Biochemistry

 Volume 11, Number 11 May 23, 1972

Influence of the Furanose on the Conformation of Adenine Dinucleoside Monophosphates in Solution†

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ABSTRACT: The influence of the 2′-hydroxyl group on nucleic acid conformation has been investigated by studying the solution conformations of six adenine dinucleoside monophosphates, rAprA, dApdA, rApdA, dAprA, $rA_{5'}p_{5'}rA$, and $dA_{5'}p_{5'}dA$, concurrently by the methods of ultraviolet (uv) hypochromicity, circular dichroism (CD), and proton magnetic resonance (pmr) spectroscopy. The pmr spectral assignments of rApdA, dAprA, and $dA_{5'}p_{5'}dA$ were made by the use of specifically deuterated dimers, dAp-8-D-rA and 8D-rApdA, deuterium-exchange studies, and molecular models; while pmr spectra of other dimers have been previously assigned. The conformational models of these dimers were constructed on the basis of these studies and were shown to be right-handed stacks with both nucleosidyl units in the anti conformation. The order of base–base stacking determined by both hypochromicity and pmr dimerization shifts is $rA_{5'}p_{5'}rA \approx dA_{5'}p_{5'}dA > dApdA \approx dAprA > rAprA \approx rApdA$. The CD spectra of the six dimers are qualitatively similar, although the amplitude in $[\theta]$ follows the order $rA_{5'}p_{5'}rA > dA_{5'}p_{5'}dA$; rAprA > dApdA ≈ dAprA > rApdA, which is different from the order of stacking found by pmr and uv measurements. The CD data may be more sensitive to the variances in the mode of the base–base orientation in different stacks, or in the proportion between the right-handed conformers and the left-handed conformers in solution. The results from this investigation together with those from previous studies indicate that the influence of the 2′-OH group of the ribose on the conformation of the ribose-containing dimers is exerted through the steric hindrance of this group and not through its hydrogen-bonding properties. Upon stacking, the ribose of the 3′-residue is compressed by the furanose of the adjacent 5′ residue (possibly by the base of the 5′ residue as well). This steric interference between the 3′-furanose group and the immediately following 5′ residue prevents an extensive overlap of the adenines in a parallel fashion. Such an interference is absent in the dimers containing a deoxyribosyl 3′ residue, such as dApdA and dAprA.

The influence of the sugar–phosphate backbone on the conformation of dinucleoside monophosphates has been studied extensively in our laboratory as a means to understanding the factors governing the properties of nucleic acids. In the preceding papers of this series, Ts'o *et al.* (1969) reported on the general conformational model of the dinucleoside monophosphates based on proton magnetic resonance (pmr) studies; Kondo *et al.* (1970) reported on the influence of different positions of the phosphodiester linkage (2′–5′, 3′–5′, and 5′–5′) on the conformation of ribosyl dimers; Tazawa *et al.* (1970) reported on the influence of the configuration of the ribosyl furanose ring (D-ribose *vs.* L-ribose) on the adenine dimers; Fang *et al.* (1971) reported on the details of the stereochemistry of the backbone of the adenine deoxyribosyl dimer, dApdA; and Miller *et al.* (1971) reported on the properties of the neutral phosphodiester of deoxyribosyl dimers (dAp(R)dA and dTp(R)dT) which provides information about the effect of the negative charge of the phosphate group and the rotational barriers of the backbone.

The contribution of the 2′-OH group to the conformation of nucleic acid, *i.e.*, the difference between ribose polynucleotides *vs.* deoxyribose polynucleotides, has been studied by many laboratories, including our own (for example, Chamberlin and Patterson, 1965; Riley *et al.*, 1966; Ts'o *et al.*, 1966; Vournakis *et al.*, 1967; Adler *et al.*, 1968, 1969; Bush and Scheraga, 1969; Maurizot *et al.*, 1969; Warshaw and Cantor, 1970; Green and Mahler, 1971; etc.). Most of these studies were based on optical methods, especially optical rotatory dispersion (ORD) and circular dichroism (CD) techniques; some of these findings will be discussed later in the paper. In

† From the Department of Radiological Sciences, The Johns Hopkins University, Baltimore, Maryland 21205. *Received December 20, 1971.* This work was supported in part by a grant from the National Institutes of Health (GM-16066-04) and a grant from the National Science Foundation (GB-8500; GB-30725X). This is paper 6 in a series entitled: "Studies of the Conformation and Interaction of Dinucleoside Mono- and Diphosphates." This paper was presented in part at the 160th National American Chemical Society Meeting, 1970, Chicago, Ill.

the present communication, four 3′–5′-linked adenine dinucleoside monophosphates (rAprA, rApdA, dAprA, and dApdA) and two 5′–5′-linked adenine dimers ($rA_{5'}p_{5'}rA$ and $dA_{5'}p_{5'}dA$) have been studied concurrently by ultraviolet (uv) hypochromicity measurement, circular dichroism, and proton magnetic resonance (pmr) techniques in the temperature range 5–60°. Conformational models were constructed on the basis of these studies. These results provide detailed stereochemical information about the influence of the 2′-OH group on the conformation of adenine dinucleoside monophosphates.

Experimental Section

Reagents for Nucleotide Synthesis. Compounds of the highest degree of purity were obtained from the following sources: deoxyadenosine, adenosine, deoxyadenosine 5′-phosphate, adenosine 3′-phosphate, Sigma Chemical Co.; 4,4′-di-*p*-methoxytrityl chloride, 2,4,6-triisopropylbenzenesulfonyl chloride, Aldrich Chemical Co.; dicyclohexylcarbodiimide, Eastman Chemical Co.; barium salt of β-cyanoethyl phosphate, Calbiochem; deuterium oxide, dimethyl-d_6 sulfoxide, Diaprep, Inc.; snake venom and spleen phosphodiesterase, Worthington Biochemical Corp.

N-Benzoyl-5′-*O*-di-*p*-methoxytrityldeoxyadenosine was prepared by the method of Schaller *et al.* (1963). Part of this material was converted to $N,O^{3'}$-dibenzoyldeoxyadenosine by treatment with benzoyl chloride followed by hydrolysis of the 5′-*O*-dimethoxytrityl group in 80% aqueous acetic acid. $N,O^{2'},O^{5'}$-Tribenzoyladenosine 3′-phosphate was prepared according to the procedure of Lapidot and Khorana (1963) and $N,O^{3'}$-dibenzoyldeoxyadenosine 5′-phosphate was obtained by the method of Ralph and Khorana (1961). A sample of adenylyl-(5′–5′)adenosine was a gift from Dr. Lloyd M. Stempel of our laboratories (Kondo *et al.*, 1970). Deoxyadenylyl-(3′–5′)-deoxyadenosine was prepared by the method of Miller *et al.* (1971).

Chromatography and Electrophoresis. Paper chromatography was carried out on Whatman No. 3MM paper by the descending technique using the following solvent systems: solvent A, 2-propanol–concentrated ammonium hydroxide–water (7:1:2, v/v); solvent C, 1-propanol–concentrated ammonium hydroxide–water (55:10:35, v/v). Paper electrophoresis was performed on a Savant flat-plate apparatus using Whatman No. 3MM paper and 0.05 M triethylammonium bicarbonate buffer (pH 7.8).

Instrumentation. Proton magnetic resonance spectra were obtained on a Varian HA-100 spectrometer equipped with a C-1024 computer of average transients, and a Varian V-6057 variable-temperature accessory. Temperature readings were calibrated using ethylene glycol and methanol standards. Chemical shifts were measured from an external tetramethylsilane capillary and are reliable to better than ±0.01 ppm, or to about ±0.02 ppm at infinite dilution. Circular dichroic spectra were recorded on a Cary 60 spectropolarimeter equipped with a 6001 CD attachment and thermojacketed cell of 1-cm path length (Optical Cell Co., Beltsville, Md.). The cell temperature was controlled by the use of a Haake Brinkman Model KT-62 constant temperature circulator. The CD spectra of the dinucleoside monophosphates (1.2 optical density units at 260 nm) were recorded in 0.05 M $NaClO_4$ (pH 7.3). Ultraviolet spectra were recorded on a Cary 15 spectrophotometer.

Assay Methods. SPLEEN PHOSPHODIESTERASE. Approximately 0.15 μmole of dinucleotide was incubated with 10 μl of enzyme solution (16 units of enzyme/ml of water) in 10 μl of 1 M ammonium acetate buffer (pH 6.5) at 37°.

SNAKE VENOM PHOSPHODIESTERASE. Approximately 0.15 μmole of dinucleotide was incubated with 80 μl of enzyme solution (5 mg of enzyme/ml of water) in 10 μl of 1 M ammonium bicarbonate buffer (pH 9.0) at 37°.

The extinction coefficient of rApdA was determined by phosphorus analysis following the procedure described by Kondo *et al.* (1970). The extinction coefficients of dAprA and $dA_{5'}p_{5'}dA$ were obtained in the following manner. Three 25-μl samples of a stock solution of the dimer were diluted to 2.0 ml with 1 M Tris-HCl buffer (pH 7.5) and the optical densities were determined at 257 nm. Five 25-μl samples from the same stock solution were each treated with 0.1 ml of 1 M HCl for a total of 60 min at 85°. The final pH of the solution was then adjusted to 7.1 using 1 M Tris-HCl buffer and the concentration of adenine produced in the hydrolysis reaction was determined. The extinction coefficient used for adenine was 13.4×10^3 at 260.5 nm (pH 7.0).

Syntheses and Characterization of Dinucleotides. ADENYLYL-(3′–5′)-DEOXYADENOSINE, rApda. $N,O^{3'}$-Dibenzoyldeoxyadenosine (1 mmole), pyridinium $N,O^{2'},O^{5'}$-tribenzoyladenosine 3′-phosphate (0.5 mmole), and pyridinium Dowex 50 ion-exchange resin (800 mg) were dried by repeated evaporation with anhydrous pyridine. The resulting gum was dissolved in 3 ml of anhydrous pyridine and the solution was treated with dicyclohexylcarbodiimide (1.4 g) for 4 days at room temperature. The reaction mixture was then treated with 50% aqueous pyridine for 3 hr followed by extraction with petroleum ether (bp 30–60°). The aqueous layer was evaporated to dryness and the resulting solid was treated with 25 ml of 15 M methanolic ammonia at 0° overnight. After evaporation of solvents the residue was dissolved in 5 ml of water and subjected to chromatography on a small DEAE-cellulose column (2.5 × 30 cm). The column was first eluted with water to remove neutral compounds followed by a linear gradient of ammonium bicarbonate (0.0–0.1 M). Fractions containing dimeric material were pooled and the solvents evaporated. Examination of this material by paper chromatography showed that the protecting groups had not been completely removed. The dimeric material was again treated with methanolic ammonia as described above. After evaporation of solvents the residue was dissolved in 30 ml of water and extracted with several (15 ml) portions of ether. The aqueous solution was concentrated and subjected to preparative paper chromatography in solvent C. Material corresponding to rApdA (770 OD_{260} units) was obtained after elution of the paper chromatograms. This material had R_F values of 2.5 (solvent A) and 1.56 (solvent C) relative to 3′-AMP which are in close agreement with those reported by Moon *et al.* (1966). The pmr spectrum of rApdA showed the four aromatic base protons (8.495(s), 8.57(s), 8.665(s), and 8.715(s) ppm from Me_4Si) and the two anomeric proton multiplets (a doublet at 6.26 ppm and a pseudotriplet at 6.80 ppm). Treatment of the dimer with snake venom phosphodiesterase gave two compounds corresponding to adenosine and deoxyadenosine 5′-phosphate.

