Circular Dichroism of Cytosine Dinucleoside Monophosphates Containing Arabinose, Ribose, and Deoxyribose*

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ABSTRACT: The ultraviolet circular dichroism and absorbance properties of several cytosine dinucleoside monophosphates were studied at neutral pH. The influence of temperature was examined relative to the appropriate mononucleotides. The oligo- and mononucleotides contained arabinose, ribose, or 2-deoxyribose, so that the effect of the sugar upon structure could be studied. Both 3'p5'- and 2'p5'-internucleotide linkages were used. Only cytidylyl(3'p5')cytidine displayed appreciable ordered secondary structure. Small amounts of asymmetric stacking were present in most of the other dinucleoside monophosphates, and were found to be in the order: cytidylyl(3'p5')cytidine >> 2'-deoxyribo-cytidylyl(3'p5')deoxyribocytidine > cytidylyl(2'p5')-cytidine > arabinocytidylyl(3'p5')arabinocytidine > arabinocytidylyl(2'p5')arabinocytidine. The possible involvement of the free 2'- or 3'-hydroxyl group in strengthening single-stranded polynucleotide structure is discussed. This involvement depends upon the position and configuration of the hydroxyl group. Rotatory properties of the component D-pentoses were observed.

The effect of salt concentration upon the circular dichroism of polyribocytidylic acid and of polydeoxyribocytidylic acid was studied in an attempt to interpret the role of the 2'-hydroxyl group.

For several years synthetic polyribonucleotides have been utilized as structural models for RNA. Circular dichroism and optical rotatory dispersion studies have shown that in neutral aqueous solution, most of these polymers, including polycytidylic acid (Fasman et al., 1964; Sarkar and Yang, 1965), exist as single-stranded helices with stacked bases. Still more recently, 3'p5'-ribodinucleoside phosphates became available for optical investigations, and many of them were shown to adopt stacked conformations (Warshaw and Tinoco, 1966; Bush and Tinoco, 1967; Brahms et al., 1967b). Such investigations gave evidence for the validity of using dinucleotides for calculating the optical and structural properties of the analogous polymers: rA(3'p5')rA (Warshaw et al., 1965; Van Holde et al., 1965) and rC-(3'p5')rC[1] (Brahms et al., 1967a) show the onset of the poly rA and poly rC helices, respectively. Similarly, RNA structure can be predicted from the optical rotatory dispersion of dinucleoside phosphates (Cantor et al., 1966).

Evidence has accumulated that the ribose 2'-hydroxyl group helps to stabilize the secondary structure of single-stranded ribonucleotides. Deoxyribonucleotides exhibit greatly diminished rotatory properties when compared with their ribo analogs. This comparison has been shown for the single-stranded forms of oligo- and polydeoxycytidylic acids (Adler et al., 1967), oligodeoxyadenylic acids (Vournakis et al., 1967), dGMP (Brahms and Sadron, 1966), and several synthetic polydeoxyribonucleotides (Ts'o et al., 1966). Similarly, ribonucleotides with 2'p5'- instead of 3'p5'-phosphodiester linkages show a decline in ordered secondary structure, as evidenced by several 2'p5'-dinucleoside phosphates (Brahms et al., 1967b), 2'p5'-oligocytidylic acids (Brahms et al., 1967a), and GMP-2' (Brahms and Sadron, 1966).

The present report is concerned with a comparison of circular dichroic properties of cytosine arabinoside dinucleoside phosphates (Wechter, 1967) and their cytidine and deoxycytidine analogs. In araC, the 2'-hydroxyl is in the β configuration instead of α (as in rC).
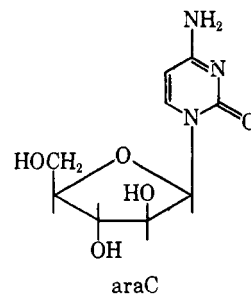
araC

The presence of the 2'-hydroxyl does not appear to be a sufficient requirement for base stacking and helix formation; these properties appear to a significant extent only in the normal 3'p5'-rC-dinucleoside phosphate.[2]

[1] Abbreviations used that are not listed in Biochemistry 5, 1445 (1966), are: rC, ribosylcytosine; dC, 2'-deoxyribosylcyto-sine; araC, 1-β-D-arabinofuranosylcytosine.

[2] Added in proof: since the submission of this paper a study of arabinose dinucleoside phosphates has appeared and similar conclusions were reached (Maurizot et al., 1968).
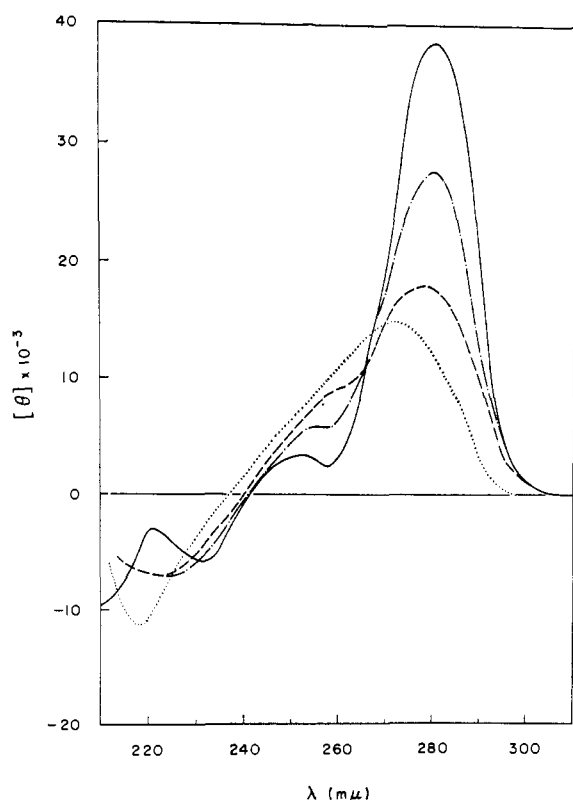
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FIGURE 1: Circular dichroism of rC(3'p5')rC at pH 8.5. 2°, ———; 31°, —·—·—; 80°, — — — — —. Circular dichrosim of rCMP-5' at 22°, · · · · ·.

