Classical Potential Energy Calculations for ApA, CpC, GpG, and UpU. The Influence of the Bases on RNA Subunit Conformations

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Synopsis

Classical potential energy calculations have been made for the ribodinucleoside monophosphates ApA, CpC, GpG, and UpU. Van der Waals', electrostatic, and torsional contributions to the energy were calculated, and the energy was minimized with the seven backbone conformational angles as simultaneously variable parameters. At the global minimum, ApA and CpC have conformations like double helical RNA: the angles ω' and ω are g^-g^-, the sugar pucker is C3'-*endo*, and the bases are *anti*. GpG and UpU, on the other hand, have the ω',ω angle pair g^-t at the global minimum, and for GpG the bases are *syn*. Energy contour maps for ω' and ω show two broad, low energy regions for ApA, CpC, and UpU: one is g^-g^-, and the second encompasses g^-t and g^+g^+ within a single low-energy contour. The two regions are connected by a path at 10–13 kcal./mole. For GpG, with bases *syn*, however, only a small low-energy region at g^-t is found. The helical 'A' RNA conformation is 8.5 kcal/mole higher for this molecule. Thus, the base composition is shown to influence the conformations adopted by dinucleoside phosphates. Comparison of calculations with experimental data, where available, show good agreement.

INTRODUCTION

The conformations of ribodinucleoside monophosphates (XpY), the dimeric subunits of RNA, are of particular interest because these are the smallest subunits which have all the conformational angles of the polymer. Consequently, it is possible that they are the conformational building blocks of the ribonucleic acids.

Previous classical potential energy calculations for guanylyl-3',5'-cytidine (GpC), provided a trial structure that was successfully used in solving the crystal structure of the Ca^{2+} salt of this molecule in the space group $P2_1$.[1] Subsequent calculations for both GpC and uridylyl-3',5'-adenosine (UpA) delineated the minimum energy conformations of
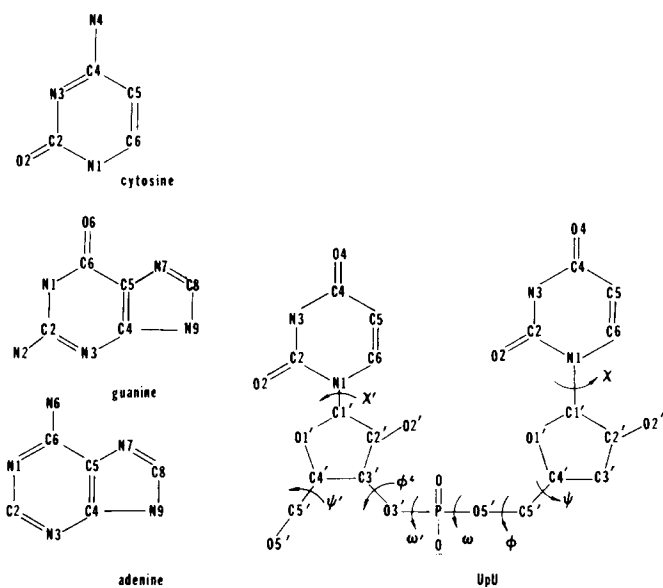
Fig. 1. The structure, numbering convention, and conformational angles are shown for UpU. The structures and numbering convention for cytosine, guanine, and adenine are also shown.

TABLE I
Notation for ω', ω Conformational Regions

ω', ω	Notation[a]
60°,60°	g^+g^+
60°,180°	g^+t
60°,300°	g^+g^-
180°,60°	tg^+
180°,180°	tt
180°,300°	tg^-
300°,60°	g^-g^+
300°,180°	g^-t
300°,300°	g^-g^-

[a] g = *gauche*; t = *trans*.

these molecules *in vacuo*.[2] These studies differed from previous potential energy calculations on nucleosides and nucleotides in that (a) a complete dinucleoside phosphate molecule is studied and (b) the energy is minimized with seven out of the eight conformational angles as simultaneously variable parameters. We found that keeping some of the backbone angles fixed spuriously alters the energy ranking of the local minima. Two important conformational regions for the dihedral angles ω' and ω (see Fig. 1) were observed. The lowest energy conformation of both GpC and UpA was like double-helical RNA, with ω' and ω in the g^-g^- conformation (see Table I). Within about 1 kcal/mole and also of wide accessibility was the g^+g^+ region.

In the present work, we calculate the *in vacuo* minimum energy conformations for adenylyl-3',5'-adenosine (ApA), cytydylyl-3',5'-cytosine (CpC), guanylyl-3',5'-guanosine (GpG), and uridylyl-3',5'-uracil (UpU). The ω',ω conformation space is also studied in detail, with the calculation of energy contour maps. We find that, with bases *anti* and sugar pucker C3'-*endo*, ApA, CpC, and UpU have two broad, low energy ω',ω conformational regions. The first is the well-known righthanded helix, g^-g^-: this is the global minimum for ApA and CpC. The second encompasses both g^-t and g^+g^+ within a single low-energy contour. The global minimum for UpU lies within this contour, in the g^-t region. GpG, by contrast, has the bases *syn*, and only one small low energy ω',ω region near g^-t. The helical RNA conformation is 8.5 kcal/mole above the global minimum for GpG. Our calculations therefore indicate that the bases influence the global minimum energy conformations of dinucleoside monophosphates *in vacuo*.

