L-$A_{3'}p_{5'}A$ and L-$A_{2'}p_{5'}A$. CHROMATOGRAPHY AND ELECTROPHORESIS. R_F values on paper chromatography and thin-layer chromatography of L-$A_{3'}p_{5'}A$ and L-$A_{2'}p_{5'}A$ together with those of D-ApA's are reported in Table I. The L-$A_{3'}p_{5'}A$ has the same R_F values as those of D-$A_{3'}p_{5'}A$ on paper chromatography in three solvent systems and on thin-layer chromatography in two solvent systems. L-$A_{2'}p_{5'}A$, however, has significantly lower R_F values than those of D-$A_{2'}p_{5'}A$ on paper chromatography in all three solvent systems, *i.e.*, 0.18 *vs.* 0.23 in solvent A, 0.44 *vs.* 0.50 in solvent B, 0.21 *vs.* 0.25 in solvent C, while the R_F values of both L- and D-$A_{2'}p_{5'}A$ on thin-layer chromatography are the same in two solvent systems. At present, it is not definitely known why the L- and D-$A_{2'}p_{5'}A$ have different R_F values on cellulose paper chromatography and have the same R_F values on silica gel thin-layer chromatography. It should be noted, however, that cellulose is optically active while silica gel is not. Therefore, there can be a resolution of these D- and L-dinucleoside monophosphates on paper as has been found for racemic mixtures of amino acids and pteridines (DeLigny *et al.*, 1963; Albert and Serjeant, 1964). The mobilities of L-ApA's and of D-ApA's on paper electrophoresis are also given in Table I. The L-$A_{3'}p_{5'}A$ and L-$A_{2'}p_{5'}A$ have the same mobilities as their respective D isomers.

PROTON MAGNETIC RESONANCE SPECTRA. The chemical shifts of the base and anomeric protons of L-ApA's and those of D-ApA's are reported in Table II. Assignments of protons of D-$A_{3'}p_{5'}A$ and D-$A_{2'}p_{5'}A$ are given in our previous paper in the series (Ts'o *et al.*, 1969) and in the preceding paper (Kondo *et al.*, 1970), respectively. As shown in Table II,

TABLE I: Chromatographic and Electrophoretic Properties of L-ApA's and D-ApA's.

Compound	Paper Chromatography, R_F			Thin-Layer Chromatography, R_F		Paper Electrophoresis, Rel Mobility to 5′-AMP
	Solvent A	Solvent B	Solvent C	Solvent A	Solvent C	
L-$A_{3'}p_{5'}A$	0.26	0.52	0.25	0.52	0.65	0.34
D-$A_{3'}p_{5'}A$	0.26	0.52	0.25	0.52	0.65	0.35
L-$A_{2'}p_{5'}A$	0.18	0.44	0.21	0.51	0.61	0.36
D-$A_{2'}p_{5'}A$	0.23	0.50	0.25	0.51	0.61	0.37

all six protons of L-$A_{3'}p_{5'}A$ and L-$A_{2'}p_{5'}A$ have the same chemical shifts as their corresponding protons in the respective D isomers.

The line widths of base protons of the L compounds in D_2O were broad, although chemical shifts could still be determined. This is presumably due to contamination by paramagnetic metal ions, since the addition of EDTA to a final concentration of 5×10^{-4} M reduced these line widths markedly (Ts'o *et al.*, 1969).

Our proton magnetic resonance studies previously showed (Ts'o *et al.*, 1969) that D-$A_{3'}p_{5'}A$ has *anti*, right-handed conformation, and that chemical shifts of the protons are very sensitive for the conformation. As shown in later sections, the circular dichroism spectra of the L dimers are exactly opposite to those of the D dimers. These circular dichroism results, together with the proton magnetic resonance data showing that the chemical shifts of the D and L dimers are identical, indicate that the conformation of these L dimers is the mirror image of the conformation of the D dimers.

ULTRAVIOLET ABSORPTION SPECTRA. The ultraviolet spectral properties of L-ApA's at pH 7 and room temperature are shown in Table III together with those of D-ApA's. The L-$A_{3'}p_{5'}A$ and L-$A_{2'}p_{5'}A$ have the same λ_{max}, the same maximal extinction coefficient, and the same hypochromicity (as compared with the monomeric units) as their respective D isomers. The 2′–5′ dimers (both D and L enantiomers) have a lower maximal molar extinction coefficient, thus a higher hypochromicity value, than the 3′–5′ dimers.

CIRCULAR DICHROISM. Spectra of L-$A_{3'}p_{5'}A$ and D-$A_{3'}p_{5'}A$ in the range of 215–300 mμ and at 21.5° are shown in Figure 2. As shown, these two spectra, within experimental error, are identical in magnitude and are opposite to each other. L-$A_{3'}p_{5'}A$ has a maximum at 250 mμ and two minima at 218 and 270 mμ, while D-$A_{3'}p_{5'}A$ has a minimum at 250 mμ and two maxima at 218 and 270 mμ, and the magnitude of $[\theta]$ of one compound at a given wavelength is the same as that of the other compound. The dependence of $[\theta]$ at 250 and 270 mμ on temperature was also examined for both compounds. Figure 3 shows that both compounds have identical dependency on temperature. These observations together with the proton magnetic resonance results suggest that the conformations of these two optical isomers are the mirror image of each other. Since D-$A_{3'}p_{5'}A$ is considered to have an *anti*-, right-handed conformation (Ts'o *et al.*, 1969; Bush and Tinoco, 1967), therefore L-$A_{3'}p_{5'}A$ would have an *anti*-, left-handed conformation.

Figure 4 shows circular dichroism spectra of L-$A_{2'}p_{5'}A$ and D-$A_{2'}p_{5'}A$ at 20.5°. Again, the two spectra have essentially the same magnitude but are opposite in sign. That is, L-$A_{2'}p_{5'}A$ has a maximum at 250 mμ and two minima at 217.5 and 270 mμ. However, while the $[\theta]$ at 270 mμ of both compounds has the same magnitude, at 250 mμ the magnitude of $[\theta]$ of L-$A_{2'}p_{5'}A$ is larger than that of D-$A_{2'}p_{5'}A$ by about 10–15%. This situation holds true at other temperatures also, as shown in Figure 5, although this difference gets

TABLE II: Chemical Shifts of the Base and Anomeric Protons of L-ApA's and D-ApA's (0.02 M) in D_2O (pD 7.6) at 28°.

Compound	Chemical Shifts (ppm from TMSi Capillary)					
	$A_{3'}p$- or $A_{2'}p$-			-$p_{5'}A$		
	H-2	H-8	H-1′	H-2	H-8	H-1′
L-$A_{3'}p_{5'}A$[a]	8.41	8.64	6.29	8.54	8.68	6.41
D-$A_{3'}p_{5'}A$	8.41	8.64	6.29	8.55	8.68	6.40
L-$A_{2'}p_{5'}A$[a]	8.20	8.61	6.58	8.61	8.44	6.25
D-$A_{2'}p_{5'}A$	8.19	8.61	6.59	8.61	8.43	6.27

[a] Spectra were measured in the presence of 5×10^{-4} M EDTA.

TABLE III: Ultraviolet Spectral Properties of L-ApA's and D-ApA's at Neutral pH and Room Temperature.

Compound	$\lambda_{max}^{pH\ 7}$[a] (mμ)	ε at λ_{max}[b] ($\times 10^3$)	Hypochromicity[c] (%)
L-$A_{3'}p_{5'}A$	258	13.5	12.3
D-$A_{3'}p_{5'}A$	258	13.6	11.9
L-$A_{2'}p_{5'}A$	258.5	13.0	15.5
D-$A_{2'}p_{5'}A$	258.5	12.9	16.1

[a] Spectra were measured in 0.01 M phosphate buffer (pH 7.0). [b] Calculated from the hypochromicity, assuming the extinction coefficients of adenosine and 2′(3′)-AMP to be 15.4×10^3 at λ_{max} (259 mμ). [c] See Experimental Section of preceding paper for the procedure of determination (Kondo *et al.*, 1970).

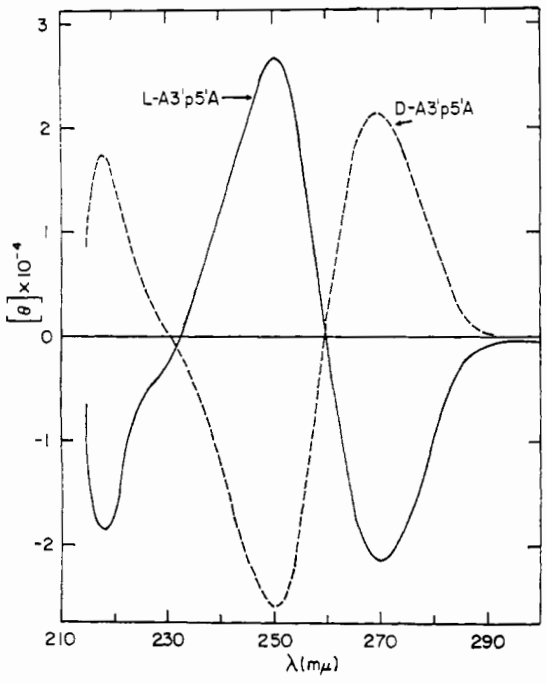

FIGURE 2: Circular dichroic spectra of L-$A_{3'}p_{5'}A$ (——) and D-$A_{3'}p_{5'}A$ (-----) in 0.05 M $NaClO_4$ (pH 7.0) at 21.5°.