FIGURE 2: Circular dichroism of dC(3'p5')dC. 1.5°, ———; 30°, —·—·—; 80° — — — — —. Circular dichrosim of dCMP-5' at 22°, · · · · ·, at pH 8.5.

Experimental Section

Materials. The araC dinucleoside phosphates and mononucleotides were a gift from Dr. W. J. Wechter (Wechter, 1967). A sample of pdCpdCp from Dr. K. Burton (Burton and Peterson, 1960) was treated with Worthington bacterial alkaline phosphatase to form dC(3'p5')dC. rC(3'p5')rC was purchased from Calbiochem, rCMP-5' from Schwarz BioResearch, and both dCMP-5' and rCMP-2',3' isomer mixture from P-L Biochemicals. Poly rC, the K$^+$ salt from Miles Chemical Co., had s = 7.18 S. Poly dC was a gift of Dr. F. J. Bollum (Bollum, 1968). The D-pentoses were bought from Calbiochem, Tris from Sigma, and salts from Fisher. Water was redistilled from glass.

Solutions and Concentrations. The pH for all studies of mono- and oligonucleotides was maintained at pH 8.5 with 0.02 M Tris-HCl in 0.08 M NaCl; this pH was chosen to ensure the nonprotonated form of cytosine. Concentrations were in the range of 0.8–1.4 × 10^{-4} M cytosine residues, corresponding to a maximum absorbance of about one for a 1-cm path length. Concentrations of solutions of araC compounds were determined by means of the extinction coefficients given by Wechter (1967), adjusted to the present solvent conditions. Extinction coefficients for rC and dC nucleotides can be found in Adler *et al.* (1967). All solutions were filtered through Millipore filters (type HAWP, 0.45-μ pore size).
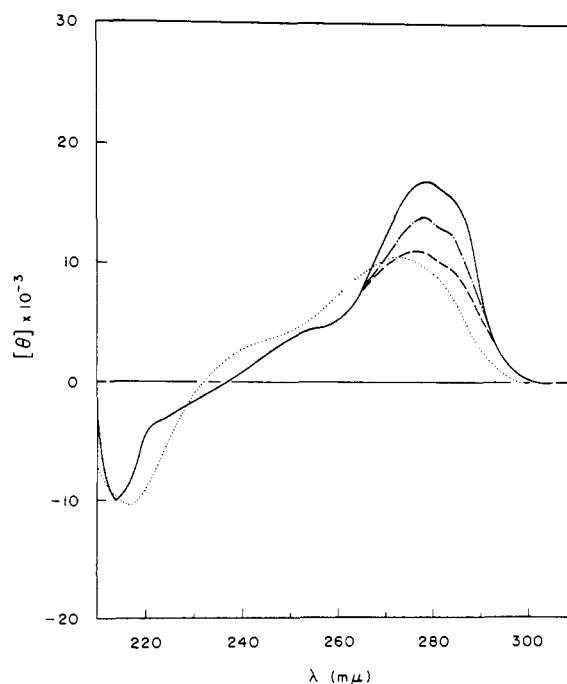
For salt-dependence studies, concentrated polymer

solutions were dialyzed exhaustively against water. The pH of the poly rC and poly dC solutions were adjusted to pH 8.5 and 10, respectively, with 0.1 M NaOH. Each polymer solution was heated briefly and slowly cooled to ensure formation of the single-stranded conformation (Fasman *et al.*, 1964). All solutions used in these experiments (polymers, salt, and water) were adjusted to pH 10.0. For circular dichroism and optical rotatory dispersion work D-pentose solutions contained 0.5% of each sugar in water, pH 8.5 ± 0.1.

Methods. All optical measurements were performed in 1-cm, jacketed, fused quartz cells (Optical Cell Co.). Aliquots of the same solution in the same cell were used for corresponding circular dichroism and absorbance determinations. Values of [θ] and ϵ were corrected for volume expansion upon heating. Cell temperature was maintained with a circulating methanol–water bath and monitored by means of a bimetallic probe (Yellow Springs Instruments) placed in the heating jacket. A Cary Model 14 spectrophotometer was used for absorbance measurements.

