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SYNTHESIS AND CONFORMATIONAL PROPERTIES OF DIRIBONUCLEOSIDE MONOPHOSPHATES CONTAINING MODIFIED NUCLEOSIDES AS FOUND IN TRANSFER RNA*

MARTIN P. SCHWEIZER**, ROOSEVELT THEDFORD*** AND JAMES SLAMA

ICN Nucleic Acid Research Institute, Irvine, Calif. 92664, and Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, N.Y. 14203 (U.S.A.)

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SUMMARY

1. The diribonucleoside monophosphates, adenylyl(3′ → 5′)N^6—(Δ^2-iso-pentenyl)adenosine (ApiPA), N^6-(Δ^2-isopentenyl)adenylyl(3′ → 5′)adenosine (iPApA) and adenylyl(3′ → 5′)pseudouridine (ApΨ) have been chemically synthesized and their solution properties studied by NMR and circular dichroism (CD) and compared with the nonsubstituted diribonucleoside monophosphates adenylyl(3′ → 5′)adenosine (ApA) and adenylyl (3′ → 5′)uridine (ApU).

2. NMR data indicates APΨ and ApU are nearly identical conformationally, while the CD results suggest somewhat greater base interaction in ApΨ.

3. ApiPA and iPApA by these criteria are both slightly less folded than ApA and the bases are interacting to a somewhat lesser extent. The relevance of these findings is discussed in terms of influence of the modified nucleosides on the structure of the anticodon loop of tRNA.

INTRODUCTION

tRNA contains numerous modified nucleosides (refs. 1, 2 and references therein). We have been interested in the role which modified nucleosides play in structural aspects of tRNA, and in this connection it appeared appropriate to determine if modified nucleosides altered conformation and interaction at the dinucleoside monophosphate level. Information regarding interaction of individual nucleosides is not

Abbreviations: ApiPA, adenylyl(3′ → 5′)N^6-(Δ^2-isopentenyl)adenosine; iPApA, N^6-(Δ^2-isopentenyl)adenylyl(3′ → 5′)adenosine; ApΨ, adenylyl(3′ → 5′)pseudouridine; iPA, N^6-(Δ^2-isopentenyl)adenosine; piPA, N^6-(Δ^2-isopentenyl)adenosine 5′-phosphate; iPAp, N^6-(isopentenyl) adenosine 3′-phosphate; CD, circular dichroism; NMR, nuclear magnetic resonance.

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** To whom inquiries should be addressed at ICN Nucleic Acid Research Institute, Irvine, Calif. 92664, U.S.A.

*** Present address: Department of Biochemistry, State University of New York at Buffalo, N.Y., U.S.A.

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difficult to obtain at the dimer level, and these findings may be considered to be representative of the nearest neighbor interactions in polynucleotides[3], although some caution in this extrapolation is advisable[4].

In studying the conformation and interactions of dinucleoside monophosphates, dinucleotides and higher order oligonucleotides, optical methods[3–5], and nuclear magnetic resonance (NMR) techniques[6–8] have been fruitfully employed. We have synthesized (Fig. 1) the modified diribonucleoside monophosphates adenylyl-