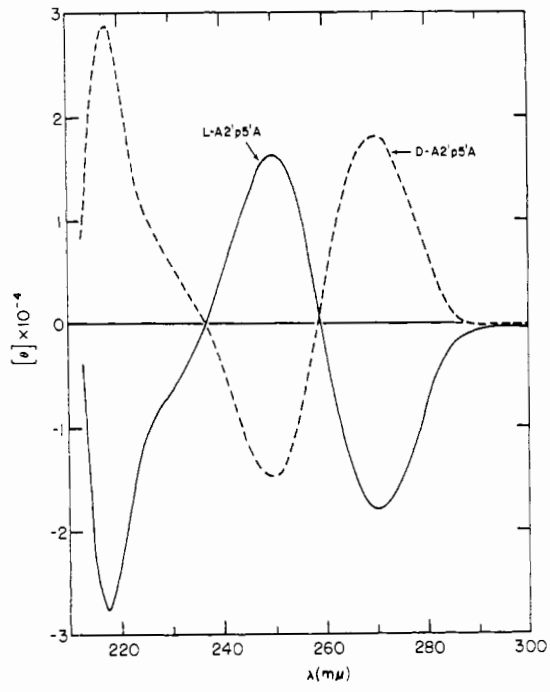

FIGURE 4: Circular dichroic spectra of L-$A_{2'}p_{5'}A$ (——) and D-$A_{2'}p_{5'}A$ (-----) in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$ at 20.5°.

smaller as the temperature increases. At present the significance of this difference is not certain even though the same result has been obtained in two separate measurements on two separate samples. On the whole, the circular dichroism spectrum of L-$A_{2'}p_{5'}A$ (Figure 4) together with the proton magnetic resonance results (Table II) again suggests that the conformation of L-$A_{2'}p_{5'}A$ is the mirror image of that of D-$A_{2'}p_{5'}A$. The circular dichroism spectra of the D-ApA's presented here has been extended about 15 mμ farther into the ultraviolet region (215 mμ) than those presented in the preceding paper (Kondo *et al.*, 1970). A discussion on the spectra of the D-ApA's has also been given in the preceding paper.

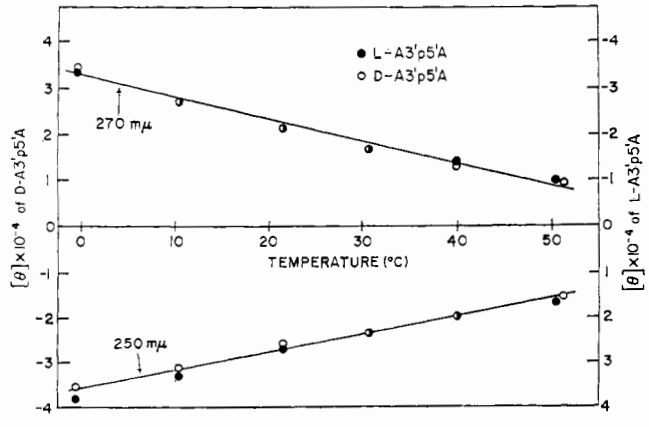

FIGURE 3: Temperature dependence of [θ] at 250 and 270 mμ of L-$A_{3'}p_{5'}A$ (●—●) and D-$A_{3'}p_{5'}A$ (○—○) in 0.05 M $NaClO_4$ (pH 7.0).

SUSCEPTIBILITY TO SPLEEN AND VENOM PHOSPHODIESTERASE. L-ApA's and D-ApA's were incubated with spleen and/or venom phosphodiesterase under the conditions described in Methods. After incubation, the mixture was analyzed by paper chromatography in solvent A which can well separate adenosine, ApA, and AMP. Results are summarized in Table IV. Under the conditions employed, L-$A_{3'}p_{5'}A$ was not hydrolyzed at all in 6 hr by spleen phosphodiesterase, which is an exonuclease specific for 3′–5′-phosphodiester (Hilmoe, 1960), while D-$A_{3'}p_{5'}A$ was hydrolyzed completely to D-3′-AMP and D-adenosine in 10 min with the same amount of enzyme. (This commercial enzyme preparation was contaminated with adenosine deaminase activity, so actually inosine was observed instead of adenosine.) Both L-$A_{2'}p_{5'}A$ and D-$A_{2'}p_{5'}A$ were completely resistant to spleen phosphodiesterase in 6 hr as expected, since this enzyme does not attack the 2′–5′-phosphodiester linkage. On the

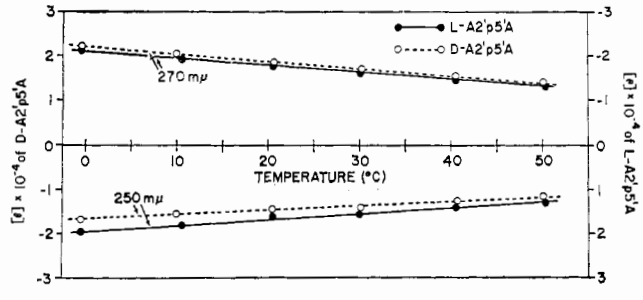

FIGURE 5: Temperature dependence of [θ] at 250 and 270 mμ of L-$A_{2'}p_{5'}A$ (●—●) and D-$A_{2'}p_{5'}A$ (○-----○) in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$.

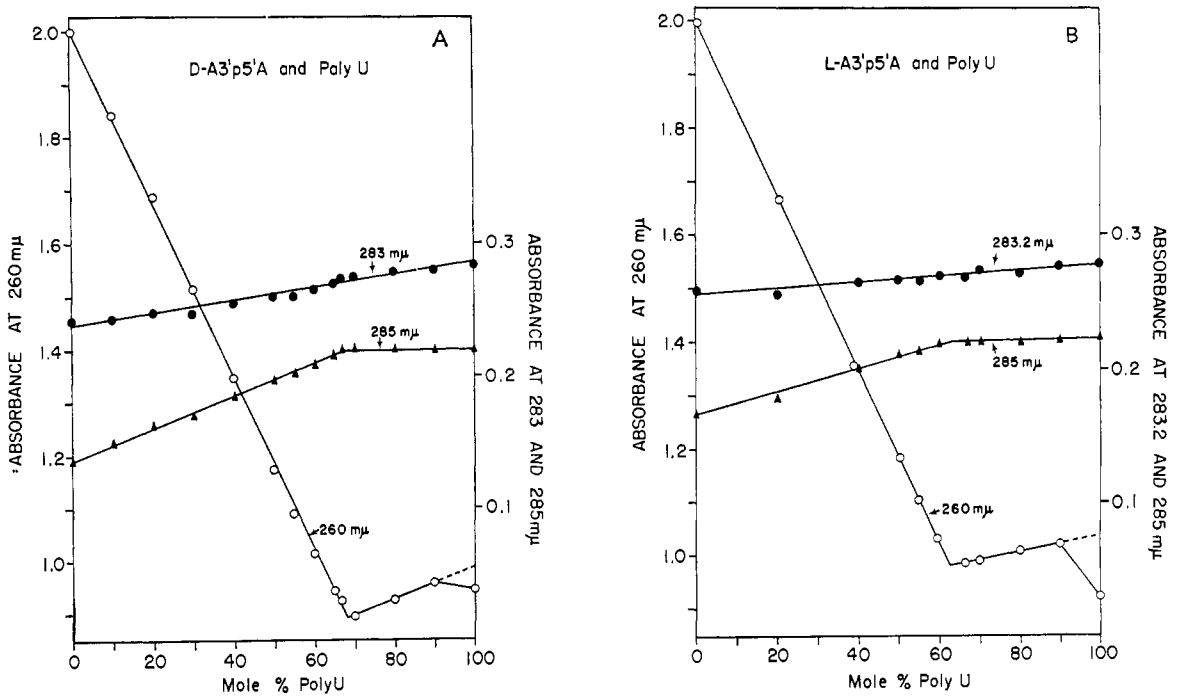

FIGURE 6: Mixing curves. (A) For D-$A_{3'}p_{5'}A$ and D-poly U at 260 mμ (○—○), 283 mμ (●—●), and 285 mμ (▲—▲). The experiments were carried out at 0° in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$. The total nucleotide concentration was 0.15 mM. (B) For L-$A_{3'}p_{5'}A$ and D-poly U at 260 mμ (○—○), 283.2 mμ (●—●), and 285 mμ (▲—▲). The experiments were carried out at −4° in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$. The total nucleotide concentration was 0.15 mM.

other hand, venom phosphodiesterase is an exonuclease which cleaves both 3′–5′- and 2′–5′-phosphodiester linkages (Sulston *et al.*, 1968). L-$A_{3'}p_{5'}A$ was degraded by this enzyme to L-5′-AMP and L-A to an extent of 11–14% in 6 hr, while D-$A_{3'}p_{5'}A$ was completely hydrolyzed to D-5′-AMP and D-A in 7 min with the same amount of enzyme. Thus, the hydrolysis rate of L-$A_{3'}p_{5'}A$ is about 400 times slower than that of D-$A_{3'}p_{5'}A$. No hydrolysis of L-$A_{2'}p_{5'}A$ occurred in 6 hr with venom phosphodiesterase, while complete hydrolysis of D-$A_{2'}p_{5'}A$ took place in 10 min. We may conclude from these observations that the L-ApA's are extremely resistant to the spleen and venom phosphodiesterase. It would be of interest to examine whether the L-ApA's could serve as inhibitors for these enzymes. For this purpose D-$A_{3'}p_{5'}A$ (or D-$A_{2'}p_{5'}A$) was incubated with spleen (or venom) phosphodiesterase both in the presence and absence of L-$A_{3'}p_{5'}A$ (or L-$A_{2'}p_{5'}A$), and the extent of hydrolysis was determined after 5 min and after 7 hr. Results are presented in Table V. As shown in the table, when the reaction mixtures were incubated for 5 min or

TABLE IV: Susceptibility of L-ApA's and D-ApA's to Spleen and Venom Phosphodiesterase.[a]

Compound	Spleen Phosphodiesterase	Venom Phosphodiesterase
L-$A_{3'}p_{5'}A$	No hydrolysis in 6 hr	11–14% hydrolysis in 6 hr
D-$A_{3'}p_{5'}A$	Complete hydrolysis in 10 min	Complete hydrolysis in 7 min
L-$A_{2'}p_{5'}A$	No hydrolysis in 6 hr	No hydrolysis in 6 hr
D-$A_{2'}p_{5'}A$	No hydrolysis in 6 hr[b]	Complete hydrolysis in 10 min

[a] Conditions for enzyme reaction are given in the Experimental Section. [b] This compound is known to be resistant to this enzyme (see text).

TABLE V: Influence of L-ApA's on the Hydrolysis of D-ApA's by Spleen and Venom Phosphodiesterase.[a]

Enzyme	ApA's	% D-ApA Hydrolyzed[b] 5 min	7 hr
Spleen phosphodiesterase	D-$A_{3'}p_{5'}A$ + L-$A_{3'}p_{5'}A$	84	104[c]
	D-$A_{3'}p_{5'}A$	83	100
Venom phosphodiesterase	D-$A_{2'}p_{5'}A$ + L-$A_{2'}p_{5'}A$	88	98[c]
	D-$A_{2'}p_{5'}A$	89	100

[a] The quantity of ApA used in these experiments was 0.2 μmole for each L and D compound. Reaction conditions are the same as those in Table IV. [b] Calculations are based on the fact that L-ApA's are completely resistant to these enzymes under the conditions used. [c] These values are considered to represent the experimental errors.

