The effect of (2'-5') and (3'-5') phosphodiester linkages on conformational and stacking properties of cytidyl-cytidine in aqueous solution

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Received 14 July 1976

ABSTRACT

Conformational properties of (2'-5') and (3'-5') CpC have been determined by proton magnetic resonance spectroscopy at 220 MHz. The ribose ring structures are predominantly ³E with the exception of the ring from the 2'-phosphate fragment of C(2'-5')pC which exhibits an ²E pucker. Bases are oriented *anti* with respect to the ribose and the conformations about C4'-C5', C5'-O5', C3'-O3' (C2'-O2') are *gg*, *g'g'*, and *g⁺g⁻*, respectively. The dimers exist as mixtures of stacked (*g⁺g⁺* and *g⁻g⁻* about the P-O(C) bonds) and unstacked species at 20°C. Stacking is estimated to be 35% in both dimers.

INTRODUCTION

Recent NMR studies of (3'-5') dinucleoside monophosphates have provided detailed information about the three-dimensional structures of these molecules in aqueous solution.²⁻⁷ Incorporation of mononucleotides into a dimer through a (3'-5') phosphodiester linkage is found to cause a series of interdependent conformational changes initiated by stacking interactions and made possible through the swivel nature of the phosphodiester backbone. Furthermore, base sequence is observed to influence the stacking and conformational properties of the dimers.⁵

In comparative CD studies of (3'-5') and (2'-5') linked dinucleoside monophosphates,⁸ it was suggested that stacking is much less marked in the latter. Subsequently, Ts'o and coworkers⁹⁻¹⁰ reasoned that the extent of stacking is comparable in both types of dimers and that the loss of selectivity in chirality of the screw axis of the stack in the (2'-5') compared to the (3'-5') dimer gives rise to the observed differences in their CD and NMR spectra. Although the (3'-5') dimer tends to form an exclusively right hand stack, it is quite possible that the (2'-5') isomer exists as a mixture of left and right hand stacks with a preference for the latter. More recent NMR studies⁵⁻⁶ have demonstrated the presence of both right and left handed stacks in the (3'-5') dimers and therefore are in conflict with the

above conclusions.

In order to explore this discrepancy further, two pyrimidine-pyrimidine dimers ((2'-5') CpC and (3'-5') CpC) were selected for a comparative study with the objective of determining the role of the phosphodiester linkage on the conformational and stacking properties of dinucleoside monophosphates. This report describes the results of these studies.

EXPERIMENTAL SECTION

A) Materials

The 2'-, 3'-, and 5'-cytosine mononucleotides and the (3'-5') and (2'-5') dicytidine monophosphates were purchased from Sigma Chemical Co. The samples were dissolved in "100%" D₂O and the solutions adjusted to a 0.01 M concentration and pD 7.4 for dimers or pD 6.0 for monomers (pD = meter reading +0.4). A trace amount of TSP (3-trimethylsilyl propionate 2,2,3,3-d₄ sodium salt) was added to the samples and served as an internal reference.

B) Syntheses of deuterated derivatives

To provide a complete assignment for the NMR spectra, deuterated derivatives of Cp2'C and Cp3'C¹¹ were synthesized using chemical procedures described in detail elsewhere.¹² Deuterated nucleotides were isolated from RNA fractions of fully deuterated blue green algae, Synchococcus lividus. The isopropylidene derivative of deuterated cytidine was subsequently synthesized and condensed with benzoylated 3'-CMP (protonated). The resultant product proved by NMR to be a mixture of Cp2'C* and Cp3'C* (star denotes the deuterated fragment). Isomerization of the 3'-CMP apparently occurs either at the benzoylation or condensation step of the reaction. To achieve a separation, the mixture of dimers was passed through a Dowex 50 W column and eluted with 0.10 N ammonium acetate at pH 4.2. The order of elution was Cp2'C* followed by Cp3'C*.

C) Measurement of spectra

Proton magnetic resonance spectra were recorded in the Fourier transform mode on a Varian HR 220 spectrometer coupled to a Nicolet FT accessory and data system. The 180°-τ-90° pulse sequence was used in order to eliminate selectively the HDO peak. All of the spectra were measured at 20 ± 2°C and calibrated by the audio side-band technique using a Hewlett Packard 4204A oscillator. Signal positions were measured relative to internal TSP with an accuracy of ± 0.002 ppm. ³¹P decoupling experiments were performed utilizing a Schomandl ND 100 M generator set at a decoupling frequency near 89 MHz.