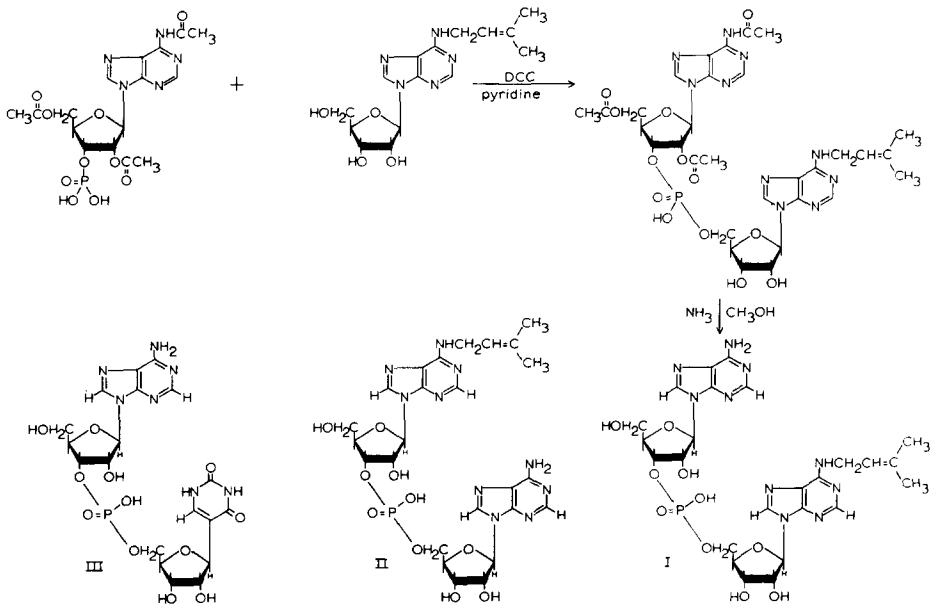

Fig. 1. Condensation method for the synthesis of modified diribonucleoside monophosphates. I, Adenylyl(3′ → 5′)N^6-(Δ^2-isopentenyl)adenosine (ApiPA); II, N^6-(Δ^2-isopentenyl)adenylyl-(3′ → 5′)adenosine (iPApA); III, adenylyl(3′ → 5′)pseudouridine (ApΨ). DCC = *N*, *N*-dicyclohexylcarbodiimine.

(3′ → 5′)N^6-(Δ^2-isopentenyl)adenosine (ApiPA, I), N^6-(Δ^2-isopentenyl)adenylyl-(3′ → 5′)adenosine (iPApA, II), and adenylyl(3′ → 5′)pseudouridine (ApΨ, III), and have studied their conformation and interactions in solution by NMR and circular dichroism (CD) and compared them to the unsubstituted counterparts ApA and ApU.

EXPERIMENTAL

Instrumentation

Ultraviolet absorption measurements were done on Cary Models 14 and 15. CD spectra were obtained at Cary Instruments, Monrovia, Calif., on a Model 61 CD spectrometer or Model 60 spectropolarimeter equipped with a CD attachment. NMR spectra were collected on a Varian A-60A; probe temperature was monitored by the ethylene glycol splitting. Chemical shifts were measured from an external tetramethylsilane capillary. No bulk susceptibility corrections were made.

Materials

N^6-(Δ^2-Isopentenyl)adenosine (iPA) was synthesized according to ROBINS *et al.*[9] We appreciate the generous gift of γ,γ-dimethyl allyl bromide from Dr. Maurice Fleysher, Roswell Park Memorial Institute. All other chemicals not specifically mentioned were of highest commercial purity.

N^6-(Δ^2-Isopentenyl)adenosine 5'-phosphate (piPA)

Phosphorylation of iPA (0.75 mmole scale) was carried out by a modification of the methoxy methylidine method of DARLIX *et al.*[10]. The desired product was separated from 2',3'-*O*-methoxymethylidine piPA and iPA on a DEAE-cellulose column (1.9 cm × 30 cm) utilizing a 0.01–0.1 M gradient (500 ml each) of triethyl ammonium bicarbonate. The fractions containing the product were combined and concentrated to dryness. Excess triethyl ammonium bicarbonate was removed by azeotroping the compound with water and ethanol, yielding piPA quantitatively as the triethylammonium salt.

Anal. Calcd. for $C_{15}H_{22}N_5O_7P \cdot 2\,H_2O$ C, 39.95; H, 5.75; N, 15.5; P, 6.89. Found: C, 39.85; H, 5.42; N, 15.46; P, 7.19 %.

N^6-(Δ^2-Isopentenyl)adenosine 3'-phosphate (iPAp)

The alkylation method of GRIMM AND LEONARD[11], used for preparation of piPA from 5'-AMP, was used to prepare iPAp, except that the N^1-isopentenyl 3'-AMP was directly rearranged to the desired N^6 isomer. The product was purified on a 1-inch cellulose powder column using isopropanol–conc. NH_4OH–water (7:1:2, by vol.) as the solvent. Yield: 0.45 mmole (15 %).

