CONFORMATIONAL CHARACTERISTICS OF DIMERIC SUBUNITS OF RNA FROM ENERGY MINIMIZATION STUDIES

MIXED SUGAR-PUCKERED ApG, ApU, CpG, and CpU

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ABSTRACT Following the procedure described in the preceding article, the low energy conformations located for the four dimeric subunits of RNA, ApG, ApU, CpG, and CpU are presented. The A-RNA type and Watson-Crick type helical conformations and a number of different kinds of loop promoting ones were identified as low energy in all the units. The ^3E-^3E and ^3E-^2E pucker sequences are found to be more or less equally preferred; the ^2E-^2E sequence is occasionally preferred, while the ^2E-^3E is highly prohibited in all the units. A conformation similar to the one observed in the drug-dinucleoside monophosphate complex crystals becomes a low energy case only for the CpG unit. The low energy conformations obtained for the four model units were used to assess the stability of the conformational states of the dinucleotide segments in the four crystal models of the $tRNA^{Phe}$ molecule. Information on the occurrence of the less preferred sugar-pucker sequences in the various loop regions in the $tRNA^{Phe}$ molecule has been obtained. A detailed comparison of the conformational characteristics of DNA and RNA subunits at the dimeric level is presented on the basis of the results.

INTRODUCTION

In the preceding article we discussed in detail the overall conformational behavior of the dimeric subunits of DNA. In order to understand the conformational properties of RNA molecules, an extensive investigation was carried out on the four dimeric subunits of RNA, namely, ApG, ApU, CpG, and CpU, representing the four general types of base sequenced (purine-purine, purine-pyrimidine, pyrimidine-purine, and pyrimidine-pyrimidine) systems. For each of these subunits the four important sugar-pucker sequences, ^3E-^3E, ^3E-^2E, ^2E-^3E, and ^2E-^2E were considered. Not only does this study provide a set of probable conformations that could be readily used for interpreting the low resolution electron density maps of tRNA molecules and RNA systems now under structural investigations, but it is also useful in assessing the conformational stabilities of various regions of the reported crystal models of the $tRNA^{Phe}$ molecule (Rao et al., 1978; Sussman and Kim, 1976; Hingerty et al., 1978; Holbrook et al., 1978). Furthermore, this study enables us to make a systematic comparison between the overall conformational features of the DNA and RNA subunit systems with similar base sequences.

METHODS

For the molecular system under investigation, the total potential energy, comprising nonbonded, hydrogen bonding, electrostatic and torsional contributions, was computed using the expressions detailed

in the preceding paper. The total potential energy function for the selected starting conformations was minimized by allowing all the seven relevant dihedral angles (Fig. 1 of the preceding paper) to vary simultaneously using Fletcher-Powell-Davidon minimization procedure (Davidon, 1959; Fletcher and Powell, 1963). The same set of 96 starting conformations used for the study of the DNA subunits (preceding paper) was considered as the starting points for the study of each of the subunits. For describing the results the same procedure adopted for designating the conformations of DNA subunits was used. The geometrical parameters used were again those of Lakshminarayanan and Sasisekharan (1970).

RESULTS

Out of the 96 starting conformations examined for each of the four units, a majority turned out to be very high energy cases. Thus, only 14, 16, 13, and 18 conformations became preferred for ApG, ApU, CpG, and CpU, respectively, within an energy limit of ΔE = 5.0 kcal/mol above the respective lowest energy state for each of the units after minimization. Thus, there is enormous restriction in the preference of conformations due to bases, a fact found for the DNA subunits also. These low energy conformations are listed in Tables I to IV. The order of preference of various conformations in each of the Tables reflects the characteristic effect of the bases present in the unit, and their sequence on the preference of backbone conformations. The important features found for each of the subunits are discussed below.

Preferred Conformations for ApG

In Table I, we note that eight conformations fall in the ^3E-^3E sugar-pucker sequence domain and six in the ^3E-^2E domain. In contrast to DNA subunits, there are no low energy conformations in the ^2E-^2E and ^2E-^3E domains for this RNA subunit. In these two sugar-pucker domains strong nonbonded and electrostatic repulsions arising from the close proximity of O(2′) atom of the 3′-nucleotide sugar with phosphate group destabilize the conformations. The preferred conformation in the ^3E-^3E domain resembles the Watson-Crick (Crick and Watson, 1954) structure with $(\omega', \omega, \psi) = (g^-, t, t)$, exhibiting good base stacking. This is at an energy level of ΔE = 0.9 kcal/mol compared with the lowest energy state (global minimum) of the unit occurring in the ^3E-^2E domain. Two more loop-promoting conformers with $(\omega', \omega, \psi) = (t, g^+, t)$ and (g^-, t, g^+) occur at ΔE = 2.6 and 2.9 kcal/mol above the global minimum. While the former can promote an open-type bend, the latter effects a sharp hair-pin bend as shown in Fig. 1. This sharp bend conformer has been designated as π-bend (Kim and Sussman, 1976) and it occurs at the AC and Tψ loop regions of tRNA^{Phe} molecule in its crystalline forms (Hingerty et al., 1978; Holbrook et al., 1978). The A-RNA type conformer (Arnott et al., 1973) lies at an energy level of ΔE = 3.9 kcal/mol, while an open-type bend conformer with $(\omega', \omega, \psi) = (g^-, t, t)$ falls at ΔE = 4.1 kcal/mol. It is interesting to note that for ApG, $\psi \simeq$ *trans* region is preferred in a majority of low energy states compared to the other two staggered ψ orientations.

