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CONFORMATIONAL CHARACTERISTICS OF THE DINUCLEOSIDE TRIPHOSPHATE pCpGp FROM ENERGY-MINIMIZATION STUDIES

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The influence of the 3′- and 5′- terminal phosphates on the conformational characteristics of the dinucleoside monophosphate CpG is described in this paper. The computed potential energy of the system is minimized with respect to the relevant 10 dihedral angles permitting the two sugar rings to adopt the alternative puckering states, 2_E and 3_E. Of the 84 conformations considered, 22 become energetically accessible. The familiar A-, B-, Z- and Watson-Crick-type backbone states of DNA subunits become low-energy forms for this RNA unit pCpGp also. The Watson-Crick-type backbone is invariably preferred in all the four sugar pucker sequences, indicating its importance in the dynamics of sugar pucker fluctuations and in the DNA-RNA association. The interphosphate geometries and the possible hydrogen-bonded states are discussed in relation to the varied folded/extended polynucleotide structures.

1. Introduction

Suggestions on the possibility of alternative structures of DNA [1–6] have given a new thrust to the research activity on the conformational characteristics of small and specific nucleotides. The proposed new structures incorporate novel local conformations of the subunits, hitherto neglected or underestimated. The existence of different kinds of conformational (A, B, C and Z) states in the polynucleotide strands, their interconversion, the types of helical twist and interstrand alignments, and finally, the overall folding have all become problems of new investigations, both in theoretical and experimental fields. In a few recent articles [7–11], we have reported our systematic theoretical studies on a number of monomeric and dimeric subunits of DNA and RNA, and a trinucleoside diphosphate unit, d(ApApA). A dinucleoside triphosphate, pCpGp, is studied in the present report. The special feature of this unit is the existence of two terminal phosphate groups enclosing a dimeric segment; the dimeric unit has the bases cytosine and guanine and this pair of bases is under extensive study under different conditions [12–14]. The conformational characteristics of the monophosphate form CpG were studied previously by us [15]. The present study on the triphosphate pCpGp brings out many salient features, specifically, the influence of the interactions of the terminal phosphates separated by a dinucleoside monosphosphate segment.

2. Methods

Fig. 1 depicts the skeleton of pCpGp with its variable dihedral angles. The potential energy of the system was considered as $E_{total} = E_{nb} + E_{es} + E_{hb} + E_{tor}$, where the subscripts nb, es, hb and tor represent, respectively, nonbonded, electrostatic, hydrogen bonding and torsional; these terms of the total energy were computed using expressions and parameters reported in our previous studies [7,8]. In the present study two additional tests were

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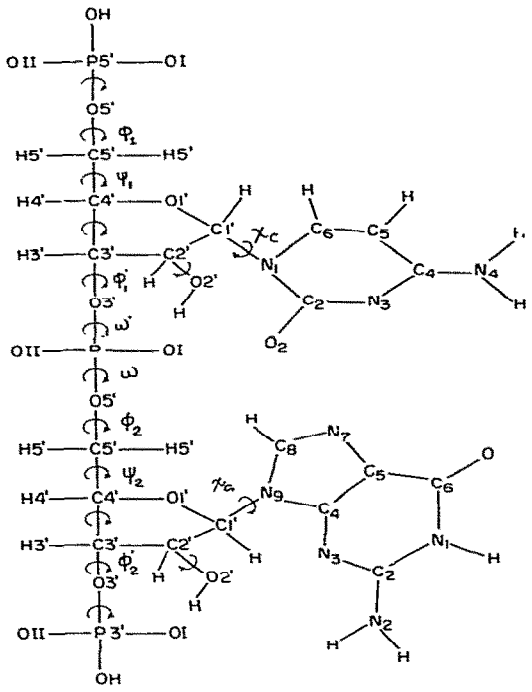

Fig. 1. The skeleton, numbering convention and variable dihedral angles for the dinucleoside triphosphate unit, pCpGp.

made, one on the value of the dielectric constant D, and the other on the type of torsional potential around the P–O bonds. Computation of electrostatic energy becomes a critical problem for systems like nucleotides, where there are highly electronegative phosphate groups. In the absence of proper counterions in a study, the only factor that has to be relied upon is the dielectric constant, whose value should be judiciously selected so as to take into account the Coulombic and Van der Waals' contributions in the correct proportion. In our theoretical studies on some modified mononucleoside diphosphate units, the interactions of the electro-negative phosphates and the neutral base drive the conformation about the $C_{3'}$–$C_{5'}$ bond predominantly toward the *trans* region [16], and this problem will be much more serious in units with three phosphate groups. To reduce this dominant preference of ψ = *trans* state, we used a value of 4 for the dielectric constant in the above-quoted work. In the present study, some trial calculations were made on pCpGp with D values of 2, 4, 6 and 8. Again, we observe that a value $D = 4$ is more suitable for dealing with the electrostatic interactions.