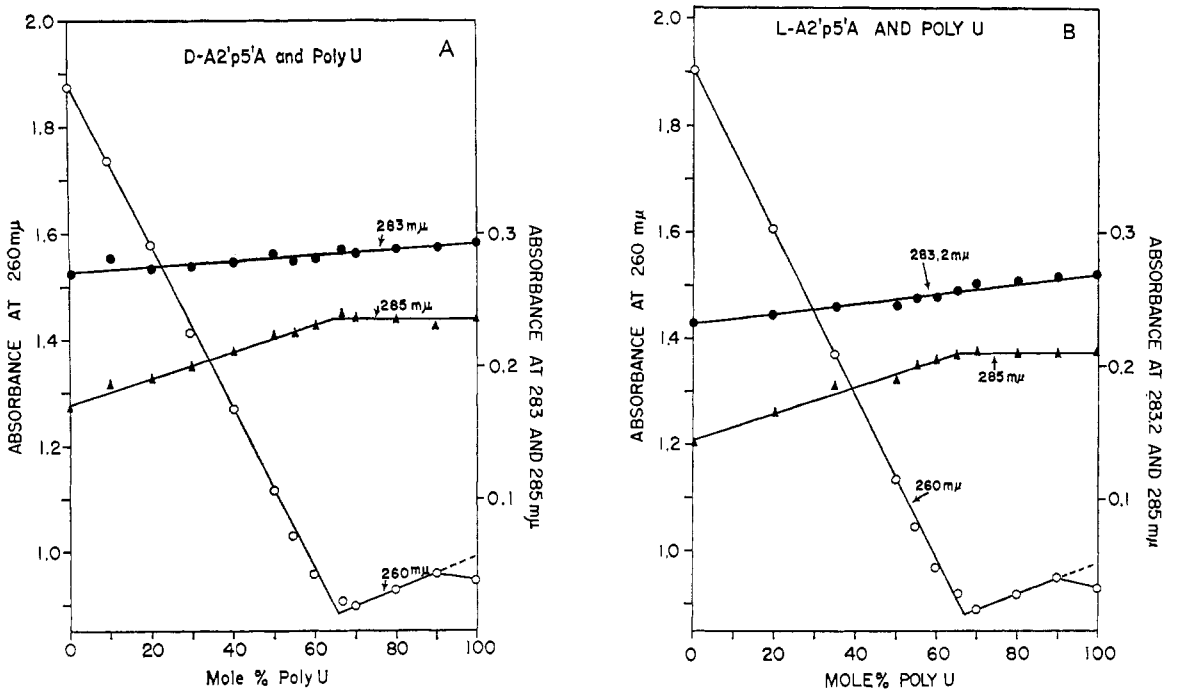

FIGURE 7: Mixing curves. (A) For D-$A_2'p_5'A$ and D-poly U at 260 mμ (○—○), 283 mμ (●—●), and 285 mμ (▲—▲). The experiments were carried out at −3° in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$. The total nucleotide concentration was 0.15 mM. (B) For L-$A_2'p_5'A$ and D-poly U at 260 mμ (○—○), 283.2 mμ (●—●), and 285 mμ (▲—▲). The experiments were carried out at −4° in 0.01 M Tris (pH 7.5)–0.01 $MgCl_2$. The total nucleotide concentration was 0.15 mM.

for 7 hr, the presence of L-ApA's did not affect the extent (rate) of hydrolysis of D-ApA's by these enzymes at all. It is concluded from these data that L-ApA's do not serve as inhibitors under the present conditions for these phosphodiesterases.

Interaction of L-*ApA's and* D-*ApA's with* D-*Poly U.* As shown in the following section, both L-$A_3'p_5'A$ and L-$A_2'p_5'A$ can interact with D-poly U to form a complex with 1A:2 U stoichiometry. While the main interest in this study is to investigate the interaction of the L-ApA's with the D-poly U, the comparison with the interaction of D-ApA's with D-poly U is a very informative approach.

STOICHIOMETRY. The ultraviolet absorption properties in mixtures of continuous variation were used to determine the stoichiometry of the interaction between the ApA's and poly U (Job, 1928; Felsenfeld and Rich, 1957). The mixing experiments were done in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$ at −4 to 0°, a condition in which the interaction is complete in 1 hr except in the case of L-$A_3'p_5'A$ where it is virtually complete. The mixing curve for D-$A_3'p_5'A$ and D-poly U at 0° and that for L-$A_3'p_5'A$ and D-poly U at −4° are very similar to each other as shown in Figure 6A,-B. For the 260-mμ absorption data in these plots, there is a discontinuity around 90 mole % of poly U. This is due to the fact that under this experimental condition, poly U by itself (at 100 mole %) forms a helical complex which is considerably hypochromic. In the presence of a small amount of ApA (say, 10 mole %) which presumably interacts with the poly U in a dispersive mode, the poly U is prevented from forming the helical complex with itself and thus loses the hypochromicity. This situation involving poly U and $A(pA)_6$ has been well studied and analyzed in a recent paper from our laboratory (Pitha and Ts'o, 1969). Other than this discontinuity at 90 mole % of poly U, the 260-mμ data show an intersection in parts A and B of Figure 6 around the 65–67 mole % of poly U. This observation clearly supports the conclusion that the stoichiometry of both ApA–poly U complexes is 1A:2U. However, the validity of this interpretation is based on the implicit assumption that there is only one type of complex existing in the mixture (Felsenfeld and Rich, 1957; Pitha and Ts'o, 1969). This situation is especially vulnerable, since in the neighborhood of 260 mμ, the extinction coefficient of poly A·poly U complex is the same as that of the mixture (0.5poly A·poly U + 0.5poly A) (Felsenfeld and Rich, 1957; Stevens and Felsenfeld, 1964). In order to ascertain that there is only one type of complex existing in the mixture, spectral data at other wavelengths were also examined. An isochromic point, which is defined as a wavelength at which the absorbance of the complex is equal to the sum of the absorbance of the noninteracting components added proportionately (Blake *et al.*, 1967), was found at 283 mμ for both 1A:2U mixtures of D-ApA and poly U, and of L-ApA and poly U. At this wavelength, the absorbance of the ApA·2poly U complex is equal to the sum of one-third absorbance of ApA and two-thirds absorbance of poly U. In other words, the difference in absorbances between the complex and the noninteracting components is zero. The absorption data obtained at this wavelength throughout the mixing experiment were found to be on a straight line in both cases (Figure 6A,B). This observation supports the conclusion that there is only one type of complex existing in the mixture. Formation of a 1A:1U complex for example, is likely to introduce changes of slope (Blake *et al.*, 1967) since the absorption of the 1A:1U complex

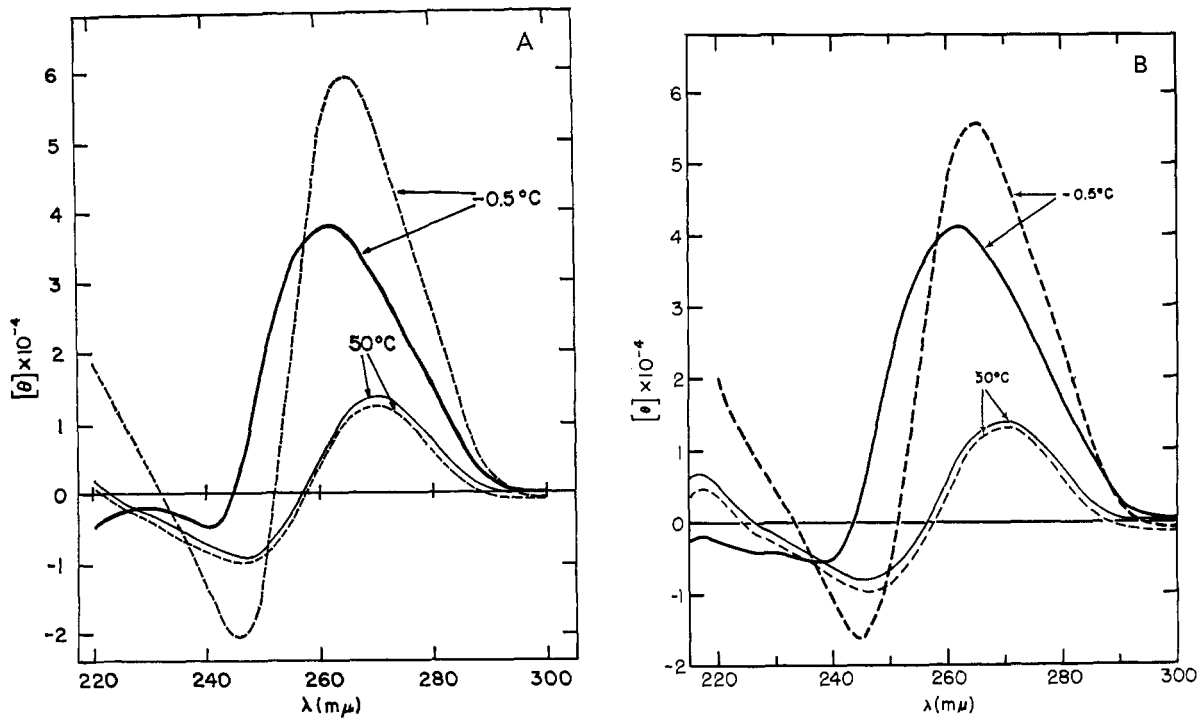

FIGURE 8: Circular dichroic spectra. (A) Of D-$A_{3'}p_{5'}A$·2D-poly U at −0.5 (——) and 50° (——) in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$. Addition spectra at −0.5 (-----) and 50° (-----) are also shown. (B) Of L-$A_{3'}p_{5'}A$·2D-poly U at −0.5 (——) and 50° (——) in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$. Addition spectra at −0.5 (-----) and 50° (-----) are also shown.

at this wavelength is likely to be different from that of the 1A:2U complex. At the wavelength of 285 mμ, the mixtures from 67 mole % to 100 mole % poly U were found to have the same absorption (Figure 6A,B). This wavelength is, therefore, the isosbestic point between the spectra of poly U and the complex of ApA·2poly U. The single intersection at 65–67 mole % poly U observed for the absorption data at 285 mμ is another support for the formation of only the 1A:2U complex. (There are other isosbestic points between the spectra of ApA's and the complex of ApA·2poly U at 282.5 and 283 mμ for D-ApA and L-ApA, respectively. These wavelengths are, however, too close to the isochromic wavelengths of the corresponding complexes, and therefore are not useful experimentally to show another intersection.) Absorption data at 280 mμ (not shown in Figure 6A or B) again give an intersection at 65–67 mole % poly U in the mixing plot. When all these ultraviolet spectral data from the mixing experiments are taken together, it is a strong indication that there is only one complex existing in the mixture which has the stoichiometry of 1A:2U. This conclusion is in agreement with the results of Cantor and Chin (1968). Based on the circular dichroism studies of 1:1 and 1:2 mixtures of $(Ap)_{0-4}A$ and poly U in 0.04 M phosphate buffer (pH 7.3)–0.5 M NaCl, they concluded that all these mixtures contain only the 1A:2U complex. Lipsett *et al.*, in an early study (1961), reported that ApA forms a 1:1 complex with poly U in 0.001 M $MgCl_2$. No experimental details were described, however. Our present observations in 0.01 M $MgCl_2$ are not in agreement with this report.