METHODS

Potential energy calculations for the molecules *in vacuo* were carried out as previously described in detail,[1] using the method of Scott and Scheraga.[3,4] Briefly, the following equation was used to evaluate the energy:

$$E = \sum_{i<j}\sum(a_{ij}r_{ij}^{-6} + b_{ij}r_{ij}^{-12}) + \sum_{i<j}\sum 332q_iq_jr_{ij}^{-1}\epsilon^{-1} +$$
$$\sum_{k=1}^{7}\frac{V_{0,k}}{2}(1 + \cos 3\theta_k) \quad (1)$$

where the double sums extend over all atom pairs whose distance varies with the dihedral angles, r_{ij} is the distance between atom pairs, q_i is the charge on atom i, a_{ij} and b_{ij} are parameters in the $6 - 12$ potential, and ϵ is the dielectric constant. The single summation extends over all seven flexible dihedral angles, where θ_k is the kth dihedral angle, and $V_{0,k}$ is the rotational barrier height for that rotation. Values for a_{ij}, b_{ij}, q_i, $V_{0,k}$, and ϵ were taken from Refs. 5 and 6. (As noted previously,[2] an error in the charges for guanosine in Ref. 6 has been corrected.) The energy was minimized with the dihedral angles as variable parameters, using a modified version of the Powell algorithm.[7]

Figure 1 gives the structure of UpU, as well as the dihedral angle definitions. The conventions of these angles follow Sussman et al.,[8] and are given again in Table II. Also shown in Figure 1 are structures of cytosine, guanine, and adenine.

Calculations were made on the Univac 1108 computer at the Georgia Institute of Technology. The molecular modeling was performed on the PDP-10/LDS-1 interactive computer graphics system at Princeton University.

Starting conformations for the minimizations were decided upon from

BROYDE ET AL.

TABLE II
Definition of Dihedral Angles

Angle[a]	Bonds
ψ'	C3'—C4'—C5'—O5'
φ'	P—O3'—C3'—C4'
ω'	O5'—P—O3'—C3'
ω	C5'—O5'—P—O3'
φ	C4'—C5'—O5'—P
ψ	C3'—C4'—C5'—O5'
χ',χ	O1'—C1'—N9—C8 (GpG, ApA)
χ',χ	O1'—C1'—N1—C6 (UpU, CpC)

[a] All angles A—B—C—D are measured clockwise from A to D when viewed along B—C. A eclipsing D is 0°.

TABLE III
Selected Minimum Energy Conformations of GpG, Dihedral Angles (degrees),
Starting Conformation/Final Conformation

χ'	φ'	ω'	ω	φ	ψ	χ	ΔE, kcal/ mole	ω',ω region
			C3' *endo* sugar pucker					
180/221	200/197	60/339	60/144	180/193	60/53	180/182	0.	g^-t
15/—1	200/181	60/24	60/77	180/198	60/57	180/185	2.2	g^+g^+
180/223	200/265	290/270	60/158	180/252	60/53	15/8	3.9	g^-t
180/218	200/219	290/316	290/289	180/168	60/66	15/30	6.8	g^-g^-
15/13	200/181	60/22	60/78	180/198	60/61	15/14	7.2	g^+g^+
180/219	200/190	60/67	60/96	180/197	60/65	180/180	7.6	g^+g^+
15/6	200/203	290/313	290/271	180/182	60/58	15/22	8.5	g^-g^-
			C2'-*endo* sugar pucker					
221/235	197/211	339/56	144/176	193/174	53/58	182/196	4.5	g^+t
223/218	265/267	270/281	158/174	252/253	53/76	8/—12	6.8	g^-t
13/35	181/227	22/315	78/160	198/206	61/57	14/—23	7.0	g^-t

observed ranges for the torsional angles in nucleosides and nucleotides,[9] taking into account the results of our earlier potential energy calculations.[2] Since calculations by Olson,[10] and by Yathindra and Sundaralingam[11] show that guanosine prefers the *syn* region for the glycosidic torsion angle, both *syn* and *anti* conformations were explored for GpG. For the other molecules, only the *anti* region was studied. The following combination of angles was used initially, except where otherwise noted: $\chi' = 15°$ *(anti)*, 180° *(syn)*; $\varphi' = 200°$; $\omega' = 60°$, 180°, 290°; $\omega = 60°$, 290°; $\varphi = 180°$; $\psi = 60°$; $\chi = 15°$ *(anti)*, 180° *(syn)*. ψ' was set at 60° as earlier work[1,2] has shown that this angle remains very close to this value. The 180° region for ω proved to be important for GpG, and therefore this region was also used as a starting conformation for the other molecules. For GpG two consecutive sets of runs were made for each trial. In the first run, only χ', χ, ω', and ω were permitted to vary, with the other angles fixed. Then the minima obtained were used as starting conformations in a simultaneous seven parameter minimization. Since no saving of computer time resulted, this procedure was not continued for the other dinucleoside phosphates.

The sugar puckers in all of the above runs were set at C3'-*endo*. Arnott[12] has noted that the C3'-*endo* pucker is necessary in the helical conformations of polyribonucleotides, in order to avoid short intramolecular distances. Furthermore, all crystal structures of ribodinucleoside monophosphates[8,13–16] and ApApA[17] are in the C3'-*endo* conformation with the exception of the 3' ribose in the 9-aminoacridine–ApU cocrystal.[18] The C2'-*endo* region was also examined, but less exhaustively. For the C2'-*endo* runs, the most important minima obtained with C3'-*endo* were used as starting conformations. In subsequent work we find that the preferred regions of ψ' depend on sugar pucker and on the ω',ω region. However, little influence on the backbone angles is noted and the energy differences are small.