RESULTS AND DISCUSSION

A) Spectral assignments

The 220 MHz spectra of 0.01 M Cp3'C^{11} at 20°, pD 7.4 and its deuterated counterpart Cp3'C^* are illustrated in Figure 1 and those of Cp2'C and Cp2'C^* , in Figure 2. Signal assignments follow directly by inspection and by comparison with the spectra of the constituent monomers. The H5 signals of $\text{Cp3}'$ - and $\text{Cp2}'$ - in the spectra of the selectively deuterated dimers appear broadened, presumably due to the presence of trace amounts of paramagnetic impurities. Since the remaining signals were not affected, no further purification of the deuterated dimers was attempted.

The H5', H5'' are assigned such that H5' is always gauche and H5'' trans to the ribose ring O1'. H5' of the -pX fragment is assigned to be at a

Figure 1. 220 MHz proton magnetic resonance spectra of (3'-5') CpC and (3'-5') CpC^* , .01 M, pD 7.4, 20°C.⁶

Figure 2. 220 MHz proton magnetic resonance spectra of (2'-5') CpC (2'-5') CpC*, .01 M, pD 7.4, 20°C.

lower field compared to H5'', whereas the opposite is assumed for the Xp- fragment, i.e., $\delta H5' < \delta H5''$.⁵ It is also assumed that no crossover of H5', H5'' signals occurs upon incorporation of monomers into dimers. A characteristic feature of Cp3'C, as in other pyrimidine (3'-5') pyrimidine dimers,⁶ is the significant magnetic nonequivalence of H5', H5'' for both Xp3'- and -3'pX fragments. These nonequivalences collapse when the (3'-5') phosphodiester backbone is replaced by a (2'-5') linkage (see Figures 1 and 2). Qualitatively, the ribose ring signals of Cp3'C appear to be uniformly distributed within a 0.35 ppm range, whereas the Cp2'C spectrum features a complete overlap of H2', H4', H5', H5'' of -2'pC and H4' of Cp2'-.

B) Chemical shifts and coupling constants

Initial sets of NMR parameters were obtained directly from the observed spectra and subsequently refined by simulations using a Varian 620i 6-spin simulation program. The final chemical shift and the spin-spin coupling constant data for Cp2'C and Cp3'C are summarized in Table 1. The effects of dimerization on chemical shifts ($\Delta\delta = \delta_{\text{monomer}} - \delta_{\text{dimer}}$) and coupling constants ($\Delta J = J_{\text{monomer}} - J_{\text{dimer}}$) are listed in Table 2. These data are utilized in the following sections in a comparative analysis of the conformational and stacking properties of the two dimers. The chemical shifts and coupling constants for the respective monomers, 2'-CMP, 3'-CMP, and 5'-CMP at pD 6.0 may be inferred from Tables 1 and 2.

TABLE 1: Proton magnetic resonance parameters of (2'-5') and (3'-5') CpC

δ^b	(2'-5')CpC		(3'-5')CpC ^a		J^c	(2'-5')CpC		(3'-5')CpC ^a	
	Cp2'-	-pC	Cp3'-	-pC		Cp2'-	-pC	Cp3'-	-pC
1'	6.002	5.858	5.782	5.882	1'2'	5.3	2.8	3.0	2.9
2'	4.749	4.167	4.461	4.213	2'3'	5.7	5.3	5.1	5.1
3'	4.440	4.240	4.453	4.300	3'4'	4.4	6.3	7.0	6.6
4'	4.119	4.137	4.327	4.247	4'5'	4.2	d	3.3	2.2
5'	3.837	4.13	3.868	4.343	4'5''	2.8	d	2.5	2.6
5''	3.892	4.12	4.004	4.121	2'P	9.1	-	-	-
					3'P	-	-	8.3	-
Δ^e	0.055	~.01	0.136	0.222	4'P	-	d	-	2.0
					5'P	-	3.6 ^f	-	4.0
					5''P	-	3.6 ^f	-	3.6
5	5.817	5.995	5.947	5.958	5'5''	-12.9	d	-13.0	-11.7
6	7.658	7.840	7.903	7.943	5'6	7.8	7.7	7.7	7.7

^aref. 6; ^b $\pm .005$ ppm; ^c ± 0.2 Hz; ^dcould not be determined due to near magnetic equivalence of H5', H5'' and overlap of five signals in this region;

