Influence of Terminal 3′ Phosphates or 2′,3′-Cyclic Phosphates on the Conformations of Oligoriboadenylates, Oligoribocytidylates, and the Corresponding Monomers†

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ABSTRACT: The circular dichroism spectra of chemically synthesized adenylate and cytidylate dinucleotides and trinucleotides bearing terminal 3′ phosphates have been compared under a variety of conditions with the spectra obtained from the corresponding oligomers with 2′,3′-terminal cyclic phosphate groups. Similar comparisons for the mononucleotides are also presented. Although the base stacking of an oligomer with a terminal cyclic phosphate might be expected to be greater than that of the corresponding oligomer with a 3′ phosphate from charge repulsion considerations, the magnitudes of the Cotton effects in the former class are always considerably smaller than those in the latter class. This suggests a decreased stacking. The implications of these observations are discussed in light of the compelling crystallographic evidence that cytidine 2′,3′-cyclic phosphate adopts an unusual sugar puckering and the syn conformation.

For a number of years, several groups have been interested in the nucleoside glycosidic torsion angle and its effects on polynucleotide conformation (Donohue & Trueblood, 1960; Sundaralingam, 1969). We have been studying this problem by using cycloadenosines as models of anti conformations [e.g., Uesugi et al. (1977)] and 8-substituted adenosines as models of syn conformations [e.g., Ikehara et al. (1978)].

However, in these cases the nucleoside residues involved remain unknown in vivo. It seemed of interest to examine some naturally occurring nucleic acids for their possible existence in the syn conformation. It should be noted that such conformations have been detected by X-ray crystallography and/or by NMR spectroscopy for deoxyguanosine (Haschemeyer & Sobell, 1965), N^2-dimethylguanosine (Brennan et al., 1972), 4-thiouridine (Saenger & Scheit, 1970), adenosine 3′,5′-cyclic phosphate (cAMP) (Watenpaugh et al., 1968; Fazakerley et al., 1977), guanosine 3′,5′-cyclic phosphate (cGMP) (Chwang & Sundaralingam, 1973), and cytidine 2′,3′-cyclic phosphate (C>p)[1] (Coulter, 1973; Lavallee & Coulter, 1973). It seems that the structural constraints of cyclic phosphates cause alterations in the preferred sugar puckers [the C(4′)-exo–C(3′)-endo conformation for 3′,5′-cyclic phosphates and the O(1′)-endo or planar conformation for 2′,3′-cyclic phosphates], reducing the rotational barrier about the glycosidic bond and hence favoring different orientations of the base moieties with respect to the furanose rings. The conformational properties of oligo- and mononucleotides with 2′,3′-cyclic phosphates are particularly interesting in view of the involvement of such compounds as intermediates in ribonuclease action. Here again, several groups have observed unusual conformational effects on binding various ribonucleotides to these enzymes (Meadows et al., 1969; Gorenstein & Wyrwicz, 1974; Ohshima & Imahori, 1971).

In the present paper, we examined the influence of a terminal 2′,3′-cyclic phosphate on mono- and oligonucleotide CD properties and compared the results with those for the corresponding molecules having terminal 3′ phosphates. The results revealed that oligo(A) and oligo(C) molecules terminated by 2′,3′-cyclic phosphates have quite different CD spectra from those not so terminated. The differences are in a direction suggesting that these molecules are less stacked than those with 3′ phosphate or with no terminal phosphate. Solvent studies provide some support for these conclusions. This suggests that the special sugar-puckering conformation of the nucleoside 2′,3′-cyclic phosphate derivatives produces perturbations in oligonucleotide stacking conformations.

Materials and Methods

All nucleotides and oligonucleotides were synthesized chemically (Ohtsuka et al., 1974; Coutsogeorgopoulos & Khorana, 1964). Full details of the synthetic methods adopted will be published elsewhere (A. F. Markham, E. Ohtsuka, and M. Ikehara, unpublished experiments). Linear oligomers with 3′ phosphates were obtained by DCC-catalyzed polymerization of properly protected 3′ nucleotides. Protected nucleotides were resolved by TEAE-cellulose (acetate form) column chromatography. After removal of protecting groups, oligonucleotides, as well as the nucleoside 3′,5′-cyclic phosphates, were further purified by paper chromatography followed by paper electrophoresis.

Nucleoside 3′,5′-cyclic phosphates were freed from contaminating traces of the 2′3′-cyclic phosphates as follows. Samples were treated with 0.1 N HCl at 20 °C for 30 min and then applied to paper chromatography after evaporation and coevaporation twice with water. Further purification was achieved by application to paper electrophoresis; 2′,3′-cyclic phosphates are fully opened to mixtures of the 2′ and 3′ phosphates whereas the cNp compounds are fully resistant.