In computing the term E_{tor}, a three-fold potential with a barrier $V_\theta = 3$ kcal/mol for C–O bonds, 3.5 kcal/mol for C–C bonds, and 1.5 kcal/mol for P–O bonds was used in our previous studies. While this treatment of C–O and C–C bond torsions was satisfactory, that of the P–O bond torsion was questioned by a reference on the grounds that a three-fold barrier will not reflect the local property properly. Therefore, we resorted to the two-term torsional function suggested by Govil [17], viz., $E_{tor}(\text{P–O}) = (V_\theta/2)(1 + \cos 3\theta) + (V'_\theta/2)(1 + \cos 2\theta)$ and took $V_\theta = V'_\theta = 1.5$ kcal/mol in the present study acording to this author. The second term in the above expression takes into account the influence of the lone-pair electrons of the P–O ester oxygens (for further details, see ref. 17). The geometrical parameters were taken from ref. 18, and the atomic partial charges from ref. 19. Following Olson [20], a term $V_1 = -166\,(q_i\alpha_j^2 + q_j\alpha_i^2)$ was included in the total energy toward the charge-induced dipole interactions between the base atoms which takes into account the base stacking feature in addition to the usual Van der Waals' and electrostatic interactions.

The technique of energy minimization was employed in the present study as was also done in our previous studies. In any function minimization study, such as the Fletcher-Powell-Davidon procedure [21], presently used, one can never be satisfied unless a good number of starting points are tried and for that matter, a system like the dinucleoside triphosphate, pCpGp, with many variables, will consume hours of computation time even in a very fast computer system. Broyde and Hingerty [22] thus restricted their study on d(pApAp) by considering only the minimum-energy conformations of d(ApA) as probable starting points. Being aware of the consequence of such restricted studies, we selected starting conformations for pCpGp in a more rigorous way: the unit has 16 potential variable dihedral angles, when the

Table 1

Probable low-energy conformations for pCpGp

State No.	Dihedral angles (°)										Relative energy (kcal/mol)	Conformational state ($\omega'\omega\psi$)
	ϕ_1	ψ_1	χ_C	ϕ_1'	ω'	ω	ϕ_2	ψ_2	χ_G	ϕ_2'		
3_E-3_E												
1	−179	49	21	−179	160	71	−178	−176	−5	−172	1.9	tg^+t
2	156	44	19	−165	−70	163	−167	175	−6	−171	2.2	g^-tt (WC)
3	175	58	11	178	57	179	−179	−175	−1	−172	2.6	g^+tt
4	177	25	33	−108	−50	−67	−170	56	48	−100	3.1	$g^-g^-g^+$ (A-RNA)
5	175	59	31	−179	177	−73	168	60	2	−173	5.1	tg^-g^+ (alt-C-DNA)
6	−177	63	13	−172	−167	−66	179	−178	3	−178	7.7	tg^-t
7	156	55	14	−170	−171	72	171	73	−3	−176	9.1	tg^+g^+
3_E-2_E												
8	−176	−57	27	−152	75	87	180	−62	24	−141	0.0	$g^+g^+g^-$
9	−176	52	25	−179	120	−84	−179	−46	22	−104	0.8	$g^+g^-g^-$
10	−178	44	30	−141	−47	173	180	180	17	−125	2.4	g^-tt (alt-WC)
11	−150	−65	31	−118	52	−134	173	173	8	−144	6.1	g^+t_st
12	−167	36	29	−140	−39	138	−158	−61	−17	−141	6.2	$g^-t_sg^-$
13	171	53	13	−169	171	73	−166	180	14	124	7.8	tg^+t
2_E-3_E												
14	176	66	31	−144	76	70	177	−176	−3	−171	0.3	g^+g^+t (Z)
15	178	72	25	−103	177	76	−174	−179	−6	−176	3.3	tg^+t
16	173	78	16	−169	−101	−69	179	177	0	−163	3.4	$g_s^-g^-t$
17	179	69	28	−116	−82	−178	−174	−176	2	−175	3.5	g^-tt (alt-WC)
18	177	67	32	−102	70	160	180	175	−5	−170	5.6	g^+tt
19	−178	69	28	−104	174	−70	159	65	−1	−179	7.4	tg^-g^+ (alt-C-DNA)
20	−172	72	22	−119	−77	−175	−176	−177	7	−128	3.7	g^-tt (alt-WC)
21	−177	69	27	−121	−170	68	−159	173	12	−122	7.3	tg^+t
22	−176	70	29	−139	84	82	−167	74	22	−110	7.8	$g^+g^+g^+$