^e $\Delta = \delta_{5'} - \delta_{5''}$; ^festimated from phosphorus decoupling

C) Conformation of the ribofuranose ring

Assuming that the ribose ring exists as an equilibrium mixture of two puckered modes, ²E and ³E, undergoing rapid interconversion,¹³⁻¹⁵ the population can be calculated from the $J_{1'2'}$ and $J_{3'4'}$ values in Table 1 to be 71% ³E and 69% ³E for Cp3'- and -3'pC in contrast to the 54% ²E and 69% ³E exhibited by Cp2'- and -2'pC. The puckering at the -pX moieties is almost identical while a significant difference occurs between the 2' and

TABLE 2: Dimerization effects on magnetic resonance parameters of (2'-5') and (3'-5') CpC

$\Delta\delta^b$	(2'-5')CpC		(3'-5')CpC ^a		ΔJ^c	(2'-5')CpC		(3'-5')CpC ^a	
	Cp2'-	-pC	Cp3'-	-pC		Cp2'-	-pC	Cp3'-	-pC
1'	-0.014	0.141	0.170	0.117	1'2'	0.1	1.3	1.2	1.2
2'	-0.025	0.154	-0.028	0.122	2'3'	-0.4	-0.3	0.2	-0.1
3'	-0.084	0.085	0.099	0.037	3'4'	0.5	-1.2	-1.4	-1.5
4'	0.006	0.133	-0.027	0.039	4'5'	0.4	-	0.8	0.2
5'	-0.024	0.07	-0.008	-0.145	4'5''	0.3	-	0.2	1.2
5''	-0.018	-0.03	-0.056	-0.028	2'P	-0.9	-	-	-
Δ^d	0	-0.10	0.048	0.117	3'P	-	-	0.2	-
					4'P	-	-	-	0
					5'P	-	0.6	-	0.2
					5''P	-	1.5	-	1.5
5	0.235	0.129	0.137	0.166	5'5''	-0.1	-	0.1	-0.1
6	0.131	0.202	-0.003	0.099	5 6	0	0	0	-0.1

^aref. 6; ^b $\Delta\delta = \delta_{\text{monomer}} - \delta_{\text{dimer}}$; ^c $\Delta J = J_{\text{monomer}} - J_{\text{dimer}}$;

^d $\Delta^d = (\delta_{5'} - \delta_{5''})_{\text{monomer}} - (\delta_{5'} - \delta_{5''})_{\text{dimer}}$

3' phosphate fragments. An analogous observation was inferred from incomplete coupling data for Ap3'C and Ap2'C by Ts'o and coworkers.⁹ Similarly, in the solid state both ribose rings of Ap3'U are in a ³E puckered mode, whereas Ap2'- exhibits ²E and -2'pU, ³E.¹⁶⁻¹⁸

Further examination of the ribose ring coupling constants in Table 1 shows that $J_{2'3'}$ is measurably larger in Cp2'- (5.7 Hz) compared to -2'pC (5.3 Hz) and Cp3'C (5.1 Hz). On the basis of the Altona-Sundaralingam pseudorotational approach¹⁴ these coupling differences are indicative of a smaller amplitude of puckering in the Cp2'- residue as compared with the remaining fragments. In striking contrast, puckering amplitudes were computed to be identical for a series of fifteen (3'-5') dimers.^{5,6} Moreover, no change in amplitude occurred in the transition from monomer $\rightarrow$ dimer.

Previous studies have demonstrated an interdependence among ribose ring conformation and stacking.^{5,6} Since Cp2'C and Cp3'C exhibit comparable stacking tendencies (*vide infra*), one would expect (a) the Cp2'- and -2'pC ribose rings to be similar in conformation to those of Cp3'- and -3'pC, respectively, (b) all rings to exist in a predominantly ³E mode, and (c) identical variation in the conformation of the monomer ribose rings toward a ³E pucker upon dimerization. The 1.3 Hz decrease in $J_{1'2'}$ of 3'-CMP and

5'-CMP, with a concomitant increase in $J_{3'4'}$ (1.3 Hz) and no significant change in $J_{2'3'}$ when incorporated into Cp2'C and Cp3'C (J values in Table 2), is consistent with a 14% shift of the ribose ring equilibrium towards 3E . On the other hand, there is no significant change of the 54% 2E population of 2'-CMP on dimerization to Cp2'C. However, dimerization is accompanied by a 0.6 Hz decrease in the sum $J_{1'2'} + J_{3'4'}$ and a 0.4 Hz increase in $J_{2'3'}$; both reflect a flattening of the 2'-CMP ribose ring upon dimerization. It is plausible that the phosphate linked at C2' in Cp2'C counteracts the effect of stacking on the ${}^2E \rightleftharpoons {}^3E$ equilibrium; the 2' phosphate linkage increases the 2E population, whereas stacking causes an increase in 3E . The net result is a decrease in the puckering amplitude.