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[1] Abbreviations used herein are as recommended by the IUPAC–IUB Commission on Biochemical Nomenclature (1970): c preceding a mononucleotide structure indicates a 3′–5′ phosphodiester linkage (e.g., cAp indicates adenosine 3′,5′-cyclic phosphate); >p at the end of an oligo- or mononucleotide structure indicates a 2′3′-cyclic phosphate (e.g., C-C-C>p is the trinucleotide with a terminal 2′,3′-cyclic phosphate); CD, circular dichroism; ORD, optical rotatory dispersion; DCC, dicyclohexylcarbodiimide; TPS, 2,4,6-triisopropylbenzenesulfonyl chloride; MeOTr, monomethoxytrityl; Bz, benzoyl. Abbreviations used in the naming of protected oligonucleotides have been described in previous communications from these laboratories [e.g., Ohtsuka et al. (1978)].

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Table I: Properties of the Synthesized Oligoribonucleotides

oligomer	PPC[a] R_f	PPC[a] R_{Np}[b]	PEP R_m[c]	Np/N ratio after BAP treatment and RNase digestion[d]
CpCp	0.42	0.86	1.01	1.08:1.00
CpC>p	0.50	1.22	0.76	
CpCpCp	0.33	0.66	1.05	2.01:1.00
CpCpC>p	0.40	0.83	0.87	
ApAp	0.20	0.63	1.02	1.15:1.00
ApA>p	0.38	1.16	0.76	1.00:1.00
ApApAp	0.10	0.30	1.05	2.14:1.00
ApApA>p	0.29	0.86	0.89	1.97:1.00

[a] 1-Propanol–concentrated NH_3–H_2O (6:1:3 v/v). [b] R_f relative to Np. [c] Relative mobility to Np (1.0) and N (0.0). [d] 2′,3′-Cyclic phosphates opened initially with 0.1 N HCl (see text).

Paper chromatography was performed in the solvent system 1-propanol–concentrated ammonia–water (6:1:3 v/v) on Whatman 3MM paper (preparative scale) or Toyo 51A paper (analytical scale). Paper electrophoresis was performed on the same materials in 50 mM triethylammonium bicarbonate buffer, pH 7.5, for 1 h with a potential gradient of 23 V/cm.

Preparation and Identification of Oligonucleotides with Terminal 2′,3′-Cyclic Phosphates. The four oligonucleotides with terminal 2′,3′-cyclic phosphates (C-C>p, C-C-C>p, A-A>p, and A-A-A>p) were obtained as byproducts in TPS-catalyzed block condensations designed to produce sequences corresponding to parts of the *Escherichia coli* $tRNA_f^{Met}$ by the diester approach (Markham et al., unpublished experiments). For example, reaction of MeOTr-BzC(Bz)-BzC(Bz)p with ibG(Bz)-ibG(Bz)$_2$ gave, inter alia, the pyrophosphate derivative of the former compound which was purified from other reactants on a column of TEAE-cellulose (acetate form). Deblocking of the pyrophosphates with 80% acetic acid and methanolic ammonia yielded equal amounts of the linear oligomer and its counterpart with a terminal cyclic phosphate. These two components are easily separated by paper chromatography and were further purified by paper electrophoresis as described above.

A sample of A-A>p was synthesized by a different route (Uesugi & Ikehara, 1977). This compound gave identical CD spectra with those of pyrophosphate-derived A-A>p and provides further support of our results.

Samples of C-C>p, C-C-C>p, A-A>p, and A-A-A>p were checked for purity by treatment with 0.1 N HCl as above (Uesugi & Ikehara, 1977). In all cases, mobilities were identical with those of corresponding marker oligonucleotides with 3′ phosphates and no resistant compounds (such as cN-Np or cN-N-Np) were detected. The structural integrity of oligomers was confirmed by first removing terminal phosphate with *E. coli* alkaline phosphatase (EC 3.1.3.1) (Worthington). The resulting oligomers were digested with either ribonuclease A (EC 3.1.4.22) (Worthington) for oligocytidylic acids or ribonuclease M (EC 3.1.4.23) (a generous gift of Dr. M. Irie; Irie, 1967) for oligoadenylic acids. The ratio of nucleotide to nucleoside components in such digestions are shown in Table I. Oligomer R_f and paper electrophoresis relative mobility values are also included in Table I. R_m values are expressed by taking the mobility of the particular mononucleotide as 1.0 and that of the nucleoside marker as 0.0. No nuclease-resistant material was observed.

UV Absorption Data. Where the molar absorptions (Inoue & Satoh, 1969; Ohtsuka et al., 1974; Janik, 1971) of oligonucleotidic compounds in the solvent systems used for optical studies do not appear in the literature, they were measured by ribonuclease digestion of the compound (~4 A_{260} units) as above and treatment of the same amount of the compound under the same conditions in the absence of enzyme. Control reactions were always included so as to check that the digestion reactions were proceeding to completion. The ϵ values for the oligomers with 2′,3′-cyclic phosphates at pH 4.0 were not determined due to a shortage of material, and the ϵ values for the corresponding oligomers with 3′ phosphates at pH 4.0 were used (Tables II and III).