8-DEUTERIOADENYLYL-(3′–5′)-DEOXYADENOSINE,8-D-rApda. Adenosine 3′-phosphate was heated at 85° in D_2O for 4 hr to give 8-deuterioadenosine 3′-phosphate. This material, after its conversion to the tribenzoyl derivative, was condensed with $N,O^{3'}$-dibenzoyldeoxyadenosine to give 8-D-rApdA in a manner similar to that described for rApdA.

DEOXYADENYLYL-(3′–5′)-ADENOSINE, dAprA. *N*-Benzoyl-5′-*O*-di-*p*-methoxytrityldeoxyadenosine (1 mmole) and dipyr-

idinium β-cyanoethyl phosphate (4 mmoles) were dried by repeated evaporation with anhydrous pyridine (four 5-ml portions). The gum was dissolved in 2 ml of dry pyridine and the solution was treated with *p*-toluenesulfonyl chloride (3.7 mmoles) for 7 hr at room temperature. The reaction solution was cooled, treated with 1 ml of ice water, and allowed to stand overnight at 27°. Saturated sodium chloride solution (3.5 ml) was added and the aqueous mixture was extracted with chloroform (three 6-ml portions). The combined chloroform extracts were evaporated to dryness. $N^1,N^6,O^{2'},O^{3'}$-Tetrabenzoyladenosine (1.2 mmoles) was added and the reactants were dried by repeated evaporation with pyridine (four 5-ml portions). The resulting gum was dissolved in 2 ml of anhydrous pyridine and the solution was treated with 2,4,6-triisopropylbenzenesulfonyl chloride (1.3 mmoles) for 16 hr at room temperature. After cooling, pyridine (1 ml) and water (10 ml) were added and the aqueous solution was extracted with ethyl acetate (three 10-ml portions). The combined extracts from ethyl acetate were dried over anhydrous sodium sulfate and then concentrated to 2 ml. The solution was applied to a silica gel column. The column was eluted with ethyl acetate followed by ethyl acetate–tetrahydrofuran (1:1, v/v). Fractions containing the fully protected dinucleoside phosphotriester were pooled and the solvents evaporated. The triester was then precipitated from acetone (3 ml) by addition of hexane (500 ml). The precipitate was collected *via* centrifugation and treated overnight with 15 M methanolic ammonia (25 ml) at 0°. After evaporation of solvents, the residue was treated with 80% aqueous acetic acid for 3 hr at room temperature. The acetic acid was removed by evaporation with ethanol. The resulting solid was dissolved in 10 ml of water and extracted with ether (three 5-ml portions). After concentration, the aqueous solution was applied to a DEAE-cellulose column (2.5 × 30 cm). The column was eluted with water followed by a linear gradient (0.0–0.25 M) of ammonium bicarbonate. Fractions containing the dinucleotide were pooled and the solvents and buffer were removed by evaporation to give 115 mg of dAprA. The dimer was further purified by preparative paper chromatography in solvent C. dAprA has R_F values of 1.93 (solvent A) and 1.37 (solvent C) relative to 3′-AMP, which are similar to those reported by Moon *et al.* (1966). The pmr spectrum showed the characteristic base proton signals (8.45(s), 8.49(s), 8.52(s), and 8.77(s) ppm from Me_4Si capillary), as well as a doublet (6.38 ppm) and a quartet (6.58 ppm) corresponding to the two anomeric protons. The presence of the 2′ and 2″ protons of the deoxyribofuranosyl moiety was observed at 2.76 and 2.96 ppm (Fang *et al.*, 1971). Treatment of dAprA with snake venom phosphodiesterase gave two compounds corresponding to adenosine 5′-phosphate and deoxyadenosine.

DEOXYADENYLYL-(3′–5′)-8-DEUTERIOADENOSINE, dAp-8-DrA. Adenosine was heated in D_2O at 85° to exchange the C-8 proton for deuterium. The deuterated nucleoside was converted to its tetrabenzoyl derivative. The dinucleoside monophosphate was then prepared in the same manner as described for dAprA.

DEOXYADENYLYL-(5′–5′)-DEOXYADENOSINE, $dA_{5'}p_{5'}dA$. $N^6,O^{3'}$-Dibenzoyldeoxyadenosine 5′-phosphate (1.13 mmoles) and pyridinium Dowex 50 ion-exchange resin were dried by evaporation with anhydrous pyridine (four 5-ml portions). Deoxyadenosine (3.4 mmoles) suspended in 18 ml of dry pyridine was added and the reaction mixture was treated with dicyclohexylcarbodiimide (1.13 mmoles, 2.33 g) for 7 days at room temperature. The reaction mixture was then treated with 50% aqueous pyridine for 16 hr. After removal of solvents, 50 ml of 7.5 M NH_4OH was added and the solution was allowed to stand for 3 days. The solvents were evaporated and the resulting solid was dissolved in water. The aqueous solution was filtered, concentrated to 3 ml, and applied to a DEAE-cellulose column (2.5 × 30 cm) in the bicarbonate form. The column was eluted with water, followed by a linear gradient of ammonium bicarbonate (0.0–0.2 M). Fractions containing the dinucleotide were pooled, and the solvents and buffer were removed by evaporation to yield 200 mg of $dA_{5'}p_{5'}dA$. This material had the same electrophoretic mobility as its ribosyl analog $rA_{5'}p_{5'}rA$. The pmr spectrum showed the base protons at 8.50(s) and 8.56(s) ppm and the anomeric protons as a pseudotriplet (6.74 ppm). The spectrum was very similar to that of $rA_{5'}p_{5'}rA$ (Kondo *et al.*, 1970). Treatment of $dA_{5'}p_{5'}dA$ with snake venom phosphodiesterase gave two compounds corresponding to deoxyadenosine and deoxyadenosine 5′-phosphate. Treatment with spleen phosphodiesterase showed the presence of a small amount of deoxyadenosine 3′-phosphate and deoxyinosine (this nuclease was contaminated with adenosine deaminase) indicating the dimer was contaminated with a small amount (<1%) of deoxyadenylyl-(3′–5′)-deoxyadenosine. For optical studies the contaminating dimer was removed by treatment of the crude dimer with spleen phosphodiesterase followed by preparative paper chromatography in solvent A. The $dA_{5'}p_{5'}dA$ was eluted from the paper and further purified by DEAE-cellulose chromatography. Fractions containing the pure dimer were pooled and the solvents and buffer were removed by evaporation. The residue was lyophilized from water.

ADENYLYL-(5′–5′)-ADENOSINE, $rA_{5'}p_{5'}rA$. *N*-Benzoyl-$O^{2'}$,-$O^{3'}$-isopropylideneadenosine (160 mg, 0.4 mmole, prepared from isopropylideneadenosine in a manner analogous to the preparation of *N*-benzoyladenosine (Schaller *et al.*, 1963)), $N,O^{2'},O^{3'}$-tribenzoyladenosine 5′-phosphate dipyridinium salt (409 mg, 0.5 mmole, prepared by benzoylation of adenosine 5′-phosphate in a manner analogous to the preparation of $N,O^{3'}$-dibenzoyldeoxyadenosine 5′-phosphate (Ralph and Khorana, 1961)) and pyridinium Dowex 50 resin (250 mg) were dried by evaporation with anhydrous pyridine (3 5-ml portions). The gum was dissolved in anhydrous pyridine (2.5 ml) and treated with dicyclohexylcarbodiimide (1.4 mmoles) followed by shaking at room temperature for 4 days. Water (2 ml) was then added and shaking was continued overnight. The reaction mixture was filtered through glass wool and thoroughly washed with pyridine. The combined filtrate and washings were concentrated to 20 ml and treated with concentrated ammonium hydroxide (30 ml) for 3 days at room temperature. The solvents were evaporated and the residue was dissolved in 0.5 N hydrochloric acid (20 ml). The pH was adjusted to 1.5 by addition of concentrated acid and the solution was heated 1 hr at 80°. After cooling the reaction mixture was neutralized with 10% aqueous tetraethylammonium hydroxide; filtered through glass wool; evaporated and the resulting syrup extracted with chloroform (three 50-ml portions). The remaining residue was dissolved in water and the solution was applied to a DEAE-cellulose column (2.5 × 40 cm) in the bicarbonate form. The column was eluted with water followed by a linear gradient of ammonium bicarbonate (0.0–0.2 M, 4 l. total volume). The dinucleotide was eluted with 0.065 M buffer and was collected. The solvents were evaporated; the residue was repeatedly evaporated with water to remove buffer and finally lyophilized from water to give $rA_{5'}p_{5'}rA$, R_F^A 0.18, R_F^C 0.23. The ultraviolet spectrum showed a λ_{max} at 259 nm and a λ_{min} at 230 nm. The pmr spectrum showed a doublet at 6.31 ppm (H-1′, $J_{1'-2'}$ = 5 cps), a

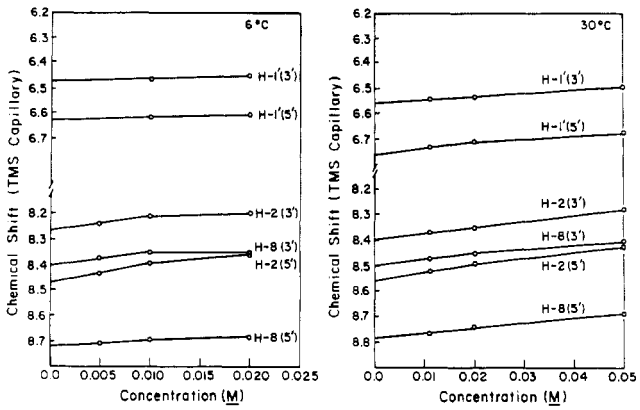

FIGURE 1: Concentration dependence of the chemical shifts of the base and anomeric protons of dApdA at 6 and 30°, in D_2O.

singlet at 8.37 ppm (H-2) and a singlet at 8.47 ppm (H-8) all measured in D_2O using an external Me_4Si capillary. For optical studies the dinucleotide was further purified by preparative paper chromatography in solvent A followed by DEAE-cellulose chromatography and lyophilization from water. The resulting white powder was completely homogeneous as judged by paper chromatography. The uv, CD, and nmr studies of this compound gave the same results as those obtained from the $rA_{5'}p_{5'}rA$ previously synthesized in our laboratory by another route (Kondo *et al.*, 1970).