In ^3E-^2E sugar-pucker sequence domain, the global minimum corresponds to $(\omega', \omega, \psi) = (g^+, g^+, t)$ which effects a hair-pin shape for the molecule as shown in Fig. 2. It is interesting to note that this conformer is similar to the left-handed Z-DNA (Wang et al., 1979) except with the sugar-pucker sequence reversed. This type of conformation has been noted in a few polydeoxynucleotides (Arnott et al., 1980) also. Furthermore, from potential energy calcula-

TABLE I

ApG: ENERGY-MINIMIZED CONFORMATIONS LYING WITHIN 5 kcal/mol ABOVE THE LOWEST ENERGY FOUND

No.	Dihedral angles (*degrees*)					Relative energy (*kcal/mol*)	Description	
	χ'	ω'	ω	ψ	χ			
^3E-^3E sugar-pucker sequence domain								
1	44	−70	155	178	25	0.9	g^-tt	(WC-Structure)
2	105	156	67	180	9	2.6	tg^+t	
3	35	−47	162	58	−1*	2.9	g^-tg^+	
4	107	159	68	−62	12	3.4	tg^+g^-	
5	104	155	−14	180	30	3.5	tg^-t	
6	107	159	−79	55	8	3.9	tg^-g^+	
7	68	−70	−89	71	37	3.9	$g^-g^-g^+$	(A-RNA)
8	98	43	166	177	10	4.1	g^+tt	
^3E-^2E sugar-pucker sequence domain								
9	126	38	93	177	47	0.0‡	$g^+g_s^+t$§	(Z − DNA)
10	51	−102	−175	72	13*	1.2	$g_s^-tg^+$	
11	30	−65	177	−176	35	3.2	g^-tt	(alt-WC)
12	27	93	158	65	25	3.4	$g_s^+tg^+$	
13	107	157	−66	−176	9	3.5	tg^-t	
14	108	162	−88	68	50	5.0	tg^-g^+	

*The ϕ' value is −104°; in other conformations it is in the *trans* region.
‡corresponds to −30.6 kcal/mol.
§s denotes skewed.

tions, Broyde et al., (1979) also find a similar conformation to be preferred for pdApdAp, but with ^3E-^3E sugar-pucker sequence. The conformation next in energy rank is the alt-π-bend with ΔE = 1.2 kcal/mol. This conformer shown in Fig. 3, however promotes only a less compact structure compared with that by π-bend with ^3E-^3E sequence shown in Fig. 1. In this conformation the dihedral angle ϕ' assumes a skewed g^- orientation in contrast to a *trans* orientation in other conformations. The alt-WC-form with $(\omega', \omega, \psi) = (g^-, t, t)$ and ^3E-^2E sugar-pucker sequence is found at an energy level of ΔE = 3.2 kcal/mol and it exhibits some base stacking property. As stated in the preceding paper the alt-WC conformer has an elongated backbone course compared with that of the conventional WC-type conformer. The rest of the conformers given in Table I are open-type loop structures.

It is interesting to note from Table I the relationship between the two groups of conformations, the first consisting of nos. 1, 3, 5, and 6 (all in ^3E-^3E sugar-pucker sequence) and the second of nos. 11, 10, 13, and 14 (all in ^3E-^2E sugar-pucker sequence). Although the conformational angles in each of the following four pairs of conformations from the two groups, namely, 1 and 11, 3 and 10, 5 and 13, and 6 and 14, are very similar, the individual members in each pair adopt different sugar-pucker sequences. This fact indicates the kind of flexibility present in the sugar unit of a dinucleotide segment in a polynucleotide chain. Among the conformations in each pair, the sugar sequences ^3E-^3E and ^3E-^2E may be adopted, depending on the requirement of a slightly shortened or elongated back-bone course at the respective site. Surprisingly, no conformation is found to lie within the considered 5.0 kcal/mol energy limit with ^2E-^2E and ^2E-^3E pucker sequences.

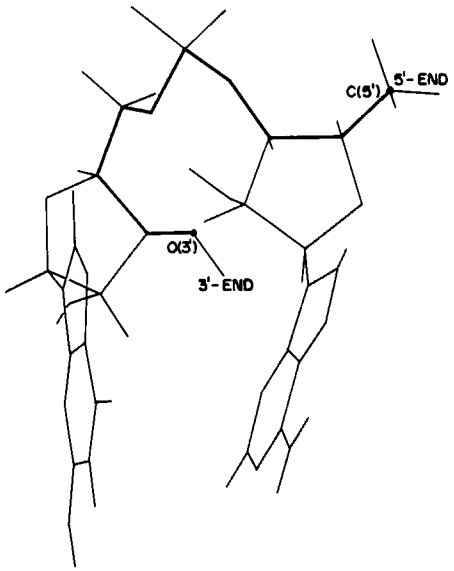

FIGURE 1

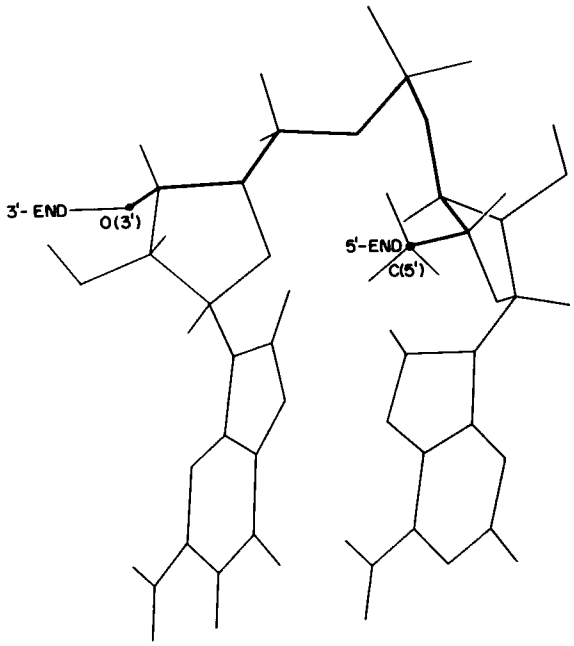

FIGURE 2

FIGURE 1 The g^-tg^+ conformation with ${}^3E$-${}^3E$ pucker sequence for ApG (No. 3 in Table I).
FIGURE 2 The preferred conformation for ApG (g^+g^+t, ${}^3E$-${}^2E$) (No. 9 in Table I). This resembles Z-DNA except with the sugar-pucker sequence reversed.