CD Measurements. CD spectra were recorded at 20 °C with a JASCO ORD/UV-5 spectropolarimeter equipped with a CD attachment. Calibration of the Cotton effect magnitude was effected with *d*-10-camphorsulfonic acid. Solutions of $A_{max} = \sim 1.3$ were prepared by using the following solvent systems: (1) methanol; (2) 0.01 M potassium phosphate buffer, pH 7.0, and 0.1 M KF; and (3) 0.05 M sodium acetate buffer, pH 4.0, and 0.01 M NaCl. Cells of 10-mm path length were used, and solvent blanks were run before and after each sample. Each spectrum was measured at least twice, and reproducible curves were obtained. UV absorptions of these solutions were measured against appropriate blanks on either a Hitachi 124 or a 323 spectrophotometer. The molar ellipticity, $[\theta]$, and molar extinction coefficient, ϵ, are presented in terms of the per residue value.

Results

The CD spectra of the various adenylate and cytidylate monomers and oligomers are in Figures 1–4, and UV and CD spectral features are collected in Tables II and III. Figure 5 illustrates the difference spectra obtained by subtracting the molar ellipticities of constituent monomers from the per residue molar ellipticities of the various oligomers at pH 7.0 or 4.0.

CD Spectra of Adenylic Acid Derivatives. As far as the three adenosine mononucleotides (Ap, A>p, and cAp) are concerned, examination of the CD spectra reveals that there is little difference either in shape or in magnitude between the three different species in the three solvent systems. The spectra at pH 7.0 are shown in Figure 1a. In the short-wavelength region (220–230 nm) Ap resembles A more closely than it resembles pA (Ikehara et al., 1977).

Examination of the dimer spectra in Figure 1b reveals the considerable quantitative difference between the CD spectra of A-Ap (Ohtsuka et al., 1974) and A-A>p at pH. 7.0. The magnitudes of the CD bands, especially that of the long-wavelength band, for A-A>p are considerably smaller than those for A-Ap.

The magnitudes of the CD bands for A-A (van Holde et al., 1965) are much larger than those for A-Ap under similar conditions (Figure 1b). Thus, the magnitudes of the A-A>p Cotton effects are significantly smaller than those of A-Ap rather than being intermediate in size between those of A-Ap and A-A (Inoue & Satoh, 1969).

Figure 2a illustrates the differences between A-A-Ap and A-A-A>p at pH 7.0. The data of Brahms et al. (1966) at pH 7.4 and 25 °C for A-A-A are also included in Figure 2a. It appears that the differences between A-A-Ap and A-A-A are much less than those between A-Ap and A-A, presumably because with increasing chain length the relative increase in charge repulsion on 3′-phosphorylation is reduced. However, the CD spectrum of A-A-A>p displays large reductions in Cotton effect magnitudes; these reductions with respect to A-A-Ap are similar to the percentage reductions on going from A-Ap to A-A>p.

CD spectra were also measured in methanol to differentiate between effects caused purely by base-stacking interactions and those due to the adoption of fixed unusual conformations in the presence of a 2′,3′-cyclic phosphate. The former should

Table II: UV Absorption and CD Spectral Properties of the Mono- and Oligoadenylic Acids

compd	conditions	UV λ_{max} (nm)	UV $\epsilon_{260} \times 10^{-4}$	CD λ (nm)	CD $[\theta] \times 10^{-4}$	CD λ (nm)	CD $[\theta] \times 10^{-4}$
Ap	pH 7	259	1.54	263.5	−0.46	226	0.20
	pH 4	258	1.54	260.5	−0.47	227	0.14
	MeOH	259	1.54	263	−0.60	225	0.22
cAp	pH 7	259.5	1.54	260	−0.57		
	pH 4	258	1.54	259	−0.50	226	0.17
	MeOH	259	1.54	258	−0.55		
A>p	pH 7	259	1.54	259	−0.48	224	0.15
	pH 4	258	1.54	260	−0.42		
	MeOH	259	1.54	263	−0.49	223	0.20
ApAp	pH 7[a]	258	1.39	273	1.00	251	−1.80
	pH 4	258	1.44	272	0.91	253	−1.57
	MeOH	259.5	1.54	263	−0.78		
ApA>p	pH 7	258	1.42	274	0.24	253	−1.26
	pH 4	258	1.44	276	0.11	253	−1.15
	MeOH	259	1.54	263	−0.85	223	0.95
ApApAp	pH 7[a]	258	1.26	270	1.90	250	−2.30
	pH 4	258	1.33	272	1.01	249.5	−1.49
	MeOH	259	1.54	263	−0.90		
ApApA>p	pH 7	257	1.36	273	0.52	252	−1.35
	pH 4	257	1.33	274	0.35	249	−0.90
	MeOH	258	1.54	263	−0.91	222	0.19

[a] Ohtsuka et al. (1974).