Results

Proton Magnetic Resonance Studies. SPECTRAL ASSIGNMENTS. The chemical shifts (δ) of the base and anomeric protons of rAprA, dApdA, rApdA, dAprA, $rA_{5'}p_{5'}rA$, and $dA_{5'}p_{5'}dA$ at 4, 30, and 60° in D_2O are shown in Table I. The concentration dependence of the chemical shifts of rAprA at about 30° has been previously reported (Ts'o *et al.*, 1969). In Figure 1 are shown the concentration dependence of the proton resonances of dApdA at both 6° and 30°. Similar concentration dependence behavior was also observed for rApdA and dAprA. The spectral assignments have been previously reported for rAprA (Ts'o *et al.*, 1969; Chan and Nelson, 1969), $rA_{5'}p_{5'}rA$ (Kondo *et al.*, 1970), and dApdA (Fang *et al.*, 1971).

The spectral assignment for $dA_{5'}p_{5'}dA$ is greatly facilitated by the fact that the spectrum at the low-field region consisted of only two base protons and one H-1′ proton. The base-proton resonances appear as two singlets (8.56 ppm and 8.50 ppm, 30°) and are similar to those of the ribofuranosyl analog, $rA_{5'}p_{5'}rA$ (Kondo *et al.*, 1970). The H-1′ resonance appears as a pseudotriplet at 6.74 ppm. The intensity of the singlet at 8.56 ppm decreases after the dimer has been heated at 85° for 4 hr in D_2O Therefore this resonance was assigned to the C-8 protons (Schweizer *et al.*, 1964; Bullock and Jardetzky, 1964) while the resonance at 8.50 ppm was assigned to the C-2 protons. As discussed in the previous paper on $rA_{5'}p_{5'}rA$, the magnetic equivalence of the same protons in both residues of the 5′–5′ dimer indicates a symmetrical conformation.

The anomeric protons of the mixed-dimer rApdA appear as a doublet (6.26 ppm, 30°) and a pseudotriplet (6.80 ppm, 30°) and were assigned on the basis of the following information: (1) the shielding effect of a cis hydroxyl group (Ts'o *et al.*, 1969) causes the H-1′ resonance of the ribofuranose to occur at significantly higher field than that of the deoxyribofuranose, and (2) the H-1′ of the ribofuranose appears as a doublet while the corresponding H-1′ of the deoxyribofuranose appears either as a quartet or as a pseudotriplet (Fang *et al.*, 1971). The doublet centered at 6.26 ppm (30°) was therefore assigned to the H-1′ of the rAp residue of the dimer, while the pseudotriplet at 6.80 ppm was assigned to the H-1′ of the pdA residue. These proton resonances of the rApdA appear at positions similar to the corresponding proton resonances in the homodimers, rAprA (6.29 ppm) and dApdA (6.76 ppm).

The base-proton resonances of rApdA appear as four singlets at 8.495, 8.57, 8.665, and 8.715 ppm (30°). For the assignment of the H-8 resonances, 8-deuterioadenylyl-(3′–5′)-deoxyadenosine was prepared. The spectrum of this deuterated dimer lacked the signal at 8.665 ppm and this resonance was therefore assigned to the H-8 of the rAp portion of the dimer. Upon heating the dimer in D_2O, the intensity of the 8.715-ppm resonance diminished. Therefore this resonance was assigned to the H-8 of the pdA portion of the dimer. The H-2 resonances were assigned after careful inspection of Corey–Pauling–Kolton models and consideration of the dimerization shift data shown in Table II. Previous work on mononucleotides (Schweizer *et al.*, 1968), ribofuranosyl dimers (Ts'o *et al.*, 1969; Chan and Nelson, 1969), and deoxyribofuranosyl dimers (Fang *et al.*, 1971) has shown that the nucleotidyl residues comprising the dimer are in an anti,anti-stacked conformation. Since the δ values of the base protons of rApdA and rAprA are very similar (Table I), we may also conclude that the nucleosidyl units in rApdA are also in the anti conformation. Examination of a Corey–Pauling–Kolton model of rApdA in the anti,anti-stacked conformation shows

TABLE I: Chemical Shifts of the Base and H-1′ Protons of Adenine–Dinucleoside Monophosphates at 4, 30, and 60° (D_2O, pD 7.4)[a] (Parts per Million from Tetramethylsilane Capillary).

Dinucleotide	Temp (°C)	($A_{3'}p$) Residue H-8	H-2	H-1′	($p_{5'}A$) Residue H-8	H-2	H-1′
rAprA	4	8.59	8.29	6.20	8.585	8.48	6.31
rApdA		8.595	8.38	6.16	8.645	8.47	6.685
dApdA		8.375	8.25	6.47	8.715	8.445	6.635
dAprA		8.38	8.28	6.47	8.71	8.38	6.25
$rA_{5'}p_{5'}rA$					8.445	8.41	6.295
$dA_{5'}p_{5'}dA$					8.465	8.435	6.69
rAprA	30	8.66	8.47	6.29	8.69	8.61	6.41
rApdA		8.665	8.495	6.26	8.715	8.57	8.80
dApdA		8.49	8.41	6.57	8.78	8.55	6.76
dAprA		8.49	8.445	6.58	8.77	8.515	6.38
$rA_{5'}p_{5'}rA$					8.525	8.50	6.38
$dA_{5'}p_{5'}dA$					8.56	8.50	6.74
rAprA	60	8.745	8.65	6.43	8.835	8.715	6.555
rApdA		8.72	8.62	6.41	8.81	8.72	6.90
dApdA		8.59	8.53	6.67	8.83	8.64	6.86
dAprA		8.60	8.58	6.67	8.84	6.62	6.49
$rA_{5'}p_{5'}rA$					8.66	8.63	6.48
$dA_{5'}p_{5'}dA$					8.67	8.63	6.85

[a] All data have been extrapolated to infinite dilution except those at 60° which were measured at 0.02 M.

that H-2 of the rAp portion is more shielded than H-2 of the pdA portion, regardless of the handedness of the dimer. The observed dimerization shift of the high-field H-2 resonance (0.19 ppm) is nearly twice as large as the low-field H-2 resonance (0.10 ppm, Table II). On this basis, the resonance at 8.495 is assigned to the H-2 of rAp residue, while that at 8.57 ppm is assigned to the H-2 of the pdA residue of the dimer. The dimerization shift data exclude a 3′-syn,5′-anti model, since both H-2 protons would be slightly shielded, and to about the same extent.

The assignment of the pmr spectrum of the mixed dimer dAprA proceeded in much the same manner as for rApdA. The anomeric proton resonances appeared as a quartet at 6.58 ppm (30°) and a doublet at 6.38 ppm (30°). The quartet was assigned to the H-1′ of the dAp portion of the dimer while the doublet was assigned to the H-1′ of the prA residue. Again the multiplicity and chemical shift values of these protons in dAprA are very similar to the corresponding protons in the homodimers dApdA (6.57 ppm) and rAprA (6.41 ppm). The base-proton resonances of dAprA appeared as four singlets at 8.445, 8.49, 8.515, and 8.77 ppm (30°). The H-8 resonances were distinguished by preparation of the specifically deuterated dimer, deoxyadenylyl-(3′–5′)-8-deuterioadenosine, which lacked the proton resonance at 8.77 ppm. Thus this resonance was assigned to the H-8 of the prA residue. Upon heating the dimer in D_2O, the intensity of the resonance at 8.49 ppm decreased. This resonance was therefore assigned to the H-8 of the dAp residue. The assignment of the H-2 resonances was again based upon inspection of Corey–Pauling–Kolton models and consideration of dimerization shift values (Table II). Both H-2 protons have large dimerization shifts. A model of dAprA in the anti, anti-stacked conformation predicts that the H-2 of the dAp portion should be more shielded relative to the H-2 of the prA portion. The higher field C-2 proton has a $\Delta\delta_D$ value of 0.24 ppm while the $\Delta\delta_D$ of the low-field H-2 is 0.17 ppm. Thus the singlet at 8.445 ppm was assigned to the H-2 of the dAp fragment and singlet at 8.515 ppm was assigned to the H-2 of the prA residue.

DIMERIZATION SHIFTS ($\Delta\delta_D$). The concept of dimerization shift ($\Delta\delta_D = \delta Np - \delta NpN'$, or $= \delta pN' - \delta NpN'$) has been found to be very informative in the evaluation of the pmr data of dimers (Ts'o *et al.*, 1969; Kondo *et al.*, 1970). Most of the $\Delta\delta_D$ originates from the nonbonded interaction, especially the base–base interaction, between the two neighboring units in the dimer. In Table II, the $\Delta\delta_D$ values for the base and anomeric protons of the six adenine dinucleoside monophosphates are shown. Since these values will be used extensively for the construction of the conformational models in a later section, only two distinct features will be briefly mentioned here. First, within the experimental error (±0.02–0.03 ppm from extrapolation to infinite dilution) and the temperature range of 4–60°, the $\Delta\delta_D$ values for $rA_{5'}p_{5'}rA$ are nearly the same as those for $dA_{5'}p_{5'}dA$; and the $\Delta\delta_D$ values for dApdA are nearly the same as those for dAprA. The $\Delta\delta_D$ values for rAprA at 30° and 60° are also the same as that for rApdA; though at 4°, there appeared to be a small but real difference (0.09 ppm). Second, the dimerization shift data clearly indicate that the conformation of dApdA is substantially variant from that of rAprA. The $\Delta\delta_D$ values for 4 protons of dApdA (H-8 and H-1′ from 3′ residue; H-2 and H-1′ from 5′ residue) are much larger than those of the corresponding protons of rAprA. On the other hand, the $\Delta\delta_D$ of H-8 from the 5′ residue of rAprA is significantly larger than that of dApdA; while the $\Delta\delta_D$ of H-2 from the 3′ residue of rAprA is about the same as that of dApdA at 4°, and became smaller than that of dApdA at 30° and 60°. These data not only indicate that the extent of base–base stacking is different between dApdA and rAprA, but also that the mode of the base–base stacking is different between dApdA and rAprA. This difference in the conformation between dApdA and rAprA is also reflected in the results from temperature and solvent studies as discussed in the following section.

TABLE II: Dimerization Shifts, $\Delta\delta_D$,[a] of the Base and H-1′ Protons of Adenine–Dinucleoside Monophosphates at 4, 30, and 60° (D_2O, pD 7.4).

Dinucleotide	Temp (°C)	($A_{3'}p$) Residue H-8	H-2	H-1′	($p_{5'}A$) Residue H-8	H-2	H-1′
rAprA	4	0.155	0.315	0.285	0.285	0.11	0.19
rApdA		0.15	0.225	0.325	0.23	0.15	0.225
dApdA		0.325	0.31	0.42	0.16	0.175	0.275
dAprA		0.32	0.28	0.41	0.16	0.21	0.25
$rA_{5'}p_{5'}rA$					0.425	0.18	0.205
$dA_{5'}p_{5'}dA$					0.41	0.185	0.22
rAprA	30	0.12	0.215	0.26	0.235	0.075	0.15
rApdA		0.11	0.19	0.29	0.19	0.13	0.16
dApdA		0.295	0.27	0.36	0.125	0.15	0.20
dAprA		0.295	0.235	0.35	0.155	0.17	0.18
$rA_{5'}p_{5'}rA$					0.40	0.185	0.18
$dA_{5'}p_{5'}dA$					0.345	0.20	0.22
rAprA	60	0.12	0.135	0.19	0.17	0.08	0.10
rApdA		0.14	0.165	0.21	0.17	0.07	0.12
dApdA		0.25	0.23	0.32	0.12	0.15	0.16
dAprA		0.24	0.18	0.32	0.155	0.175	0.17
$rA_{5'}p_{5'}rA$					0.335	0.165	0.18
$dA_{5'}p_{5'}dA$					0.28	0.16	0.17

[a] $\Delta\delta_D = \delta A_p - \delta ApA$; or $\delta pA - \delta ApA$. Data from Table I.