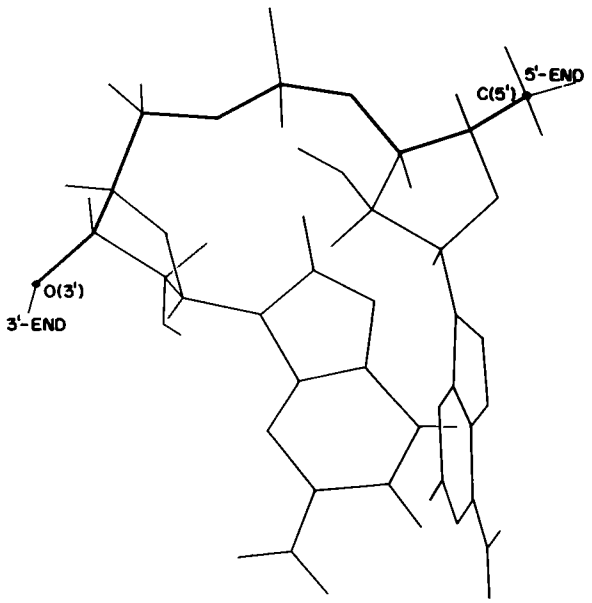

FIGURE 3 The alt-π bend conformation with ${}^3E$-${}^2E$ pucker sequence for ApG (No. 10 in Table I).

Preferred Conformations for ApU

Table II lists the low energy conformations for this unit. The lowest energy conformer (global minimum) corresponds to the ^3E-^2E domain as noted for ApG. There are seven low energy conformations in the ^3E-^3E domain and eight in the ^3E-^2E domain. An interesting feature noted here is the occurrence of a low energy conformation in the ^2E-^2E domain also. The preferred conformation in the ^3E-^3E domain is a bent conformer with $(\omega', \omega, \psi) = (t, g^+, g^+)$ at ΔE = 0.6 kcal/mol. The WC-type conformer comes next in energy rank and it exhibits good base-stacking property. The loop type tg^-g^+ conformer and the A-RNA conformer fall at energy levels of ΔE = 1.1 and 1.5 kcal/mol, respectively. The base orientations are *high anti-anti* in the two loop type conformations and *anti-anti* in the two helical type conformations. The predicted A-RNA type conformer is similar to that observed in the crystal structures of ApU (Seeman et al., 1976), and GpC (Rosenberg et al., 1976; Hingerty et al., 1976). The π-bend conformer lying at ΔE = 3.4 kcal/mol has a skewed ϕ' ($-110°$) orientation in contrast to the other preferred conformations. In one of the remaining nonhelical conformations with $(\omega', \omega, \psi) = (g^+, t, g^-)$, we note a skewed ϕ (115°) orientation, in contrast to the *trans* orientation observed in other low energy conformations. This skewed orientation may be due to the presence of the hydrogen bond-type interaction between the C(2′) hydroxyl group of the 5′-nucleotide residue and one of the oxygen atoms of the 5′-phosphate.

TABLE II

ApU: ENERGY-MINIMIZED CONFORMATIONS LYING WITHIN 5 kcal/mol ABOVE THE LOWEST ENERGY FOUND

No.	Dihedral angles (*degrees*)					Relative energy (*kcal/mol*)	Description	
	χ'	ω'	ω	ψ	χ			
^3E-^3E sugar-pucker sequence domain								
1	106	160	60	53	6	0.6	tg^+g^+	
2	41	−100	157	160	23	1.0	g^-tt	(WC-structure)
3	108	158	−62	57	6	1.1	tg^-g^+	
4	1	−68	−68	60	7	1.5	$g^-g^-g^+$	(A-RNA)
5	5	−56	154	57	2*	3.4	g^-tg^+	(π-bend)
6	112	172	−67	169	19	3.6	tg^-t	
7	127	92	−168	−24	22‡	4.0	$g_s^+tg^-$	
^3E-^2E sugar-pucker sequence domain								
8	10	−73	−179	170	25	0.0§	g^-tt	(alt-WC)
9	108	159	65	56	36	0.2	tg^+g^+	
10	109	159	−64	59	36	0.6	tg^-g^+	
11	110	160	−63	160	33	2.9	tg^-t	
12	112	−59	−170	60	28*	3.4	g^-tg^+	(alt-π-bend)
13	107	158	84	−50	40	3.5	tg^+g^-	
14	24	64	−169	−54	42‡	4.7	g^+tg^-	
15	102	59	179	166	34	4.9	g^+tt	
^2E-^2E sugar-pucker sequence domain								
16	69	−142	−65	50	49	2.6	tg^-g^+	

*The ϕ' value is around $-110°$; in other conformations it has a value in the *trans* region.
‡The ϕ value is around 115° (skewed).
§Corresponds to −42.0 kcal/mol.

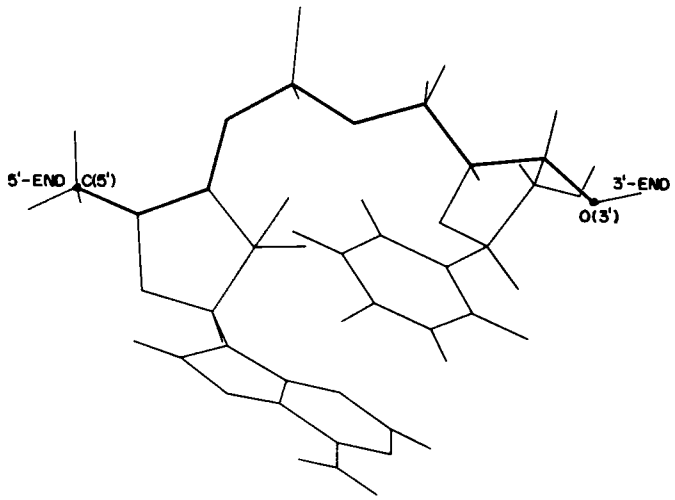

FIGURE 4 The alt-WC-Conformer with ^3E-^2E sequence for ApU (No. 8 in Table II). This promotes good base stacking.