Circular dichroism and optical rotatory dispersion data were obtained with a Cary Model 60 recording spectropolarimeter, equipped with a 6001 circular dichroism accessory, and set for a half-band width of 15 Å. All nucleotide spectra were run with a 3-sec time constant. Care was taken that the temperature of the Pockels cell did not rise above 30°, and that this component was kept dry; the latter condition entailed nitrogen flushing at 10 ft^3/hr for experiments below 25°. The circular dichroism buffer blank changed slightly with temperature at λ <225 mμ; this was corrected for. All circular dichroism and absorbance heating curves were
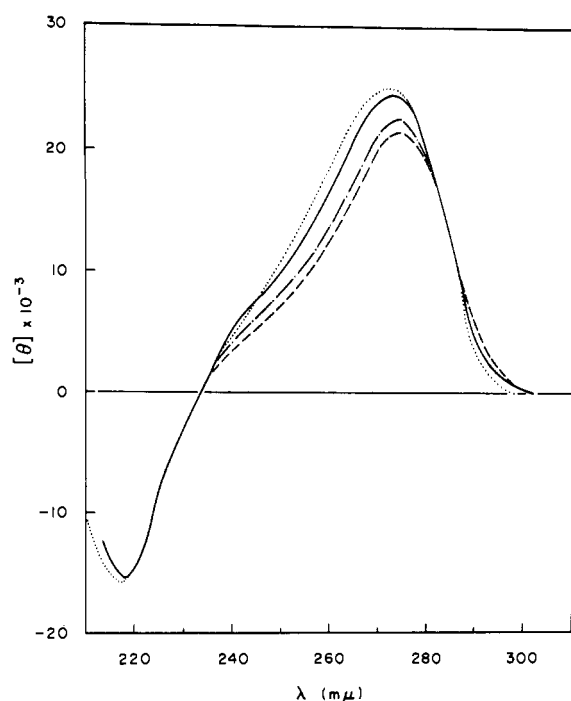
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FIGURE 3: Circular dichroism of araC(3'p5')araC. 3°, ——; 30°, —·—·—; 80°, —————. Circular dichroism of araCMP-5' at 22°, ·····, at pH 8.5.

FIGURE 4: Circular dichroism of araC(2'p5')araC. 3°, ——; 40°, —·—·—; 80°, —————, at pH 8.5.

reversible upon recooling to 20°, indicating the absence of hydrolysis or degradation.

Circular dichroism results are reported in terms of [θ] (residue ellipticity per mole of cytosine residue), optical rotatory dispersion in terms of [M'] (molar rotation corrected for refractive index). Both parameters have units of (deg cm²)/dmole. The circular dichroism curves could be measured to ±0.0005 deg in the wavelength range 230–310 mμ; this corresponds to an experimental error of ±500 in [θ]. Signal-to-noise ratios always exceeded 30 at circular dichroism peaks. Wavelength readings were reproducible to ±0.5 mμ.

Results and Discussion

Circular Dichroism of Dinucleoside Monophosphates. Circular dichroism data for dinucleoside monophosphates containing cytosine were taken at pH 8.5 from about 2 to 80°. Representative curves are given in Figures 1–4. There was no dichroic signal above 310 mμ; below 210 mμ the low signal-to-noise ratio precluded accurate data. Curves for the corresponding mononucleotides are included for comparison. At all temperatures data for araCMP-2' and for araCMP-3' were identical with those for araCMP-5', within experimental error. Similarly, an rCMP-2',3' isomer mixture gave the same circular dichroism results as rCMP-5'. The curves for rC(3'p5')rC (Figure 1) agree well with those obtained by Brahms *et al.* (1967a).

At high temperature, all the dinucleoside phosphates approach the appropriate mononucleotide limit for the magnitude of their circular dichroism peak near 270 mμ (although not necessarily for the position of this peak or for the shape of their circular dichroism spectra). At
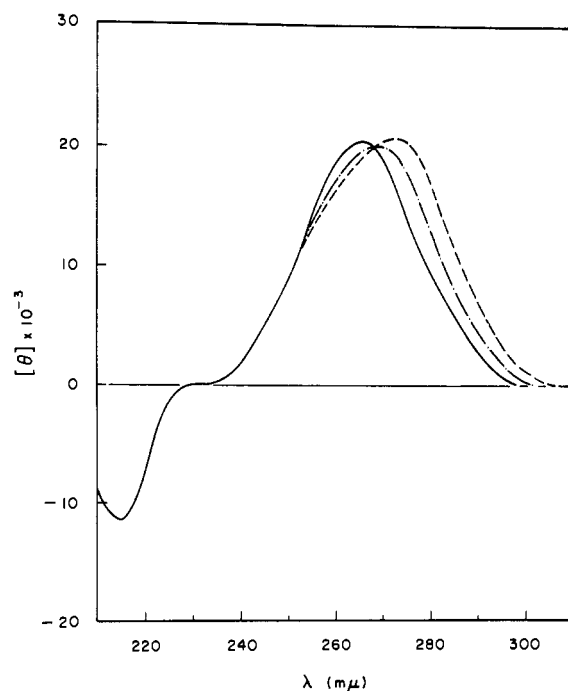
lower temperatures progressive formation of intramolecular helical structure is shown by an increase in the longest wavelength circular dichroism peak; this increase is especially pronounced in rC(3'p5')rC, and is nil in araC(2'p5')araC. All of the circular dichroism curves are seen to be nonconservative (Bush and Brahms, 1967) in the wavelength region studied. A second, smaller positive circular dichroism peak is apparent in the 240–255-mμ region for several of the compounds: dCMP, the 3'p5'-linked dinucleoside phosphates, poly-rC, and poly-dC. This shoulder grows with temperature for rC(3'p5')rC. AraCMP, rCMP, and the 2'p5'-linked dinucleoside phosphates (see Brahms *et al.* (1967a) for the rC compound) do not exhibit this shoulder although neutral cytidine itself is thought to undergo two π → π* transitions in the 240–280-mμ region (Miles *et al.*, 1967).