Pseudouridine 5'-phosphate (pΨ)

Pseudouridine (122 mg, 0.5 mmole; commercial mixture 65 % β-ribofuranosyl and 35 % other isomers) was phosphorylated as above for piPA. The product was purified from starting material on a DEAE-cellulose column as described for piPA. It was shown to be the β-anomer by NMR as described below in RESULTS AND DISCUSSION.

Adenylyl(3' → 5')N^6-(Δ^2-isopentenyl)adenosine (ApiPA)

Syntheses of the diribonucleoside monophosphates were generally accomplished by a slightly modified procedure as described by FOLLMANN[12], shown in Fig. 1 for ApiPA. Purification procedures were slightly altered. After hydrolysis of (3' → 3') and (3' → 2') dinucleoside phosphates, the product mixture was concentrated to dryness and redissolved in water (5 ml). After adjusting the pH of the aqueous solution to 8.0 with NH_4OH, it was placed on a preconditioned DEAE-cellulose column (bicarbonate form, 1-inch × 20 inch) and separated from nucleosides and mononucleotides by elution with a linear gradient (1 l each) of triethylammonium bicarbonate (0.01–0.1 M, pH 7.5) at a flow rate of 30 ml/h. The desired product came off the column in Tubes 51–75. These tubes were combined and lyophilized 4 times in order to remove the excess triethylammonium bicarbonate, yielding 150 mg (40 %, based upon N^6, O^2, O^5-triacetyladenosine 3'-phosphate) of the desired product as the triethylammonium salt.

Anal. Calcd. for $C_{31}H_{48}O_{10}P_1$ C, 48.0; H, 6.19; N, 19.85; P, 4.0. Found: C, 47.90; H, 6.10; N, 19.16; P, 3.80 %.

The 3' → 5' linkage in ApiPA was demonstrated by degradation to adenosine 2',3'-phosphates and iPA using mild alkali, to adenosine and piPA using venom phosphodiesterase and to 3'-AMP and iPA using spleen phosphodiesterase.

Adenylyl(3′ → 5′)pseudouridine (ApΨ)

The procedures for synthesis of ApΨ were essentially the same as those described above for ApiPA. Yield: 10 % of ApΨ (triethylammonium salt), based upon N^6, O^2, O^5-triacetyladenosine 3′-phosphate. ApΨ was characterized by alkaline hydrolysis and phosphodiesterase degradation. Digestion by spleen phosphodiesterase followed by chromatography of the hydrolysis mixture revealed the presence of α-pseudouridine, as well as β-pseudouridine. From these results, it was estimated that about one-half of the ApΨ synthesized contained α-pseudouridine. The dimer mixtures containing both α- and β-pseudouridine were separated by 48 h preparative chromatography in isopropanol–conc. NH_4OH–water (7:1:2, by vol.) on 3MM paper. Data presented below concern the ApΨ containing β-pseudouridine.

Phosphorous microanalytical data for ApΨ: Calcd., 5.09. Found: 4.77 %.

N^6-(*Δ*^{2}-*Isopentenyl)adenylyl(3′ → 5′)adenosine (iPApA)*

Synthesis of this diribonucleoside monophosphate in low yield (5 mg, approx. 2 %) was accomplished in a similar manner as described above. iPAp (0.5 mmole) was acetylated according to the general method of LAPIDOT AND KHORANA[13] yielding 2′,5′-di-*O*-acetyl-isopentenyladenosine 3′-phosphate. The acetylated nucleotide was not isolated and the entire mixture was converted to the pyridinium salt.

This compound was condensed with adenosine in the usual manner. iPApA, isolated and purified according to the procedure outlined for ApiPA, was characterized by enzymatic degradation to its constituents.

RESULTS AND DISCUSSION

(*I*) *NMR studies on modified mononucleotides*

(*A*) *Self-association of piPA and iPAp*

Previous NMR studies[14–16] have demonstrated that nucleosides and mononucleotides associate in aqueous solution *via* vertical stacking of base rings. The stacking interaction is manifested in resonance lines shifting upfield with concentration especially the base protons and the anomeric H-1′. We have determined that these protons in piPA and iPAp display upfield concentration dependent chemical shifts about 10–15 % greater than 5′-AMP. This increase in association is undoubtedly due to the hydrophobic isoprene group.