The alt-WC-conformer is the global minimum for ApU and it exhibits reasonable stacking property as seen from Fig. 4. Two more loop-promoting conformations with $(\omega', \psi) = (t, g^+)$ but differing in their ω orientation (g^+ in conformer 9 and g^- in 10) turn out to be equally probable cases. Such backbone conformational states are noted in the ^3E-^3E domain also (Nos. 1 and 3). The π-bend in the ^3E-^2E domain has an energy of ΔE = 3.4 kcal/mol and it

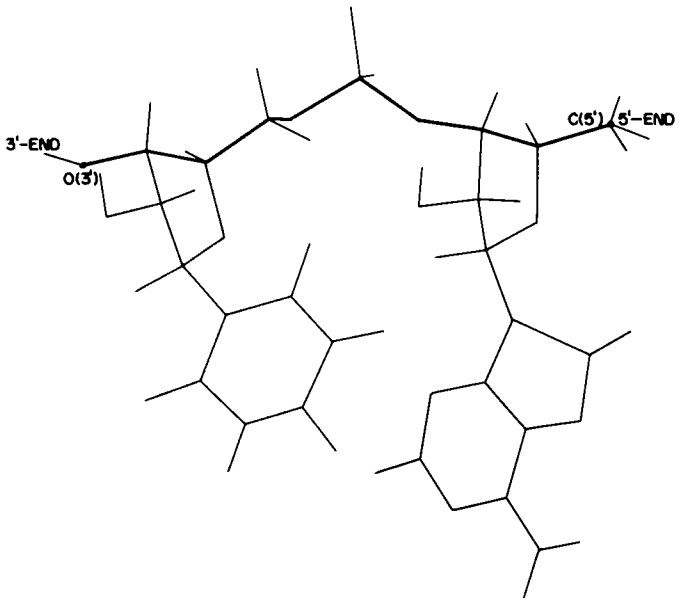

FIGURE 5 The extended loop conformation tg^-g^+ with ^2E-^2E pucker sequence predicted for ApU (No. 16 in Table II).

has $\phi' = -110°$; The g^+tg^- conformer in this domain (No. 14) also has the unusual ϕ orientation around 115° as noted in the ^3E-^3E domain.

The only conformer preferred in the ^2E-^2E domain having $(\omega', \omega, \psi) = (t, g^-, g^+)$ is again an open-type loop conformation (see Fig. 5). This conformer is less compact than those resulting from ^3E-^3E and ^3E-^2E sugar-pucker sequences with the same backbone state. As noted for ApG the ^2E-^3E sugar sequence is not preferred for this unit also. The conformation observed in the drug-dinucleotide complex ApU-9 amino acridine cocrystal (Seeman et al., 1975) having ^2E-^3E sequence is noted at an energy level of 7.8 kcal/mol compared with the global minimum.

Preferred Conformations for CpG

In contrast to ApG and ApU, the lowest energy conformation (global minimum) for CpG lies in the ^3E-^3E domain (See Table III) and it resembles the A-RNA form. The WC-type conformer has an energy of only $\Delta E = 0.4$ kcal/mol. Both these helical type conformations exhibit good base stacking. The rest of the five conformations in the ^3E-^3E domain are different types of loop structures which include the π-bend.

The most preferred among the six low energy cases in the ^3E-^2E domain is the alt-WC-conformer and it has an energy of only $\Delta E = 0.5$ kcal/mol. The Z-DNA type g^+g^+t conformer (Wang et al., 1979) which was found to be the most preferred case for ApG, acquires an energy of $\Delta E = 3.4$ kcal/mol for CpG. An interesting and unique feature noted for this unit is the preference of alt-A-RNA conformer with backbone like A-RNA, but with ^3E-^2E sugar-pucker sequence at an energy level of 3.9 kcal/mol. This conformer (shown in Fig. 6) elongates the backbone as base-to-base separation compared with the A-RNA

TABLE III

CpG: ENERGY-MINIMIZED CONFORMATIONS LYING WITHIN 5 kcal/mol ABOVE THE LOWEST ENERGY FOUND

No.	Dihedral angles (*degrees*)					Relative energy (*kcal/mol*)	Description	
	χ'	ω'	ω	ψ	χ			
^3E-^3E sugar-pucker sequence domain								
1	20	−73	−68	57	16	0.0*	$g^-g^-g^+$	(A-RNA)
2	24	−53	164	176	7	0.4	g^-tt	(WC-structure)
3	30	92	174	51	−2	0.5	g^+tg^+	
4	16	71	−117	−65	12	1.4	$g^+t_2g^-$	
5	25	17	127	173	13	4.4	$g_1^+t_1t$	
6	26	153	−69	−62	11	4.9	tg^-g^-	
7	30	−43	170	53	5‡	5.0	g^-tg^+	(π-bend)
^3E-^2E sugar-pucker sequence domain								
8	23	−54	177	179	25	0.5	g^-tt	(alt-WC)
9	14	29	117	178	34	3.4	$g^+g_1^+t$	(Z-DNA)
10	22	38	178	−63	30	3.7	g^+tg^-	
11	12	−48	−68	56	74	3.9	$g^-g^-g^+$	(alt-A-RNA)
12	26	127	−65	−55	34	4.3	$t_2g^-g^-$	
13	26	162	63	−61	33	5.0	tg^+g^-	

*Corresponds to −26.7 kcal/mol.

‡The ϕ' value is around 105°; in other conformations it has a value in the *trans* region.

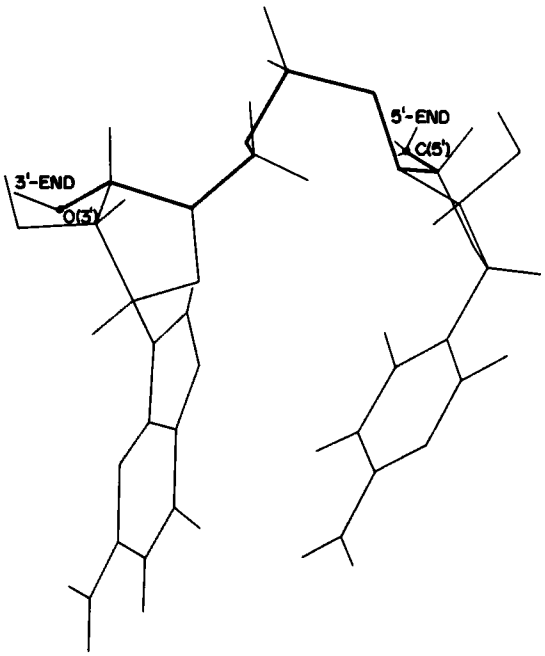

FIGURE 6 The alt-A-RNA conformation predicted for CpG having ^3E-^2E pucker sequence (No. 11 in Table III). This promotes an elongated backbone compared with that of A-RNA conformer.

conformer and destacks the bases. When properly base paired and with considerable alteration in the χ and ϕ angles, this conformation can provide enough room for the chromophoric drug molecules to intercalate between the adjacent base pairs (Berman et al., 1978). Crystal structures of a few drug-dinucleoside phosphate complexes reveal such a feature (Tsai et al., 1977; Jain et al., 1977; Westhof and Sundaralingam, 1980) and the base sequence in these dinucleoside Phosphates is pyrimidine-purine type. The present study thus provides a clue as to the preference of Py-Pu base sequence over the other types for the drug intercalation phenomenon in polynucleotides. Unlike ApG and ApU, no *high anti* base orientation is noted in the 3'-nucleotide residue, which is due to the presence of pyrimidine base in that residue.