Figure 5 summarizes the effect of temperature upon the first circular dichroism peak. The data on rC(2'p5')-rC were taken from Brahms *et al.* (1967a); residue ellipticities were calculated in this case with the formula, [θ] ≈ 3305(ϵ_l − ϵ_r). Only rC(3'p5')rC shows appreciable ordered (probably helical) conformation, as evidenced by the considerably larger [θ] for the dimer than for the monomer (even at 80°), and by the steep "melting" of [θ] with temperature. By these criteria, the other dinucleoside phosphates have little base stacking, even at 2°. (More asymmetry would probably be apparent at even lower temperatures.) The amount of ordered conformation can be put in the sequence: rC(3'p5')rC ≫ dC(3'p5')dC > rC(2'p5')rC > araC(3'p5')araC > araC(2'p5')araC. The last compound shows no stacking at all, by these criteria.

The arabinose dinucleoside phosphates contain even less helix than dC(3'p5')dC, in which the 2'-hydroxyl
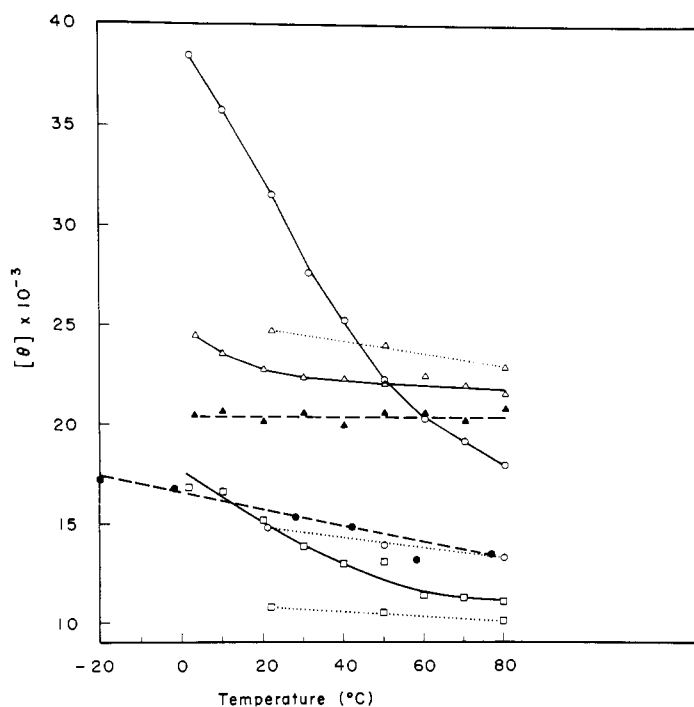
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FIGURE 5: Circular dichroism–temperature profiles for dinucleoside phosphates and mononucleotides. All data were taken at the longest wavelength circular dichroism peak, which usually varied with temperature. The solvent was 0.02 M Tris buffer, pH 8.5, with 0.08 M NaCl. araC(3'p5')araC, △—△; araC(2'p5')araC, ▲--▲; rC(3'p5')rC, O—O; dC(3'p5')dC, □—□; araCMP-5', △···△; rCMP-5', O···O; dCMP-5', □···□. Data for rC(2'p5')rC, ●--●, were taken from Brahms *et al.* (1967a), in which the solvent was 0.01 M Tris (pH 7.5–8.0) with 4.7 M KF.

group is totally lacking. We can conclude that this hydroxyl must be in the ribose orientation in order to promote strong base stacking in ribonucleotides.

This analysis is based on the assumption that all the dinucleoside phosphates, if fully stacked, would have similar geometries. However, the circular dichroism curves of the dimers differ somewhat in shape. This difference may be due only partially to the different intrinsic monomer Cotton effects, to nonconservative interactions, and to the various extents of stacking. It may, in addition, reflect differences in the geometry of base stacking, which could vary with the nature of the sugar, the type of phosphodiester linkage, and the temperature. Because of possible differences in helix geometry, and because of lack of circular dichroism data on the fully stacked forms of the dimers, no quantitative comparisons have been made. However, the circular dichroism curve shapes are similar enough, and the temperature dependence of rC(3'p5')rC is sufficiently greater than the others, so that the main conclusion would still be valid.

Figures 4 and 5 show that the circular dichroism of araC(2'p5')araC is anomalous in its response to heating. Instead of a decrease in [θ] and a small shift in peak wavelength, this compound exhibits no change at all in [θ], while λ_{peak} undergoes a large red shift (see Table I).