Table III: UV Absorption and CD Spectral Properties of the Mono- and Oligocytidylic Acids

compd	conditions	UV λ_{max} (nm)	UV $\epsilon_{270} \times 10^{-4}$	CD λ (nm)	CD $[\theta] \times 10^{-4}$	CD λ (nm)	CD $[\theta] \times 10^{-4}$
Cp	pH 7	271	0.90	274	1.24	222	−0.78
	pH 4	276.5	1.05	280	0.81		
	MeOH	272	0.90	274	1.83	221	−1.20
cCp	pH 7	270	0.90	273	1.50	219	−0.83
	pH 4	275	1.05	277	1.25		
	MeOH	272	0.90	274	1.89	221	−1.15
C>p	pH 7	267	0.90	273	0.48	217	−1.00
	pH 4	272	1.05	277	0.31		
	MeOH	267	0.90	278	0.50	217	−1.14
CpCp	pH 7	267	0.84	278	1.89		
	pH 4	272.5	0.98	285	1.59		
	MeOH	269	0.90	274	1.71	218	−1.30
CpC>p	pH 7	267	0.85	278	1.02		
	pH 4	272.5	0.98	280	0.76		
CpCpCp	pH 7	269	0.81	279	2.27		
	pH 4	275.5	0.96	286	1.78		
	MeOH	269	0.90	277	1.39	220	−0.83
CpCpC>p	pH 7	269	0.82	279	1.28		
	pH 4	274	0.96	284	0.87		
	MeOH	269	0.90	276	1.18	217	−0.89

not be observed in this solvent whereas the latter should persist (Ohtsuka et al., 1974; Cantor et al., 1969). The spectra for A-Ap and A-A>p in methanol (Figure 1d) are similar to those of the monomers, indicating that base-stacking interactions are the main factors responsible for the conservative dimer spectra at pH 7.0. However, we note that the spectra of both A-Ap and A-A>p in methanol give negative CD maxima ~50% larger than those of Ap or A>p in methanol. Similarly, the conservative type spectrum observed in aqueous media is destroyed for A-A-Ap and A-A-A>p in methanol (Table III) and the two CD spectra are essentially the same.

We also note (Figures 1c and 2b) that similar results to those at pH 7.0 are observed in the pH 4.0 system for the adenylate dimers and trimers. Although there may be considerable base protonation at this pH (Topal & Warshaw, 1976; Ogasawara & Inoue, 1976), it is interesting that the CD spectral effects persist under conditions where the dissociation state of a 3′ phosphate is the same as that of a 2′,3′-cyclic phosphate.

CD Spectra of Cytidylic Acid Derivatives. Figure 2c shows the CD spectra of cCp, Cp, and C>p at pH 7.0, and the crossover points display shifts to longer wavelength in this order. The Cotton effect maximum at around 274 nm for cCp is 21% greater than that of Cp, while that of C>p is decreased to only 39% of that of Cp (Table III). At pH 4.0 (Figure 2d), the position of the CD crossover points follows the same pattern as at pH 7.0, but the difference between cCp and Cp is more marked. The same difference between the spectra of Cp and C>p was observed in methanol (Figure 3a), with the magnitude of the C>p positive Cotton effect decreased to 27% of that of the Cp Cotton effect.

The CD spectra of C-Cp and C-C>p at pH 7.0 are presented in Figure 3b along with a spectrum derived from the data of Brahms et al. (1976) for C-C in 4.7 M KF at pH 7.5–8.0. The magnitude of the positive Cotton band of C-C is almost twice that of C-Cp. On the other hand, the corresponding magnitude of C-C>p is only half that of C-Cp. There are no wavelength shifts here (Figures 3b and 5c). For the trimer in the pH 7.0 system (Figure 3d), there is again a reduction in the positive Cotton effect magnitude to about half of the C-C-Cp value on going to C-C-C>p with no significant wavelength shift.

In methanol (Table III), C-Cp displays a CD spectrum similar to that of Cp, though the magnitude of the positive band is slightly smaller. The CD spectrum of C-C>p in this solvent was not measured due to a shortage of material. C-C-Cp and C-C-C>p also display monomer-like spectra with decreased positive bands with respect to C-Cp and even larger decreases with respect to Cp (Table II). Again, as in the adenylate series, similar reductions in Cotton effect magnitudes to those at pH 7.0 are observed at pH 4.0 (Figures 3c and 4a) for C-C>p and C-C-C>p with respect to C-Cp and C-C-Cp.

Discussion

Conformation of Nucleoside 2′,3′-Cyclic Phosphates. According to the results of Miles et al. (1968, 1970), the sign and magnitude of monomer CD bands are determined by the glycosidic torsion angle and the sugar-puckering form. In the case of pyrimidine nucleosides particularly (Miles et al., 1970), differences in sugar puckering seem to produce relatively large differences in the magnitudes of the CD bands in the long-wavelength region (B_{2u} band). The calculated rotational strength–torsion angle diagram (Miles et al., 1970) suggests that cytidine will show a positive B_{2u} band when the glycosidic torsion angle (χ) is between −80 and +80° and that the 3′-endo sugar conformation will give larger positive bands than the 2′-endo conformation. Cytidine in a syn conformation should show a negative B_{2u} band.