TEMPERATURE AND SOLVENT EFFECTS. The effect of temperature on the dimerization shifts of the six dimers is also shown in Table II. The $\Delta\delta_D$ values of rAprA are more temperature sensitive than the corresponding $\Delta\delta_D$ values of dApdA. Between 4 and 60°, the $\Delta\delta_D$ of the H-2 and H-1′ of the rAp portion and the H-8 and and H-1′ of the prA portion varies from 58 to 33%. The temperature variation of $\Delta\delta_D$ for the six protons of dApdA is less than 26% in the temperature range 4–60°. In fact, the dimerization shifts of both rApdA and rAprA appear to be more temperature sensitive than the $\Delta\delta_D$ values of both dAprA and dApdA.

The value of $\Delta\delta_D$ is relatively temperature independent for the dimer $rA_{5'}p_{5'}rA$ at the 4–60° range (Kondo *et al.*, 1970). The deoxyribodimer $dA_{5'}p_{5'}dA$ behaves in a similar manner. The $\Delta\delta_D$ values of the base protons of this deoxy 5′ dimer are very similar at all temperatures, and also similar to the values for the $rA_{5'}p_{5'}rA$ in this temperature range.

The chemical shifts of the dimer base protons and anomeric protons in dimethyl-d_6 sulfoxide are shown in Table III. Dimethyl sulfoxide has been shown to disrupt base stacking in nucleic acids (Ts'o *et al.*, 1969; Kondo *et al.*, 1970; Crawford *et al.*, 1971). The similarity of the chemical shift values of all these dimers to those of the corresponding monomers and to each other is a good indication that the dimers are not stacked in this solvent. This study clearly indicates that the differences in dimerization shifts of these six adenine dimers are due to differences in conformation.

TABLE III: Chemical Shifts of the Base and H-1′ Protons of Adenine–Dinucleoside Monophosphates in Me_2SO-d_6 at 30° (Parts per Million from Tetramethylsilane Capillary).

	($A_{3'}p$) Residue				($p_{5'}A$) Residue			
Dinucleotide	H-8	H-2	H-1′	$-NH_2$	H-8	H-2	H-1′	$-NH_2$
rAprA	8.70	8.48	6.23	7.67	8.78	8.50	6.29	7.59
dAprA	8.69	8.49	6.68		8.78	8.51	6.29	
dApdA	8.69	8.51	6.68		8.80	8.52	6.72	
$rA_{5'}p_{5'}rA$					8.83	8.50	6.23	7.60
$dA_{5'}p_{5'}dA$					8.79	8.49	6.72	7.56

TABLE IV: Temperature and Solvent Effect on the Coupling Constants of the H-1′ Protons of Adenine–Dinucleoside Monophosphates (D_2O, pD 7.4) (Parts per Million from Tetramethylsilane Capillary).

	rAprA		rApdA		dAprA		dApdA		$rA_{5'}p_{5'}rA$	$dA_{5'}p_{5'}dA$
Temp (°C)	$J(3')$	$J(5')$	$J(3')$	$J(5')$	$J(3')$	$J(5')$	$J(3')$	$J(5')$	$J(5')$	$J(5')$
5	2.5	2.0	3.5	5.6	8.8, 4.8	6.0	8.8, 5.6	6.5	4.3	6.5
30	3.2	3.5	4.0	6.0	8.0, 5.5	5.7	8.5, 5.5	6.4	4.6	6.5
60	4.5	4.1	4.5	6.3	8.1, 6.0	5.3	7.9, 6.0	6.7	4.7	7.0
Me_2SO-d_6 (30°)	6.8	5.2			7.5, 5.2	5.2	7.9, 5.7	6.1	5.7	7.1

COUPLING CONSTANTS BETWEEN H-1′ AND H-2′ (OR H-2″). The coupling constants $J_{1'-2'}$ for the ribosyl dimers and $J_{1'-2'}$, $J_{1'-2''}$ or $0.5J_{1'-2'}+ {}_{1'-2''}$ for the deoxyribosyl dimers are presented in Table IV. Recently Fang *et al.* (1971) reported an extensive analysis of the coupling constants of the sugar protons in dApdA. The coupling constants $J_{1'-2''}$, $J_{1'-2''}$, $J_{2'-3'}$, and $J_{2''-3'}$ of both the dAp and pdA portions were obtained by first-order analyses of 100- and 220-MHz spectra. The various dihedral angles were obtained by use of the Karplus equation and examination of molecular models. The J values were found to be (1) fairly temperature and solvent (Me_2SO-d_6) independent, and (2) quite similar to the values of the constituent monomers which were also temperature and solvent independent. The result of this work suggested that the furanose conformation of 3′-dAMP and the dAp portion of dApdA is C(2′)-endo (envelope) or C(2′)-endo–C(3′)-exo (twisted form), while that of 5′-dAMP and the dpA portion of dApdA is a rapid equilibrium between C(2′)-endo and C(3′)-endo.

Several groups have studied the effects of temperature and solvent on $J_{1'-2'}$ of rAprA (Hruska and Danyluk, 1968; Chan and Nelson, 1969; Ts'o *et al.*, 1969; Kondo *et al.*, 1970; Fang *et al.*, 1971). Unlike the deoxyribosyl dimer case, the (H-1′)–(H-2′) coupling constants of rAprA are much smaller than those of the constituent monomers. As shown in Table IV, the (H-1′)–(H-2′) coupling constants of rAprA increase in the temperature range 5–60°. In Me_2SO-d_6 the J values are large and similar to those of the corresponding monomers. Thus in aqueous solution, the furanose conformation of the monomer and the conformation of the furanose in the dimer rAprA is not the same. Furthermore, the results indicate that the furanose conformation of rAprA is influenced by the stacking of the bases, while the furanose conformation of dApdA is not (Fang *et al.*, 1971).

The J values of both the deoxyribosyl and ribosyl portions of the mixed dimer dAprA are essentially temperature invariant. On the other hand, the coupling constants of the rAp residue in the mixed dimer rApdA increase with increasing temperature, although this increase is smaller than that observed for the parent dimer rAprA. This is because at low temperature, the $J_{1'-2'}$ value of the rAp residue in rApdA is larger than that of rAp in rAprA. Contrarily, the $J_{1'-2'}$ value of the pdA residue in rApdA is more temperature dependent than that of dApdA. This is due to the fact that the $J_{1'-2'}$ value of the pdA residue in the mixed rApdA at low temperature is smaller than that of the parent dApdA. These results on the 3′–5′ dimers suggest that the dependency of the furanose conformation of the rA residue on temperature or on base stacking in the dimer can be listed in the following increasing order: dAprA < rApdA < rAprA. Similarly, the dependency of the furanose conformation of the dA residue on temperature or on base stacking in the dimer can be listed as: dApdA ≈ dAprA < rApdA. This summary indicates that the furanose conformation in a dimer will be sensitive to temperature and base stacking, if this dimer has a ribosyl nucleotide at the 3′ residue as rAp.

The coupling constant of the 5′–5′ deoxy dimer $dA_{5'}p_{5'}$-dA is not sensitive to temperature and is very similar to that of dApdA (Table IV). The coupling constant of the 5′–5′ ribosyl dimer $rA_{5'}p_{5'}rA$ is slightly temperature dependent. The $J_{1'-2'}$ values of $rA_{5'}p_{5'}rA$ are larger than those of the prA residue in rAprA, but smaller than those of 5′-rAMP (Ts'o *et al.*, 1969).

Ultraviolet-Absorbance Studies. The molar extinction coefficients and the hypochromicity values of the six dimers are listed in Table V. The values for rApdA, dAprA, and $dA_{5'}$-$p_{5'}dA$ were determined by chemical or enzymatic degradation techniques (Experimental Section). The molar extinction coefficients of rAprA, $rA_{5'}p_{5'}rA$ (Kondo *et al.*, 1970), and dApdA (Miller *et al.*, 1971) have been previously published.

TABLE V: Molar Extinction Coefficients of Adenine Dinucleoside Monophosphates at Neutral pH and Room Temperature.

Dinucleotide	λ (nm)	Molar Extinction Coef (10^3)	% Hypochromicity
rAprA[a]	258	13.6	11.7
rApdA	257	13.3	13.7
dAprA	257	12.5	17.6
dApdA[b]	258	12.7	16.5
$rA_{5'}p_{5'}rA$[a]	259	12.0	22.1
$dA_{5'}p_{5'}dA$	259	11.5	24.3

[a] Data from Kondo *et al.* (1970). [b] Data from Miller *et al.* (1971).

The data clearly indicate that the per cent hyprochromicity of rAprA is significantly smaller than that of dApdA. From this measurement, the results suggest that intramolecular base stacking in dApdA is significantly higher than in rAprA. This suggestion is consistent with the proton magnetic resonance studies.

The mixed-dimer rApdA has approximately the same hypochromicity value as rAprA, while the value of dAprA is similar to that of dApdA. In both cases, the extent of hypochromicity, and therefore the extent of stacking, appears to be determined by the furanose of the 3′ residue of the dimer; the dimer which has a ribose nucleotide (rAp) at the 3′ residue has less hypochromicity and therefore less stacking.

The 5′–5′ isomers $dA_{5'}p_{5'}dA$ and $rA_{5'}p_{5'}rA$ appear to have the largest amount of base stacking of the six dimers studied. The extent of stacking is approximately the same in both 5′–5′ dimers, however. The large extent of base overlap in the $rA_{5'}p_{5'}rA$ has been noticed and discussed previously (Kondo *et al.*, 1970).

Circular Dichroism Studies. Figure 2 shows the circular dichroism spectra of rAprA, dApdA, rApdA, and dAprA at 3° in the wavelength range 230–300 nm. The shapes of all four curves are quite similar. All show a peak at 272 nm, a trough at about 250 nm, and a crossover point at approximately 258 nm. The spectra of $rA_{5'}p_{5'}rA$ and $dA_{5'}p_{5'}dA$ are shown in Figure 3. Again the peak occurs at 272 nm, while the trough and crossover point occur at 254 and 264 nm, respectively.

Although qualitatively similar, the spectra or the four 3′–5′ dimers differ quantitatively. The ribosyl dimer rAprA shows the greatest [θ] values in both peak and trough approximately twice as large as the deoxyribosyl dimer dApdA (2.8×10^4 *vs.* 1.8×10^4 at 272 nm, and -3.2×10^4 *vs.* -2.2×10^4 at 250 nm).

The mixed dimers rApdA and dAprA also show quantitative differences in their CD spectra. The magnitude of the molecular ellipticity from peak to trough of dAprA is approximately twice as large as that of rApdA (2.2×10^4 *vs.* 1.1×10^4 at 272 nm, and -2.3×10^4 *vs.* -1.7×10^4 M at 252 nm). At 3 ± 1°, the magnitude of [θ] from peak to trough of the four 3′–5′ dimers can be arranged in the following decreasing order: rAprA > dAprA ≈ dApdA > rApdA.