RESULTS
Minimum Energy Conformations of GpG, UpU, CpC, and ApA
GpG

Twenty-four different starting conformations were examined for GpG with the ribose pucker C3'-*endo* and $\psi = 60°$. Table III shows selected low-energy conformations found for this molecule. At the global minimum, the glycosidic torsion angles χ' and χ are both *syn*, the ω',ω angle pair are in the g^-t region and ψ is in the 60° range. To examine further ψ, the global minimum angles were employed as a starting conformation except that ψ was initiated at 180° and 300°. Conformations resulting from these trials were of higher energy than for the 60° region. In agreement with earlier calculations on 5'-GMP,[11] we also find that χ for the 5' base is near 180° when ψ is near 60°. This is outside the usually observed *syn* range of 210–260°. The unusual behavior of χ for 5' *syn* bases has been attributed[10,11] to unfavorable steric interactions between the base and the 5' phosphate when χ is in its more normal region. With both bases *syn*, we find only one low-energy ω',ω region, namely g^-t. The g^+g^+ and g^-g^- regions are of higher energy. The A form of RNA conformation (C3'-*endo*, bases *anti*, and ω',ω g^-g^-) has a conforational energy 8.5 kcal/mole higher than the global minimum.

Some calculations were also made with the riboses set at C2'-*endo*. The lowest energy conformation again found χ' and χ *syn*. Unlike C3'-*endo*, the ω',ω angle pair are in the g^+t range. The lowest minimum energy conformation obtained for C2'-*endo* ribose pucker is 4.5 kcal/mole higher than the lowest energy conformation found with C3'-*endo*.

UpU

Results for UpU are given in Table IV. The lowest energy conformation is again found in the g^-t region of ω',ω, with bases *anti* and sugar pucker C3'-*endo*. The A form helical RNA (g^-g^-) range is 1.3 kcal/mole above the global minimum. The lowest energy conformation for the C2'-*endo* pucker is 1.8 kcal/mole above that of C3'-*endo*. The ω',ω

BROYDE ET AL.

TABLE IV
Minimum Energy Conformations of UpU, Dihedral Angles (degrees),
Starting Conformation/Final Conformation

χ'	φ'	ω'	ω	φ	ψ	χ	ΔE, kcal/ mole	ω',ω region
			C3'-*endo* sugar pucker					
11/33	200/194	60/340	60/128	180/189	60/63	15/7	0.	g^-t
15/6	200/204	180 290/317	290/271	180/180	60/59	15/20	1.3	g^-g^-
15/33	200/274	290/328	180/158	180/195	60/64	15/16	2.0	g^-t
15/7	200/197	60/80	180/163	180/169	60/55	15/19	7.8	g^+t
15/42	200/188	180/213	60/59	180/171	60/58	15/25	9.2	tg^+
15/38	200/260	290/273	60/61	180/173	60/60	15/25	10.0	g^-g^+
15/44	200/197	60/93	290/308	180/182	60/62	15/35	10.3	g^+g^-
			C2'-*endo* pucker					
6/33	204/290	317/201	271/305	180/118	59/60	20/72	1.8	tg^-
33/33	274/289	328/327	158/188	195/176	64/64	16/33	4.8	g^-t
33/26	194/205	340/344	128/169	189/195	63/50	7/11	6.5	g^-t

TABLE V
Minimum Energy Conformations of ApA, Dihedral Angles (degrees),
Starting Conformation/Final Conformation

χ'	φ'	ω'	ω	φ	ψ	χ	ΔE, kcal/ mole	ω',ω region
			C3'-*endo* sugar pucker					
15/7	200/205	180 290/311	290/273	180/179	60/58	15/18	0.	g^-g^-
15/14	200/180	60/19	60/81	180/195	60/64	15/18	1.0	g^+g^+
15/54	200/196	290/333	180/146	180/194	60/52	15/10	1.9	g^-t
15/8	200/227	290/320	60/110	180/199	60/78	15/19	3.1	g^-g^+
15/106	200/190	60/47	180/182	180/175	60/58	15/63	3.5	g^+t
15/55	200/188	180/212	60/58	180/176	60/60	15/25	14.0	tg^+
15/84	200/196	60/93	290/307	180/183	60/61	15/35	14.7	g^+g^-
			C2'-*endo* sugar pucker					
14/−18	180/201	19/253	81/202	195/188	64/69	18/7	1.8	g^-t
54/45	196/226	333/317	146/158	194/200	52/62	10/−19	2.8	g^-t
7/43	205/288	311/325	273/233	179/167	58/58	18/−28	5.1	g^-t

angle pair is tg^-. A higher energy conformation resulted when this minimum was used as a starting conformation with $\psi = 180°$ instead of 60°.

ApA

Results for ApA are given in Table V. Three important minima occur for C3'-*endo* pucker. The global minimum is the helical RNA region, g^-g^-. g^+g^+ is 1 kcal/mol higher, and g^-t is 1.9 kcal/mole above the global minimum. For C2'-*endo*, the lowest energy minimum is 1.8 kcal/mole above that of the C3'-*endo* global minimum, and ω',ω is g^-t.

CpC

Three important minima for ω',ω are obtained for C3'-*endo* (see Table VI): the helical RNA region, g^-g^-, is the global minimum, with g^-t 1.1 kcal/mole above it, and g^+g^+ at 2.4 kcal/mole. The lowest energy C2'-*endo* conformation is 5.8 kcal/mole above the C3'-*endo* global minimum and the ω',ω pair is g^-g^-.