The magnitudes of the positive CD bands for C>p are much smaller than those for Cp in the three different solvent systems. These results can be explained in two ways: (1) C>p has a high content of the syn conformation in a glycosidic conformational equilibrium and/or (2) the sugar-puckering conformation of C>p is not favorable for generation of a positive band. X-ray crystallographic studies show that C>p takes an O(1′)-endo or planar sugar conformation and a syn glycosidic conformation (χ = ~−110°; Coulter, 1973). These somewhat unusual conformations are due to the constraints imposed by the 2′,3′-cyclic phosphate group and therefore seem likely to also be favored in solution. Examination of Corey–Pauling–Koltun (CPK) models shows that in these

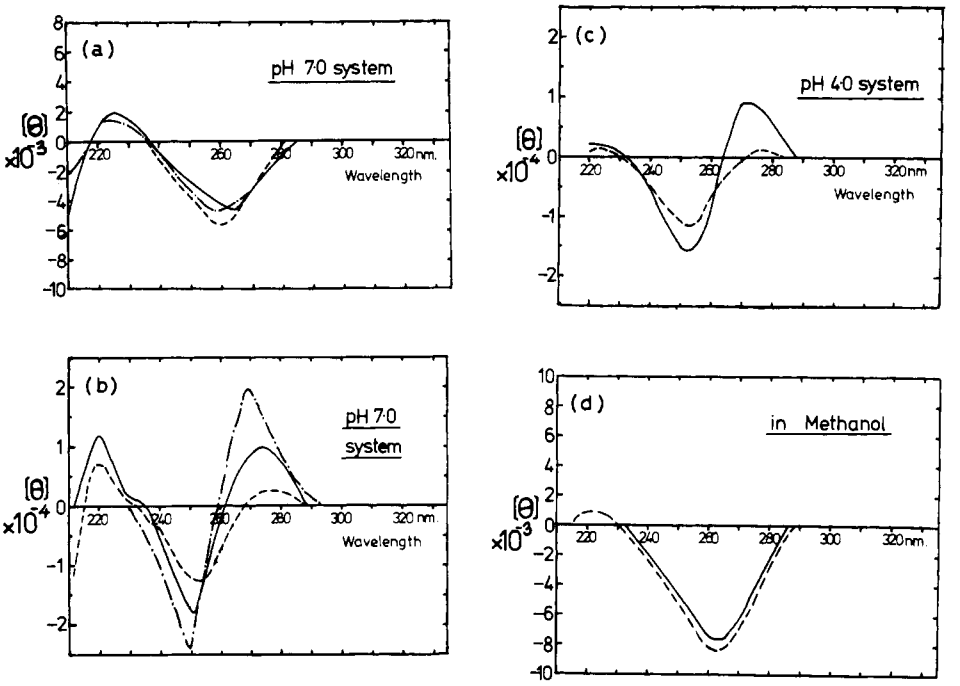

FIGURE 1: CD spectra of the adenylic acids (a) [(—) Ap; (---) cAp; (-·-) A>p] and the diadenylic acids (b–d) [(—) A-Ap; (---) A-A>p; (-·-) A-A].