D) Relative orientation between base and ribose rings

The sugar-base torsion angle of (3'-5') dimers in the solid state almost exclusively occupies the anti domain.¹⁸⁻²¹ Similarly, Ap2'U exhibits an anti orientation in both nucleotidyl residues with χ_1 (55°) > χ_2 (5°).^{17,22} It is assumed that in aqueous solution monomers and dimers exist as a mixture of anti and syn conformers; however, on the basis of Mn^{++} ion binding,²³ pH effects,²⁴ ${}^{13}C$ - 1H coupling,²⁵ and four bond 1H - 1H coupling,²⁶ the anti conformer is predominant, whereas the results from NOE²⁷ and spin-lattice relaxation (T_1) studies²⁸ have been interpreted in terms of a distinct preference for the syn conformer. In the absence of evidence suggesting otherwise, and in line with the X-ray crystallographic data, it is assumed that the anti orientation is prevalent in both Cp3'C and Cp2'C, and no attempt is made at the present to determine this angle.

As shown elsewhere,⁶ incorporation of pyrimidine monomers into dimers causes a decrease in the glycosyl torsion angle, the origin of the perturbation in χ_{CN} being the base-base interaction. The temperature dependence study of Cp3'C shows that H6 moves upfield while H5 moves downfield with temperature. A similar trend has been observed in Cp2'C.²⁹ Such results, as proposed by Prestegard and Chan,³⁰ are consistent with a destacking of the bases and an increase in χ_{CN} approaching the values for the constituent monomers with increasing temperature.

E) C4'-C5' (ψ_1, ψ_2) and C5'- ϕ_5' (ϕ_2)²²

The populations of gg ($\psi_1, \psi_2 = 60^\circ$) and g'g' ($\phi_2 = 180^\circ$) were calculated using expressions developed earlier.³¹ Both dimers show overwhelming preference for gg and g'g', ranging from 70-90% with no significant difference between Cp2'C and Cp3'C. This result is in agreement with single crystal structure data. Although the conformation about C4'-C5' (ψ_2) of

-2'pC cannot be determined since the appropriate coupling constants are not extractable from the 220 MHz spectra (see Table 1), it is reasonable to assume that the gg population is also prevalent about this bond. This conclusion is based on the earlier work on (3'-5') dinucleoside monophosphates and the structure of Ap2'U in the solid state.¹⁷ Furthermore, the existence of a direct correlation between the conformation about C4'-C5' and C5'-O5' of nucleotides has been demonstrated such that a high g'g' population (C5'-O5') is always associated with a high gg population about C4'-C5'.³²

A survey of the J values in Table 2 shows that there is a decrease of 1.0 Hz in the sum $J_{4'5'} + J_{4'5''}$ and 1.9 Hz in $J_{5'p} + J_{5''p}$. Thus, dimerization causes an increase of approximately 10% in gg and g'g' populations manifesting a loss in flexibility about the C4'-C5' and C5'-O5' bonds.

F) C3'-O3' and C2'-O2' (ϕ_1')