TABLE VI
Minimum Energy Conformations of CpC Dihedral Angles (degrees)
Starting Conformation/Final Conformation

| χ'	φ'	ω'	ω	φ	ψ	χ	ΔE, (kcal/ mole)	ω',ω region
			C3'-endo sugar pucker					
15/32	200/193	$^{180}_{290}$/288	290/277	180/175	60/61	15/47	0.	g^-g^-
15/30	200/196	290/339	180/128	180/189	60/63	15/10	1.1	g^-t
15/20	200/178	60/22	60/81	180/194	60/64	15/40	2.4	g^+g^+
15/15	200/186	60/79	180/169	180/180	60/49	15/25	8.0	g^+t
15/36	200/187	180/213	60/59	180/172	60/54	15/37	9.4	tg^+
15/36	200/260	290/273	60/59	180/174	60/56	15/38	9.9	g^-g^+
15/36	200/195	60/93	290/308	180/183	60/61	15/43	10.3	g^+g^-
			C2'-endo sugar pucker					
32/45	193/245	287/280	277/288	175/169	61/55	47/45	5.8	g^-g^-
20/44	178/198	22/12	81/107	194/193	64/70	40/36	7.4	g^+g^+
30/44	196/198	339/12	128/107	189/193	63/70	10/36	7.4	g^+g^+

In summary, the lowest energy conformations of CpC and ApA are similar to A RNA. However, the lowest energy conformations of GpG and UpU differ from the A form RNA helix in ω' and ω. In addition, the bases are *syn* for GpG. The other angles are found within the ranges generally favored by nucleotides.[2,5,6,9,11,19–21] The helical RNA conformation is particularly disfavored for GpG, while for UpU it is a low-energy conformation. Figure 2 shows three of the four molecules at their respective global minima. The global minimum of ApA has the bases stacked in parallel arrangement and the sugar oxygens O1' point in the same direction. The conformation of CpC, not shown, is similar to ApA. UpU and GpG both have their bases tilted and their O1's point away from each other. For all molecules examined, the lowest energy conformations obtained with the C2'-endo pucker are less favorable than their C3'-endo counterparts. Although an exhaustive study of the C2'-endo conformations was not made, this result is supported by the structural data from dinucleoside monophosphates and ApApA.

Minimum Energy ω',ω Regions Calculated for Dinucleoside Phosphates

Figure 3 summarizes all the minimum energy regions found in the present work for the C3'-endo ribose pucker, as well as all regions obtained earlier for UpA and GpC.[1,2] The conventional notation for the staggered ethane-like conformations (Table I) is used to describe the minimum energy regions. It should be noted, however, that this description is used to cover a rather wide range. The 20° minimum for the g^+g^+ (20°,80°) region and the 340°,130° minimum for g^-t are closer to eclipsed than staggered. Scott and Scheraga[3] found similar eclipsed conformations to be of low energy in their calculations on hydrocarbons. Even when the sum of the van der Waals' radii used to calculate the parameter b in the Lennard–Jones potential, Eq. (1), is increased by 0.2 Å
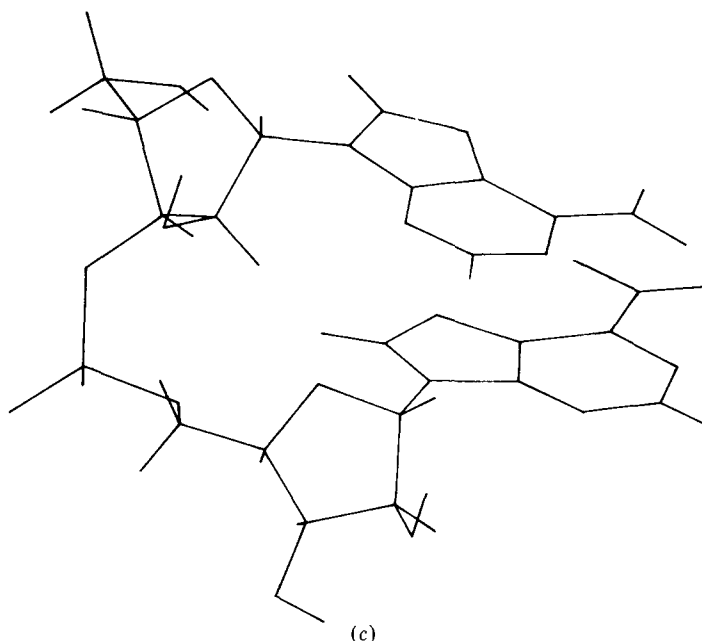
(a)

(b)

Fig. 2 (continued)