two terminal phosphate hydroxyl oxygens are treated as united-atom entities (fig. 1). The end phosphate groups were kept in staggered states around the O–P bonds such that the P–OH bond is *trans* to the O–C bond, thus eliminating two variables. Similarly, in the sugar rings the 2′-hydroxyl bond was kept in the *gauche*$^-$ position (in accordance with crystal-state observation) to the $C_{2'}$–$C_{3'}$ bond, again eliminating two variables. The sugar ring was treated as a fixed system in the 3_E or 2_E state, thus eliminating the two ring ψ' angles from the variables' set. Thus, the remaining ten variables ϕ_1, ψ_1, χ_C, ϕ_1', ω_1', ω_2, ϕ_2, ψ_2, χ_G and ϕ_2' alone were considered, taking the following values as the best starting locations for them: χ_C, $\chi_G = 40°$; ϕ_1', $\phi_2' = -130°$; ϕ_1, $\phi_2 = 180°$; ψ_1, ω, $\omega' = 60°$, $180°$, $-60°$; and $\psi_2 = 60°$. The possible combinations of these values yield 27 ($1 \times 1 \times 1 \times 1 \times 3 \times 3 \times 3 \times 1 \times 1 \times 1$) conformations for a specific sugar pucker sequence. For the four sugar pucker sequences, 3_E-3_E, 3_E-2_E, 2_E-3_E and 2_E-2_E, 108 conformations are thus possible, of which we excluded the 24 conformations with $(\omega', \omega) = (g^+, g^-)$ as these are not observed in any of the experimental studies reported hitherto. The remaining 84 conformations were considered as starting points for the minimization study. For each of the starting points, the computed total energy was minimized by allowing the ten dihedral angles to vary simultaneously so as to meet the condition that the difference in energies of two consecutive interations is less than or equal to 0.05 kcal/mol, and the gradient for each variable is less than 0.5. To standardise the energy values of the two different sugar pucker states, the internal energies of sugar rings were computed and added to the final minimized energy values as detailed in the article of Ponnuswamy and Thiyagarajan [23]. All the hydrogens, except the terminal phosphate hydroxyl ones, were considered explicitly.

3. Results

Out of the 84 starting conformational states considered, only 22 states moved toward an energy difference of $\Delta E = 10$ kcal/mol with respect to the lowest energy located. These 22 probable conformational states are listed in table 1. The conformation of the lowest energy (global minimum) is in the 3_E-2_E sugar pucker domain. A unique feature of this global minimum-energy conformation is the adoption of the g^- region by both the 3′-side and 5′-side ψ angles, keeping the three phosphorus atoms at more or less equal distances. This conformation produces a sharp turn in the backbone as shown in fig. 2. Apart from this global minimum state, the 3_E-2_E sugar pucker domain contains five other low-energy conformations. Characteristically, this sugar pucker domain permits the $\psi = g^-$ orientation by the 5′-terminal phosphate in three conformations and this ψ orientation is not noted in any of the low-energy conformations associated with the other three kinds of sugar pucker domains. Of the six low-energy conformations in the 3_E-2_E domain, the one with backbone dihedral angles very close to those of the Watson-Crick structure (which we designate as alt-WC DNA) is of particular interest. This type of backbone introduces good base stacking (see table 2).

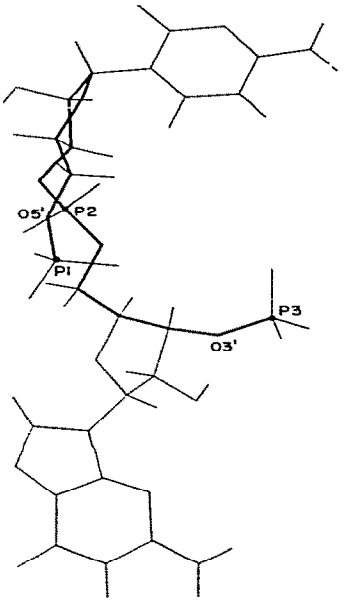

Fig. 2. The global energy minimum conformation predicted for pCpGp ($g^+g^+g^-$, 3_E-2_E). This conformation (No. 8 in table 1) could produce a sharp turn in the backbone.