(*B*) *Mononucleotide conformation*

The intramolecular field effect of an ionized 5′-attached phosphate group has been utilized to obtain information about 5′-nucleotide conformation in solution[14]. Base protons in the vicinity of the phosphate are deshielded when ionization occurs, presumably due to coulombic attraction between the negative phosphate and the particular hydrogen. Since the deshielding was found to occur in 5′-phosphates specifically at H-8 of purine nucleosides and H-6 of pyrimidine derivatives, it was concluded that the 5′-mononucleotides were predominantly in the *anti* conformation. Deshielding effects of similar magnitude were observed both when comparing nucleoside with nucleotide monoanion and nucleotide mono- and dianions. H-8 of piPA and H-6 of pΨ are shifted to lower field 0.12–0.13 ppm due to phosphate ionization, which suggests that these 5′-mononucleotides are predominantly *anti*. The fact that H-6 is

phosphate deshielded is the confirmation that the pΨ product isolated from the phosphorylation of pseudouridine is the β-anomer although some α-anomer of pseudouridine was present in the starting material.

Ionization of a phosphate group in the 3'-position has little or no effect on base proton chemical shifts[14] since the intervening space between the protons and the phosphate is too great. However, it is believed that position of the phosphate has little effect upon basic nucleoside conformation; thus, iPAp is probably also in the *anti* range.

(II) Studies on the modified diribonucleoside monophosphates

(A) Ultraviolet spectroscopy

In Fig. 2 are presented the ultraviolet spectra of the three modified dimers in acidic, neutral and basic solution. The pH dependencies are similar to those of the constituent chromophores and the wavelength maxima are intermediate between them. The long wavelength shoulder observed for Ap× in basic solution (292 mn) is characteristic for ionization of N^3H or N^1H in pseudouridine. A similar spectrum has been eported by VENKSTERN AND BAEV[17] for ApΨp(3') isolated from tRNA digests.

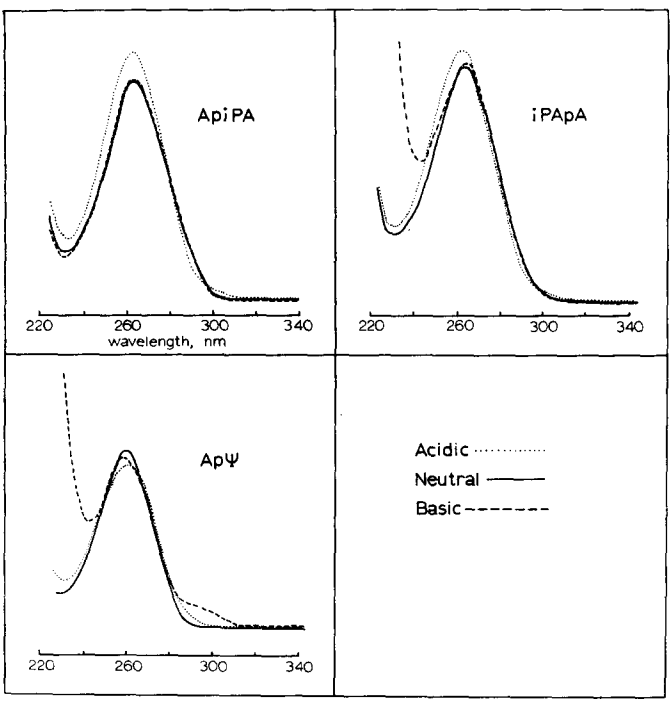

Fig. 2. Ultraviolet spectra of modified diribonucleoside phosphates. Acidic, pH 1–1.5; neutral, pH 6–7; basic, pH 11–12.

(B) NMR investigation

Proton resonances in the 60 MHz spectra of the modified dimers were assigned by noting the similarity with spectra of the well-characterized unsubstituted dimers[6,7].