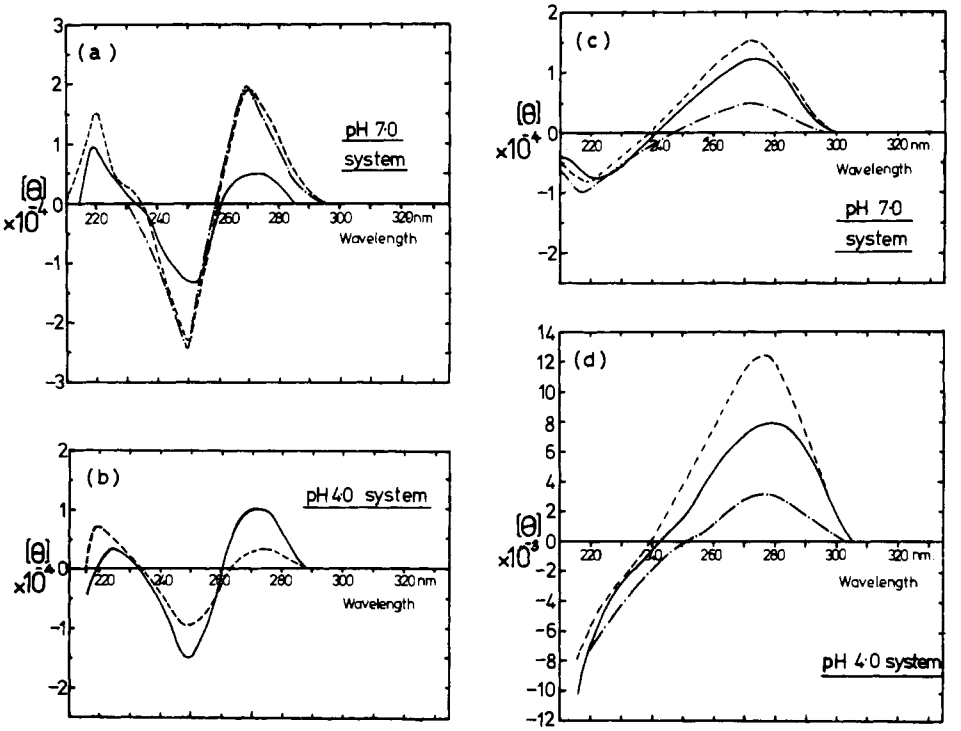

FIGURE 2: CD spectra of the triadenylic acids (a) [(---) A-A-Ap; (—) A-A-A>p; (-·-) A-A-A] and (b) [(—) A-A-Ap; (---) A-A-A>p] and the cytidylic acids (c and d) [(—) Cp; (---) cCp; (-·-) C>p].

conformations (especially the O(1′)-endo conformation) the average distance between the base and sugar is greater than in the 3′-endo conformation. The same is true for the 2′-endo with respect to the 3′-endo conformation. Therefore, it might be expected that a O(1′)-endo or planar sugar conformation would also reduce the rotational strength of the B_{2u} band. On the other hand, Cp is assumed to prefer a 3′-endo sugar puckering (60%) from ^{1}H NMR studies (Davies & Danyluk, 1975). The special sugar conformation of C>p also relieves repulsion between O(2) and H(2′), producing rotational freedom about the glycosidic bond and allowing a syn conformation in crystals. Thus, the combined effects of sugar puckering and glycosidic conformation could well reduce the magnitude of the positive CD band of C>p.

A>p may adopt similar sugar-puckering conformations, and similar effects to those discussed above are to be expected. However, in this case, it is considered that Ap itself favors a 2′-endo conformation (Davies & Danyluk, 1975) and a syn conformation (Son & Chachaty, 1973). Moreover, it has been suggested by Miles et al. (1968) that the rotational strength of adenosine at 260 nm will be negative in sign when the glycosidic torsion angle (χ) lies within a broad region (0–200°)

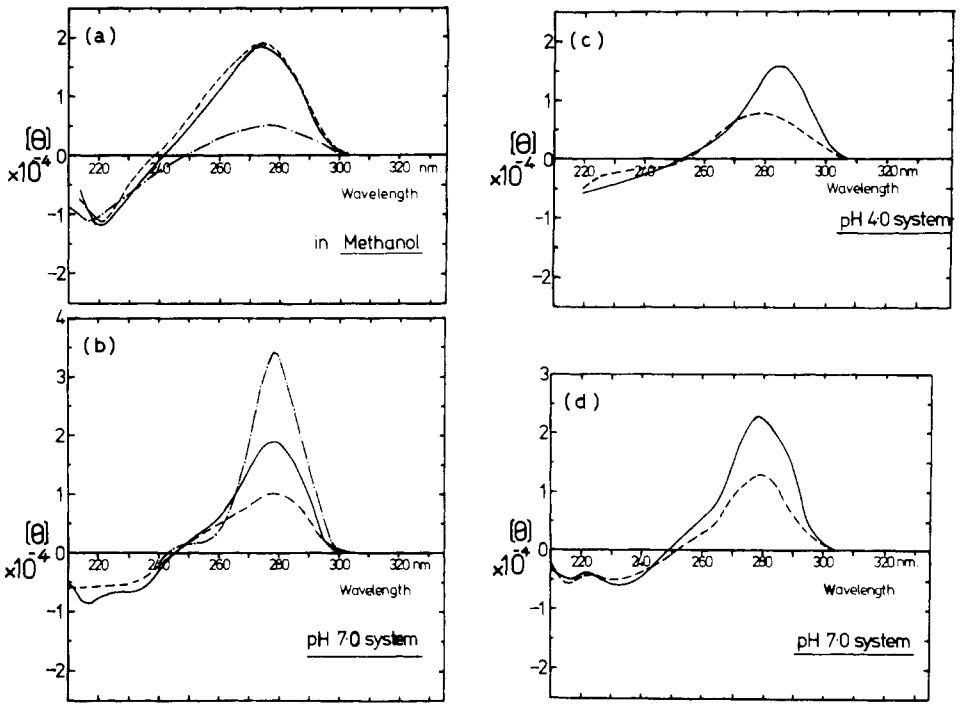

FIGURE 3: CD spectra of the cytidylic acids (a) [(—) Cp; (---) cCp; (-·-) C>p, the dicytidylic acids (b and c) [(—) C-Cp; (---) C-C>p; (-·-) C-C], and the tricytidylic acids (d) [(—) C-C-Cp; (---) C-C-C>p].