Ultraviolet Absorption of Dinucleoside Monophosphates. Absorption spectra of the two arabinose dinucleoside monophosphates were run from 10 to 80°. Values of the maximum extinction coefficients and the corresponding peak wavelengths are displayed in Figure 6. The 2'p5' and 3'p5' isomers give identical results upon

heating: a 1-mμ red shift and a hypochromicity of 4%. Therefore, the unusual circular dichroism heating behavior of araC(2'p5')araC cannot be explained by large changes in its absorption spectrum. The data in Figure 6 are similar to those for dC(3'p5')dC (Adler *et al.*, 1967). The temperature dependence of the circular dichroism of araC(2'p5')araC may reflect some conformational transition, different from normal gradual unstacking of bases, which could cause a greater change in
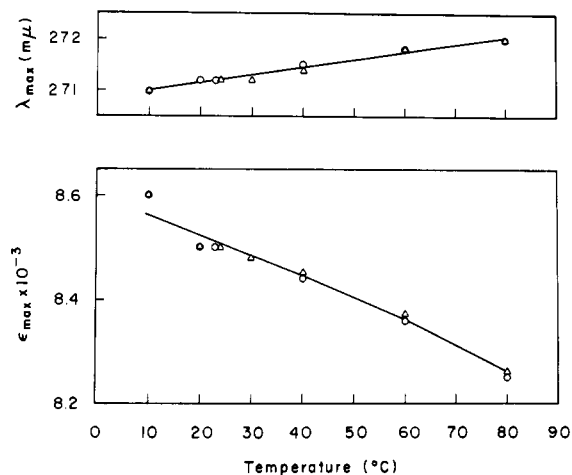

FIGURE 6: Ultraviolet absorption–temperature profiles for arabinosylcytosine dinucleoside phosphates. Upper curve shows position of the longest wavelength absorption peak; lower curve shows corresponding extinction coefficient. araC(3'p5')araC, O—O; araC(2'p5')araC, △—△, at pH 8.5.

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TABLE I: Transition Wavelengths[a] for Cytosine Nucleotides.

Compound	Absorption, λ_{max} (mμ)		Circular Dichroism, λ_{peak} (mμ)	
	2°	80°	2°	80°
Poly rC	268.5	271	276[c]	
Poly dC	268.5	271	278[c]	
rC(3'p5')rC	269.5	271.5	280	278
rC(2'p5')rC[b]	269.5		275	276[d]
dC(3'p5')dC	270.5	272	278	276
araC(3'p5')araC	271	272	273	274
araC(2'p5')araC	271	272	265	271.5
rCMP-5'	272	272.5	272[c]	274
rCMP-2',3' mixture	270[c]		271[c]	
dCMP-5'	271.5	273	272.5[c]	
araCMP-5'	271[c]		272[c]	272
araCMP-3'	271[c]		271[c]	
araCMP-2'	271[c]		271[c]	

[a] Only the longest wavelength peak, in millimicrons is tabulated. [b] Data are taken from Brahms et al. (1967a); the solvent was 4.7 M KF. [c] 22°. [d] 28°.

λ_{peak} than in [θ]; this could be a change in geometry. Alternatively, if a dinucleoside phosphate is viewed as a torsional oscillator, instead of as a mixture of its stacked and unstacked forms, then the temperature dependence of its circular dichroism or optical rotatory dispersion can be different from that of its absorption spectrum (Davis and Tinoco, 1968; Glaubiger et al., 1968). Thus, araC(2'p5')araC may be relevant as evidence for Tinoco's oscillator model.

Table I lists the wavelengths for the first circular dichroism and absorption transitions of the cytosine compounds under consideration. Only araC(2'p5')araC shows an appreciable temperature dependence. Complete absorption temperature profiles for rC(3'p5')rC and dC(3'p5')dC can be found in Adler et al. (1967); Brahms et al. (1967a) contains an absorption spectrum for rC(2'p5')rC. Table I shows that, for all mononucleotides, the circular dichroism peak wavelength is equal to the absorption wavelength, to within 1 mμ. This is not true for the dinucleoside monophosphates and polymers. If exciton interaction between chromophores on a single-stranded helical molecule produces circular dichroism band splitting, then λ_{max} for absorption does not coincide with λ_{max} for any circular dichroism band. In cases where the rotational strength for a given transition is locally conserved, such as with ApA (Van Holde et al., 1965), the actual wavelength for the circular dichroism transition crossing point can be estimated from the monomer absorption maximum. However, such an analysis is not possible for cytosine compounds because of their nonconservative rotatory properties, further complicated by the possibility of overlapping electronic transitions in the near-ultraviolet region (Miles et al., 1967), even though exciton splitting is apparent in oligocytidylic acids (Brahms et al., 1967a). In the present case, the quantity ($\lambda_{peak,\ circular\ dichroism}$ — $\lambda_{max,\ absorption}$) vanishes for mononucleotides, is greatest for rC(3'p5')-

rC, and perhaps can be used as a qualitative, empirical measure of exciton splitting. If this quantity is applied to the dinucleoside monophosphates in Table I as a criterion of base stacking, then the same sequence of conformational stability is obtained as in the previous (circular dichroism) section. Because of the nonconservative Cotton effects, this wavelength difference should be used with caution; for example, it would indicate considerable stacking for rC(3'p5')rC even at 80°.