(1) *Intermolecular interaction.* Dinucleoside monophosphates composed of two purine nucleosides such as adenosine have been shown to self-associate in dimer stacks[6,7] which is manifested in substantial upfield movement of proton chemical shifts with concentration increase in ^2H_2O. This type of behavior is noted in Table I for ApiPA base, H-1′ and methyl protons over the concentration range 0.0–0.08 M. Comparisons with ApA over the same range illustrates that the concentration induced shifts for ApiPA are somewhat greater, which probably reflects the influence of the hydrophobic isoprene groups in enhancing dimer aggregation.

TABLE I

DEPENDENCE OF δ UPON CONCENTRATION (^2H_2O, p^{2}H 7.4, 28–31°)

δ values at infinite dilution are extrapolated.

		H-8(5′)	*H-8(3′)*	*H-2(5′)*	*H-2(3′)*	*H-1′(5′)*	*H-1′(3′)*	=C(*CH*$_3$)$_2$
ApA*	$\Delta\delta$ (0.0–0.08 M)	0.10	0.13	0.21	0.20	0.06	0.08	
ApiPA	$\Delta\delta$ (0.0–0.08 M)	0.12	0.18	0.25	0.26	0.15	0.11	0.28
		H-8	*H-2*	*H-6*	*H-5*	*H-1′(5′)*	*H-1′(3′)*	
ApU	$\Delta\delta$ (0.0–0.05 M)	0.03	0.04	0.01	0.01	0.01	0.01	
ApΨ	$\Delta\delta$ (0.0–0.04 M)	0.02	0.07	0.04		**	0.05	

* From ref. 6.

** H-1′ of pseudouridine in ApΨ was obscured by the H^{2}HO resonance.

As noted earlier[6–8], dinucleoside monophosphates composed of both purine and pyrimidine nucleosides have considerably lower self-association tendencies as monitored by concentration-dependent chemical shifts. Such observations also generally hold true for ApΨ, as contained in the lower part of Table I, even though the concentration range under consideration was only 0.0–0.04 M because of limited material.

(2) *Intramolecular interaction as monitored by NMR.* Previous proton magnetic resonance studies[6–8] have elucidated the nature of the intramolecular base-stacking interaction in diribonucleoside monophosphates. From these results a model was suggested for the folded conformation of dimers, with both nucleosides in the *anti* conformation and the bases stacked. This general model predicts that H-8 of the 5′-attached nucleoside and H-2 of the 3′-attached nucleoside should be strongly shielded by the ring current anisotropy of the adjacent base. Experimentally one verifies this by comparisons of chemical shifts in the dimers with shifts of these same protons in mixtures of the monomers. These $\Delta\delta$ values are "dimerization shifts" and reflect the difference in proton magnetic environment between monomer and dimer states. Thus in Table II are listed $\Delta\delta$ values for ApA, ApiPA, ApU and ApΨ, all 0.02 M in ^2H_2O, p^{2}H 5.9, 35°. Here one observes that $\Delta\delta_D$ values for ApiPA are generally less than ApA. For H-8(5′), $\Delta\delta_D$ is nearly halved, which indicates that there is less time average overlap by the 3′-adenosine. This is illustrated in the upper half of Fig. 3. ApiPA probably exists in a slightly less tightly folded conformation than ApA. This may be caused by insertion of the isoprene chain between the bases, since the $\Delta\delta_D$ for the

TABLE II

COMPARISON OF THE PROTON CHEMICAL SHIFTS IN DINUCLEOSIDE MONOPHOSPHATES AND MIXTURES OF THEIR MONOMERIC CONSTITUTENTS* "DIMERIZATION SHIFTS", $\Delta\delta_D$ (ppm) = $\delta_{Monomer} - \delta_{Dimer}$

	H-8(5')	*H-8(3')*	*H-2(5')*	*H-2(3')*	*H-1'(5')*	*H-1'(3')*	$=C(CH_3)_2$
Ap+pA → ApA	0.17	0.08	0.06	0.20	0.06	0.14	
Ap+piPA → ApiPA	0.10	0.11	0.05	0.16	0.03	0.24	0.07
	H-8	*H-2*	*H-6*	*H-5*	*H-1'(A)*		
Ap+pU → ApU	0.03	0.05	0.21	0.29	0.06		
Ap+pΨ → ApΨ	0.02	0.03	0.22		0.03		

* 0.02 M in ^2H_2O, p^{2}H 5.9, 35°.