Table 2

Base stacking geometry predicted for the RNA subunit pCpGp

Sugar pucker	Conformational state	Base stacking energy (kcal/mol)	Mean distance between base planes (Å)	Angle between base planes (°)	Overlapping area (%) [a]
3_E-3_E	A-RNA	−1.913	3.9	35	35
	WC	−2.960	3.3	10	94
3_E-2_E	alt-WC	−1.766	4.0	31	65
2_E-2_E	tg^+t	−0.870	4.8	32	12

[a] Overlapping is 100% when the base with smaller area is completely overlapped by the base of larger area.

The 3_E-3_E sugar pucker domain contains seven low-energy conformations, of which the Watson-Crick A-RNA and alt-C-DNA type (C-DNA has a 2_E-2_E sequence) structures are noteworthy. The best preferred conformation of this domain has $(\omega', \omega, \psi) = (t, g^+, t)$. Such a backbone course is favored in the helical structures formed with mixed sugar puckers (A + B genus) [24]. This conformation is shown in fig. 3. The Watson-Crick type and the A-RNA conformers exhibit good base stacking (table 2), whereas the other conformations do not show any base stacking character.

In the 2_E-3_E sugar pucker domain, we note six low-energy conformations of which the Z-DNA type structure is the best one (fig. 4). The Z-DNA type conformer does not show any base stacking property [3,5]. Interestingly, the other five conformations in this domain are less stable by more than 3 kcal/mol with respect to the global energy minimum state. The tg^-g^+ conformer in this do-

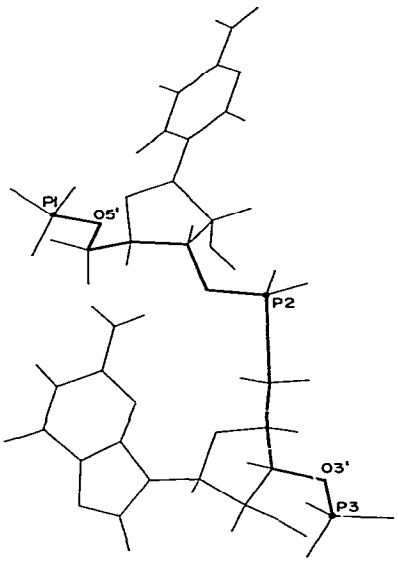

Fig. 3. The lowest energy conformation (tg^+t) predicted for pCpGp in 3_E-2_E sugar pucker domain (No. 1 in table 1).

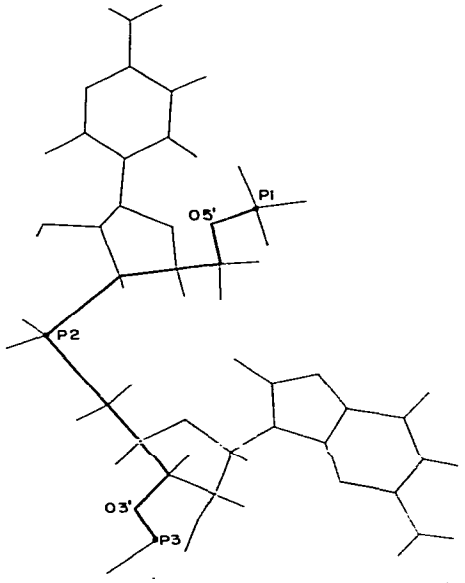

Fig. 4. The lowest energy conformation (g^+g^+t) predicted for pCpGp in 2_E-3_E domain. This type of backbone is preferred in Z-helical structures.

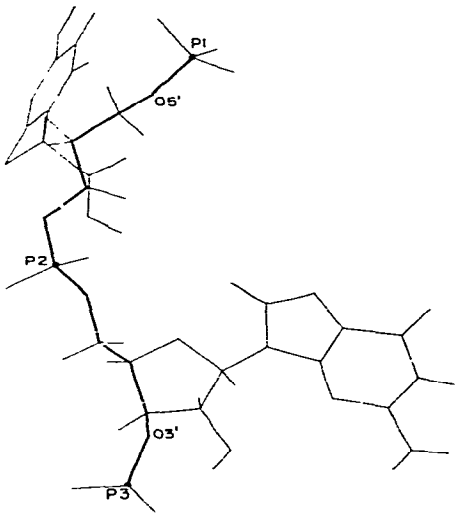

Fig. 5. The alt-WC ($g^- tt$) conformation predicted to be the best preferred in the 2_E-2_E domain.

main is the one observed in the crystal structure of dpApTpApT [25].