The effect of temperature on the molecular ellipticities of the four 3′–5′ dimers are shown in Figure 4. It should be noted that while the slopes of [θ] *vs.* temperature of dApdA, dAprA, and rApdA are about the same, the slope of [θ] *vs.* temperature of rAprA is distinctly steeper (Figure 4). As the result of the difference in thermo sensitivity, the magnitude of [θ] of rAprA becomes smaller than that of dAprA or becomes equal to that of dApdA at 50–60°.

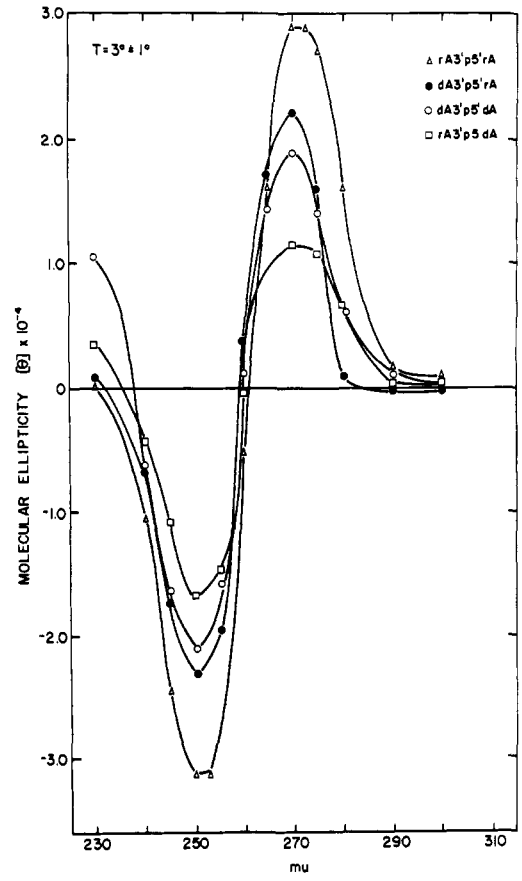

FIGURE 2: Circular dichroic spectra of rAprA (Δ), dAprA (●), dApdA (○), and rApdA (□) in 0.05 M $NaClO_4$ (pH 7.3) at 3 ± 1°.

The spectra in Figure 3 show that at 3° the magnitude of [θ] of $rA_{5'}p_{5'}rA$ is about twice that of $dA_{5'}p_{5'}dA$ (2.15×10^4 *vs.* 1.15×10^4 at 272 nm, and -2.0×10^4 *vs.* -0.75×10^4 at 255 nm). The slope of [θ] *vs.* temperature, however is about the same for both 5′–5′ dimers (Figure 5). From 2° to 60°, the magnitude of [θ] of $rA_{5'}p_{5'}rA$ is always substantially larger than that of $dA_{5'}p_{5'}dA$.

The CD properties of the adenine–dinucleoside monophosphates have been studied previously by various laboratories, including our own. The CD properties of rAprA have been reported by Van Holde *et al.* (1965), Maurizot *et al.* (1969), and Kondo *et al.* (1970). The CD properties of dApdAp have been reported by Warshaw and Cantor (1970), and those of pdApdA have been reported by Bush and Scheraga (1969). Bush and Scheraga also found that at 23° (pH 7.4, 0.01 M Tris), the magnitude of [θ] of rAprA is much larger than that of dAprA, in agreement with our results (Figures 1 and 4). In a brief report, Maurizot *et al.* (1969) have compared the CD properties of rAprA, rApdA, dApdA, and dAprA in 4.7 M KF from −20° to about 60–70°. Their results from 0° to 60° are in agreement with ours, *i.e.*, the order of the magnitude of the CD bands (one positive and one negative) was rAprA > dAprA ~ dApdA > rApdA, and the CD bands of rAprA had a greater dependence on temperature than those of dApdA. While the [θ] values reported here are comparable to those published previously, the values in this paper are probably more accurate since

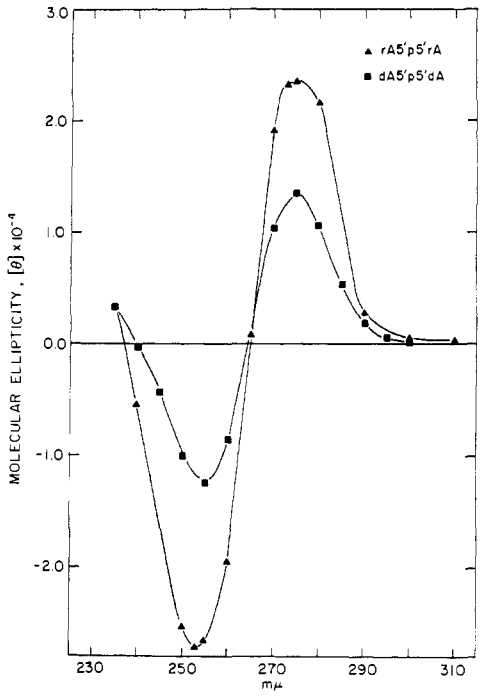

FIGURE 3: Circular dichroic spectra of $rA_{5'}p_{5'}rA$ (▲) and $dA_{5'}p_{5'}dA$ (■) in 0.05 M $NaClO_4$ (pH 7.3) at 3 ± 1°.

they were calculated on the basis of extinction coefficients determined experimentally. In several previous reports, the $[\theta]$ values were calculated using the extinction coefficient of rAprA and not from the actual compounds. A comprehensive study on the comparison of the CD properties between ribosyl *vs.* deoxyribosyl dimer has been made by Warshaw and Cantor (1970), and their results will be discussed later.

Discussion

Conformational Models. The general features of the conformational model of rAprA and $rA_{5'}p_{5'}rA$ have been discussed extensively in the preceding papers in this series (Ts'o *et al.*, 1969; Kondo *et al.*, 1970). Both nucleosidyl residues in the dimer are in the anti conformation, and the screw axis of the stack is right handed. Independently, Chan and Nelson

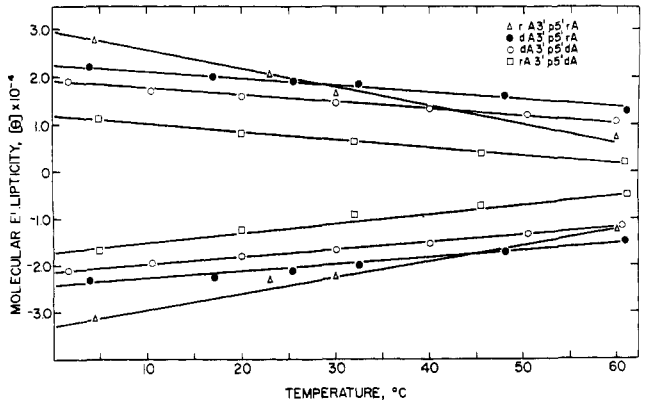

FIGURE 4: The temperature dependence of the molecular ellipticity, $[\theta]$, at the peak (272 nm) and trough (252 nm) of rAprA (Δ) and at the peak (270 nm) and trough (250 nm) of dAprA (●), dApdA (○), and rApdA (□) in 0.05 M $NaClO_4$ (pH 7.3).

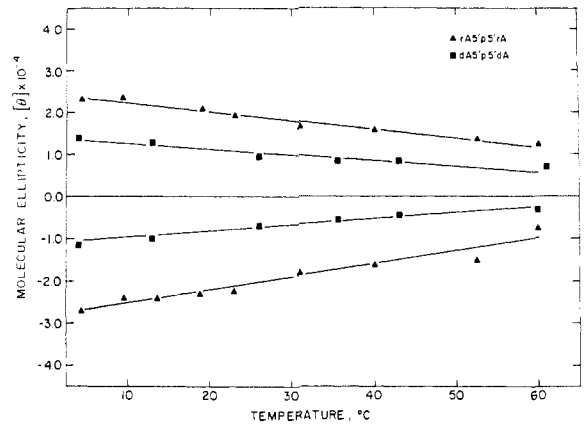

FIGURE 5: The temperature dependence of the molecular ellipticity, $[\theta]$, at the peak (275 nm) and trough (252 nm) of $rA_{5'}p_{5'}rA$ (▲) and at peak (275 nm) and trough (255 nm) of $dA_{5'}p_{5'}dA$ (■) in 0.05 M $NaClO_4$ (pH 7.3).

(1969) also came to this same conclusion from their extensive pmr studies on rAprA. These same general features were found to be true also for the conformation of dApdA (Fang *et al.*, 1971; Miller *et al.*, 1971). The specific relaxation effect of Mn^{2+} on H-8 of the pdA residue in pmr studies the upfield shift of H-8 of the p(R)dA residue in the alkyl phosphotriester of dApdA *vs.* (dAp(R)dA) the same proton in dApdA and the shielding patterns of the base protons, have established that the nucleosidyl units in dApdA are in the anti conformation. The extensive shielding of H-1′ and endo H-2″ in the dAp residue conclusively indicates that the screw axis of the stack is right handed. Quantitative calculations of the CD pattern of rAprA support a model of anti,anti, right-handed stack as in DNA or RNA (Bush and Tinoco, 1967; Johnson and Tinoco, 1969). The CD patterns of all six adenine dinucleoside monophosphates are similar, as indicated in this and other studies. Therefore, CD studies suggest that all these adenine dimers most likely have the same basic conformation. Similarly, the dimerization shift values of rApdA are nearly the same as those of rAprA, those of dAprA the same as those of dApdA, and those of $dA_{5'}p_{5'}dA$ the same as those of $rA_{5'}p_{5'}rA$. Therefore, the pmr studies also indicate that all these adenine dimers have the anti,anti, right-handed conformation.

Potentially, the pmr data can provide information about the conformation in greater detail. In the consideration of the relationship of pmr data to the geometry of the dimer conformation, we shall assume that the values of $\Delta\delta_D$, the parameter for the measurement of base–base interaction, originate entirely from the influence of the diamagnetic anisotropy of the neighboring adenine. The isoshielding zones of adenine due to the ring current effect have been recently calculated by Giessner-Prettre and Pullman (1970), following the previous approach of Waugh and Fessenden (1957), and Johnson and Bovey (1958). The published curves are for protons located in a plane 3.4 Å away from the adenine surface. These isoshielding curves have been used for defining the conformation of rAprA, $rA_{2'}p_{5'}rA$, $rA_{5'}p_{5'}rA$, rAprC, and $rA_{2'}p_{5'}rC$ (Kondo *et al.*, 1970). This study also indicates that the distances of 3–4 Å from the plane of the bases are applicable for model construction in accordance with the nmr data, while the distances of 2 and 5 Å are not. In Figure 6, the schematic presentation of the front view of the conformational model for rAprA is shown. The bases are taken

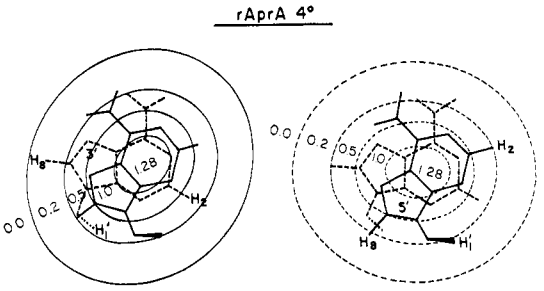

FIGURE 6: Schematic presentation of the conformational model of rAprA as viewed in the 5′ to 3′ direction. The dimensions of the bases and the magnetic isoshielding zones are from the work of Giessner-Prettre and Pullman (1970). The bases are parallel to each other with a distance of 3.4 Å (bases with solid lines are above bases with dotted lines) and to the plane of the paper with the H-1′ protons out of the plane. The relative orientation between the two bases was determined by consideration of the pmr data at 4°; the isoshielding zone values of Giessner-Prettre and Pullman; and backbone constraint determined by building Corey–Pauling–Koltun and Kendrew models. The nucleotidyl units have an anti conformation and the screw axis is right handed, *i.e.*, the axis advances upward from the plane of the paper in a counterclockwise manner.