Rotatory Properties of D-Pentoses. As can be seen in Figure 5, the peak ellipticities of araC compounds, at 80°, are intrinsically larger than those of rC and dC derivatives, although their ultraviolet absorption spectra are very similar. This relation is valid even for the mononucleotides, where the optical activity is not derived from any helical molecular structure, but simply from electronic interaction between the cytosine chromophore and the asymmetric centers on the sugar ring. In pyrimidine nucleotides there is a barrier to free rotation about the C–N glycosidic bond; this barrier is caused by interference between the 2-carbonyl group of the pyrimidine ring and the 2'-β and 5'-β substituents on the furanose ring. Examination of molecular models shows that the hindrance is greater for arabinose than for ribose or deoxyribose compounds. This difference could lead to greater rigidity of the arabinose nucleotides and, hence, augmented rotatory properties (Emerson et al., 1967). However, even ribose nucleotides show an exclusive preference for the *anti* configuration, with the 2-C=O pointed away from the sugar ring (Shefter et al., 1964; Bugg and Marsh, 1967). Therefore, an alternative explanation for the greater rotatory strength of araC nucleotides may lie in the asymmetry of the arabinose moiety itself.

To examine this question we studied the optical rotatory dispersion and circular dichroism of D-ribose, D-arabinose, and D-2-deoxyribose (which is the same as D-2-deoxyarabinose). Each pentose was dissolved in
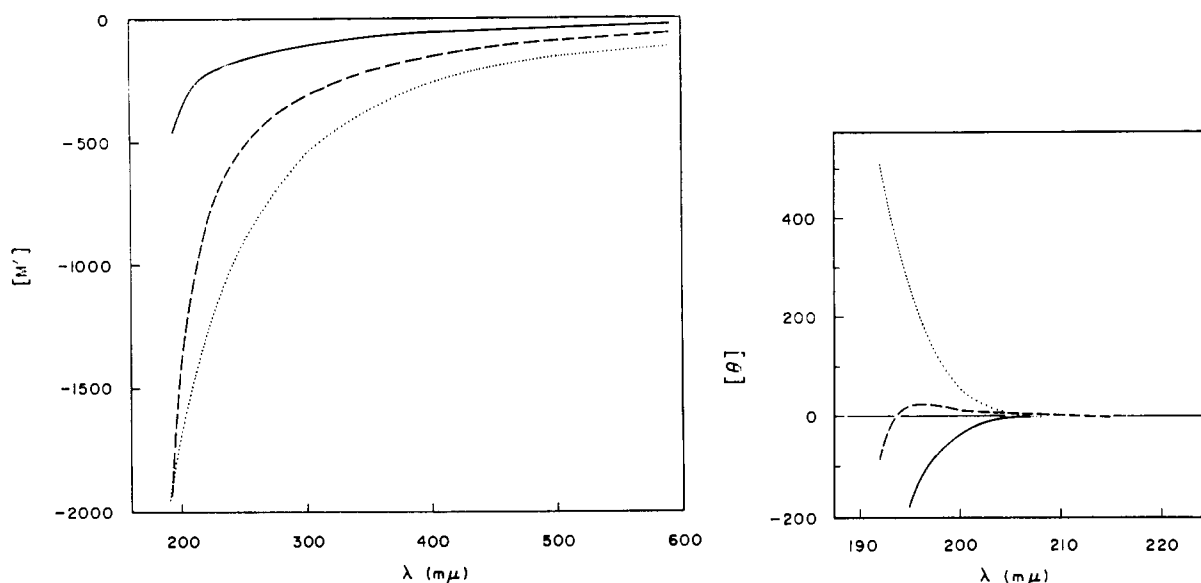
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FIGURE 7: Optical rotatory properties of pentoses at 22°. Top curves show optical rotatory dispersion, bottom curves give circular dichroism. The solvent is water (pH 8.5); sugar concentrations 0.5%. D-Ribose, ——; 2-deoxy-D-ribose, – – – –; D-arabinose, · · · · · ·.

water at pH 8.5 and was allowed to stand until the equilibrium mixture of 1α and 1β isomers was attained. (There was no change in optical rotatory dispersion or circular dichroism over the course of 30 hr.)

The results are given in Figure 7. All of the optical rotatory dispersion curves are negative in the visible and near-ultraviolet regions; the signs of the circular dichroism curves vary. There is no contradiction here; the circular dichroism reflects only the highest wavelength transitions, while the optical rotatory dispersion curve is the rotatory sum of all asymmetric electronic transitions. There is a correlation between the optical rotatory dispersion and the circular dichroism data in that arabinose exhibits the greatest magnitude for both rotatory properties. Thus, arabinose may be intrinsically more asymmetric than either ribose or deoxyribose, and this would affect the rotations associated with all of its absorption bands. This asymmetry may help account for the high ellipticities of the unstructured araC mononucleotides and dinucleoside monophosphates.

Molecular Models. The central problem of the present study can be stated as: What role is played by the 2'-hydroxyl group in stabilizing the base-stacked, helical conformation of ribose oligo- and polynucleotides? Why does destabilization occur when this hydroxyl group is missing (in deoxyribonucleotides and in ribonucleotides with 2'p5' linkages)? Finally, why is there even less helical structure when this hydroxyl group is pointed up (like the heterocyclic ring) and not down, relative to the pentose plane?