(c)
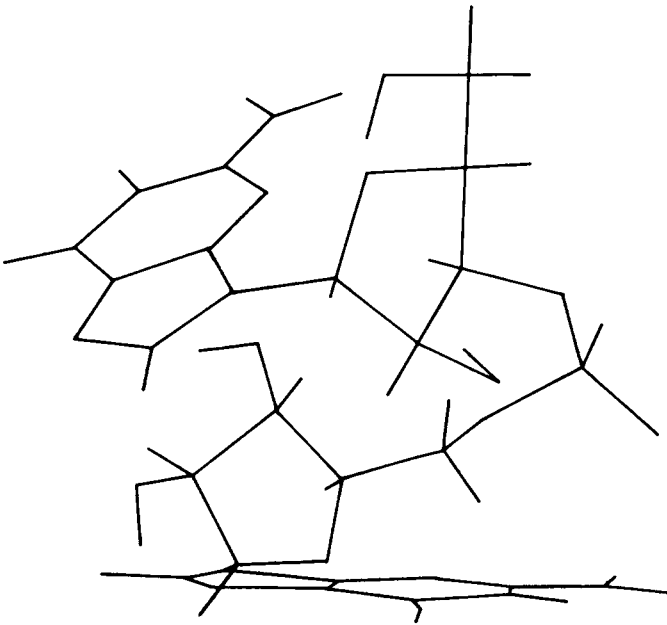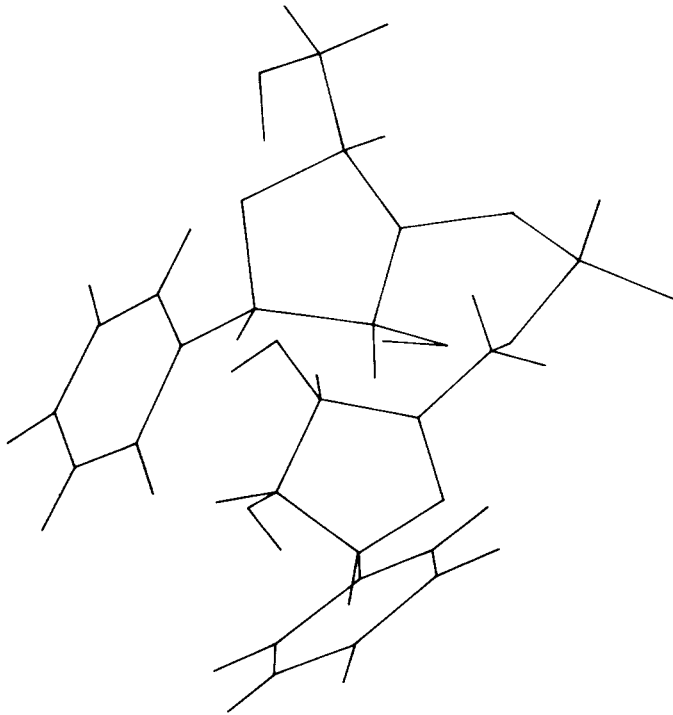
Fig. 2. The lowest energy *in vacuo* conformations for (a) GpG, (b) UpU, and (c) ApA are drawn. See Tables III, IV, and V for torsion angles. All ribose puckers are C3'-endo.

(to prevent other favorable interactions from causing a violation of the van der Waals' contact distance) the minima still remain in the same vicinity. Local minima closer to the conventional staggered conformations are found (78°,88°), but at somewhat higher energy[2] (see Table VII).

Three low-energy regions (0–2.5 kcal/mole) are apparent. g^-g^-, the A form RNA conformation, is the global minimum for GpC, UpA, CpC, and ApA. It is of low energy for all XpY's examined except GpG, where it occurs at 8.5 kcal/mole. g^+g^+ occurs as a minimum for all molecules except UpU. g^-t is the global minimum for GpG and UpU, and lies within 2 kcal/mol for CpC and ApA. This conformation could introduce a bend if incorporated into helical RNA.

Energy Contour Maps of the ω',ω Angle Pair

To obtain information on the conformational space accessible to the ω',ω angle pair, energy contour maps for these angles were calculated. The maps are obtained by fixing all conformational angles except ω' and ω at values near the low-energy minima (see Tables III–VI). Then the energy is computed over 18° intervals of ω' and ω, for a total of 400 points. Fixing six of the eight conformational angles permits the calculation of the energy maps within a reasonable amount of computer time. However, the choice of angles may influence the appearance of the con-

360

CpC (10.8)──┐ ┌─UpU (10.3)
UpA (14.8)──┤ ├─ApA (12.6)
GpG syn (15.1)─┘ └─GpC (11.1)

GpG syn (15.1)
CpC   (0.0)
ApA   (0.0)
GpG   (8.5)

UpA (0.0)
GpC (0.0)

UpU (1.3)
UpA (0.0)

288

──GpC (5.0)
──UpA (1.9)

UpA (6.0)

UpA (4.6)

──GpG (5.9)

216

UpA (6.2)
GpC (7.7)

GpG syn (0.0)
ApA   (1.9)
UpU   (2.0)
UpU   (0.0)
CpC   (1.1)

ApA(3.5)─●

UpU(7.8)
CpC(8.0)

ω (degrees)

144

CpC(2.4)
GpC(1.1)
UpA(0.5)
GpG(7.2)
ApA(1.0)

GpG(23.0)
CpC(10.0)
UpA(10.8)

ApA (11.9)
UpU (9.2)
GpC(14.5)
GpG syn(15.0)

GpC (1.7)
ApA (3.1)

GpG syn (10.9)

CpC (9.9)
UpU(10.0)

72

GpG syn (7.6)
GpC   (7.7)
UpA   (4.2)

0   72   144   216   288   360

ω' (degrees)

Fig. 3. A summary of minimum energy conformations in ω',ω space calculated for six XpY's: UpU, GpG, CpC, ApA, UpA, and GpC. The conformations for UpA and GpC were calculated previously.[2] Bases are *anti* except where it is designated otherwise. The numbers in parenthesis represent ΔE, the energy difference between the local minimum shown and the global minimum energy conformation for that molecule.