In the 2_E-2_E domain, three conformations come within the 10 kcal/mol energy limit, of which two have energies $\Delta E > 7$ kcal/mol. An observation in this sugar pucker domain is the occurrence of the conformation with $(\omega', \omega, \psi) = (g^+, g^+, g^+)$, which could produce a sharp left-handed turn in the backbone such as that in Z-DNA. The tg^+t conformation alone in the 2_E-2_E domain exhibits a reasonable base stacking property (table 2). The lowest energy conformation in this domain is depicted in fig. 5.

'. Discussion

1. Preferred backbone states

The Watson-Crick-type backbone happens to one of the best low-energy states invariably in the four sugar pucker domains. In our earlier ies on dimeric subunits, we frequently ob- ed that this backbone state is energetically referred over the A-RNA backbone state. The Watson-Crick backbone again intrinsically exhibits good base stacking (except for the 2_E-2_E state) and hydrogen-bond-promoting character. Hence, the Watson-Crick-type backbone state could be a stable form for RNA polymers do well. It is noted that the A-helical backbone structure facilitates RNA-DNA interaction [26]. Recently, in an attempt to redefine polynucleotide helical structures, Chandrasekaran et al. [27] indicated the Watson-Crick structure as a possible conformational state for the DNA-RNA hybrid poly(dI)·poly(C). This observation from X-ray fiber diffraction studies and our present result from energy studies suggest that the presence of a Watson-Crick-type backbone in the strands will also facilitate RNA-DNA interaction. The Z-DNA-type conformation (fig. 4) for pCpGp is the second most preferred conformation. Our previous studies [15] on RNA dimeric units also predicted this conformation as one of the lowest energy cases for purine-purine and pyrimidine-purine systems. These results predict that a backbone like the Z-type is also a plausible conformation for RNA polymers.

4.2. Preferred conformational domains

In our study on modified mononucleoside diphosphates, we demonstrated that the addition of a 3'-terminal phosphate often drives the ψ angle toward the *trans* region [16]. The results of pCpGp also emphasise this fact: out of the 22 low-energy conformations, 14 have $\psi = trans$ orientation. Broyde and Hingerty [22] arrived at a similar conclusion in their study on d(pApAp). The preferred values of ϕ' indicate that this angle varies from 170 to 260° and, irrespective of the type of base present, it usually assumes values greater than 210° in 2_E sugars, and values less than 210° in 3_E sugars. This specificity is exhibited by both the 3'-terminal and the in-chain ϕ' angles. However, ψ and ϕ do not show any such dependence on the mode of sugar pucker.

4.3. Sugar pucker effect

In our studies on dimeric subunits of RNA, we noted that the sugar pucker 2_E-3_E was not a

favorable state and no conformation was predicted in this domain within an energy limit of 5 kcal/mol over the lowest energy state located. Interestingly, the present results on pCpGp indicate that the addition of the 5′-terminal phosphate brings in the 2_E pucker at 3′-ribose as a favorable case. The 22 low-energy conformations for pCpGp are distributed in the four sugar pucker domains, respectively, seven in 3_E-3_E, six each in 3_E-2_E and 2_E-3_E, and three in 2_E-2_E. The energies of the conformations indicate that 2_E-2_E is the least preferred domain. In the 3_E-2_E domain, three out of the six low-energy conformations have the $\psi = g^-$ orientation and such a preference of ψ is observed in the 3_E-2_E segments of the crystal structures of tRNA-Phe molecules [28–31]. For a similar backbone course, the 3_E-2_E sugar state sometimes brings the phosphates closer when compared to the 3_E-3_E state and consequently forced ψ to move toward the g^- region. Another interesting point to be noted is the base-base gap in the alt-WC conformer. In this conformation a wider vertical gap is introduced between the bases due to the flipping of the 3′-sugar from the 3_E to the 2_E state (normal WC to alt-WC) without large alterations in ϕ' and ϕ orientations; this gap provides enough room for heavy drugs of complex shapes to intercalate between the bases.