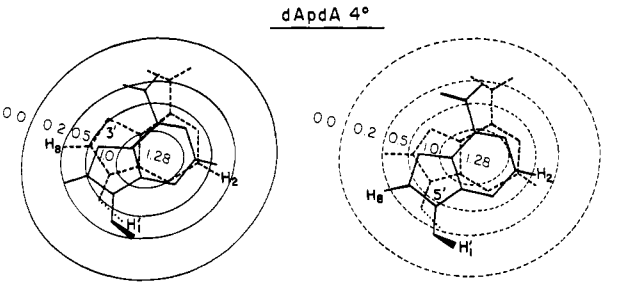

FIGURE 7: Schematic presentation of conformational model of dApdA. Explanation is the same as that for Figure 6.

to be parallel with a vertical distance of 3.4 Å. This model is constructed on the basis of the $\Delta\delta_D$ values at 4° (Table II) and of the isoshielding curves of Giessner-Prettre and Pullman (1970). The isoshielding zones of the 3′ residue (rAp) at the bottom is shown in Figure 6a, while the isoshielding zones of the 5′ residue (prA) at the top is shown in Figure 6b. The arrangement of the two residues is not only governed by the consideration of matching the $\Delta\ \delta_D$ values of 6 protons to two appropriate sets of shielding, but also by the constraint of the backbone as indicated by both Corey–Pauling–Kolton and Kendrew models. The location of the H-1′ is less certain since they are not in the same plane as the base protons. The same approach is adopted to construct the schematic presentation of dApdA in Figure 7. It should be noted that these static models are only the representation of the time-average results of a dynamic phenomenon.

A comparison between the model of rAprA (Figure 6) and that of dApdA (Figure 7) reveals interesting differences. First, the extent of the base–base overlap is significantly larger in dApdA than in rAprA. Second, the bases are almost parallel to each other in dApdA, while the bases are definitely oblique to each other in rAprA. For instance the angle between the two C(4)–C(5) bonds in dApdA is about 15° (0° would indicate a parallel orientation between two adenines), while this angle is 45–50° in rAprA. The first difference explains why four protons out of six in dApdA have higher $\Delta\delta_D$ values than those in rAprA. The second difference explains why the $\Delta\delta_D$ value (0.285 ppm) of H-8 of (prA) in rAprA is significantly higher than that (0.16 ppm) for the same proton in dApdA; this H-8 is the only proton in rAprA more shielded than its counterpart in dApdA (Table II). As shown in Figures 6 and 7, the H-8 in the prA residue is closer to the six-membered ring than the H-8 in the pdA residue because of the oblique arrangement. Such a situation would cause the H-8 in the prA residue to be in a higher shielding zone.

Since the $\Delta\delta_D$ values of dAprA are practically identical with those of dApdA, the conformational model of dAprA should be the same as that of dApdA (Figure 7). Similarly, the model of rApdA is probably close to that of rAprA (Figure 6); however, the $\Delta\delta_D$ values of these two dimers at this temperature (4°) are close but not the same. Again, the $\Delta\delta_D$ values of $dA_{5'}p_{5'}dA$ are the same as those of $rA_{5'}p_{5'}rA$, therefore the model of $dA_{5'}p_{5'}dA$ should be the same as the model of $rA_{5'}p_{5'}rA$, which has been constructed by the same approach and published previously (Kondo *et al.*, 1970). At higher temperature, the movement of the bases away from each other takes place through rotations in the phosphodiester linkage. As a result, the $\Delta\delta_D$ values decrease at higher temperature. In our previous pmr study on the adenine–dinculeoside alkyl phosphotriesters, it was shown that the two adenine planes are continuously rotating relative to each other (Miller *et al.*, 1971). At low temperature and in aqueous solution, the rotation rate is slow and the base planes tend to spend a long time in a stacked conformation; while at high temperature, the rotation rate becomes fast and the base planes spend more time away from each other. This study and our pmr study on adenine ribosyl dimers (Kondo *et al.*, 1970) clearly indicate that the "two-states" model is not applicable for the treatment of the temperature effect on the conformation of dinucleoside monophosphates.

Correlation among the Results fron Uv Absorbance, Circular Dichroism, and Pmr Studies. Hypochromicity is the measurement of base–base interaction in a dimer by the uv-absorbance method, while the amplitude in mean residue ellipticity $[\theta]$ between the peak and the trough of the CD curve serves the same purpose in CD studies (Kondo *et al.*, 1970). In this section, these parameters from optical measurements are compared to the $\Delta\delta_D$ values from the pmr study which has been used for the construction of conformational models in the preceding section.

The hypochromicity results are in good agreement with the $\Delta\delta_D$ values. The agreement can be examined in two aspects. First, within experimental error, the hypochromicity of rAprA is essentially the same as rApdA, dAprA the same as dApdA, and $rA_{5'}p_{5'}rA$ the same as $dA_{5'}p_{5'}dA$ (Table V). As shown in Table II, this situation also holds for $\Delta\delta_D$ values. Thus, the hypochromicity data and the $\Delta\delta_D$ values both indicate that each member of these three pairs of dimers, *i.e.*, rAprA and rApdA, dApdA and dAprA, $rA_{5'}p_{5'}rA$ and $dA_{5'}p_{5'}dA$, has the same conformation as the other in the pair. Second, the hypochromicity of these three pairs can be arranged in the following decreasing order (Table II): $rA_{5'}p_{5'}rA$ (same as $dA_{5'}p_{5'}dA$) > dApdA (same as dAprA) > rAprA (same as rApdA). This is the same order for the extent of the overlap of the bases in the dimer, as shown by the conformational models constructed from the $\Delta\delta_D$ values (Figures 6 and 7; Kondo *et al.*, 1970). Therefore, the hypochromicity data support the conformational models derived from the pmr study.

As for the CD results, the situation is much more complex.

Not only do the amplitudes in $[\theta]$ between the peak and the trough of these six dimers not follow the order of hypochromicity (or the order of base–base overlap in the conformational models), but also the pair members in two pairs (*i.e.*, rAprA *vs.* rApdA; $rA_{5'}p_{5'}rA$ *vs.* $dA_{5'}p_{5'}dA$) do not have similar amplitudes. For instance, while the per cent of hypochromicity and the $\Delta\delta_D$ values (therefore the conformational model) of $rA_{5'}p_{5'}rA$ are very similar to those of $dA_{5'}p_{5'}dA$, the amplitude of $[\theta]$ of $rA_{5'}p_{5'}rA$ is about twice as large as that of $dA_{5'}p_{5'}dA$ (Figures 3 and 5). The differences in amplitude among these six adenine homodimers can be attributed to the following three factors: (1) the averaged distance between the two transition dipoles of the two adenines; (2) the averaged angle θ (not $[\theta]_{CD}$) between the two transition dipoles; (3) the averaged proportion of the right-handed conformer *vs.* the left-handed conformer in the population. These factors have been discussed extensively in our previous paper on the conformation of the adenine ribosyl dimers with different positions of phosphodiester linkage (Kondo *et al.*, 1970). It was found that while the hypochromicity data and the pmr results are in good agreement for the order of base–base interaction among the three adenine dimers ($rA_{5'}p_{5'}rA$ > $rA_{2'}p_{5'}rA$ > $rA_{3'}p_{5'}rA$), the order from CD results differs ($rA_{3'}p_{5'}rA$ > $rA_{5'}p_{5'}rA$ > $rA_{2'}p_{5'}rA$). This variance was reconciled by the consideration that the modes of base–base overlap are different among these three dimers. As shown from these conformational models, while the extent of base–base overlap is larger in $rA_{5'}p_{5'}rA$ and $rA_{2'}p_{5'}rA$ than in $rA_{3'}p_{5'}rA$, the angle ($\theta_{transition}$) between the in-plane transition dipoles for π–π^* transition (which is the same as the angle between the geometrical principal axis of the bases in the case of a homodimer) may be smaller in $rA_{2'}p_{5'}rA$ and in $rA_{5'}p_{5'}rA$ than that in $rA_{3'}p_{5'}rA$. Since the amplitude in the CD curve is very sensitive to the θ value (a function of $\sin\theta\cos\theta$ for a dipole approximation), $rA_{3'}p_{5'}rA$ could have a larger amplitude than the other two dimers even if it has a smaller extent of base–base overlap in its stack.

This same reasoning may be applicable in the comparison between the rAprA and dApdA. From the hypochromicity data (~12% *vs.* ~17%; Table V) and from the conformational models (Figures 6 and 7), the degree of the base–base overlap is certainly more extensive in the dApdA than in rAprA; yet the amplitude of the CD curve of rAprA is significantly larger than that of dApdA at low temperature (by ~30% at 0°, ~20% at 25°). In the preceding section, it was pointed out that the bases are almost parallel to each other in dApdA, while the bases are definitely oblique to each other in rAprA. As a consequence, the θ transition (same as the angle between the two C(4)–C(5) bonds) is only about 15° in dApdA, but about 45–50° in rAprA. This substantial difference in θ transition most likely is the factor which causes the amplitude of rAprA to be larger than that of dApdA even though dApdA has a larger base–base overlap. The contribution of the third factor, *i.e.*, the proportion of the right-handed conformer *vs.* that of the left-handed conformer in solution, cannot be evaluated at present.