TABLE VII

Comparison of Calculated *In Vacuo* Minimum Energy Conformation with Conformations of RNA Subunits in Crystals

Molecule	χ'	φ'	ω'	ω	φ	ψ	χ	ΔE,[a] kcal/ mole	Ref.
GpC[b] (exptl)-2-12	217—227	287—291	279—295	165—179	45—59	14—38		16
GpC (exptl)	13	209	291	284	186	51	25		15
GpC (calcd)	4	205	296	279	182	57	24	0.0	1
UpA (exptl)	12	206	81	82	203	55	37		8,13
UpA (calcd)	5	180	19	81	193	69	6	0.5	2
UpA (calcd)	7	180	78	88	233	59	31	4.0	2
$A^1pA^2pA^3$									
A^1pA^2 (exptl)	7	223	281	297	160	c	24		17
ApA (calcd)	7	205	311	273	179	58	18	0.0	this work
A^2pA^3 (exptl)	24	207	76	92	186	c	21		17
ApA (calcd)	14	180	19	81	195	64	18	1.0	this work

[a] ΔE is the difference in energy between global minimum and local minima.

[b] Four different GpC conformers per unit cell. Range of observations is given.

[c] ψ was given as *gauche—gauche* (60° region).

tour maps. To evaluate this point, energy maps were obtained for CpC with $\chi' = 30°$, $\chi = 30°$; $\chi' = 15°$, $\chi = 15°$; and $\chi' = 30°$, $\chi = 45°$. This examines the effect of small changes in base orientation on ω',ω conformation space.

Conformational energy maps for CpC and UpU are shown in Figure 4. The energy map for GpG is given in Figure 5. The overall features of the maps for ApA (not shown), UpU, and CpC are similar to those previously published for GpC and UpA.[2] Differences in detail, however, are noted. There are two broad low-energy regions. The upper region corresponds to the g^-g^- range. The lower region encompasses both g^-t and g^+g^+ in one contour here. The g^-t region was outside the low-energy contours for GpC and UpA. For UpU the lower region, which contains the global minimum, is wider than for ApA and CpC. For GpG, the ω',ω map shows a single low-energy region near g^-t when both bases are *syn.* With the bases *anti,* the map for GpG (not shown) is similar to those shown in Figure 4.

An interesting finding from these maps is that the two low-energy regions of ApA, UpU, and CpC are connected by paths at 10–13 kcal/mole.

ω' (degrees)

(a)
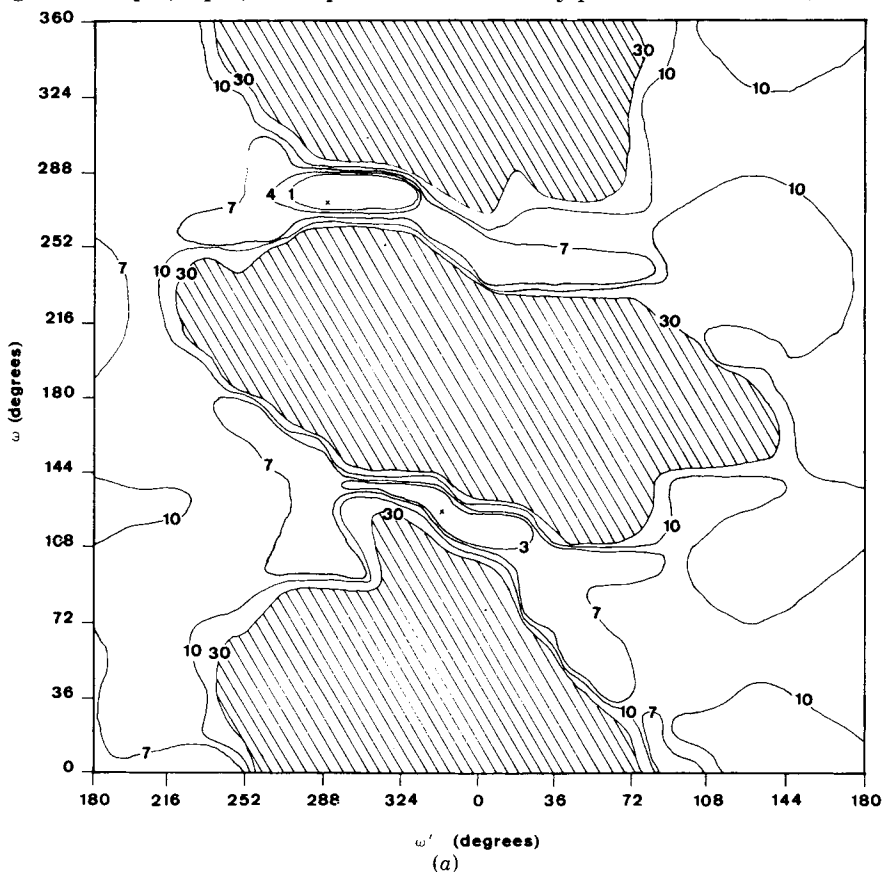
Fig. 4 (continued)
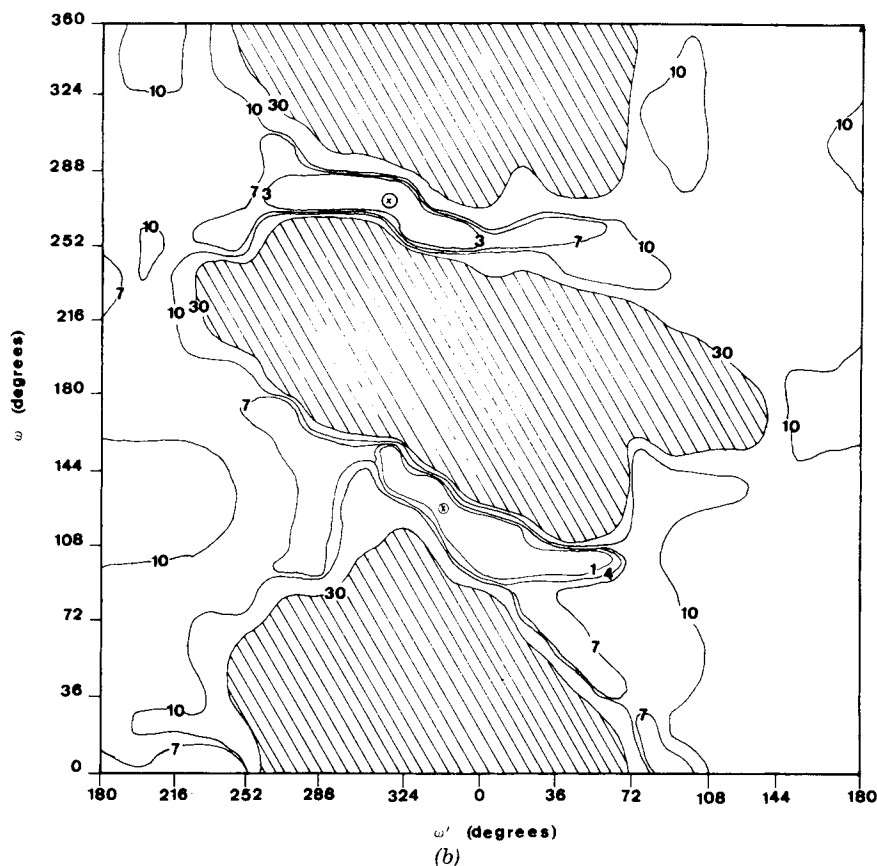
Fig. 4. Energy contour maps of ω' vs. ω. The two lowest energy conformations are designated by x's. Shaded areas indicate $\Delta E \geq 30$ kcal/mole. (a) CpC with remaining angles fixed at $\chi = 15°$, $\psi' = 60°$, $\varphi' = 200°$, $\varphi = 185°$, $\psi = 60°$, and $\chi' = 30°$. (b) UpU with fixed angles the same as CpC except $\chi' = 15°$.