Table 3

Interphosphate geometries of the low-energy conformations

Conformational state [a]	Sugar pucker	Distance (Å)			Angle (°) (P_1-P_2-P_3)
		P_1-P_2	P_2-P_3	P_1-P_3	
tg^+t	3_E-3_E	6.21	7.17	12.18	139.2
	3_E-2_E	6.07	7.31	12.19	139.0
	2_E-3_E	6.95	7.20	13.49	157.8
	2_E-2_E	7.01	7.41	13.47	149.4
g^-tt (WC-DNA)	3_E-3_E	6.04	7.22	12.30	145.9
	3_E-2_E	5.23	7.33	10.80	120.3
	2_E-3_E	6.97	7.20	13.18	147.3
	2_E-2_E	7.05	7.42	12.90	132.0
g^+tt	3_E-3_E	6.44	7.18	10.72	104.1
	3_E-2_E	4.73	7.47	7.37	69.2
	2_E-3_E	6.90	7.21	11.20	105.7
tg^-g^+ (C-DNA)	3_E-3_E	6.35	6.45	11.42	132.4
	2_E-3_E	6.94	6.54	13.20	170.4
$g^-g^-g^+$ (A-RNA)	3_E-3_E	6.32	6.73	12.78	170.1
tg^-t	3_E-3_E	6.33	7.21	9.46	88.4
tg^+g^+	3_E-3_E	6.25	7.24	12.14	135.1
$g^+g^+g^-$	3_E-2_E	5.13	6.20	6.61	69.7
$g^+g^-g^-$	3_E-2_E	6.05	5.77	8.35	89.8
$g^-t_sg^-$	3_E-2_E	4.89	6.24	8.96	107.2
g^+g^+t (Z-DNA)	2_E-3_E	7.00	7.15	11.43	108.7
g^-g^-t	2_E-3_E	7.21	7.13	11.76	111.5
$g^+g^+g^+$	2_E-2_E	7.13	6.75	8.65	76.8
ttt [b]	3_E-3_E	7.24	6.25	12.14	135.1
	3_E-2_E	7.72	6.25	12.53	133.8
	2_E-3_E	7.24	6.98	14.10	176.1
	2_E-2_E	7.20	6.98	14.17	176.8

[a] See table 1 for the dihedral angles of the respective states.
[b] Reference states computed (with $\omega' = \omega = \psi = 180°$) for comparison.

4.4. Phosphate effect and interphosphate geometries

Theoretical studies on mononucleosides and dinucleoside monophosphates have correctly predicted the importance of the $\psi = g^+$ orientation, but failed to bring to light the importance of the $\psi = trans$ orientation; the $\psi = trans$ state emerges as an equally probable case when a phosphate is added at the 3′-terminus. The $\psi = trans$ conformation is often suggested to be the case in many of the fiber diffraction studies on polynucleotides. In a dinucleoside triphosphate, such as pCpGp, each base has one 3′- and one 5′-phosphate, and hence it is a good model system to be investigated for purpose of extrapolating the results of the subunit system to polynucleotides. The presence of the 5′-phosphate in a few specific situations forces the ϕ' orientation to the g^- region. This $\phi' = g^-$ state is observed in left-handed DNA helices. The conformational changes noted between the internal and terminal G residues in the crystal structure of the hexamer d(CpGpCpGpCpG) [3] and also the differences noted between the middle dinucleoside and terminal backbone segments in the crystal structure of d(pApTpApT) [25] thus could be explained on the basis of the terminal phosphate effect.

Table 3 lists the interphosphate geometries for the 22 low-energy conformations. We note from these data that the 2_E-3_E sugar pucker sequence produces relatively more elongated backbones than the other sequences and this accounts for the observation of such sugar pucker combinations mostly in loops and bends in tRNA-Phe crystal