As has been proposed recently,⁵⁻⁷ the most likely conformational status about C3'-O3' and C2'-O2' bonds in monomers and dimers is a mixture of g^+ ($\phi_1' = 300^\circ$, P-O3' (or P-O2') trans to C3'-C4' (C2'-C3')) and g^- ($\phi_1' = 180^\circ$, P-O3' (P-O2') trans to C2'-C3' (C1'-C2')) forms. Evidence has also been presented for the existence of a coupling between ribose ring conformation and the $g^+ \rightleftharpoons g^-$ equilibrium; namely, the 3E pucker is associated with g^- and 2E with a g^+ orientation.⁶ On this basis, the conformation about C3'-O3' in Cp3'C would be predicted to be in a predominantly g^- orientation. In Cp2'C, the Cp2'- ribose ring consists of an approximately 50-50 mixture of 2E and 3E , and, therefore, the ratio of g^+ to g^- is expected to approach unity. Although it is difficult to estimate the contributions from g^+ and g^- to the overall dimer conformation, one can determine the H3'-C3'-O3'-P and H2'-C2'-O2'-P dihedral angles from the Karplus relation, $J_{HP} = 18.1 \cos^2\theta - 4.8 \cos\theta$, and the J_{HP} coupling constants in Table 1. These angles are computed to be $\pm 35^\circ$ ($\phi_1' = 275^\circ$ or 205°) and $\pm 31^\circ$ ($\phi_1' = 271^\circ$ or 209°) in Cp3'- and Cp2'-, respectively. It should be noted (Table 2) that while no change in $J_{H3'-P}$ of 3'-CMP (8.3 Hz) occurs upon dimerization to Cp3'C, there is a significant increase from 8.3 Hz to 9.2 Hz in $J_{H2'-P}$ of 2'-CMP compared to Cp2'C. The latter is interpreted in terms of a $\sim 4^\circ$ decrease in dihedral angle. Therefore, in addition to any perturbation of the $g^+ \rightleftharpoons g^-$ equilibrium that may occur, there is a noticeable change in the magnitude of ϕ_1' in 2'-CMP upon dimer formation.

G) P-O3' or P-O2' (ω_1') and P-O5 (ω_1)

Single crystal structural studies and theoretical calculations for dinucleoside monophosphates have demonstrated the existence of several

minimum energy conformations arising solely from a variation of the ω_1'/ω_1 phosphodiester backbone dihedral angles.³³⁻³⁵ These conformations include the $\underline{g}^-\underline{g}^-$ right hand stack ($\omega_1'/\omega_1 \sim 270^\circ$), the $\underline{g}^+\underline{g}^+$ left hand stack ($\omega_1'/\omega_1 \sim 90^\circ$), and several extended orientations of the backbone. The degree of base overlap is significantly greater in $\underline{g}^-\underline{g}^-$ than in $\underline{g}^+\underline{g}^+$. In the solid state the ω_1'/ω_1 angles of Ap2'U and Ap3'U are in a $\underline{g}^-\underline{g}^-$ orientation so that in both cases the bases are stacked in a right-handed fashion. In recent NMR studies of (3'-5') dimers, the chemical shift trends of the 5',5'' protons were utilized to distinguish between the two stacked forms. It was argued that in the $\underline{g}^-\underline{g}^-$ stack H5' of the 5' phosphate fragment is exposed to a deshielding by O2' of the 3' fragment. Therefore, selective downfield shifts of H5' compared to H5'' upon incorporation of a 5' monomer into a dimer are indicative of the presence of a $\underline{g}^-\underline{g}^-$ stack. The H5',H5'' chemical shifts of the 3' fragment, on the other hand, are only influenced in the $\underline{g}^+\underline{g}^+$ orientation by the O2' and O3' of the adjacent ribose ring and by the neighboring base ring anisotropy. In pyrimidine-pyrimidine dimers, the latter effect is small;³⁶ therefore, these dimers, when in a $\underline{g}^+\underline{g}^+$ conformation, show a deshielding of H5' and H5'' with the downfield shift of H5'' being significantly greater because of its closer proximity to O2' and O3'.

The basic conclusion was that the (3'-5') dimers exist as a mixture of the two stacked and extended forms. As will be shown in a later section, Cp2'C also exists as a mixture of stacked and unstacked species. The discussion below is limited to providing evidence for the presence of the two stacked forms, and no attempt will be made to estimate the composition of the stacked species in the two dimers.

In Cp3'C, the -3'pC H5' is deshielded by 0.15 ppm and H5'' by 0.03 ppm (Table 2). As discussed by Kondo and Danyluk,⁷ a small deshielding, in this case $\sim .03$ ppm and comparable for both protons, is expected due to a greater deshielding effect of the phosphate group in a locked-in conformation about ω_1'/ω_1 . The additional 0.12 ppm deshielding of H5', leading to an increased chemical shift difference between H5' and H5'', is a consequence of the close proximity of Cp3'- O2' in the $\underline{g}^-\underline{g}^-$ right hand stack orientation of the backbone. The Cp3'- H5'' is also found to be deshielded by .06 ppm, and examination of models demonstrates that this is possible only in a $\underline{g}^+\underline{g}^+$ stack since H5'' is relatively close to O2' and O3' of -3'pC. These trends can be observed in Figures 3(a) and 3(b) which show Cp3'C in the $\underline{g}^-\underline{g}^-$ and $\underline{g}^+\underline{g}^+$ stack, respectively.