Figure 7A,B presents the ultraviolet spectral data of the mixtures of D-$A_{2'}p_{5'}A$ and D-poly U, and of L-$A_{2'}p_{5'}A$ and D-poly U in the continuous variation experiments performed at −3 and −4°, respectively. Again, the analyses of the spectral properties of these mixing experiments are identical with those discussed above for the experiments of mixing the $A_{3'}p_{5'}A$'s with poly U. The results observed in Figure 7A,B are the same as those in Figure 6A,B. Therefore, D-$A_{2'}p_{5'}A$ and L-$A_{2'}p_{5'}A$ also form only one type of complex with D-poly U in the mixture and the stoichiometry of the complex is 1A to 2U.

CIRCULAR DICHROISM OF 1A·2U COMPLEXES. Circular dichroic spectra of D-$A_{3'}p_{5'}A$·2D-poly U in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$ at −0.5 and 50° are shown in Figure 8A together with the addition spectra which are obtained from the sum of the spectra of the two separate components at both temperatures. At −0.5°, the measured spectrum of the complex is quite different from the addition spectrum. The λ_{max} is shifted from 265 to 262 mμ, the λ_{min} is shifted from 245.5 to 240 mμ, and both $[\theta_{max}]$ and $[\theta_{min}]$ are decreased substantially by the formation of the complex. This observation indicates the existence of the complex.

The spectral positions of λ_{max} and λ_{min} reported here are very close to those reported by Cantor and Chin (1968); however, the $[\theta_{max}]$ value (3.80×10^4) observed in 0.01 M $MgCl_2$ at −0.5° in the present experiment is higher than the $[\theta_{max}]$ value (3.06×10^4) observed in 0.5 M NaCl at 3° in their experiment. At 50°, on the other hand, the measured spectra are quite similar to the addition spectra showing that there is no (or very little) complex formation at this temperature.

Figure 8B shows the measured circular dichroism spectra of L-$A_{3'}p_{5'}A$·2D-poly U at −0.5 and 50° together with the addition spectra. At −0.5°, the measured spectrum of the complex is quite different from the addition spectrum as

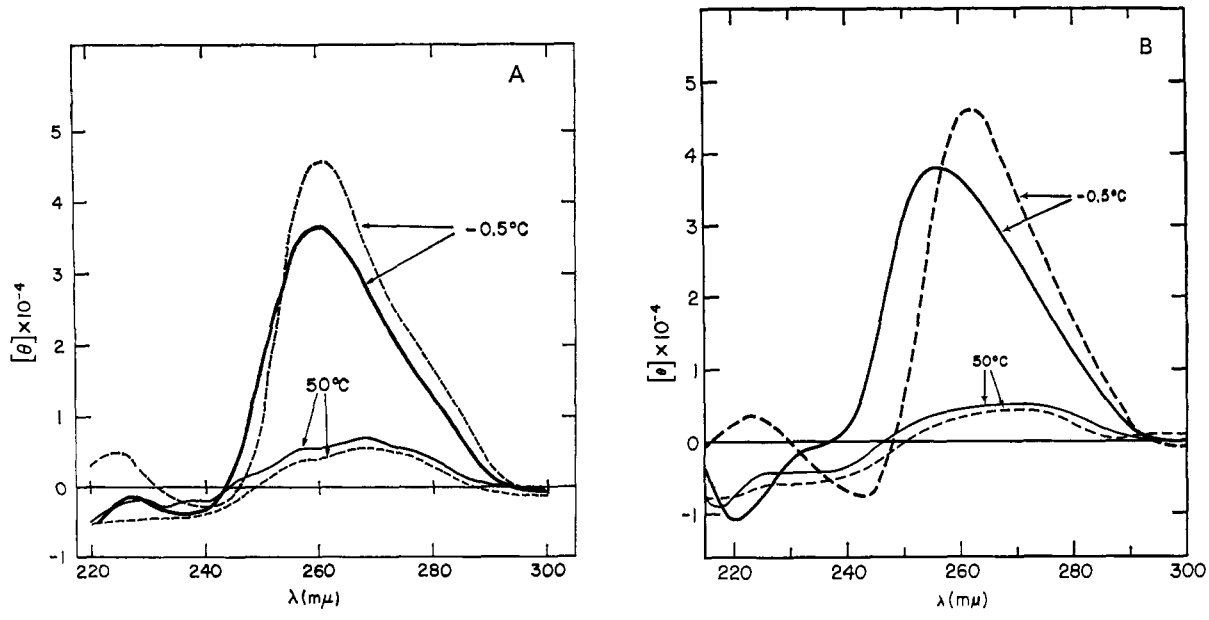

FIGURE 9: Circular dichroic spectra. (A) Of D-$A_{2'}p_{5'}A$·2D-poly U at −0.5 (——) and 50° (——) in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$. Addition spectra at −0.5 (-----) and 50° (-----) are also shown. (B) Of L-$A_{2'}p_{5'}A$·2D-poly U at −0.5 (——) and 50° (——) in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$. Addition spectra at −0.5 (-----) and 50° (-----) are also shown.

indicated by the hypsochromic shift of λ_{max} and λ_{min} and the reduction of the $[\theta_{max}]$ and $[\theta_{min}]$. This comparison of the circular dichroism spectra indicates the formation of complex between L-$A_{3'}p_{5'}A$ and poly U which is in agreement with the conclusion drawn from the mixing experiments reported above. At 50°, the observed spectrum is similar to the addition spectrum, indicating that at this elevated temperature, the complex of L-$A_{3'}p_{5'}A$·2D-poly U has been dissociated.

Figure 9A,B shows the measured circular dichroism spectra and the addition spectra of D-$A_{2'}p_{5'}A$·2D-poly U complex and of L-$A_{2'}p_{5'}A$·2D-poly U complex, respectively. At −0.5°, the measured spectra are quite different from the addition spectrum which indicates the formation of the complex as discussed above for the interaction of $A_{3'}p_{5'}A$'s with poly U. At 50°, the observed spectra are similar to the addition spectra which shows that the complex of $A_{2'}p_{5'}A$·2D-poly U has been dissociated at this elevated temperature.

The comparison of the circular dichroism spectra measured at −0.5° of the complex of D-$A_{3'}p_{5'}A$·2D-poly U and the complex of L-$A_{3'}p_{5'}A$·2D-poly U is presented in Figure 10A. As shown, the spectra of these two complexes are very similar. It should be noted that Cantor and Chin (1968) have shown that the circular dichroism spectrum of D-$A_{3'}p_{5'}A$·2D-poly U complex is almost identical with that of D-poly A·2D-poly U complex. Thus, the circular dichroism spectral studies suggest that the overall conformation of the complex of L-$A_{3'}p_{5'}A$·2D-poly U resembles closely those of the D-$A_{3'}p_{5'}A$·2D-poly U and of the D-poly A·2D-poly U. The comparison of the circular dichroism spectra, measured at −0.5°, of the complex of D-$A_{2'}p_{5'}A$·2D-poly U and the complex of L-$A_{2'}p_{5'}A$·2D-poly U is presented in Figure 10B. Again, the overall features of the circular dichroism spectra of these two complexes are very similar in the region above 240 mμ, even though the difference between these two spectra is larger than the difference between the two spectra presented in Figure 10A, especially in the region below 240 mμ. Actually, the circular dichroism spectra of the four complexes presented in Figure 10A,B closely resemble each other, except that in the region below 240 mμ the spectrum of L-$A_{2'}p_{5'}A$·2D-poly U complex appears to be unlike the others. These comparative circular dichroism studies strongly imply that the helical conformations of these four ApA·2D-poly U complexes share many major common features.

THERMAL STABILITY OF THE COMPLEXES. In 0.01 M $MgCl_2$–0.01 M Tris (pH 7.5) the complex of D-$A_{3'}p_{5'}A$·2D-poly U has a relatively sharp thermal transition profile as indicated by both the ultraviolet (Figure 11A) and circular dichroism (Figure 11B) measurements with a T_m value of 13.6–13.8°. The L-$A_{3'}p_{5'}A$·2D-poly U also has a moderately sharp transition profile, but the T_m value (5.6–5.8°) is about 8° lower (Figure 11A,B). Poly U itself has about the same T_m (5.5°) in the same solvent. However, the transition observed in the ultraviolet absorption (Figure 11A) of the mixture L-$A_{3'}p_{5'}A$ and D-poly U at 5.6° is definitely due to the A–U complex because of the large hypochromicity observed for this transition. The addition transition curve, which is the sum of the absorption profile of the same concentration of ApA and poly U measured separately over a −5 to 30° temperature range, is also presented in Figure 11A. The comparison of the measured transition profile and the addition transition profile clearly indicates that the transition observed is that of the L-$A_{3'}p_{5'}A$·2D-poly U complex. The transition at 5.8° observed in Figure 11B from the circular dichroism measurement is also that of the A–U complex and not that of the poly U self-complex. As discussed in the above section, the circular dichroism spectrum of the 1:2 mixture of L-$A_{3'}p_{5'}A$ and D-poly U measured at −0.5° is quite different from the addition spectrum obtained from