A path connecting the low-energy region of GpG to the next higher energy domain at 7 kcal/mole also exists, at about 15 kcal/mole.

Small changes in the orientation of the bases can affect the ω',ω conformational energy map. Figure 4b shows the energy map for CpC with $\chi' = 30°$, $\chi = 30°$. The global minimum is located in the g^-g^- region with the second minimum in the g^-t region. This is the result obtained previously where the backbone torsional angles were varied simultaneously (Table VI). Figure 6a depicts the low-energy contours with $\chi' = 30°$ and $\chi = 45°$. g^-g^- remains the lowest energy region and covers about the same area as in Figure 4c. However, the g^-t region is substantially changed. The low-energy conformation found in the g^-t region of Figure 4c ($\omega' = 339°$, $\omega = 128°$) is not within a low-energy contour in Figure 6a. This is perhaps not surprising since this g^-t low-energy conformation has $\chi = 10°$ rather than 45° (Table VI). When χ' and χ are both set at 15° (Fig. 6b), g^-t becomes the lowest energy re-
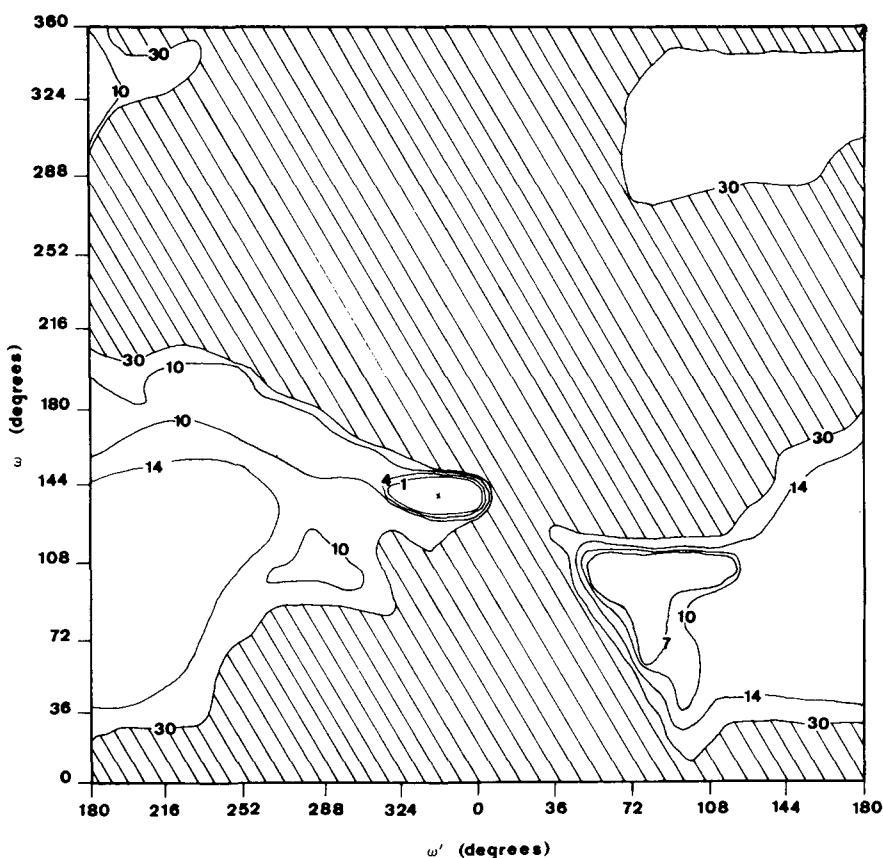
Fig. 5. Energy contour map of ω' vs. ω for GpG with bases *syn*. Lowest energy conformation is indicated by an x. Stipled areas denote $\Delta E \geq 30$ kcal/mole. Angles are fixed at $\chi' = 220°$, $\psi' = 60°$, $\varphi' = 200°$, $\varphi = 185°$, $\psi = 60°$, $\chi = 180°$.