Table 4

Hydrogon bonds in the low-energy conformations of pCpGp

Conformation [a]	Donor	Acceptor	Distance (Å)	Conformation [a]	Donor	Acceptor	Distance (Å)
^3_E-^3_E				^2_E-^3_E			
tg^+t	O2′(2)	OI(3)	3.23	g^+g^+t	O2′(2)	OI(3)	3.28
	O2′(1)	OI(2)	3.30		O2′(1)	OI(2)	3.01
g^+tt	O2′(2)	OI(3)	3.21		O2′(1)	O2(C)	3.24
	O2′(1)	OI(2)	3.08	tg^+t	O2′(2)	OI(3)	3.14
g^-tt	O2′(2)	OI(3)	3.24		O2′(1)	O2(C)	3.01
	N4(C)	N7(G)	3.42	$g_s^-g^-t$	O2′(2)	OI(3)	3.35
$g^-g^-g^+$	O2′(2)	OI(3)	2.97		O2′(1)	OII(2)	2.88
tg^-g^+	O2′(2)	OI(3)	3.07		O2′(1)	O2(C)	2.87
	O2′(1)	OII(2)	3.22	g^-tt	O2′(2)	OI(3)	3.16
tg^-t	O2′(2)	OI(3)	3.08		O2′(1)	O2(C)	3.11
	O2′(1)	OII(2)	3.43	g^+tt	O2′(2)	OI(3)	3.16
tg^+g^+	O2′(2)	OI(3)	3.13		O2′(1)	O2(C)	3.24
	O2′(1)	OII(2)	3.46	tg^-g^+	O2′(2)	OI(3)	3.04
					O2′(1)	O2(C)	3.14
^3_E-^2_E				^2_E-^2_E			
$^+g^+g^-$	O2′(2)	OI(3)	2.80				
	O2′(2)	N3(G)	3.35	g^-tt	O2′(2)	OI(3)	2.87
g^-g^-	O2′(2)	N3(G)	3.33		O2′(2)	N3(G)	2.98
tt	O2′(2)	OI(3)	3.29		O2′(1)	O2(C)	3.02
	O2′(2)	N3(G)	3.20	tg^+t	O2′(2)	OI(3)	3.22
$t_s t$	O2′(2)	OI(3)	2.70		O2′(2)	N3(G)	3.05
	O2′(2)	N3(G)	3.00		O2′(1)	O2(C)	3.07
$t_s g^-$	O2′(2)	N3(G)	2.59	$g^+g^+g^+$	O2′(2)	N3(G)	3.33
g^-t	O2′(2)	OI(3)	3.48		O2′(1)	OI(2)	2.95
	O2′(2)	N3(G)	3.05		O2′(1)	O2(G)	3.14

[a] See table 1 for the dihedral angles of the respective states.

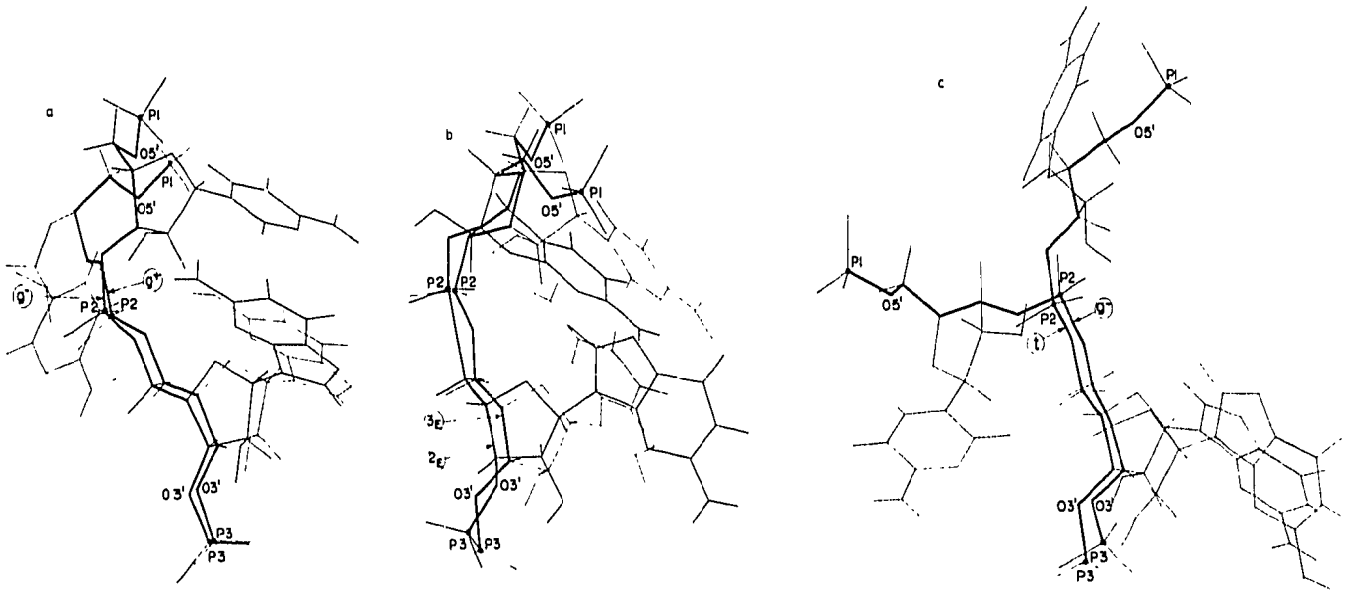

Fig. 6. (a) In this diagram the effect of change in ω' from g^- to g^+ is shown (Nos. 2 and 3 in table 1). (b) This sketch shows the change in backbone course due to the change in sugar pucker at the 5′-side for the backbone course, $g^- tt$ (Nos. 2 and 10 in table 1). (c) The reversal in the chain direction due to the change in ω from the t to the g^- region in the 2_E-3_E domain is depicted in this diagram (Nos. 16 and 17 in table 1).