Courtauld models were constructed for the cytosine dinucleoside monophosphates listed in Table II. Each molecular model was arranged so that (1) both glycosidic bonds were in the *anti* configuration, which has been found valid from X-ray studies of rCMP-3' (Bugg and Marsh, 1967) and of rA(2'p5')rU (Shefter *et al.*, 1964), and from optical rotatory studies of pyrimidine

nucleosides (Emerson *et al.*, 1967). Then (2) each model was twisted to form the first step of a single-stranded, right-handed helix; the bases were stacked perpendicular to the helical axis, with a vertical distance between base planes of 3.4 Å, and a rotation angle of 35–40° between successive cytosine axes. These parameters were chosen from calculations performed on rA(3'p5')rA (Warshaw *et al.*, 1965; Van Holde *et al.*, 1965), rC(3'p5')rC (Brahms *et al.*, 1967a), and other dinucleoside phosphates (Bush and Tinoco, 1967).

All five of the cytosine dinucleoside phosphate models, regardless of type of pentose or internucleotide linkage, were capable of assuming this stacked, helical conformation without strain. With each model in this conformation, the free 2'- or 3'-hydroxyl group (of the 3'-phosphate- or 2'-phosphate-linked sugar) was examined for its ability to stabilize the stacked structure through hydrogen-bond formation. Several oxygen atoms were considered as possible electron donors. Rotation was allowed around the phosphate group bonds, when sterically possible. The dC compound, of course, has no free 2'-OH. The 2'-OH groups of the 5'-linked sugars were not examined as possible hydrogen bonders; these groups are identical in rC(3'p5')rC and in rC(2'p5')rC, and thus cannot account for the difference in secondary structure between these compounds.

Table II summarizes the allowable hydrogen bonds which could confer rigidity upon the asymmetric conformations. Evidence for hydrogen bonds involving the cytosine carbonyl oxygen has been advanced by Ts'o (Ts'o *et al.*, 1966; Schweizer *et al.*, 1968). Brahms and Sadron (1966) showed that a phosphate oxygen might hydrogen bond to the 2'-OH of GMP. We have considered these two types of oxygen atoms, plus the ring oxygen of the 5'-linked sugar. (In no case is a hydrogen bond sterically feasible between the hydroxyl group of interest and the base carbonyl of the 5'-linked cytidine.)

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TABLE II: Interactions Shown Possible from Courtauld Models of Dinucleoside Phosphates.

| Compound | Secondary Structure[a] | Possible Hydrogen Bonds between Cytidyl Moiety's Free Hydroxy[b] and Oxygen from | | |
		2-C=O of Same Cytidyl	Phosphate[c]	Ether of Next Sugar[d]
rC(3'p5')rC	+++	+	+	+
rC(2'p5')rC	+	−	+	−[e]
dC(3'p5')dC	+	−	−	−
araC(3'p5')araC	+	+	+	+
araC(2'p5')araC	−	−	−	−[e]

[a] As shown by circular dichroism and its temperature dependence. [b] The 2'-OH or 3'-OH of the 3'-P or 2'-P linked sugar. [c] Three of the phosphate oxygens (excluding the 2'- or 3'-esterified one). [d] The 5'-esterified sugar ring oxygen. [e] Hydrogen bond can form, but only at the cost of good overlap of bases.

From this study of models, no obvious explanation emerges for the different stabilities of helical structure in the various dinucleoside monophosphates (see the section on circular dichroism for the sequence of stabilities). Even if two simultaneous hydrogen bonds are proposed, for example, to the carbonyl and to the phosphate (Witzel and Barnard, 1962), the difference between rC(3'p5')rC and araC(3'p5')araC cannot easily be rationalized. Furthermore, the problem remains that dC-(3'p5')dC with no 2'-OH shows greater base stacking than does rC(2'p5')rC or either of the araC dinucleoside monophosphates; the answer does not appear to be steric hindrance caused by the 2' substituents on arabinose. There appears to be some special factor in rC-(3'p5')rC which greatly promotes ordered secondary structure. Since dC(3'p5')dC also exhibits noticeable base stacking, the factor is not likely to be the 2'-OH group. The intrinsic free energy of base stacking should be identical for all the cytosine dinucleoside monophos-

phates under consideration, unless the stacking geometries are quite different for the various dimers. It is difficult to imagine any significant differences in hydrophobic interactions. However, specific hydrogen bonding of solvent water molecules to a part of the nucleotide structure may be sterically possible in only some oligonucleotides, and cannot be ruled out as the stabilizing factor. Clearly, further work is needed on the forces stabilizing single-stranded nucleotide structure.