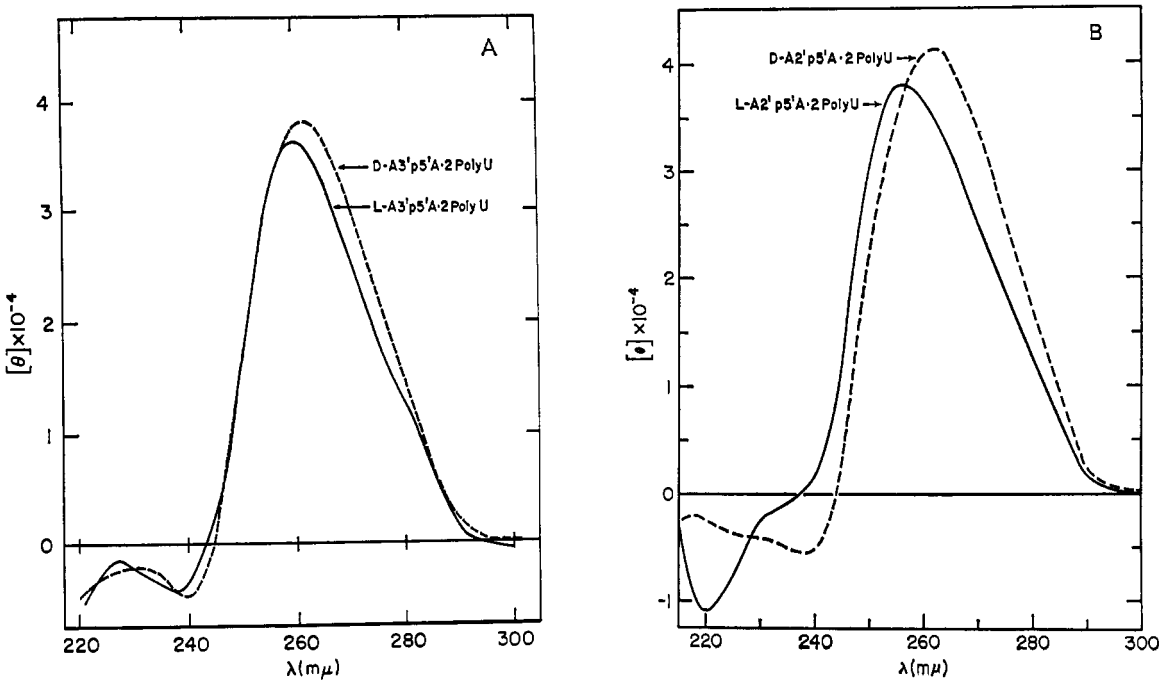

FIGURE 10: Circular dichroic spectra. (A) Of D-$A_{3'}p_{5'}A$·2D-poly U (-----) and L-$A_{3'}p_{5'}A$·2D-poly U (——) at −0.5° in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$. (B) Of D-$A_{2'}p_{5'}A$·2D-poly U (-----) and L-$A_{2'}p_{5'}A$·2D-poly U (——) at −0.5° in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$.

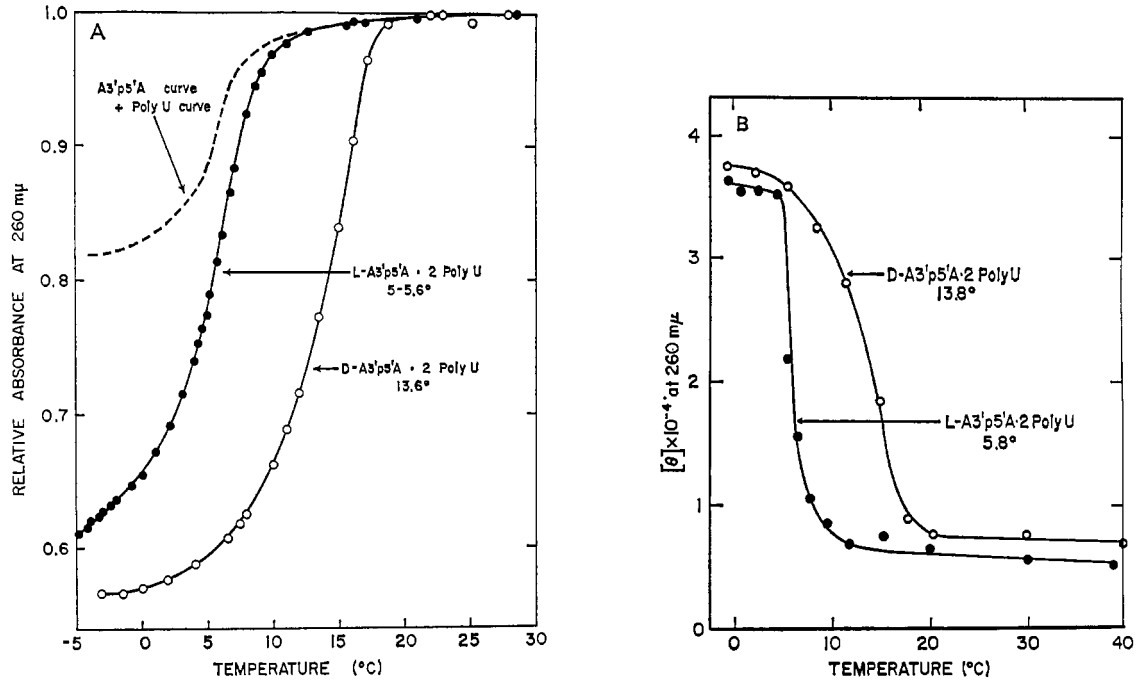

FIGURE 11: The melting of D-$A_{3'}p_{5'}A$·2D-poly U (O—O) and L-$A_{3'}p_{5'}A$·2D-poly U (●—●). (A) Followed by ultraviolet absorbance at 260 mμ in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$. Total nucleotide concentration is 0.15 mM. The addition transition curve (-----) is also shown. Relative absorbance is expressed as the ratio of the absorbance at a given temperature to the absorbance plateau of the melted complex at high temperature. (B) Followed by circular dichroism at 260 mμ in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$. Total nucleotide concentration is 0.15 mM.

the sum of the absorption of the separate components. The hypochromicity of the melting of the L-$A_{3'}p_{5'}A$·2D-poly U complex appears to be less than that of the D-$A_{3'}p_{5'}A$·2D-poly U complex. At present, it is not certain whether this is entirely due to the incompleteness of the complex formation even at −5°, which would affect the computation of the hypochromicity, or whether the intrinsic property of the L-$A_{3'}p_{5'}A$·2D-poly U complex also contributes to the decreased hypochromicity.

Figure 12A,Bs show the transition profiles of the D-$A_{2'}p_{5'}A$·

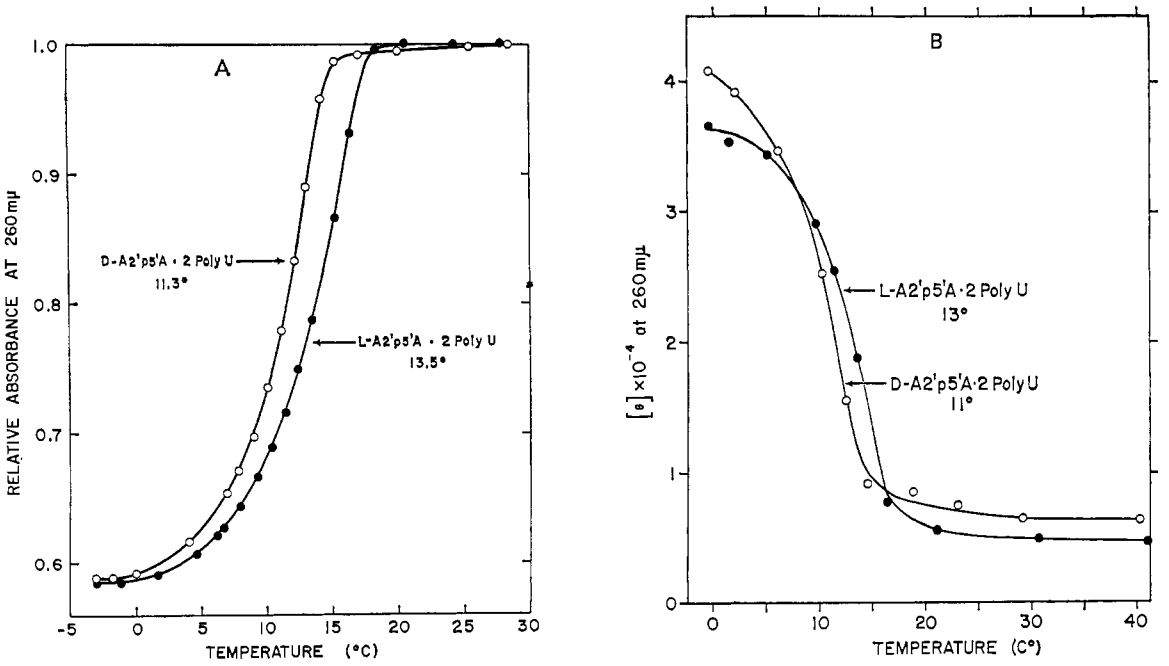

FIGURE 12: The melting of D-$A_{2'}p_{5'}A$·2D-poly U (O—O) and L-$A_{2'}p_{5'}A$·2D-poly U (●—●). (A) Followed by ultraviolet absorbance at 260 mμ in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$. Total nucleotide concentration is 0.15 mM. Relative absorbance is expressed as the ratio of the absorbance at a given temperature to the absorbance plateau of the melted complex at high temperature. (B) Followed by circular dichroism at 260 mμ in 0.01 M Tris (pH 7.5)–0.01 M $MgCl_2$. Total nucleotide concentration is 0.15 mM.

2D-poly U complex and of the L-$A_{2'}p_{5'}A$·2D-poly U complex in ultraviolet measurement and in circular dichroism measurement, respectively. These two complexes have very similar T_m values and transition profiles. In fact, the T_m (13–13.5°) of the L-$A_{2'}p_{5'}A$·2D-poly U complex is 2° higher than the T_m (11–11.3°) of the D-$A_{2'}p_{5'}A$·2D-poly U complex. The T_m values of these four complexes in 0.01 M $MgCl_2$–0.01 M Tris (pH 7.5) are summarized in Table VI. The T_m values (~13°) of the D-$A_{3'}p_{5'}A$·2D-poly U complex and of the L-$A_{2'}p_{5'}A$·2D-poly U are about the same and are slightly higher than the T_m (~11°) of the D-$A_{2'}p_{5'}A$·2D-poly U, while the T_m of the complex L-$A_{3'}p_{5'}A$·2D-poly U (~5.5°) is the lowest. The T_m values reported here are comparable with those reported by Michelson and Monny (1967) for D-$A_{3'}p_{5'}A$·2D-poly U complex (13.5°) and for D-$A_{2'}p_{5'}A$·2D-poly U complex (10.6°). Their measurements were done in 0.1 M NaCl–0.01 M $MgCl_2$–0.05 M sodium cacodylate (pH 7.5) and with twice as much nucleotide as our experiments.