Preferred Conformations for CpU

The main features noted for CpU from Table IV are, (*a*) an extended loop promoting conformer with $(\omega', \omega, \psi) = (t, g^+, g^+)$ and ^3E-^2E sugar sequence is the global minimum structure; (*b*) the WC-type and A-RNA type conformers are placed at 0.1 and 0.8 kcal/mol, respectively; (*c*) as in CpG no *high anti* base orientation is noted; and (*d*) most interestingly, this unit prefers ^2E-^2E sugar-pucker sequence in four low energy conformations, compared with only one in ApU and none in ApG and CpG.

DISCUSSION

Apart from the specific points brought out in the Results section a number of general conformational features of RNA systems could be delineated from Tables I–IV. Except for

TABLE IV

CpU: ENERGY-MINIMIZED CONFORMATIONS LYING WITHIN 5.0 kcal/mol ABOVE THE LOWEST ENERGY FOUND

No.	Dihedral angles (*degrees*)					Relative energy (*kcal/mol*)	Description	
	χ'	ω'	ω	ψ	χ			
^3E-^3E sugar-pucker sequence domain								
1	21	−69	174	158	14	0.1	g^-tt	(WC-structure)
2	11	−75	−63	50	8	0.8	$g^-g^-g^+$	(A-RNA)
3	22	52	165	−20	23*	2.9	$g^+tg_s^-$	
4	25	163	66	51	7	3.4	tg^+g^+	
5	10	−64	139	57	8	3.7	$g^-t_sg^+$	(π-bend)
6	25	161	−63	57	6	3.9	tg^-g^+	
^3E-^2E sugar-pucker sequence domain								
7	25	169	65	55	35	0.0‡	tg^+g^+	
8	23	54	−175	−55	42*	0.1	g^+tg^-	
9	25	162	−67	59	36	0.4	tg^-g^+	
10	20	−79	142	53	35	0.4	$g^-t_sg^+$	
11	27	164	−63	166	35	2.9	tg^-t	
12	25	165	82	−57	40	3.1	$tg_s^+g^-$	
13	26	167	90	173	34	3.4	tg_s^+t	
14	22	−82	178	−52	40	3.8	$g_s^-tg^-$*	
^2E-^2E sugar-pucker sequence domain								
15	34	−127	−59	59	28	1.7	$t_sg^-g^+$	
16	38	−153	72	171	28	3.7	tg^+t	
17	35	−148	67	57	36	4.2	$t_sg^+g^+$	
18	31	−148	45	−63	37	4.7	$t_sg^+g^-$	

*The ϕ value is around 120°; in other conformations it is in the usual *trans* orientation.
‡Corresponds to −35.4 kcal/mol.

CpG, the lowest energy conformations (global minima) for the other units lie in the ^3E-^2E sugar-pucker sequence domain. The A-RNA type and WC-type helical and the π-bend conformational states become, in general, low energy cases in all the units. We also note the characteristic effects of the bases on the conformational properties of the backbone and on base stacking. The general conformational features of the RNA subunits when compared with the corresponding DNA sub-units, reveal many correlations.

Effect of Base Sequence on the Sugar Pucker

An analysis of the number of predicted low energy conformations for each of the four subunits reveals that there are eight and six conformations in the ^3E-^3E and ^3E-^2E sugar-pucker sequence domains for ApG, seven and eight conformations for ApU, seven and six for CpG, and six and eight for CpU. The ^2E-^2E domain is associated with only one conformation for ApU and four for CpU and this domain is not at all preferred for ApG and CpG. The ^2E-^3E domain, on the other hand, is completely absent in all the predicted low energy conformations for all the four units. These results indicate that the ^3E-^3E and ^3E-^2E sequences are more or less equally favored in all the units, but the ^2E-^2E sequence is preferred only occasionally depending on the base sequence.

The nonpreference of ^2E-^2E and ^2E-^3E sequences in the case of RNA subunits could be

attributed to the destabilization caused by the strong nonbonded and electrostatic repulsions arising between the hydroxyl group of the sugar of the 3'-nucleotide and the phosphate group, due to their close proximity.

The sugar-pucker sequence effect on the directional behavior of the backbone is clearly demonstrated by a few conformations with similar backbone dihedral angles, but with different sugar-pucker sequences. It is noted that for a particular type of backbone conformation the preference of ^3E-^2E sugar-pucker sequence over the ^3E-^3E may be due to the requirement of a slightly elongated backbone course. Similarly, the ^2E-^2E sequence, usually results in an extended open-type loop structure (see Table V of the preceding paper for the end-to-end distances of various backbone conformations each with different sugar-pucker sequence). Apart from the base sequence effect on the preference of sugar pucker sequence, another striking correlation is noted in the interdependence of ϕ and χ orientations. In almost all the low energy conformations for the four units with $\psi \simeq 60°$ or 180°, the angle ϕ assumes a value in the *trans* region. However, in a few conformations predicted for ApU and CpU with $\psi \simeq -60°$ (Nos. 7, 13, and 14 in Table II and Nos. 3, 8, and 14 in Table IV) ϕ assumes a skewed orientation around 120°. This specific feature is exhibited only by ApU and CpU which have a pyrimidine base in the 5'-nucleotide residue (it is not found in ApG and CpG). This indicates that the type of base (purine or pyrimidine) in the 5'-nucleotide residue has a definite influence on the orientation of ϕ depending on the ψ orientation. Olson and Flory