Effect of Salt on Circular Dichroism of Cytosine Polynucleotides. The dependence of the circular dichroism of single-stranded poly rC and poly dC upon salt concentration was examined as a test for the involvement of phosphate groups in helix stabilization. The reasoning proceeds as follows. In all polynucleotides at alkaline pH, repulsion is expected among neighboring charged phosphate groups. When the ionic strength is made very low, the lack of charge shielding should increase this repulsion, partially destabilize any secondary structure, and cause a decrease in circular dichroism signal. This effect would be expected for both poly dC and poly rC, in the absence of other considerations. However, if there are hydrogen bonds in poly rC between the 2'-hydroxyl groups and phosphate oxygens, then a decrease in salt
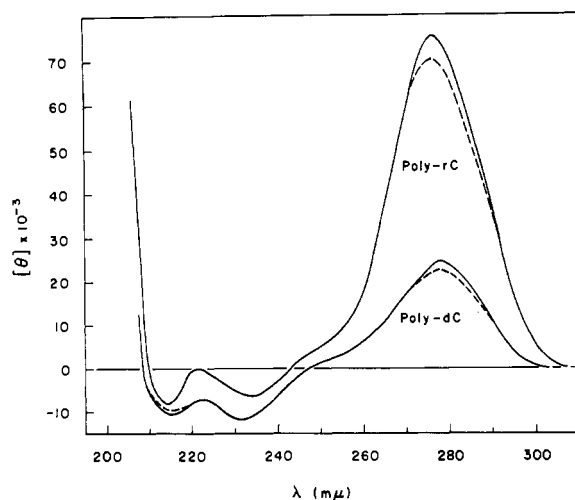

FIGURE 8: Circular dichroism of poly-rC and poly-dC. Effect of salt concentration at pH 10.0. 2 M NaCl, ——; 10^{-4} M salt, - - - - -. Upper curves, poly-rC; lower curves, poly-dC.
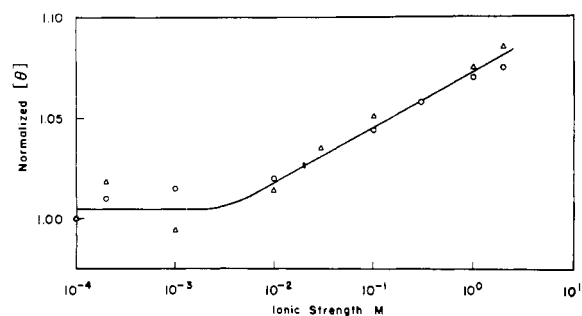

FIGURE 9: Effect of NaCl concentration upon circular dichroism magnitude of single-stranded cytosine polymers. Poly rC peak at 276 mμ, O—O; poly dC peak at 278 mμ, △—△. Normalized [θ] is defined as the ellipticity at a given NaCl concentration divided by θ at 10^{-4} M salt.

concentration would increase the effective charge on the phosphate oxygen, thus leading to stronger hydrogen bonds and a greater rigidity of structure. Because of this balancing of repulsion and attraction in poly rC, a greater destruction of helix at low ionic strength would be predicted for poly dC than for poly rC.

A pH of 10.0 was chosen for this experiment to ensure that poly dC would remain in its uncharged, single-stranded form. The pK_a of poly dC is 7.4 in 0.1 M NaCl (Inman, 1964). However, this pK rises as the salt concentration is lowered; a solution of poly dC at pH 8.5 in 10^{-3} M NaCl was largely double stranded, as seen from its ultraviolet spectrum. A similar pK shift for poly rC was responsible for the changes in optical rotatory dispersion seen by Fasman et al. (1964) in a preliminary salt-dependence study at pH 7. At pH 10.0, with polymer concentrations of 1.6×10^{-4} M cytosine residues, neither poly rC nor poly dC showed signs of double-stranded helix formation. The circular dichroism spectra, at all ionic strengths, were, in shape, identical with the circular dichroism at pH 8.5, ionic strength 0.1 M; the circular dichroism peak wavelength remained at 276 mμ for poly rC and 278 mμ for poly dC.

Figure 8 shows the circular dichroism curves. The larger magnitude of [θ] for poly rC than for poly dC is similar to the larger [m'] in optical rotatory dispersion (Ts'o et al., 1966; Adler et al., 1967). Poly rC shows a gradual 7% decrease in its circular dichroism peak as the ion concentration is lowered from 2 to 10^{-4} M. This small salt effect is similar to that seen for other single-stranded ribonucleotides: poly I (Brahms and Sadron, 1966), poly A and dinucleoside phosphates (Cantor et al., 1966), and oligocytidylic acids (Brahms et al., 1967a). It is much smaller than the destabilizing effect of low salt upon double-stranded DNA (Schildkraut and Lifson, 1965). This result alone could show that phosphate–phosphate repulsion might be balanced by phosphate–hydroxyl attraction in single-stranded poly rC.

However, poly dC exhibits exactly the same 7% decrease in [θ]. The comparison between the two polymers can be seen in the semilog plot of Figure 9. Therefore, the results of these experiments are evidence against a stabilizing 2'-hydroxyl–phosphate hydrogen bond in polyribonucleotides. The small magnitude of the salt effect probably shows that neighboring phosphates are sufficiently far apart, in single-stranded nucleotides, that their repulsion is not an effective force.

Conclusions

Circular dichroism and absorbance properties of cytosine arabinoside dinucleoside monophosphates were examined, and compared with those of the riboside and deoxyriboside analogs. The araC compounds exhibit much less ordered secondary structure than rC(3'p5')-rC, and less even than dC(3'p5')dC. The building of molecular models failed to clarify why only the natural, 3'p5'-linked ribose dinucleoside phosphates should develop significant base stacking. A stabilizing hydrogen

bond between the 2'-hydroxyl group and a phosphate oxygen seems unlikely.

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