Discussion

In the preceding paper (Kondo *et al.*, 1970), the influence of the position of the phosphodiester linkages, *i.e.*, 2′–5′, 3′–5′, and 5′–5′, on the conformation of the dinucleoside monophosphate has been compared concurrently by three physicochemical methods. The bases in these three types of dimers were shown to have different geometrical relationships. In the present paper, comparison is made between dimers joined by the same type of phosphodiester linkage which, however, are built with enantiomeric pentose units, *i.e.*, D-ribose and L-ribose. Thus, L-$A_{3'}p_{5'}A$ is compared with D-$A_{3'}p_{5'}A$ and L-$A_{2'}p_{5'}A$ is compared with D-$A_{2'}p_{5'}A$. In the comparison between these enantiomers, certain properties are observed to be the same as expected. For instance, the ultraviolet absorption spectra (Table III), the proton magnetic resonance spectra (Table II), the R_F values in silica gel thin-layer chromatography, mobilities in electrophoresis (Table I), etc., are the same for each of the members in the pair. In fact, these similarities are adopted as part of the structural proof for the synthetic L-ApA(s). As for the circular dichroism studies, the spectra of the L-ApA(s) are opposite in sign but the same (or nearly the same within experimental error) in magnitude as those of the corresponding D-ApA(s) (Figures 2–5). All these observations confirm the expectation that the conformation of the

TABLE VI: Melting Temperature, T_m, of ApA·2Poly U Complexes as Measured by Ultraviolet Absorption and Circular Dichroism.[a]

Complex	T_m (°C)	
	Ultraviolet	Circular Dichroism
L-$A_{3'}p_{5'}A$·2poly U	5.6	5.8
D-$A_{3'}p_{5'}A$·2poly U	13.6	13.8
L-$A_{2'}p_{5'}A$·2poly U	13.5	13[b]
D-$A_{2'}p_{5'}A$·2poly U	11.3	11[b]

[a] In 0.01 M Tris (pH 7.5), 0.01 M $MgCl_2$, ApA 5×10^{-5} M, poly U 1×10^{-4} M. [b] T_m measurement is less accurate. See Figures 11B and 12B.

L-ApA(s) is the mirror image of that of the corresponding D-ApA(s). Therefore, while the conformation of the D dimers is an *anti,anti*, right-handed stack (Kondo *et al.*, 1970), the conformation of the L dimers is an *anti,anti*, left-handed stack.

The first interesting difference observed between the enantiomers is that the L-ApA(s) are either completely or extremely resistant to hydrolytic attack of spleen and venom phosphodiesterase (Table IV). In addition, these L dimers are not inhibitory to the action of these enzymes under the present conditions. The observations suggest that the interaction between these enzymes and the dinucleoside monophosphate involves at least a three-point spatial relationship. This type of geometrical relationship involving three points in space endows the enzyme with the specificity to distinguish between the enantiomers. Similar results have been obtained by Holý and Šorm (1969) who reported that the 2′,3′-cyclic phosphates of various L-ribonucleosides are resistant to pancreatic RNase A (EC 2.7.7.16), RNase T1 (EC 2.7.7.26), and RNase T2 (EC 2.7.7.24). They also found that D-$G_{3'}p_{5'}$L-A and D-$G_{3'}p_{5'}$L-C are resistant to snake venom phosphodiesterase (EC 3.1.4.1). Shimizu *et al.* (1967) found that L-5′-AMP and L-5′-IMP are resistant to snake venom 5′-nucleotidase (EC 3.1.3.5) and Chassy and Suhadolnik (1967) found that β-L-2′-deoxyadenosine is resistant to the adenosine deaminase (EC 3.5.4.4) from calf intestinal mucosa. In all of the examples mentioned above, L compounds are resistant to enzymes. On the other hand, certain L compounds have been reported to serve as substrates for some enzymes. *E. coli* alkaline phosphatase (EC 3.1.3.1) dephosphorylated 2′(3′)-phosphates of various L-nucleosides including L-uridine, L-cytidine, L-adenosine, L-guanosine, etc. (Holý and Šorm, 1969). Phosphotransferases both from carrot and human prostate phosphorylated L-uridine (Wu and Chargaff, 1969). The adenosine deaminase (EC 3.5.4.4) from takadiastase deaminated L-adenosine, although at a very slow rate (Minato *et al.*, 1965). The adenosine deaminase from calf intestinal mucosa was shown to act on α-L-2′-deoxyadenosine (Chassy and Suhadolnik, 1967). Thus, the above studies show that the three-dimensional stereochemical arrangement between the base, the sugar, and possibly the phosphate group of the substrate might be important for the activities of certain enzymes, and not for other enzymes. Therefore, comparative studies of enzyme action on enantiomers of nucleosides and nucleotides can be useful in elucidating the mode of action of those enzymes.

The second important observation is on the interaction of D-poly U with the enantiomeric ApA(s). It is certain that the D-poly U can form a complex with L-ApA(s) having the same stoichiometry (2U:1A) as the complex of D-poly U with D-ApA(s). In addition, the circular dichroism spectra of the four poly U–ApA complexes are very similar to each other (Figure 10); in particular, the circular dichroism spectra of D-$A_{3'}p_{5'}$A·2D-poly U and L-$A_{3'}p_{5'}$A·2D-poly U are nearly identical (Figure 10A). This observation suggests that the overall conformations of these helical complexes are very similar. These complexes, however, have different T_m values (5–14°) in the same Mg^{2+} buffer solution, indicating small differences in their stabilities with respect to the temperature perturbation. We shall examine the thermodynamics of the formation of these complexes, drawing support from the knowledge obtained from calorimetric studies on the A·U complexes. From this analysis, we are able to investigate the question of chirality of these dinucleoside monophosphates in a quantitative manner.

The thermodynamics of the complex formation between ApA and poly U is analyzed in accordance with the following formulation and equations

$$\text{ApA(free)} + 2\text{poly U} \rightleftharpoons \text{ApA(stacked)}\cdot 2\text{poly U} \quad \text{(I)}$$

$$\text{ApA(free)} \rightleftharpoons \text{ApA(stacked)} \quad \text{(II)}$$

$$\text{ApA(stacked)} + 2\text{poly U} \rightleftharpoons \text{ApA(stacked)}\cdot 2\text{poly U} \quad \text{(III)}$$

Process I represents the overall process of the formation of the ApA·2poly U complex. For the present purpose, the change of states of ApA in the process of complex formation is divided into two steps. In the first step (process II) the ApA in the "free" state is changed into a "stacked" state. In the free state, ApA is not complexed and assumes a conformation determined by the energies of the isolated molecule in solution, while in the stacked state, ApA is still not complexed but assumes a conformation identical with that in the ApA·2poly U complex. Thus, the ApA in the stacked state is ready to combine with the poly U (process III) to form the helical complex without any further change in conformation. Therefore, process I is the sum of process II and process III and the standard free-energy change in process I, ΔF_I, is the sum of ΔF_{II} and ΔF_{III}, the standard free-energy change in processes II and III, respectively

$$\Delta F_I = \Delta F_{II} + \Delta F_{III} \quad (1)$$

We assign ΔF_I^D to be the standard free-energy change in process I for the formation of D-$A_{3'}p_{5'}$A·2D-poly U complex, ΔF_I^L to be the standard free-energy change in process I for the formation of L-$A_{3'}p_{5'}$A·2D-poly U complex, and ΔF_I^{D-L} to be the difference between ΔF_I^D and ΔF_I^L as

$$\Delta F_I^D = \Delta F_I^L + \Delta F_I^{D-L} \quad (2)$$

At the melting temperature of D-$A_{3'}p_{5'}$A·2D-poly U complex, T_m^D

$$\Delta F_I^D = \Delta H_I^D - T_m^D \Delta S_I^D = 0 \quad (3)$$

where ΔH and ΔS are the corresponding enthalpy and entropy changes. Similarly, at the melting temperature of L-$A_{3'}p_{5'}$A·2D-poly U complex, T_m^L

$$\Delta F_I^L = \Delta H_I^D - T_m^L \Delta S_I^D - \Delta F_I^{D-L}{}_{(T_m L)} = 0 \quad (4)$$

In formulating eq 4, we have implicitly assumed that ΔH_I^D is constant with respect to temperature variation (or $\Delta C_p = 0$).

This assumption is essentially valid for the following reasons. (1) The difference in temperature between T_m^D and T_m^L is small; (2) in the complex formation of the polymers, *i.e.*, poly A·2poly U, the ΔH is dependent on temperature to a certain extent ($\Delta C_p = -63 \pm 84$ cal/mole-deg, sodium salt; -116 ± 39 cal/mole-deg, potassium salt) and this phenomenon is explained on the basis of the effect

of temperature and salt on the poly A conformation (Krakauer and Sturtevant, 1968; Rawitscher *et al.*, 1963; Ross and Scruggs, 1965). The effect of temperature on the conformation of poly A is certainly larger than the effect of temperature on the conformation of ApA. Subtracting eq 3 from eq 4, we obtain

$$\Delta F_{\mathrm{I}}{}^{\mathrm{D-L}}{}_{(T_{\mathrm{m}}\mathrm{L})} = (T_{\mathrm{m}}{}^{\mathrm{D}} - T_{\mathrm{m}}{}^{\mathrm{L}})\Delta S_{\mathrm{I}}{}^{\mathrm{D}} \quad (5)$$

The values for $T_{\mathrm{m}}{}^{\mathrm{L}}$ and $T_{\mathrm{m}}{}^{\mathrm{D}}$ can be obtained from Table VI. The $\Delta S_{\mathrm{I}}{}^{\mathrm{D}}$ values for the adenosine·2poly U complex formation and for the poly A·2poly U complex formation have been computed from the ΔH determined calorimetrically and from the relationship $\Delta H/\Delta S = T_{\mathrm{m}}$. The $\Delta S_{\mathrm{I}}{}^{\mathrm{D}}$ value for the formation of the monomer·polymer complex was found to be -42 cal/mole-deg (Scruggs and Ross, 1970) and the $\Delta S_{\mathrm{I}}{}^{\mathrm{D}}$ value for the formation of the polymer·polymer complex was found to be -34 to -36 cal per mole-deg (Ross and Scruggs, 1965; Neuman and Ackermann, 1967; Krakauer and Sturtevant, 1968). The $\Delta S_{\mathrm{I}}{}^{\mathrm{D}}$ value for the dimer·polymer complex is likely to be smaller than that for the monomer·polymer complex and is likely to be larger than that for the polymer·polymer complex. Therefore, for the analyses of the $A_{3'}p_{5'}A$·2poly U complexes, a value for $\Delta S_{\mathrm{I}}{}^{\mathrm{D}}$ of $-(40 \pm 2)$ is adopted for eq 5

$$\Delta F_{\mathrm{I}(5^\circ)}{}^{\mathrm{D-L}} = (8.5^\circ)(-40 \text{ cal/mole-deg}) = -340 \pm 20 \text{ cal/mole} \quad (6)$$