For the difference in CD amplitude between rAprA *vs.* rApdA, as well as between $rA_{5'}p_{5'}rA$ *vs.* $dA_{5'}p_{5'}dA$, the situation is even more subtle. As far as the per cent of hypochromicity is concerned, the pair members in these two pairs have the same extent of base–base interaction within experimental error. As far as the $\Delta\delta_D$ values are concerned, the pair members in these two pairs are very *similar*, although not identical. The differences in $\Delta\delta_D$ values between rAprA *vs.* rApdA are more significant than those between the $rA_{5'}p_{5'}rA$ *vs.* $dA_{5'}p_{5'}dA$. These differences, however, are not sufficiently large for us to construct distinctly different conformational models for each pair member in the pair in our present state of theoretical understanding and experimental results. All the data indicate that the averaged distance between the bases must be very similar for each pair member in the pair, especially since the hypochromicity measurement is a function of $1/R^3$ where R is the average distance (Kondo *et al.*, 1970). Therefore, the significant variance in CD between the pair members in the pair has to be explained on the basis of factors 2 and 3, *i.e.*, a difference in the mode of base–base stacking leading to a difference in angle $\theta_{transition}$, and a difference in proportion between the right-handed conformer and the left-handed conformer in the population. Since the $\Delta\delta_D$ values of $rA_{5'}p_{5'}rA$ and $dA_{5'}p_{5'}dA$ are so close to each other, the difference in the mode of stacking must be rather subtle or slight. Therefore, the consideration of the proportion between the right-handed conformer and the left-handed conformer in solution is likely to be the important factor in explaining the difference between the two 5'–5' dimers. At present, no definite stereochemical reason can be proposed to explain why $rA_{5'}p_{5'}rA$ would have proportionally more right-handed conformers than $dA_{5'}p_{5'}dA$, since the 2'-OH groups are located outside the stack. However, this question is related to the furanose conformation, especially whether the C(2') atom is endo or exo. The furanose conformation of $dA_{5'}p_{5'}dA$ may not be the same as that of $rA_{5'}p_{5'}rA$. While the difference in the proportion between the right-handed conformer and the left-handed conformer will have a significant influence on the amplitude of the CD curve (but not peak and trough spectral position), it will have no effect on the percentage of hypochromicity and the $\Delta\delta_D$ values of the base protons. This situation will satisfy the requirement of the present data and resolve the apparent conflict. However, the difference in the proportion between these two conformers could lead to some difference in $\Delta\delta_D$ values of H-1' and H-2' (trans to H-1'). The magnitude cannot be accurately estimated yet and this question should be studied further, especially since the difference in the $\Delta\delta_D$ values of H-1' between $dA_{5'}p_{5'}dA$ and $rA_{5'}p_{5'}rA$ is not significant experimentally (Table II).

On the other hand, the CD results are in good agreement with the uv absorbance and pmr data in two respects. First, the amplitude of dApdA and that of dAprA in CD are nearly the same (Figures 2 and 4); this observation is in accord with the results from hypochromicity and from $\Delta\delta_D$ values which are also the same for these two dimers. Second, the CD data indicate that the temperature effect on the amplitude of rAprA is significantly larger than that on other dimers (Figure 4). This result is in agreement with the $\Delta\delta_D$ values at different temperatures (Table II).

Influence of the 2'-Hydroxyl Group on the Conformation. The chief purpose of this paper is to investigate the influence of the 2'-OH group on the conformation of dinculeoside monophosphate. Through this study, ultimately we hope to learn about the difference, and the cause of this difference, between the conformation of DNA and that of RNA.

To this end, the conformation of six adenine dinucleoside monophosphates were studied and compared concurrently by uv absorbance, CD, and pmr methods. Four of them are 3'–5' dimers, which represent all possible combinations of the ribosyl and deoxyribosyl nucleotide units, *i.e.*, rAprA, rApdA, dAprA, and dApdA. Two of them are 5'–5' dimers which have been selected for the following special reasons. In the 5'–5' ribosyl dimer, the 2'-OH group can no longer

reach the phosphate group, since the phosphate linkage has been moved from the 3′ position to the 5′position. Therefore, the 2′-OH group in the 5′–5′ dimer can only interact with the base, if indeed this group can interact with any other component of the molecule at all. In addition, according to the conformational model (Kondo *et al.*, 1970), the two 2′-OH groups are located at the outside of the stack of $rA_{5'}p_{5'}rA$, and should not provide steric hindrance to the stacking. Therefore, it appears that comparison of $dA_{5'}p_{5'}dA$ and $rA_{5'}p_{5'}rA$ may yield valuable information about the interaction of the 2′-OH group.

There are two major developments in this area which should be discussed at the outset. The first is the study on the 2′-*O*-methyl derivatives in which the hydrogen-bonding properties of the 2′-OH group are now blocked. The amplitude of the CD curve of AmpAm is smaller than that of rAprA in 4.7 M KF at temperatures below 40–50° (Bobst *et al.*, 1969b). Also, at 23° and 0.01 M Tris, the amplitude of the CD curve of AmpA is smaller than that of rAprA (Bush and Scheraga, 1969). Since dApdA also has a smaller amplitude than rAprA, and in view of the problem in the interpretation of CD, this observation does not lead to a simple conclusion. At the level of polynucleotides, however, the interpretation is unambiguous. The CD spectrum of the poly(Am) in the single-stranded state at neutral pH is very similar to that of poly(rA) (Bobst *et al.*, 1969a). On the other hand, the ORD and CD patterns of single poly(rA) are distinctly different from those of poly(dA) (Ts'o *et al.*, 1966; Adler *et al.*, 1969). This observation indicates that the conformation of poly(Am) resembles that of poly(rA), but differs from that of poly(dA). This conclusion is supported by pmr results. The chemical shifts of protons in AmpAm have been compared to those in ApA at 37° and were found to be closely similar (Bobst *et al.*, 1969b). This study on the polynucleotide yielded even more conclusive information. The chemical shifts and polymerization shifts of poly(rA), poly(Am), and poly(dA) from 20 to 75° clearly indicate that the conformation of poly(Am) closely resembles that of poly(rA), but differs distinctly from that of poly(dA) (Alderfer *et al.*, 1971). Parenthetically, the conformation of the polynucleotides indicated by the pmr data has the same characteristics as indicated in the study on dinucleoside monophosphates reported here. For example, the arrangement of the bases in poly(dA) is in a more parallel fashion, while that in poly(rA) and poly(Am) is oblique. Since the 2′-*O*-methyl group exerts a similar influence on conformation as the 2′-OH group, the above studies clearly show that the hydrogen-bonding properties are not the means by which the 2′-OH group influences the conformation of the polynucleotides. It should be noted that these studies on the 2′-*O*-methyl derivatives do not show whether or not the 2′-OH group would form hydrogen bonding with other components (for instance, bases or phosphate group) in the molecules; these studies do indicate, however, that if such hydrogen-bonding takes place, then this bonding does not exert a major influence on the conformation of the polynucleotides.

The second major development is the knowledge obtained about the furanose conformation through the pmr study on the coupling constants of the furanose protons. In the preceding paper of this series, four coupling constants, $J_{1'-2''}$ (cis), (trans), $J_{2'-3'}$ (cis), and $J_{2''-3'}$ (trans), of the furanose of both the dAp and pdA residues in dApdA and of 3′-dAMP and 5′-dAMP were determined (Fang *et al.*, 1971). The data show that the dAp residue in dApdA and 3′-dAMP have similar coupling constants, and that their furanose conformation is that of C(2′)-endo (envelope form) or C(2′)-endo–C-(3′)-exo (twisted form), while the pdA residue in dApdA and the 5′-dAMP also have similar coupling constants and their furanose conformation is that of a rapid equilibrium between C(2′)-endo and C(3′)-endo. In dilute solution, the $J_{1'-2'}$ values of 5′-rAMP and 5′-dAMP are insensitive to temperature variation as well as insensitive to change of solvent from D_2O to Me_2SO-d_6. However, the $J_{1'-2'}$ of 5′-rAMP in aqueous solution is sensitive to the concentration of nucleotides or to addition of purine (Ts'o *et al.*, 1969), while under identical conditions, the $J_{1'-2'}$ of 5′-dAMP is *insensitive* to both nucleotide concentration and the presence of purine (Fang *et al.*, 1971). This phenomenon was attributed to the influence of the neighboring molecules in the stack formation; apparently, the formation of stacks has a large effect on the furanose conformation (as monitored by $J_{1'-2'}$) of 5′-rAMP, but has *no* effect on 5′-dAMP. At the dimer level, the $J_{1'-2'}$ values of rAprA are much smaller than those of the corresponding mononucleotides at or below room temperature, increase at elevated temperature, and approach those of the mononucleotides when dissolved in a destacking solvent such as Me_2SO-d_6 (Hruska and Danyluk, 1968; Ts'o *et al.*, 1969; Bangerter and Chan, 1969; Fang *et al.*, 1971). On the other hand, the values of $J_{1'-2'}$ (cis) and $J_{1'-2''}$ (trans) of dApdA are not sensitive to temperature, or to the change of solvent from D_2O to Me_2SO-d_6; and as mentioned before, the four coupling constants in dApdA are very similar to those of the corresponding mononucleotides (Fang *et al.*, 1971). These data indicate that the formation of intermolecular stacks of the 5′-rAMP or the formation of intranucleotidyl stacking of rAprA causes a change in the furanose conformation, while the stacking of 5′-dAMP or dApdA does not cause a change in the furanose conformation. This is a fundamental difference in stereochemistry between the backbone of the adenine ribosyl dinucleotide *vs.* that of the adenine deoxyribosyl dinculeotide.

The conformational models of dApdA and rAprA presented here (Figures 6 and 7) clearly show that the averaged modes of stacking of the two dimers are quite different. After an extensive survey of the CD properties of 16 ribodinculeoside monophosphates and their corresponding deoxyribosyl derivatives, Warshaw and Cantor (1970) concluded that "the geometry of the base-stacked conformation of the ribo compounds must differ substantially from that of deoxy compounds." Indeed, according to the models presented here, the bases are stacked in a more parallel manner and also more extensively in dApdA than in rAprA. This conclusion was also supported by the observation that the rate of hydrolysis of rAprAp by micrococcal nuclease is three times faster than that of dApdAp; this nuclease has a known preference for disordered polynucleotides (Sulkowski and Laskowski, 1970).

Our present study also indicates that the influence of the 2′-OH group on the conformation of the dimer can be observed only when the 2′-OH group is located next to a 3′-phosphodiester linkage. In the case of the 5′–5′ dimer, the 2′-OH groups are *not* located next to a 3′-phosphate linkage; thus, the stacks of $dA_{5'}p_{5'}dA$ and $rA_{5'}p_{5'}rA$ have very similar conformations and stabilities, indicated by the uv and pmr data. The comparison between these two ribosyl and deoxyribosyl 5′–5′ dimers reveals that the possible hydrogen bonding of the 2′-OH group to the bases, if it occurs, has little influence on the conformation or stability; this conclusion is in accord with that derived from the study on 2′-*O*-methyl derivatives discussed above. Furthermore, the con-

formation of these dimers are found to be influenced substantially by the 2'-OH group located next to the 3'-phosphate linkage. Thus, among these four 3'–5' dimers, rAprA and rApdA belong to one group which has conformational properties of ribosyl compounds, while dApdA and dAprA belong to another group which has properties of deoxyribosyl compounds. Though the precise stereochemical reason for these observations remains to be investigated, we have cited in our preceding paper the proposal of Bangerter and Chan (1969) that the nonbonded repulsion between the 2'-OH group in the rNp portion of the ribosyl dimer and the base and the ether oxygen of the furanose in the prN portion of the dimer may provide a steric hindrance to stacking in ribosyl dinucleoside monophosphate; such a hindrance, if it does in fact exist, should be considerably reduced in the case of deoxyribosyl dinucleoside monophosphate (Fang *et al.*, 1971).