TABLE V
BASE-STACKING GEOMETRIES PREDICTED FOR THE DINUCLEOSIDE MONOPHOSPHATES

	Type of conformer	Sugar-pucker sequence	Mean distance between base planes	Angles between base planes	Overlapping area*	Stacking property‡
			(*Å*)	(*degrees*)	(%)	
ApG	WC-structure	^3E-^3E	3.6	19	30	good
	alt-π-bend	^3E-^2E	4.2	20	35	reasonable
	alt-WC	^3E-^2E	3.6	9	40	good
	A-RNA	^3E-^3E	4.0	40	10	poor
ApU	alt-WC	^3E-^2E	3.5	13	50	good
	WC-structure	^3E-^3E	3.8	19	25	reasonable
	alt-π-bend	^3E-^2E	4.3	32	12	poor
	A-RNA	^3E-^3E	3.5	13	50	good
CpG	A-RNA	^3E-^3E	4.0	33	10	Poor
	WC-structure	^3E-^3E	3.7	20	18	reasonable
	alt-WC	^3E-^3E	3.6	30	15	reasonable
	Z DNA	^3E-^2E	3.7	35	25	reasonable
	alt-A-RNA	^3E-^2E	4.8	56	22	poor
CpU	A-RNA	^3E-^3E	3.5	20	28	good

*The extent of base overlapping is said to be 100% when the area of two similar bases get completely overlapped and also when the base with smaller area is completely overlapped by the base of larger area.
‡The base stacking is said to be good when the mean distance between base planes is in the range of 3.5–4.0 Å, the angle between them is within 20° and the extent of base overlap is >30%. The terms 'reasonable' and 'poor' are used relatively with respect to the definition given for good base stacking.

(1972) have demonstrated the (ϕ-ψ) interdependence. Here we notice the influence of base sequence on ϕ which may be due to a combination of shorter range ϕ-ψ and ψ-χ interdependencies. We also note a few conformations having ϕ' in a skewed g^- orientation ($-110°$). The alt-π-bend (No. 10 in Table I) for ApG, the π-bend, and its mixed sugar counterpart for ApU (Nos. 5 and 11 in Table II), the A-RNA and its mixed pucker counterpart for CpG (Nos. 1 and 11 in Table III) and the π-bend for CpU (No. 5 in Table IV), all assume ϕ' values in the region of $-100°$ to $-120°$, in contrast to the rest of the conformations.

The alt-A-RNA form with ^3E-^2E sequence stands to be a low energy case only for CpG. A similar conformation with considerable deviations in χ and ϕ was observed in the case of drug-dinucleotide cocrystals (Jain et al., 1977; Tsai et al., 1977; Westhof and Sundaralingam, 1980) which also have py-pu type base sequence. The present study, therefore, throws ample light on the influence of the base sequence type on the behaviour of the backbone of the RNA subunits.

Base Orientations

An analysis of the preferred orientations of the bases in the subunit systems shows that a number of loop-type conformations predicted for ApG and ApU (Nos. 2, 4–6, 8, 9, 13, and 14 in Table I, and nos. 1, 3, 6, 7, 9–13, and 15 in Table II) assume mainly a ***high anti-anti*** base orientation. However, no such feature is noted in the case of CpG and CpU. These results clearly exhibit the propensity of a purine base in the 3′-nucleotide residue of the dinucleotide monophosphate to have ***high anti*** orientation. The absence of such an orientation in the 5′-nucleotide residue, irrespective of the presence of purine or a pyrimidine base, indicates indirectly the characteristic effect of the 5′-phosphate of the nucleotide unit. This 5′-phosphate influence which restrains the base in a nucleotide to remain in the *normal anti* region was already demonstrated by our earlier studies and also by studies of Sundaralingam (Sundaralingam, 1973; Thiyagarajan and Ponnuswamy, 1979*a*).

Base-Stacking Properties

The stacking interactions present in the nucleic acid systems depend on the nature of the base sequence and, perhaps, also on the sugar-pucker sequence. Hence, we analyzed the base-stacking properties exhibited by the predicted low energy conformations. Table V lists these properties. It is noted from this Table that apart from the two helical type conformers, A-RNA and WC-state, a few loop-promoting conformations and the mixed sugar-pucker states of the helical conformers also exhibit recognizable base-stacking feature. The base stacking, if considered on the whole for each of the units, is weaker for the py-py base sequence than the py-pu case. The pu-pu and pu-py sequences exhibit more or less similar stacking, but stronger compared to the sequences mentioned above. These predictions are consistent with the solution conformations of the dinucleoside monophosphates (Ts'o, et al., 1969; Lee and Tinoco, 1977) and also with our study on the DNA subunits, described in the preceding article. However, Ezra et al., (1977) predict from their high resolution NMR studies on the solution conformations of dinucleoside monophosphates that the stacking interaction decreases in the order of pu-py, pu-pu, py-py, and py-pu. With regard to the effect

of sugar-pucker sequence on the base-stacking feature, it is noted that the ^3E-^3E promotes better stacking than that of ^3E-^2E sequence.

An Assessment of the Conformational Stabilities of the Dinucleotide Segments in the tRNA^{Phe} Models

With the use of the predicted low energy conformations for the four subunits of RNA we made an analysis of the conformational stabilities of all the dinucleotide segments along the stretch of the backbone of the tRNA^{Phe} molecule in its two crystalline forms (Rao et al., 1978 [MSN], Sussman and Kim, 1976, [DUK], Helbrook et al., 1978 [MIT], Hingerty et al., 1978 [MRC]). Such an attempt has the following limitations:

(*a*) It is not proper to extrapolate the results of the subunit system directly to the polymer systems.

(*b*) The present study did not consider all the kinds of nucleotides that occur in the tRNA^{Phe} molecule and as a crude approximation it is to be assumed that the influence of the actual base sequence does not differ much from that of the respective general base sequences (pu-pu, pu-py, py-pu, and py-py).

(*c*) The addition of terminal phosphates may alter the conformational priorities of the backbone (Yathindra and Sundaralingam, 1974, 1975; Thiyagarajan and Ponnuswamy, 1979*a*; Broyde and Hingerty, 1979).