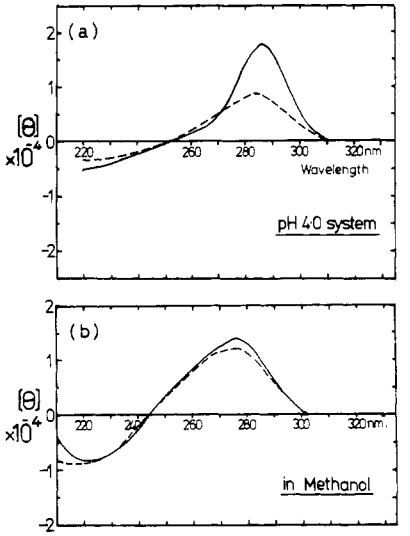

FIGURE 4: CD spectra of the tricytidylic acids: (—) C-C-Cp; (---) C-C-C>p.

including the anti, high anti (Prusiner et al., 1973), and a part of the syn regions. In fact, 8-methyladenosine, which is believed to favor a syn conformation, shows an almost identical CD spectrum with that of adenosine (Ikehara et al., 1977).

Effect of a 3'-Terminal Phosphomonoester Group. Comparison of the present results for A-Ap and C-Cp with the data (Topal & Warshaw, 1976) for A-A and C-C at neutral pH shows that the magnitudes of the long-wavelength CD bands are reduced to about 40 and 60% of those for A-A and C-C, respectively, by introduction of a 3'-terminal phosphate dianion. These values were calculated from the difference spectra (Figure 5), which afford a better estimate of base-stacking interactions than the original spectra. The CD spectra of oligonucleotides contain contributions from the interaction of the bases and from the interaction of base chromophores with the asymmetric environments of the sugar moieties. The former contribution might be expected to be directly observable from the CD difference spectra since the contributions from base–sugar interactions should be closely approximated by the spectra of the appropriate monomers.

At the trimer level, A-A-Ap and A-A-A (Brahms et al., 1966) show almost identical CD spectra (Figure 2), but the magnitude of the long-wavelength CD band of C-C-Cp is ~40% of that estimated for C-C-C (Brahms et al., 1967) (calculated from the difference spectra). It appears that stacking in oligocytidylic acids is much more sensitive to phosphate repulsion than that in oligoadenylic acids, in which the effect of a 3'-terminal phosphate group is negligible at the trimer level. This difference may be due to the difference in stacking ability between adenine and cytosine residues.

Effect of a Terminal 2',3'-Cyclic Phosphate Group. Consideration of charge repulsion effects as discussed above points to oligonucleotides with terminal 2',3'-cyclic phosphates being intermediate cases between 3'-phosphorylated and 3'-unphosphorylated oligomers. However, the results for oligomers with 2',3'-cyclic phosphate groups clearly show that this is not the case. The difference spectra at pH 7.0 (Figure 5) show that cyclization of the 3'-terminal phosphate causes approximately a 60% reduction in the magnitude of the long-wavelength CD band regardless of the chain length or the base. It is rather surprising to observe that A-A-A>p still shows a 60% reduction of CD band magnitude with respect to A-A-Ap in which the destacking effect of the 3'-terminal phosphate group is negligible. A reduction in the magnitudes of the CD bands for oligonucleotides can reflect destacking of the bases or changes in the geometry of stacking (Bush, 1974). An example of the latter effect is found in dA-dA, which shows smaller CD bands but larger hypochromicity and ^{1}H NMR dimerization shifts than A-A (Kondo et al., 1972). It is also interesting to note that A-dA, which has hypochromicity comparable to that of A-A, shows markedly decreased CD bands with respect to A-A and even to dA-dA (Kondo et al., 1972). Moreover, the pdA residue of A-dA favors a 2'-endo puckering which allows a base–sugar geometry similar to those encouraged by an O(1')-endo or planar conformation. In the case of ribodinucleoside monophosphates,