models. A perusal of the interphosphate angles suggests that the 3_E-2_E sugar pucker sequence, in comparison with the other sequences, could produce sharper turns in the backbones, whereas the 2_E-3_E pucker sequence could produce extended backbones. These observations emphasise the fact that the flipping of the sugar pucker between the 3_E and 2_E modes of 3′-ribose alters the backbone course appreciably.

4.5. Hydrogen-bonded structures

Our earlier theoretical study [11] and a recent NMR study by Bolton and Kearns [32] described the association features of a water molecule with the 2′-OH group in RNA subunits and also the influence of solvent on backbone folding. In the present study we have analysed the intrastrand hydrogen bonds in the resultant 22 low-energy conformations. Table 4 lists the noted hydrogen bonds. In sorting out the hydrogen-bonded states, only the X···Y distance was considered and the linearity condition of X–H···Y was not imposed, since it could be achieved with minor alterations of the angles. In conformity with solution studies, we note in many low-energy conformations that the ribose oxygen atoms O2′(1) and O2′(2) form hydrogen bonds with one of the 3′-phosphate oxygens. These hydrogen bonds are, however, broken when the backbone angle ω' takes up the g^- orientation as this condition keeps the central phosphate group away from the 2′-OH of the first ribose unit. Further, these specific hydrogen bonds are also not feasible when ϕ' assumes values greater than 200°. The Watson-Crick-type conformation alone permits interbase hydrogen bonding. Intranucleotide hydrogen bonds are noted between one of the acceptor atoms (N_3 for A and O_2 for C) of the bases and the 2′-OH group in all the conformations associated with the 2_E sugar state. The 2_E sugar pucker, usually, brings the 2′-OH group nearer to the base unit.

Flexibility in the backbone structures

A comparison of the backbone states of the various conformations listed in table 1 provides clues as to the flexibility inherent in a few specific cases. Fig. 6 superposes three pairs of conformations which add information to this aspect. Conformers 2 and 3 of table 1 superposed in fig. 6a differ in their energies by only 0.4 kcal/mol while the backbone conformation changes from $g^- tt$ to $g^+ tt$; this change in ω' from the g^- to the g^+ region folds the chain by bringing the phosphates closer and turns the 5′-base away from the helical axis. The $g^+ tt$ conformer is observed at the bend connecting the D and a-c stems in the tRNA-Phe model of Holbrook et al. [30]. Conformers 2 and 10 of table 1 have a similar backbone but different sugar pucker combinations. The change takes place at the expense of only 0.2 kcal/mol energy; since the change in sugar pucker is at the 5′-side, the backbone course is not greatly altered (fig. 6b). Model building indicates that the flipping of 5′-sugar to 2_E from 3_E and a small increase in angle ϕ'_1 push the bases apart and out of plane. To bring the bases back into plane with each other, ω' moves from -70 to $-47°$ and χ_G also moves from -6 to 170°. The resultant conformer is well suited for drug intercalation. Conformers 16 and 17 shown in fig. 6c show that the change in ω from t to g^- reverses the direction of the chain at the central phosphate and pushes the 5′-part of the chain away from the helical axis. All the three kinds of changes in the backbone illustrated in fig. 6 take place with minimal energy changes and hence they may have profound influence on the flexibility of polynucleotides incorporating local regions in these conformational states.

5. Conclusions

The present theoretical study indicates that the flexibilities not only in ω' and ω, but also in ϕ' and ψ parameters have their say in deciding the preferred backbone conformations of polynucleotides. The inclusion of the terminal phosphates in a monophosphate unit results in a phosphate-sugar pucker sequence dependence; the missed 2_E-3_E sugar pucker domain in the studies of dinucleoside monophosphate units is brought back to play by the additional phosphates. The 3′-terminal phosphate also enhances the occurrence of the ψ = *trans* state. The present study also indicates that the

Z-type backbone state originally predicted for DNA is possible for RNA molecules also. The Watson-Crick-type backbone state seems to be one of the favorable conformations for DNA-RNA interaction.

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