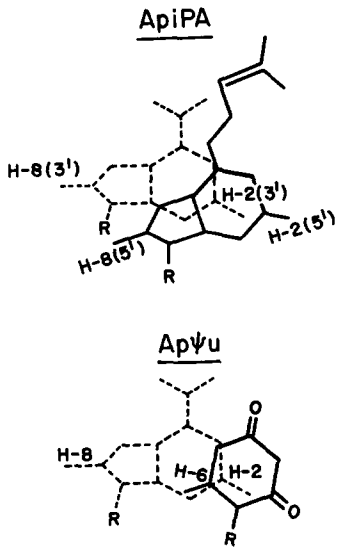

Fig. 3. Models for the time average base–base interaction in ApiPA and ApΨ.

methyl protons is significant. Additional NMR data which indicates less folding in ApiPA is the magnitude of the $J_{1'-2'}$ coupling constants. In ApA for example, $J_{1'-2'}$ values are about 3 Hz, whereas in the mononucleotides, the corresponding couplings are about 5.6–6 Hz. This decrease may be attributed to a combination of stacking and changes in ribose conformation[6,18] in the dimer. At elevated temperatures the dimer unfolds and the $J_{1'-2'}$ approach those of the monomers. In ApiPA, the $J_{1'-2'}$ values are 4.2 Hz for both H-1', H-2' pairs.

As may be seen in Table II, the dimerization shifts for ApΨ are nearly the same as for ApU. The H-6 proton in both dimers is significantly shielded in the dinucleoside monophosphate which indicates overlap of the uracil ring by the adjacent adenine, as shown in the lower half of Fig. 3. In this model of base overlap in ApΨ it is assumed that the pΨ moiety is in the *anti* conformation. As was reasoned for piPA in ApiPA, this assumption is based upon the fact that pΨ is *anti* and not likely to change from mononucleotide to dinucleotide. Additional evidence supporting *anti* pΨ in ApΨ is

the magnitude of the $J_{1'-6}$ coupling constant (0.8 Hz) which is essentially the same in the dimer as in the nucleoside[19], which is also the same in the 5′-mononucleotide. Since this coupling constant would be expected to change if there were any changes in sugar-base torsion angle, the similar $J_{1'-6}$ magnitude in nucleoside, 5′-mononucleotide and ApΨ signifies that the conformation in all three cases is the same.

Regarding intramolecular base–base interaction in iPApA, it is of interest to compare the chemical shifts of this dimer with the sequence isomer, ApiPA. Although there was insufficient iPApA to study concentration dependence of the chemical shifts, it is reasonable to assume similar behavior as exhibited by ApiPA. In terms of the mononucleotides piPA and iPAp, the only shift differences observed were due to the 5′-attached phosphate. Thus at a given concentration and temperature, any chemical shift differences noted between iPApA and ApiPA not due to position of phosphate attachment should reflect differences in base–base interaction in the dimers.

In a 0.018 M ^2H_2O solution, at 33°, iPApA protons display the following chemical shifts: H-8(5′), 8.70 ppm; H-8(3′) and H-2(5′) (merged), 8.64; H-2(3′), 8.50 and C–C$(CH_3)_2$, 2.21. Under identical conditions, the corresponding shifts for ApiPA are 8.72, 8.61, 8.49 and 2.09 ppm. The notable difference is in the methyl shifts, with the methyl protons resonating 0.12 ppm to lower field in iPApA. Examination of the models for ApiPA and iPApA clearly shows that in ApiPA rotation of the isoprene group about the N^6–CH_2 bond brings the methyl protons in close proximity to the adenine of the 3′-attached adenosine, but that similar rotation in iPApA does not result in any close approach of methyl groups to the adenine of 5′-attached adenosine. These observations support the validity of the folded, base stacked conformation proposed for ApA and other dinucleoside monophosphates[23–25].