Figure 3. Perspective drawing: (a) (3'-5')CpC in g^-g^- conformation
 (b) (3'-5')CpC in g^+g^+ conformation
 (c) (2'-5')CpC in g^-g^- conformation
 (d) (2'-5')CpC in g^+g^+ conformation

Cp2'C is shown in Figures 3(c) and 3(d) in the g^-g^- and g^+g^+ conformations. Comparison of 3(a) and 3(c) allows one to predict any perturbations in the chemical shifts that might occur in going from a 3'-5' g^-g^- to a 2'-5' g^-g^- linkage. All dihedral angles are similar in both figures, the only exception being a 3E pucker in Cp3'- (3a) and a 2E pucker in Cp2'- (3c). One would expect to observe the following trends in chemical shifts:

1. larger positive dimerization shifts of the base protons in Cp2'C due to the greater degree of base-base overlap;
2. larger upfield shifts of the ribose ring protons (H1', H2', H3', and H4') in -2'pC compared to the -3'pC due to the closer approach of the neighboring base in the former;
3. smaller dimerization shifts of the ribose ring protons in Cp2'- compared to Cp3'- due to the increased distance of the neighboring base from the Cp2'- ring;
4. no deshielding of the exocyclic H5' in -2'pC since there is no close approach of this proton to oxygens of the neighboring

ribose ring as in -3'pC. In fact, H5' is expected to be shielded in -2'pC because it comes closer to the cytosine base of the 3' phosphate fragment, and;

5. no effects on the chemical shift of H5'' from -2'pC, except for a small deshielding due to the locked phosphate group. This would then lead to a collapse of the H5', H5'' magnetic non-equivalence at the 5' phosphate terminal compared to 5'-CMP or Cp3'C.

A survey of the chemical and dimerization shifts in Tables 1 and 2 demonstrates that all of the trends discussed above are indeed observed. Thus, (a) $\Delta\delta_{\text{base}}$ in Cp2'C (0.13-0.24 ppm) > $\Delta\delta_{\text{base}}$ of Cp3'C (0.0-0.17 ppm), (b) $\Delta\delta_{\text{ribose -2'pC}}$ (0.09-0.15 ppm) > $\Delta\delta_{\text{ribose -3'pC}}$ (0.04-0.12 ppm), (c) $\Delta\delta_{\text{ribose Cp2'-}}$ (-0.08 to .006 ppm) < $\Delta\delta_{\text{ribose Cp3'-}}$ (-0.03 to 0.17 ppm) (d) H5' of -2'pC is shielded by 0.07 ppm while -3'pC H5' is deshielded by 0.15 ppm, (e) $\Delta\delta_{\text{H5'' -2'pC}} \sim \Delta\delta_{\text{H5'' -3'pC}}$ (-.03 ppm), and (f) the chemical shift difference of the -2'pC compared to the monomer (Δ' , see Table 2) decreases by .10 ppm whereas in -3'pC it increases by 0.12 ppm. The evidence is overwhelmingly in favor of the presence of the $\underline{g^-g^-}$ right-hand stacked conformation in Cp2'C.

It is emphasized that none of the above chemical shift trends would be observed if Cp2'C was exclusively in a $\underline{g^+g^+}$ orientation (see Figures 3(b) (Cp3'C in $\underline{g^+g^+}$) and 3(d) (Cp2'C in $\underline{g^+g^+}$)); however, one would expect a downfield shift of H3' in Cp2'- compared to 2'-CMP since it becomes exposed to a deshielding effect of O3' from -2'pC. This is characteristic for the $\underline{g^+g^+}$ orientation only. In fact, H3' of Cp2'- is deshielded by .08 ppm (Table 2). Furthermore, as expected, the deshielding of H5'' of Cp2'- (.02 ppm) is not as pronounced as in Cp3'- (.06 ppm). Although H5'' may be deshielded by O2' in Cp2'C, the deshielding is balanced by an increased shielding from the neighboring base of -2'pC. The $\underline{g^+g^+}$ stacked orientation must therefore contribute significantly to the overall conformation of the dimer.