From eq 1 and 2, we obtain

$$\Delta F_{\mathrm{I}}{}^{\mathrm{D-L}} = \Delta F_{\mathrm{II}}{}^{\mathrm{D-L}} + \Delta F_{\mathrm{III}}{}^{\mathrm{D-L}} \quad (7)$$

where

$$\Delta F_{\mathrm{II}}{}^{\mathrm{D-L}} = \Delta F_{\mathrm{II}}{}^{\mathrm{D}} - \Delta F_{\mathrm{II}}{}^{\mathrm{L}}$$

and

$$\Delta F_{\mathrm{III}}{}^{\mathrm{D-L}} = \Delta F_{\mathrm{III}}{}^{\mathrm{D}} - \Delta F_{\mathrm{III}}{}^{\mathrm{L}} \quad (8)$$

In examining the formulation of these three processes, we shall assume that

$$\Delta F_{\mathrm{III}}{}^{\mathrm{D-L}} = 0 \text{ or } \Delta F_{\mathrm{III}}{}^{\mathrm{D}} = \Delta F_{\mathrm{III}}{}^{\mathrm{L}}$$

In this important assumption which will be discussed later, we view that once the ApA exists in a correct stacked state, the standard free-energy change of process III is not related to the backbone of the ApA dimer. Therefore, we assign most of the difference in the standard free-energy change for the overall process (process I) between these two complexes, $\Delta F_{\mathrm{I}}{}^{\mathrm{D-L}}$, to the difference in standard free-energy change of process II, *i.e.*

$$\Delta F_{\mathrm{I}}{}^{\mathrm{D-L}} = \Delta F_{\mathrm{II}}{}^{\mathrm{D-L}} \quad (9)$$

$\Delta F_{\mathrm{II}}{}^{\mathrm{D-L}}$ describes the difference in standard free-energy change of the following two processes

$$\mathrm{ApA^D(free)} \rightleftharpoons \mathrm{ApA^D(stacked)} \qquad \text{II (D)}$$

$$\mathrm{ApA^L(free)} \rightleftharpoons \mathrm{ApA^L(stacked)} \qquad \text{II (L)}$$

Since the circular dichroism spectrum of the D-$A_{3'}p_{5'}A$·2D-poly U complex is almost identical with that of the poly A·2poly U complex (Cantor and Chin, 1968), the chirality of the helix of the dimer·polymer complex is expected to be that of the polymer·polymer complex. From the X-ray diffraction study, it is known that the pattern of poly A·poly U is very similar to that of helical RNA duplex (Sasisekharan and Sigler, 1965; Davies, 1967; Arnott, 1970). The helical RNA duplex, such as that of reovirus RNA, has been reported to be right handed (Arnott *et al.*, 1967a,b). The correct X-ray pattern and the interpretation for poly A·2poly U complex has not been published (Davies, 1967). Since the circular dichroism spectrum of poly A·2poly U is nearly identical with that of poly A·poly U (Wolfe *et al.*, 1969), we shall adopt the view that the chirality of poly A·2poly U complex is right handed; therefore, the chirality of D-$A_{3'}p_{5'}A$·2D-poly U complex is also right handed. Since the circular dichroism spectrum of L-$A_{3'}p_{5'}A$·2D-poly U is almost the same as that of D-$A_{3'}p_{5'}A$·2D-poly U, we suggest that the chirality of L-$A_{3'}p_{5'}A$·2D-poly U is right handed as well. From this consideration, we can further define process II (D) and process II (L) as

$$\mathrm{ApA^D(free)} \rightleftharpoons \mathrm{ApA^D(right\text{-}handed\ stack)} \qquad \text{II (D)}$$

$$\mathrm{ApA^L(free)} \rightleftharpoons \mathrm{ApA^L(right\text{-}handed\ stack)} \qquad \text{II (L)}$$

From the consideration that the enantiomers are mirror images of each other, the standard free energy, F, for the conformational states of the enantiomers will be the same as

$$F(\mathrm{ApA^D(right\text{-}handed\ stack)}) = F(\mathrm{ApA^L(left\text{-}handed\ stack)})$$
$$F(\mathrm{ApA^D(left\text{-}handed\ stack)}) = F(\mathrm{ApA^L(right\text{-}handed\ stack)})$$
$$F(\mathrm{ApA^D(free)}) = F(\mathrm{ApA^L(free)}) \quad (10)$$

Thus

$$\Delta F_{\mathrm{II}}{}^{\mathrm{D-L}} = [F(\mathrm{ApA^D(right\text{-}handed\ stack)}) - F(\mathrm{ApA^D(free)})] - [F(\mathrm{ApA^L(right\text{-}handed\ stack)}) - F(\mathrm{ApA^L(free)})] = -[F(\mathrm{ApA^D(left\text{-}handed\ stack)}) - F(\mathrm{ApA^D(right\text{-}handed\ stack)})] = -\Delta F_{\mathrm{IV}}$$

where F_{IV} describes the standard free-energy change of process IV which is

$$\mathrm{ApA^D(right\text{-}handed\ stack)} \rightleftharpoons \mathrm{ApA^D(left\text{-}handed\ stack)} \quad \text{(IV)}$$

Therefore, the $-\Delta F_{\mathrm{I}(5^\circ)}{}^{\mathrm{D-L}}$ (= 340 cal/mol) can be considered as the standard free-energy change of process IV, *i.e.*, the equilibrium between the stacked conformation of D-$A_{3'}p_{5'}A$ having a right-handed turn to the stacked conformation having a left-handed turn. The orientation of the bases in the stacked conformation is that of the $A_{3'}p_{5'}A$·2poly U helix. From the relationship of $-RT \ln K = 340$ cal/mol (eq 6), the K_{IV} value (5°) of process IV is calculated to be 0.54, or the distribution of the population at 5° is calculated to have 34% of the D-$A_{3'}p_{5'}A$ in the left-handed stack. Since the entropy of $\mathrm{ApA^D}$(right-handed stack) should be identical with the entropy of $\mathrm{ApA^D}$(left-handed stack) therefore the ΔS_{IV} ($\Delta S_{\mathrm{II}}{}^{\mathrm{D-L}}$ or $\Delta S_{\mathrm{I}}{}^{\mathrm{D-L}}$, eq 9 and 11) is zero. It follows

that ΔF_{IV} (ΔF_{II}^{D-L} or ΔF_{I}^{D-L}) equals ΔH_{IV} (ΔH_{II}^{D-L} or ΔH_{I}^{D-L}). From this consideration, we can calculate the K value of process IV at 25° by the relationship of $\ln K = [(-340 \text{ cal/mol})/R \cdot 298°]$. K_{IV} is found to be 0.57, or the distribution is calculated to be about 36% in left-handed stacks at 25°.

We shall now examine the most important assumption in this analysis, *i.e.*, $\Delta F_{III}^{D} = \Delta F_{III}^{L}$. In essence, this assumption states that the differences in free-energy states for the formation of the complexes are the same when the geometry of the bases in $A_{3'}p_{5'}A$ is the same and the helical turn (right handed) of the axis is the same, regardless of whether the backbone is made of D-ribose or L-ribose. Since the hydrogen bonding, base stacking, phosphate repulsion, etc., are unchanged, this assumption should be essentially correct. In support of the above statement, model building study (CPK model) does not reveal any major stereochemical hindrance in the formation of a right-handed L-$A_{3'}p_{5'}A$·2D-poly U complex. However, if ΔF_{III}^{D} (negative) is larger than ΔF_{III}^{L} (negative), or ΔF_{III}^{D-L} is negative instead of zero, then the ΔF_{II}^{D-L} (negative) or ΔF_{IV} (positive) will be a smaller value. Under this condition, K_{IV} approaches unity or the distribution will be more in favor of the left-handed stack. On the other hand, if ΔF_{III}^{D} (negative) is smaller than ΔF_{III}^{L} (negative) or ΔF_{III}^{D-L} is positive instead of zero, then the ΔF_{II}^{D-L} (negative) or ΔF_{IV} (positive) will be a larger value. Then K_{IV} approaches zero or the distribution will be less in favor of the left-handed stack. If the assumption that $\Delta F_{III}^{D-L} = 0$ is not entirely correct, then it appears likely that the free-energy change for the formation of the natural complex with D-$A_{3'}p_{5'}A$ may be larger than the free-energy change for the formation of the unnatural complex with L-$A_{3'}p_{5'}A$. Thus, the situation that ΔF_{III}^{D-L} is negative may occur. Under this condition, the distribution may be even more in favor of the left-handed stack than presently calculated.

From the X-ray diffraction (Arnott, 1970) we learn that poly A·poly U helix has a geometry of 11 bases/turn. Following the previous arguments about the similarities of the circular dichroism spectra among poly A·poly U complex, poly A·2poly U complex and $A_{3'}p_{5'}A$·2poly U complex, we shall assume that the $A_{3'}p_{5'}A$·2poly U complex also has a geometry of 11 bases/turn. In this conformation, the intersection of the principal axes of the neighboring adenines will have an angle of about 33°. Therefore, the conformation of D-$A_{3'}p_{5'}A$ in the right-handed stack defined above will also have an intersection of the principal axes of the two adenines at an angle of about 33°. Process IV can be redefined as

$$A_{3'}p_{5'}A^{D}(\text{right-handed stack } (33°)) \rightleftharpoons A_{3'}p_{5'}A(\text{left-handed stack } (33°))$$

The above thermodynamic analyses show that the difference in free energy (or enthalpy) between the conformation of D-$A_{3'}p_{5'}A$ in a 33° right-handed stack to the conformation of D-$A_{3'}p_{5'}A$ in a 33° left-handed stack is about 340 cal/mole, or that the distribution of D-$A_{3'}p_{5'}A$ between the 33° right-handed stack and the 33° left-handed stack is about 2:1. In the preceding paper (Kondo *et al.*, 1970), the conformational model proposed for the D-$A_{3'}p_{5'}A$ is the right-handed stack with the intersection angle of about 60° for the principal axes of the two adenines. At present, the standard free-energy change of process V cannot be estimated. Since

$$A_{3'}p_{5'}A^{D}(\text{right-handed stack } (60°)) \rightleftharpoons A_{3'}p_{5'}A^{D}(\text{left-handed stack } (60°)) \quad \text{(V)}$$

the circular dichroism and proton magnetic resonance data both indicate the predominance of the right-handed form (see Kondo *et al.*, 1970), the F_V is likely to be larger than 340 cal/mole.