(C) CD studies

We present in Fig. 4 the comparative CD spectra of the modified dimers and their unsubstituted counterparts. The spectra of the latter are identical to those reported earlier[4,5]. Examination of the data in Fig. 4 reveals some interesting features

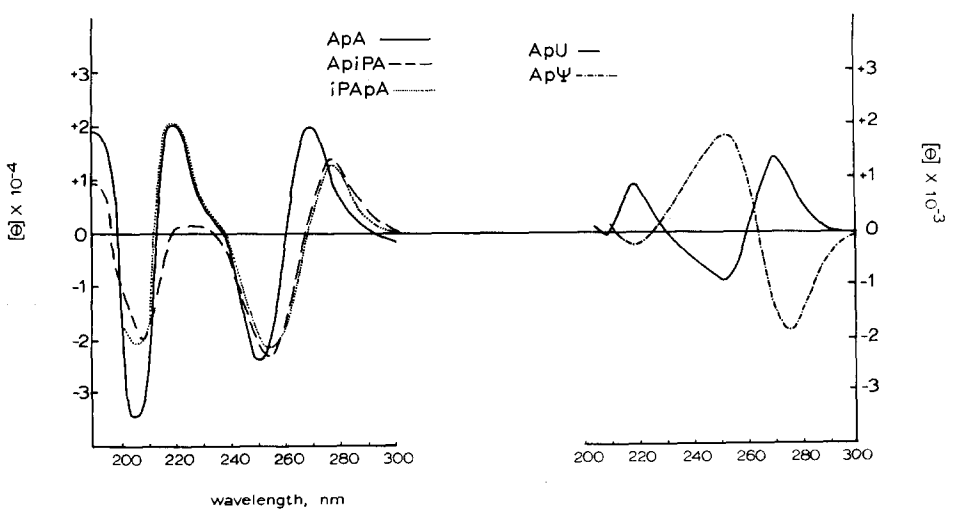

Fig. 4. CD Spectra of diribonucleoside phosphates. Solution concentration: 1.5–2.0 absorbance units in 0.01 M phosphate buffer, pH 6.7, containing 0.001 M $MgCl_2$ and 0.1 M NaCl.

of the modified dimers. First, with regard to the degenerate long wavelength transitions of the purine dimer, the $[\theta]_{peak}$ values for both ApiPA and iPApA are reduced to about 65–67 % of those found for ApA. These data may cautiously be taken as an indication of reduced base stacking.

It is curious to note that whereas both ApA and iPApA display short wavelength degenerate transitions at about 214–217 nm, and single Cotton effects at about 230–233 nm, the curve for ApiPA in this region appears to represent two single Cotton effects of opposite sign, a positive transition at about 230 nm and a negative transition at about 218 nm. While the origin of these effects is not known, the short wavelength behavior is a result of base interaction in the dimers and the effects are species specific.

Mirror image CD spectra are obtained for ApU and ApΨ, with larger $[\theta]$ values for ApΨ. In the former, the usual positive peak is observed in the 270 nm region, whereas in ApΨ, the long wavelength Cotton effect is negative, in agreement with the earlier data of ZAVIL'GEL'SKII AND LI[20] for ApΨ isolated from tRNA digests. Inasmuch as the sign of the Cotton effect is related to the angular relationship between transition moments of the interacting bases, the negative Cotton effect could be due an opposite helical sense in the stacked dimer. This explanation was advanced by the Russian workers. Alternatively, the change in sign of rotation may simply be due to the fact that the uracil base has been rotated through an angle of 120° from ApU to ApΨ, as proposed by SCOTT AND ZAMECNIK[21].

In conclusion, NMR data shows that ApU and ApΨ are similar in terms of conformation and base–base interaction in aqueous solution. The somewhat larger $[\theta]$ for ApΨ may indicate a slightly larger degree of base interaction than in ApU.