Interestingly, the existence of a mixture of $\underline{g^-g^-}$ and $\underline{g^+g^+}$ can account for the trend in the ribose ring dimerization shifts of -2'pC, i.e., $\Delta\delta\text{H1}' \sim \Delta\delta\text{H2}' \sim \Delta\delta\text{H4}'$ (0.13-0.15 ppm) > $\Delta\delta\text{H3}'$ (0.085 ppm) in Table 2. In a $\underline{g^-g^-}$ orientation, all four protons are expected to be shielded by the cytosine base of the 2'-phosphate moiety (Figure 3(c)), but in a $\underline{g^+g^+}$ orientation (Figure 3(d)) only H3' will be deshielded because of its close proximity to O3' of Cp2'-. Apparently, the effect of the base on H3' is greater than

that of O3'. Thus, the net result is an upfield shift of H3' which is significantly less than for H1', H2', and H4'.

H) Base-base interactions

Based on upfield chemical shifts of base and anomeric protons, it has been argued that dinucleoside monophosphates exist in solution as an equilibrium mixture of stacked and extended conformers,^{9,37} however, more recent studies^{5,6} have demonstrated that upfield shifts alone cannot be used to monitor stacking. Observed differences in upfield shifts of base protons may be a consequence of either a difference in the extent of stacking or in the degree of overlap of bases.³⁸ Since the ribose ring conformation is not likely to be affected by the latter possibility, it provides a more reliable index for stacking than chemical shifts. A change in the ribose ring equilibrium towards an ³E pucker is indicative of bases in a stacked configuration.⁵

In Table 2, the base protons of both dimers are shifted upfield compared to the monomers ($\Delta\delta = 0-0.24$ ppm) with the $\Delta\delta_{\text{base}}$ Cp2'C (0.13-0.24 ppm) on the average greater than the upfield shifts of the base proton signals in Cp3'C (0-0.17 ppm). On the other hand, the -2'pC and -3'pC ribose ring coupling constants are almost identical. The ring structures and the extent of base-base interaction must therefore be comparable in these two dimers. Using procedures developed earlier, the amount of stacked species is computed to be 35% in Cp2'C and Cp3'C. Hence, the greater upfield shifts of base protons in Cp2'C must be due to the greater degree of overlap of the two bases when the dimer is in a $\underline{g}^-\underline{g}^-$ (ω_1'/ω_1) stacked orientation (Figure 3(c)). The Cp2'- and Cp3'- ribose rings are excluded from the above computations, since these rings are also effected by the phosphate group linked at the C2' or C3' (vide supra), so they cannot be used in comparison of dimers with different phosphodiester linkages, i.e., (2'-5') vs (3'-5').

SUMMARY

Proton magnetic resonance evidence has been presented which indicates that (1) the position of the phosphate group and base-base stacking can profoundly alter the structure of the sugar rings in dimers, (2) the phosphodiester linkage has little or no effect on the extent of base-base stacking as proposed by Ts'o and coworkers,⁹⁻¹⁰ and (3) (2'-5') and (3'-5') dimers exist in solution as an equilibrium mixture of the $\underline{g}^-\underline{g}^-$, $\underline{g}^+\underline{g}^+$, and several extended orientations of the phosphodiester backbone. Although the percent stacked species ($\underline{g}^+\underline{g}^+ + \underline{g}^-\underline{g}^-$) is the same in the two types of

dimers, i.e., 35% for Cp2'C and Cp3'C, it is likely that the ratio g^+g^+/g^-g^- differs, and this would account for CD results reported by Brahms and coworkers;⁸ however, the contribution of each of the two conformers and therefore any changes in the g^+g^+/g^-g^- ratio cannot be estimated at the present.

ACKNOWLEDGMENT

The authors are indebted to Drs. H. L. Crespi and J. J. Katz of the Chemistry Division, Argonne National Laboratory, for supplying fully deuterated algae. This work was supported by the U.S. Energy Research and Development Administration.

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- 11 For convenience, the following shorthand notation is used:
Cp3'C = (3'-5')CpC; Cp2'C = (2'-5')CpC;
Cp3'- = 3' phosphate fragment of Cp3'C;
-3'pC = 5' phosphate fragment of Cp3'C;
Cp2'- = 2' phosphate fragment of Cp2'C;
-2'pC = 5' phosphate fragment of Cp2'C
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