(*d*) Some of the conformations may receive additional stability via solvent-solute–long range–tertiary–ionic interactions; for example, we have earlier demonstrated the possible role played by the solvent-solute interactions in the stability of nucleic acid systems (Ponnuswamy and Thiyagarajan, 1978; Thiyagarajan and Ponnuswamy, 1979*b*).

For the present analysis, we mainly consider two aspects, namely, (*a*) the occurrence of the specific sugar-pucker sequences, and (*b*) the energy states of the specific base-sequenced dinucleotide units. For the analysis of the latter aspect we assumed that the theoretical predictions for ApG, ApU, CpG, and CpU could reasonably be compared with the crystal observations on all kinds of pu-pu, pu-py, py-pu and py-py sequences, respectively, in the tRNA^{Phe} molecule in order to have an approximate impression on the overall stability of its conformation.

According to the predictions made in Tables I–IV, the ^3E-^3E and ^3E-^2E sugar-pucker sequences are more or less equally favored, but the ^2E-^2E and ^2E-^3E are least favored. However, the number of occurrences of ^3E-^2E in tRNA^{Phe} crystals is very low (6 to 8 times) when compared to ^3E-^3E which occurs 57 to 72 times. This discrepancy between the theoretical prediction and the experimental observation could be explained from the knowledge of the least probable nature of the ^2E-^2E and ^2E-^3E sequences, even at the dimeric level. Although the ^3E-^2E is a stable sugar sequence for a dimeric unit, if a third unit is linked on the 3′-side of the dimer with a sugar unit of ^3E or ^2E state, the resulting trimer unit will contain a sugar sequence ^2E-^3E or ^2E-^2E on its 3′-side, which were predicted to be the least stable cases. Hence, the occurrence of ^3E-^2E sequence is drastically constrained in poly-ribonucleotides due to the severe restriction on the occurrence of the two sequences ^2E-^2E and ^2E-^3E.

From the results so far described on the dimeric subunits we can state that a polymer chain with a long sequence of ^3E pucker states, with occasional presence of one or two ^2E sugar

pucker units, is the structurally best probable case, and we note such a feature in the $tRNA^{Phe}$ molecule in its crystalline forms.

With regard to the second kind of analysis, the following observations are made. A majority of the dimeric segments in each of the four models adopt the A-RNA-type conformation which is found to be a low energy case for all subunits considered in the present study. It is noted that 38, 55, 47, and 44 dinucleotide segments in the $tRNA^{Phe}$ adopt this conformation, respectively, in the models of MSN, MRC, MIT and DUK. The remaining 37, 20, 28 and 31 dimeric segments in the respective models with non-A-RNA–type conformations only were considered for comparison with the theoretical predictions; also, we have not included the dimeric segments with ^2E-^2E and ^2E-^3E sequences as they are least stable according to the present study. We note that 13 out of 25, 6 out of 11, 12 out of 20, and 11 out of 24 cases in the respective models could be associated with the low energy conformations listed in Tables I to IV. In the MSN model, 5 of the nonaccountable cases adopt the phosphodiester conformations $(\omega', \omega) = (g^-, g^+)$ or (g^+, g^-) which were demonstrated as high energy states by a number of studies on dinucleoside phosphates (Yathindra and Sundaralingam, 1974; Olson and Flory, 1972). Considering the MRC model, we notice such skewed phosphodiester conformations at P21 and P48, which again could not be accounted as low energy cases. The alt-A-RNA-type conformational state found at the phosphodiester P60 could not be accounted as a low energy case as the base sequence at that site is pyrimidine-pyrimidine; such a conformation was predicted to be a low energy case only for CpG, but not for other subunits. In the MIT model there are five phosphodiesters which assume $(\omega', \omega) = (g^+, g^-)$ or (g^-, g^+) orientation, which are unstable according to the present study. The other two disagreeing cases in this model are the conformations found at P16 and P25 which have $(\omega', \omega, \psi) = (t, g^+, t)$ and (g^+, t, t), respectively. Although these two conformations become preferred in other base sequenced units, they are not preferred for ApU, and hence are unaccountable as low energy states. Thus, whenever there are disagreements between our predictions and the experimental results, we notice entirely different base sequences compared to the model subunits studied here.

Comparison with other Experimental Results

In this section the experimental data available for a few dimeric subunits of RNA are compared with our theoretical predictions. The available crystallographic data are listed in Table VI. Except two cases, all the structures listed in this Table are accountable as low energy cases according to the present study. The exceptions are the two conformations corresponding to the UpA crystal (Rubin et al., 1972) having $(\omega', \omega, \psi) = (t, g^- g^+)$ and (g^+, g^+, g^+) and the conformation of ApU-9 amino acridine complex crystal (Seeman et al., 1975). From their potential energy calculations on UpA, Broyde et al., (1974) account for both the observed conformations for that molecule. The disagreement with the former case may be attributed to the difference in the base sequence in the theoretically investigated unit, namely, CpG, for which these two conformations became high energy states and that with the latter case may be attributed to the additional tertiary interactions stabilizing the conformation.

It has been reported (Suck et al., 1973) that the ApApA molecule in its crystalline form