Both NMR and CD data suggest that ApiPA and iPApA are less folded, with slightly reduced base–base interaction than in ApA. In terms of relevance to tRNA anticodon segments, the results obtained here suggest that in the less compact convolves formation, the hydrophobic isoprene is more readily available for its function in stabilizing the anticodon–codon interaction. LEONARD *et al.*[22] have recently concluded from data on model dimers containing adenine and N^6-isopentenyladenine bases linked through flexible trimethylene bridges that the isoprene group helps to stabilize the stacked conformation of the anticodon loop as originally proposed by FULLER AND HODGSON[23]. The data presented here are not in conflict with the conclusions presented by these authors. ApiPA and iPApA, containing the natural diribosyl phosphate linkage, appear to be strongly stacked and are only slightly less folded than ApA. Undoubtedly in tRNA anticodon loops, adenosine and iPA are involved in maintenance of the stacked conformation. ApΨ apparently is similar to ApU in terms of base interaction. Pseudouridine may stabilize the tRNA–messenger complex due the availability of the additional hydrogen at N-1.

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REFERENCES

1 D. B. DUNN AND R. H. HALL, in H. A. SOBER, *Handbook of Biochemistry*, The Chemical Rubber Co., Cleveland, Ohio, 1968, p. G-3.
2 H. G. ZACHAU, *Angew. Chem. Intern. Ed.*, 8 (1969) 711.
3 C. R. CANTOR, S. R. JASKUNAS AND I. TINOCO, *J. Mol. Biol.*, 20 (1966) 39.
4 C. A. BUSH AND H. A. SCHERAGA, *Biopolymers*, 7 (1969) 395.
5 J. BRAHMS, J. C. MAURIZOT AND A. M. MICHELSEN, *J. Mol. Biol.*, 25 (1967) 481.
6 P. O. P. TS'O, N. S. KONDO, M. P. SCHWEIZER AND D. P. HOLLIS, *Biochemistry*, 8 (1969) 997.
7 S. I. CHAN AND J. H. NELSON, *J. Am. Chem. Soc.*, 91 (1969) 168.
8 B. W. BANGERTER AND S. I. CHAN, *J. Am. Chem. Soc.*, 91 (1969) 3910.
9 M. J. ROBINS, R. H. HALL AND R. THEDFORD, *Biochemistry*, 6 (1969) 1837.
10 J. L. DARLIX, H. P. M. FROMAGEOT AND P. FROMAGEOT, *Biochim. Biophys. Acta*, 145 (1967) 517.
11 W. A. H. GRIMM AND N. J. LEONARD, *Biochemistry*, 6 (1967) 3625.
12 H. FOLLMANN, *Tetrahedron Letters*, 22 (1967) 2113.
13 Y. LAPIDOT AND H. G. KHORANA, *J. Am. Chem. Soc.*, 85 (1963) 3852.
14 M. P. SCHWEIZER, A. D. BROOM, P. O. P. TS'O AND D. P. HOLLIS, *J. Am. Chem. Soc.*, 90 (1968) 1042.
15 O. JARDETZKY, *Biopolymer Symp.*, 1 (1964) 501.
16 A. D. BROOM, M. P. SCHWEIZER AND P. O. P. TS'O, *J. Am. Chem. Soc.*, 89 (1967) 3612.
17 T. V. VENKSTERN AND A. A. BAEV, *Spectra of Nucleic Acid Compounds*, Plenum Press, New York, 1968, p. 45.
18 F. E. HRUSKA AND S. S. DANYLUK, *J. Am. Chem. Soc.*, 90 (1968) 3266.
19 F. E. HRUSKA, A. A. GREY AND I. C. P. SMITH, *J. Am. Chem. Soc.*, 92 (1970) 4088.
20 G. B. ZAVIL'GEL'SKII AND L. LI, *Molekulyarnaya Biologiya*, 1 (1967) 323.
21 J. F. SCOTT AND P. C. ZAMECNIK, *Proc. Natl. Acad. Sci. U.S.*, 64 (1969) 1308.
22 N. J. LEONARD, H. IWAMURA AND J. EISINGER, *Proc. Natl. Acad. Sci. U.S.*, 64 (1969) 352.
23 W. FULLER AND A. HODGSON, *Nature*, 215 (1967) 817.