The $J_{1'-2'\ (2'')}$ values of the six dimers (Table IV) relate a revealing story. The data strongly suggest that the decrease in $J_{1'-2'\ (2'')}$ values of the dimer *vs.* that of the monomer represent the compression of the furanose ring due to stacking. This compression can be released at elevated temperature or in a destacking solvent such as Me_2SO-d_6. In dApdA and dAprA, the $J_{1'-2'\ (2'')}$ values of all four furanoses are larger and similar to those of the mononucleotides, as well as temperature independent and solvent independent. This observation implies that the furanoses in these dimers are not compressed in stacking; perhaps this is the reason why the bases in dApdA and dAprA can have a parallel, extensive overlap. In rAprA and rApdA, the $J_{1'-2',\ (2'')}$ values of the three riboses are significantly smaller than those of the mononucleotides and are temperature dependent and solvent dependent. This observation implies that the riboses in these dimers are being compressed in stacking. Thus, the 2'-OH group in these riboses may provide a steric hindrance in forcing the bases to have an oblique and less extensive mode of stacking in rAprA and rApdA. It is interesting to note that the $J_{1'-2'}$ of the prA residue in rAprA indicates that the ribose in this 5' portion is being compressed. Since the C-2' atom and the 2'-OH group of the 5'-nucleosidyl unit are located outside the stack (Figure 6, Ts'o *et al.*, 1969), these groups should not provide any stereochemical hindrance to stacking. We have proposed, therefore, that the change of $J_{1'-2'}$ of the prA residue may reflect not the change of the C-2' position, but rather the C-1' atom instead (Kondo *et al.*, 1970). From this point of view, this change of $J_{1'-2'}$ of the prA residue may be taken as evidence that the O(1')–C(1') region is being compressed by the 2'-OH group of the rAp residue in the dimer. The average value of $J_{1'-2'}$ and $J_{1'-2'}$ of the pdA residues in rApdA is slightly smaller than that of the monomers (6.6–6.7 cps, Fang *et al.*, 1971) or that of the dApdA (6.5 cps), especially at 5° (5.6 cps, Table IV). This J value is also somewhat temperature dependent. Again, we propose that this phenomenon may be due to the compression of the O(1')–C(1') region of the deoxyribose in the pdA residue by the 2'-OH group of the ribose in the rAp residue. As for the 5'–5' dimers, the $J_{1'-2'\ (2'')}$ value of $dA_{5'}p_{5'}dA$ indicates that the deoxyribose is not compressed. On the other hand, the $J_{1'-2'}$ value of $rA_{5'}p_{5'}rA$ shows that the ribose is somewhat compressed upon stacking. While the origin of this stereochemical obstruction is not obvious since the 2'-OH group is located outside the stack, these data do suggest that the furanose conformation of the ribose and of the deoxyribose may not be the same in these two dimers. This suggestion is supported by the observation that the CD properties of these two 5'–5' dimers are different; we have attributed this difference to variation in the proportion of left-handed confomer *vs.* right-handed conformer in the population, a situation which can be brought about by a subtle difference in furanose conformation.

In summary, the uv, pmr, and, possibly, the CD data on these six adenine dinucleoside monophosphates indicate that the influence of the 2'-OH group of the ribose on the conformation of the ribose-containing dimers is exerted through steric hindrance of this group and not through its hydrogen-bonding properties. Upon stacking, the ribose of the 3' residue is compressed by the furanose of the adjacent 5' residue (possibly by the base of the 5' residue as well). This steric interference between the 3'-furanose group and the immediately following 5' residue prevents an extensive overlap of the adenines in a parallel fashion. Such an interference is absent in the dimers containing a deoxyribosyl 3' residue, such as dApdA and dAprA.

Acknowledgment

We thank Miss Helen M. Holmes for her technical assistance in obtaining certain CD spectra.

References

Adler, A. J., Grossman, L., and Fasman, G. D. (1968), *Biochemistry 7*, 3836.

Adler, A. J., Grossman, L., and Fasman, G. D. (1969), *Biochemistry 8*, 3846.

Alderfer, J. L., Tazawa, I., Tazawa, S., and Ts'o, P. (1971), *Biophys. Soc. Abstr. 11*, 207a.

Bangerter, B. W., and Chan, S. I. (1969), *J. Amer. Chem. Soc. 91*, 3910.

Bobst, A. M., Cerutti, P. A., and Rottman, F. (1969a), *J. Amer. Chem. Soc. 91*, 1246.

Bobst, A. M., Rottman, F., and Cerutti, P. A. (1969b), *J. Amer. Chem. Soc. 91*, 4603.

Bullock, F. J., and Jardetzky, O. (1964), *J. Org. Chem. 29*, 1988.

Bush, C. A., and Scheraga, H. A. (1969), *Biopolymers 7*, 395.

Bush, C. A., and Tinoco, I., Jr., (1967), *J. Mol. Biol. 23*, 601.

Chamberlin, M. J., and Patterson, D. L. (1965), *J. Mol. Biol. 12*, 410.

Chan, S. I., and Nelson, J. H. (1969), *J. Amer. Chem. Soc. 91*, 168.

Crawford, J. E., Chan, S. I., and Schweizer, M. P. (1971), *Biochem. Biophys. Res. Commun. 44*, 1.

Fang, K. N., Kondo, N. S., Miller, P. S., and Ts'o, P. O. P. (1971), *J. Amer. Chem. Soc. 93*, 6665.

Giessner-Prettre, C., and Pullman, B. (1970), *J. Theor. Biol. 27*, 87 and 341.

Green, G., and Mahler, H. R. (1971), *Biochemistry 10*, 2200.

Hruska, F. E., and Danyluk, S. S. (1968), *J. Amer. Chem. Soc. 90*, 3266.

Johnson, C. E., Jr., and Bovey, F. A. (1958), *J. Chem. Phys. 29*, 1012.

Johnson, W. C., Jr., and Tinoco, I., Jr. (1969), *Biopolymers 7*, 727.

Kondo, N. S., Holmes, H. M., Stempel, L. M., and Ts'o, P. O. P. (1970), *Biochemistry 9*, 3479.

Lapidot, V., and Khorana, H. G. (1963), *J. Amer. Chem. Soc. 85*, 3857.

Maurizot, J. C., Brahms, J., and Eckstein, F. (1969), *Nature (London) 222*, 559.

Miller, P. S., Fang, K. N. Kondo, N. S., and Ts'o, P. O. P. (1971), *J. Amer. Chem. Soc. 93*, 6657.
Moon, M. W., Nishimura, S., and Khorana, H. G. (1966), *Biochemistry 5*, 937.
Ralph, R. K., and Khorana, H. G. (1961), *J. Amer. Chem. Soc. 83*, 2926.
Riley, M., Maling, B., and Chamberlin, M. J. (1966), *J. Mol. Biol. 20*, 359.
Schaller, H., Weimann, G., and Khorana, H. G. (1963), *J. Amer. Chem. Soc. 85*, 3821.
Schweizer, M. P., Broom, A. D., Ts'o, P. O. P., and Hollis, D. P. (1968), *J. Amer. Chem. Soc. 90*, 1042.
Schweizer, M. P., Chan, S. I., Helmkamp, G. K., and Ts'o, P. O. P. (1964), *J. Amer. Chem. Soc. 86*, 696.
Sulkowski, E., and Laskowski, M., Jr. (1970), *Biochim. Biophys. Acta 217*, 538.
Tazawa, I., Tazawa, S., Stempel, L. M., and Ts'o, P. O. P. (1970), *Biochemistry 9*, 3499.
Ts'o, P. O. P., Kondo, N. S., Schweizer, M. P., and Hollis, D. P. (1969), *Biochemistry 8*, 997.
Ts'o, P. O. P., Rapaport, S. A., and Bollum, F. J. (1966), *Biochemistry 5*, 4153.
Van Holde, K. E., Brahms, J., and Michelson, A. M. (1965), *J. Mol. Biol. 12*, 726.
Vournakis, J. N., Poland, D., and Scheraga, H. A. (1967), *Biopolymers 5*, 403.
Warshaw, M. M., and Cantor, C. R. (1970), *Biopolymers 9*, 1079.
Waugh, J. S., and Fessenden, R. W. (1957), *J. Amer. Chem. Soc. 79*, 846.

Modification of Chromatin by Trypsin. The Role of Proteins in Maintainance of Deoxyribonucleic Acid Conformation†

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ABSTRACT: Limited tryptic digestion of chromatin from rabbit liver or calf thymus leads to cleavage of *ca.* 55 peptide bonds per 100 DNA base pairs, equal to the number of lysyl + arginyl residues which are freely titratable in the native nucleoprotein complex. Nearly all the proteins of chromatin possess regions which are not bound to DNA, and hence are susceptible to tryptic proteolysis. The peptides which remain bound to DNA in the residual complex, tryptic chromatin, appear to occupy the same binding sites that they occupied in the native complex, and amount to 45–70% of the total protein initially present. Consequent to tryptic digestion, chromatin exhibits only slight alterations in thermal denaturation, but has an increased flow dichroism, increased specific viscosity, and a circular dichroism pattern apparently identical with that of protein-free DNA. These findings are interpreted as resulting from loss of the specific conformation which characterized DNA in chromatin, a conformation thought to be formation of a supercoil. In conjunction with recent results demonstrating the binding of histones to DNA at their amino and carboxyl termini, but not in their central regions, these results are discussed as indicating stabilization of the DNA supercoil in chromatin through the formation of histone bridges.

The conformation of DNA in chromatin, the nucleoprotein complex in eukaryotic cells, differs from the conformation of the protein-free nucleic acid in the B form in aqueous solution. Measurements of circular dichroism (Permogorov *et al.*, 1970; Shih and Fasman, 1970; Simpson and Sober, 1970), flow birefringence, flow dichroism (Ohba, 1966), light scattering, and viscosity (Zubay and Doty, 1959) have all suggested that the DNA of chromatin is shortened in overall length, and/or that there is a reduced perfection of base stacking for nucleic acid. These findings have been interpreted to be consistent with the formation of a supercoiled structure for DNA when it is complexed with proteins in chromatin; and this postulated structure has been supported by electron microscopic (Ris, 1967; Itzhaki and Rowe, 1969) and X-ray diffraction (Pardon *et al.*, 1967; Richards and Pardon, 1970) studies. Although protein is important in the stabilization of the supercoiled conformation, little evidence has been presented to indicate the mechanism through which the histone fractions maintain the native conformation of DNA in chromatin.

Recently, Boublik and collaborators (1970) have obtained evidence by nuclear magnetic resonance study of histone–DNA complexes that the central region of the slightly lysine-rich histone, FII, and the lysine-rich histone FI, are in a helical conformation, and are not bound to the nucleic acid. In contrast, the more highly basic amino-terminal and carboxyl-terminal regions of these two histones appear to be bound to DNA firmly. We concurrently have approached the binding of histones to DNA in chromatin by acetylation of the free lysyl residues of the histones in chromatin (Simpson, 1971) and localization of the modified residues in the sequence of the histone fractions. Preliminary results are consistent with the binding mode proposed for the FII histone by Boublik *et al.* (1971), so far.

The current investigation has attempted to ascertain the possible role of histones which possess nonbound central regions in the conformational stabilization of DNA in chromatin. Since trypsin requires a free basic amino acid for hydrolysis of a polypeptide, tryptic digestion of proteins bound

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