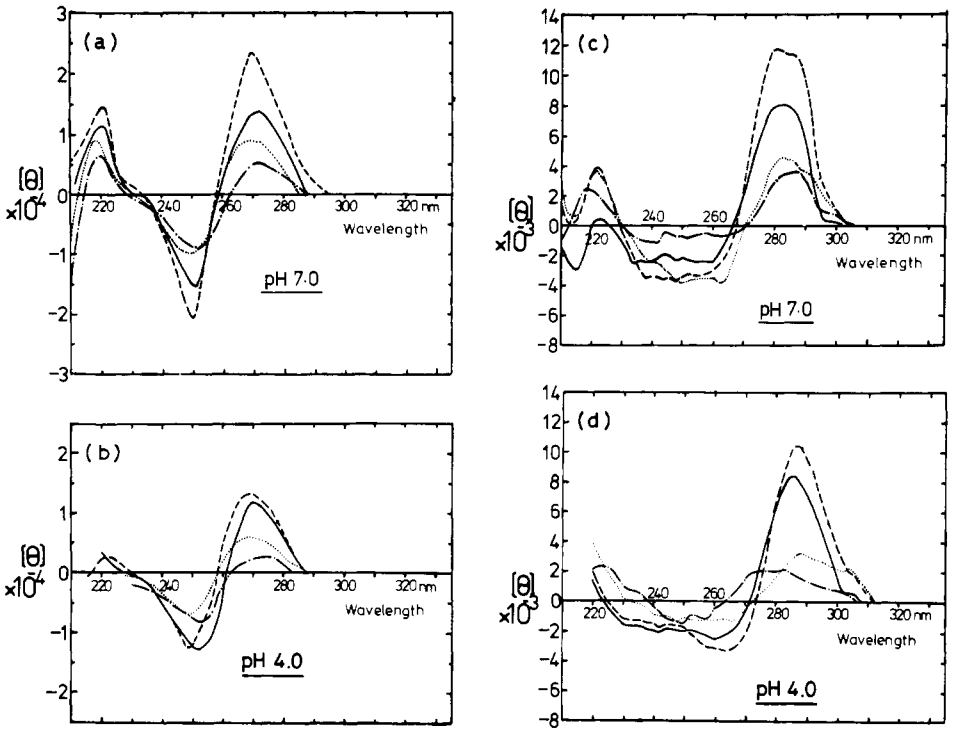

FIGURE 5: CD difference spectra in the oligoadenylic acids (a and b) [(---) A-A-Ap; (···) A-A-A>p; (—) A-Ap; (—·—) A-A>p] and the oligocytidylic acids (c and d) [(---) C-C-Cp; (···) C-C-C>p; (—) C-Cp; (—·—) C-C>p]. The oligomer CD spectrum was subtracted by the average CD spectrum of the component monomers.

it is known that the sugar-puckering conformations of both nucleoside residues are affected by stacking interactions (Hruska & Danyluk, 1968a; Ts'o et al., 1969; Lee et al., 1976). The 3′-linked nucleoside and also the 5′-linked nucleoside tend to prefer the 3′-endo conformation more strongly than the corresponding monomers upon stacking. Therefore, it may be necessary for the 5′-linked nucleoside to take the 3′-endo conformation so as to achieve maximum stacking. In the case of A-A>p, this process may be prohibited because the furanose ring of the 5′-linked component does not have sufficient flexibility. The situation may be similar to the case of A-dA because the pdA residue is expected to prefer a 2′-endo conformation more strongly than does a pA residue (Davies & Danyluk, 1974). In fact ^{1}H NMR studies of these dimers show that the coupling constants between H-1′ and H-2′ of the pA>p (Hruska & Danyluk, 1968a) and pdA (Kondo et al., 1972) residues remain very similar to those of the monomers, suggesting little change in the furanose conformations. Brief reports on ^{1}H NMR studies of A-A>p have been published (Hruska & Danyluk, 1968a,b), and this dimer was assumed to take a stacking conformation similar to A-A.

Similar arguments can also be applied to the cytidylic acid series. The long-wavelength CD band of dC-dC is much smaller than that of C-C (Adler et al., 1968), but the hypochromicity of the deoxy dimer is only slightly smaller than that of the ribo dimer (Adler et al., 1967). The ϵ values shown in Table II indicate that A-A>p and A-A-A>p have significantly smaller hypochromicities than those of A-Ap and A-A-Ap, respectively. C-C>p and C-C-C>p have slightly reduced hypochromicities. In conclusion, the effect of a terminal 2′,3′-cyclic phosphate group on oligonucleotide conformation may involve destabilization of the ordinary, anti-anti, right-handed stacking by a combination of unfavorable sugar puckering and altered glycosidic conformational preferences.

Conclusions

As discussed above, the peculier CD properties of mono- and oligonucleotides with terminal 2′,3′-cyclic phosphate groups can be ascribed to the special sugar-puckering conformation imposed by the cyclic phosphate group and the resulting flexibility in glycosidic conformation. We believe that the majority of the population of nucleoside 2′,3′-cyclic phosphate residues takes an O(1′)-endo or a planar conformation, as found in crystals of C>p (Coulter, 1973), whereas a tendency toward ordinary 3′-endo or 2′-endo puckering has been proposed by Lapper & Smith (1973) from ^{1}H and ^{13}C NMR studies. The most important finding in this paper is that a special furanose conformation in the 3′-terminal residue can greatly destabilize base stacking even in a trimer.

After submission of this paper, we noted a recent publication in which the conformations of C>p and U>p were investigated by ^{1}H NMR spectroscopy using lanthanide shift reagents (Lavallee & Myers, 1978). It was concluded that both compounds take a syn conformation and an O(1′)-endo puckering in aqueous solution.

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