While the above thermodynamic analyses can also be applied to the study of $A_{2'}p_{5'}A$·2poly U complex, the situation may not be the same. First of all, the T_m of D-$A_{2'}p_{5'}A$·2poly U complex is about 2° lower than that of D-$A_{3'}p_{5'}A$·2poly U complex. This is not surprising since the arc of $A_{2'}p_{5'}A$ is larger than the arc of $A_{3'}p_{5'}A$ or $U_{3'}p_{5'}U$ (Kondo *et al.*, 1970). The intersection angle of the principal axes of the neighboring bases is larger for the 2′–5′ dimer than that for the 3′–5′ dimer. The helix formed by the 2′–5′ phosphodiester linkage will tend to have fewer bases (fewer than 11) per turn. In a double-stranded helix where one strand consists of 3′–5′ linkage and the other strand 2′–5′ linkage, the constraint and distortion on the conformation is rather self-evident upon examination of the CPK model. Thus, the lowering of the T_m of the D-$A_{2'}p_{5'}A$·2poly U complex is to be expected. This phenomenon has been previously observed by Lipsett *et al.* (1961) and by Michelson and Monny (1967). Interestingly, the complex of L-$A_{2'}p_{5'}A$·2poly U has a T_m higher than that of D-$A_{2'}p_{5'}A$·2poly U complex but equal to that of D-$A_{3'}p_{5'}A$·2poly U complex (Table VI). These differences are rather small (2°), but are larger than the experimental error which is estimated to be less than 1°.

A direct application of the thermodynamic analyses invoked above for $A_{3'}p_{5'}A$ to the problem of $A_{2'}p_{5'}A$ will lead to the results that $\Delta F_{VI(13°)}$ for the following process is negative (−80 cal/mole).

$$A_{2'}p_{5'}A^{D}(\text{right-handed stack } (33°)) \rightleftharpoons A_{2'}p_{5'}A^{D}(\text{left-handed stack } (33°)) \quad \text{(VI)}$$

This would mean that the distribution of D-$A_{2'}p_{5'}A$ is about 55% in the left-handed stack and 45% in the right-handed stack. At present, the number of bases per turn in the $A_{2'}p_{5'}A$·2poly U helix is unknown but is unlikely to be much smaller than ten since the poly U conformation is the dominating factor. For the sake of discussion, we shall just assume an intersection of 33° (11 bases/turn) for the two principal axes in the adjacent adenines of the D-$A_{2'}p_{5'}A$ in the right-handed stack conformation as shown in process VI. From the preceding paper (Kondo *et al.*, 1970), the conformational model proposed for D-$A_{2'}p_{5'}A$ is the right-handed stack with the intersection angle of about 80° for the principal axes of the two adenines. It is not theoretically impossible that while the ΔF_{VI}^{D} has a small negative value the ΔF_{VII}^{D} may have a small positive value, process VII being

$$A_{2'}p_{5'}A^{D}(\text{right-handed stack } (80°)) \rightleftharpoons A_{2'}p_{5'}A^{D}(\text{left-handed stack } (80°)) \quad \text{(VII)}$$

The other possibility in bringing the present T_m analyses in harmony with the conformational model for D-$A_{2'}p_{5'}A$

proposed in the preceding paper is that ΔF_{III}^{D-L} is positive instead of zero (or ΔF_{III}^{D} (negative) is smaller than ΔF_{III}^{L} (negative)). When ΔF_{III}^{D-L} is larger than 80 cal/mole, then the ΔF_{II}^{D-L} can become negative or ΔF_{VI} can become positive. In order for the population of D-$A_{2'}p_{5'}A$(right-handed stack (33°)) to be twice as large as the population of D-$A_{2'}p_{5'}A$-(left-handed stack (33°)) in process VI as in the case of D-$A_{3'}p_{5'}A$ in process IV, ΔF_{III}^{D-L} has to be around 400 cal/mole. There are subtle differences in the ribosyl backbone structure between the D–D complex and the L–D complex. When the strands are maintained in an antiparallel manner, in the D–D complex the direction of the ribosyl ring oxygens in the A strand is opposite to the direction of the ribosyl ring oxygens in the U strand; while in the L–D complex, the direction of the ribosyl ring oxygens is the same for both A and U strands. However, at present, we are unable to describe the stereochemical reasons why in process III, ΔF_{III}^{D} (negative) would be smaller than ΔF_{III}^{L} (negative) in the formation of $A_{2'}p_{5'}A \cdot 2$poly U complex.

Regardless of the quantitative aspect of this estimation, the experimental results clearly indicate that L-$A_{2'}p_{5'}A$ and D-poly U can form a complex which has a stability against temperature perturbation even slightly greater than that of the D-$A_{2'}p_{5'}A \cdot 2$D-poly U complex. Since the stack of L-$A_{2'}p_{5'}A$ is most likely right handed in the complex because of the similarity between the circular dichroism spectra of the D–D complex and the L–D complex, the T_m results suggest that L-$A_{2'}p_{5'}A$ can form a right-handed stack easily (in fact the apparent suggestion is that L-$A_{2'}p_{5'}A$ can form a right-handed stack more easily than D-$A_{2'}p_{5'}A$). From the symmetry consideration of the enantiomers, therefore, the comparison between the T_m results on both $A_{3'}p_{5'}A$ and $A_{2'}p_{5'}A$ suggest that D-$A_{2'}p_{5'}A$ can form a left-handed stack with greater ease than D-$A_{3'}p_{5'}A$. This same conclusion was proposed in the first paper in this series (Ts'o *et al.*, 1969) upon examination of the CPK model of $A_{2'}p_{5'}C$ as compared with the model of $A_{3'}p_{5'}C$. Thus, from this comparative study on D-$A_{3'}p_{5'}A$ and L-$A_{3'}p_{5'}A$ *vs.* D-$A_{2'}p_{5'}A$ and L-$A_{2'}p_{5'}A$, we may safely conclude that the D-2′–5′ dimer can form a left-handed stack more readily than the D-3′–5′ dimer. In other words, *the selectivity in chirality of the screw axis of the stack of the 2′–5′ dimer is less than the selectivity of the 3′–5′ dimers.*

As students of the structure and conformation of nucleic acids, we often wonder why in nature the nucleic acid is built with a 3′–5′phosphodiester linkage instead of a 2′–5′-phosphodiester linkage, especially for RNA. Since the selectivity in chirality of the 3′–5′ dimer stack is higher than that of the 2′–5′ dimer stack, it is reasonable to predict that the selectivity in the conformation of the 3′–5′ polymer is higher than that of the 2′–5′ polymer, or the entropy state of the 3′–5′ polymer is lower than the entropy state of the 2′–5′ polymer. If we consider that the existence of a biological system depends on a high degree of selectivity (or low state of entropy), then the nucleic acid built with a 3′–5′ linkage may be preferred over the nucleic acids built with a 2′–5′ linkage in the process of evolution. Furthermore, these two types of nucleic acid are unlikely to coexist, since the stability of the double-stranded helix consisting of both 3′–5′ strands will be higher than the stability of the double-stranded helix consisting of one 3′–5′ strand and one 2′–5′ strand.

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Conformational Studies on Transfer Ribonucleic Acid. Fluorescence Lifetime and Nanosecond Depolarization Measurements on Bound Ethidium Bromide*

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ABSTRACT: Ethidium bromide has been used as a fluorescent probe of the conformation of unfractionated yeast tRNA and purified yeast $tRNA^{Phe}$. A variety of types of data suggest that the dye binds by intercalation and that there is one principal binding site for the dye on $tRNA^{Phe}$ in the absence of magnesium. Time-dependent fluorescence depolarization measurements show that in the presence of magnesium both tRNA samples behave as a prolate ellipsoid with an axial ratio of 2.0 to 3.0. This is consistent with previous estimates of the size and shape of tRNA. In the absence of magnesium the unfractionated tRNA sample becomes considerably elongated and resembles a prolate ellipsoid with an axial ratio of 4.6. This change is accompanied by a shift in the circular dichroism spectrum indicative of disruption of base pairs. No such changes in overall conformation are observed for yeast $tRNA^{Phe}$. Nevertheless there is evidence for small changes in this purified sample as a function of magnesium concentration. There are revealed by studies of the quenching of the fluorescence of the Y base of $tRNA^{Phe}$ by ethidium bromide. Addition of ethidium bromide has no effect on the lifetime of Y either in the absence or presence of magnesium.

However, studies of static fluorescence show that the Y base is drastically quenched by ethidium bromide in the absence of magnesium. Only slight quenching occurs if magnesium is present. These results suggest that ethidium binds very close to Y if no magnesium is present, and very far away if there is magnesium in solution.

Applications of fluorescence lifetime and nanosecond depolarization techniques to the study of protein conformations have been very successful in recent years. These studies can yield such valuable information as the polarity at specific regions of a macromolecule, the rotational relaxation times and thus size and shape parameters (Wahl and Timasheff, 1969; Tao, 1969), and distances between chromophores attached to the macromolecule (Stryer, 1968). Although steady-state fluorescence measurements have long been used for the same type of studies, their interpretation is less direct than the time-resolved methods, and often requires tedious concentration and viscosity-dependent experimentation.

This paper describes conformational studies on unfractionated yeast tRNA and yeast $tRNA^{Phe}$ following much the same lines of approach as those described above. The conformation of tRNA is of much interest in view of the dependence of its biological activity on its structural integrity (Adams *et al.*, 1967; Lindahl *et al.*, 1966). Much information already exists on possible secondary structures (Madison, 1968; Cramer *et al.*, 1968; Cantor *et al.*, 1966) and overall size and shape of tRNA (Lake and Beeman, 1968; Krigbaum and Godwin, 1968; Adams *et al.*, 1967; Ninio *et al.*, 1969; Beardsley and Cantor, 1970). This can greatly assist the interpretation of nanosecond depolarization results. Previous studies have suggested that tRNA may resemble a prolate ellipsoid. This offers the possibility of observing more complex rotational relaxation behavior than has been found for typical globular proteins. Most tRNAs are known to undergo structural changes which are dependent on the magnesium

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