TABLE VI
COMPARISON OF THE PREDICTED LOW ENERGY CONFORMATIONS WITH CRYSTALLOGRAPHIC DATA

Molecule	Reference	Pucker sequence	χ'	ϕ'	ω'	ω	ϕ	ψ	χ	Energy state	Remark
					(degrees)					*kcal/mol*	
Fiber											
A-RNA	1	^3E-^3E	14	−158	−66	−66	−174	49	14	3.9	Table I, No. 7
										1.5	Table II, No. 4
										0.0	Table III, No. 1
										0.9	Table IV, No. 2
Crystal											
ApApA	2										
A_1PA_2 portion		^3E-^3E	7	−137	−79	−63	−160	53	24	3.9	Table I, No. 7
A_2PA_3 portion		^3E-^3E	24	−153	76	92	−174	61	21	*	
ApU_1	3	^3E-^3E	7	−147	−67	−72	177	57	29	1.5	Table II, No. 4
		^3E-^3E	4	−139	−76	−65	168	58	29	1.5	Table II, No. 4
GpC	4	^3E-^3E	13	−149	−68	−75	−176	50	32	1.5	Table II, No. 4
GpC_1	5	^3E-^3E	8	−138	−66	−69	−179	47	33	1.5	Table II, No. 4
GpC_2		^3E-^3E	4	−143	−69	−67	172	57	21	1.5	Table II, No. 4
GpC_3		^3E-^3E	1	−136	−70	−74	167	63	25	1.5	Table II, No. 4
GpC_4		^3E-^3E	7	−144	−72	−77	−179	52	28	1.5	Table II, No. 4
UpA_1	6	^3E-^3E	20	−137	165	−88	−168	53	49	*	
UpA_2		^3E-^3E	10	−158	84	84	−158	53	36	*	
ApU‡	7	^2E-^3E	76	−138	100	86	−158	63	72	*	
CpG_1§	8	^3E-^2E	29	−134	−79	−74	−150	72	101	3.9	Table III, No. 11
CpG_2§		^3E-^2E	21	−135	−69	−69	−136	55	109	3.9	Table III, No. 11
UpA_1§	9	^3E-^2E	26	−153	−74	−69	−124	52	99	3.9	Table III, No. 11
UpA_2§		^3E-^2E	24	−142	−58	−84	−130	70	110	3.9	Table III, No. 11
CpA‖	10	^3E-^2E	12	−152	−76	−67	−137	62	84	3.9	Table III, No. 11

*Not accountable as low energy cases.
‡Complexed with 9-amino acridine.
§Complexed with Ethidium Bromide.
‖Complexed with Proflavine.
Reference 1, Arnott et al. (1973); 2, Suck et al. (1973) 3, Seeman et al. (1976); 4, Rosenberg et al. (1976); 5, Hingerty et al. (1976); 6, Rubin et al. (1971); 7, Seeman et al. (1975); 8, Jain et al. (1977); 9, Tsai et al. (1977); 10, Westhof and Sundaralingam (1980).

adopts two types of conformations, namely, the A-RNA type and the $g^+g^+g^+$ bend type. Of these two, the A-RNA type helical conformer is accountable as a low energy case in the present study. However, the $g^+g^+g^+$-bend conformer remains as a high energy case.

Comparison of the Conformational Characteristics of DNA and RNA Subunits

It has been of great interest for research workers in the field of nucleic acids to positively distinguish the conformational characteristics of ribonucleic acids from those of deoxyribonucleic acids as these two members of the nucleic acid family adopt very different secondary and tertiary structures. A few points on this aspect can be highlighted from the studies on the dimeric subunits of RNA and DNA presented in this and in the preceding papers. The investigations made by us confine to four DNA subunits, dApdA, dApdT, dTpdA and dTpdT and four RNA subunits ApG, ApU, CpG, and CpU. Although it is not proper to draw any

definite conclusions with this limited data, we unambiguously note some general features with regard to the specificities of the two families of subunits and they are given below:

(*a*) The ^3E-^3E sugar-pucker sequence is noted to be the most favored state in both the RNA and DNA subunits as we note more number of local minima in this domain for most of the units studied. In the case of RNA subunits the ^3E-^3E sugar sequence is also equally preferred as the ^3E-^2E sequenced state, but ^2E-^3E and ^2E-^2E sequences are highly prohibited. On the other hand in the DNA subunits it is noted that the two sugar sequences ^3E-^3E and ^2E-^2E are more or less equally favored again as reflected by the number of local minima, but to a lesser degree than in the case of ^3E-^2E sequence. The ^2E-^3E sequence is also adoptable and is actually present in a few conformations, but as a least preference. The prohibited nature of the ^2E-^2E and ^2E-^3E sugar sequences in the RNA subunits explains the very limited occurrence of ^2E sugar-pucker states in the crystal structures of $tRNA^{Phe}$ molecules. Since all the four types of sugar puckerings are adoptable by the DNA subunits, it could be stated that the mixture of ^2E and ^3E sugar-pucker states will be more in polydeoxyribonucleotides and any study focussed on the chain dimensions of the polydeoxynucleotides and their tertiary folding should consider this aspect for explaining the experimental observations.

(*b*) The occurrence of a *high anti* base orientation in the 3′-nucleotide residue in most of the nonhelical conformations having either ^3E-^3E or ^3E-^2E sugar sequence and its complete absence in the 5′-nucleotide residues is a common feature noted in both the families of the subunit systems. This reveals that the influence of the 5′-phosphate group is very similar in both RNA and DNA subunits. In the case of RNA subunits the *high anti* base orientation is not adopted in the 5′-nucleotide residue of the four dinucleoside monophosphates. However, one conformation close to the C-DNA-type structure predicted for the two DNA subunits, dApdA and dTpdA, adopts a *high anti* base orientation. The absence of this type of base orientation in the case of RNA subunits could be attributed to the unstable nature of the conformations having ^2E-^2E sugar sequence.

(*c*) Another interesting common feature in both the families of subunit systems is the adoption of a conformation having $(\omega', \omega, \psi) = (g^-, g^-, g^+)$ and ^3E-^2E mixed sugar-pucker sequence. This specific conformation is found to be a low energy state only for CpG in the case of RNA subunits and only for dTpdA in the case of DNA subunits. In both cases, the base sequence is of the type, pyrimidine-purine. As noted already, this conformation is capable of producing an extended backbone course. This is consistent with the experimental observations that the pyrimidine-purine base sequence is a pre-requisite for the formation of intercalative complex crystals of drug and dinucleoside monophosphate.

(*d*) The flexibility around the C(5′)—O(5′) bond seems to be another feature to be mentioned. A good number of low energy conformations predicted for the two RNA subunits ApU and CpU adopting ψ values around $-60°$ invariably assume the conformation about C(5′)—O(5′) bond in the vicinity of $\phi = 120°$. This skewed orientation of ϕ may probably be due to the hydrogen bond-type interactions between the C(2′) hydroxyl group of the 3′-nucleotide residue with one of the oxygen atoms of the 5′-phosphate. The low energy conformations for these units however, do not adopt ϕ values in this region with $\psi = 60°$ or 180°. Interestingly no such ϕ orientation is noted in any of the low energy conformations predicted for the four subunits of DNA.

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