gion. Additionally, both the g^-t and g^-g^- regions change in shape. The other features of the energy maps in Figures 6a and 6b are similar to Figure 4c. These results show that the location and order of the low-energy conformations are affected by small changes in base orientations. This points out the importance of varying all dihedral angles simultaneously in calculating minimum energy conformations. Holding some angles fixed can rearrange the order of the minimum energy conformations.

DISCUSSION

Influence of Bases on Dinucleoside Monophosphate Conformation

The results indicate that the bases can affect the conformation of dinucleoside phosphates. This influence can be noted in the lowest ener-
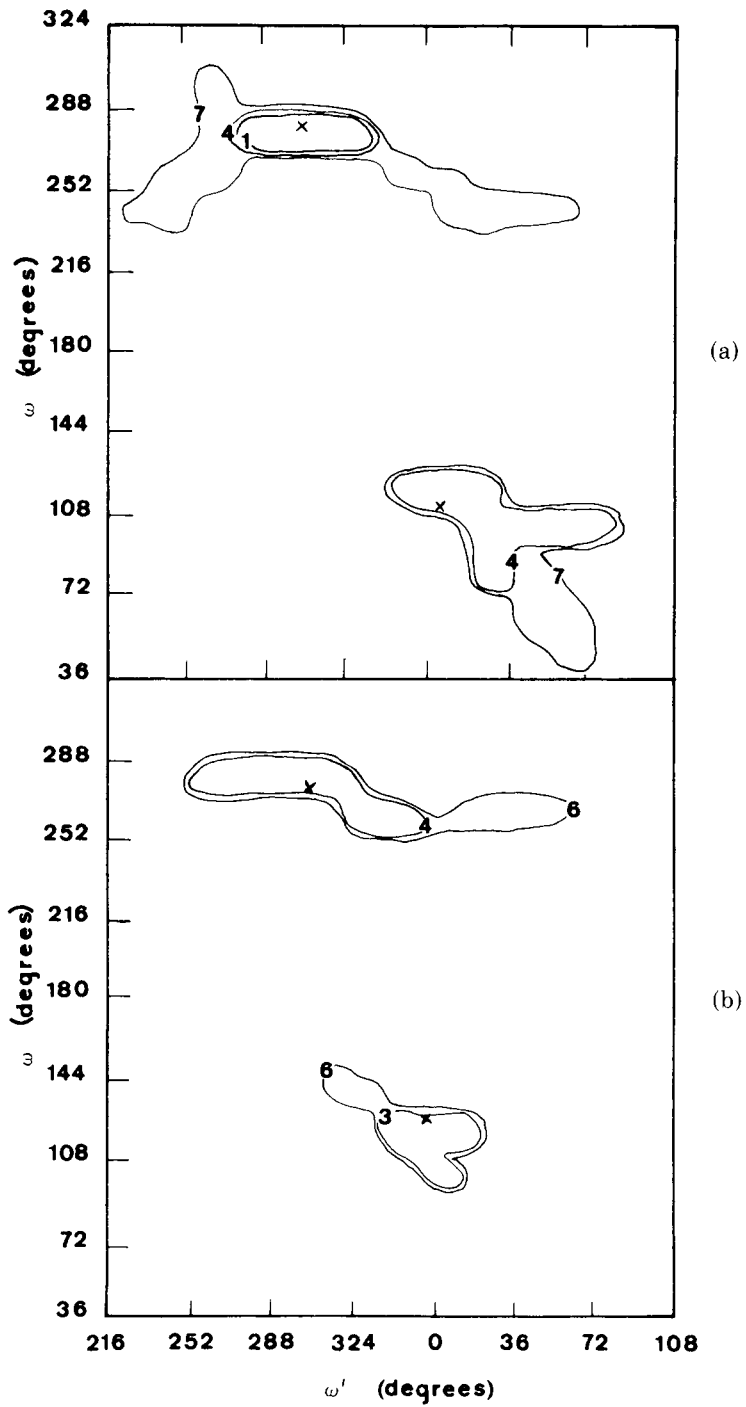
Fig. 6. Low energy contours of ω' vs. ω for CpC. Remaining torsion angles are fixed at positions given in Figure 4b except for χ' and χ. (a) $\chi' = 30°$, $\chi = 45°$; (b) $\chi' = 15°$, $\chi = 15°$.

gy conformations and the size and shape of the conformational regions accessible to the molecules.

The lowest energy conformation of GpG is particularly unusual. The two *syn* conformations of the bases appear to be stabilized by different interactions. The 3' base has a favorable interaction between N3 and H(O5'), while the 5' base is stabilized by an interaction between phoshate oxygen and H(N2). It seems that both of these are needed for the *syn* conformation to be the global minimum. For GpC, *syn–anti* conformations, with $\omega',\omega\ g^-g^-$ or g^-t, are over 5 kcal/mole above the helical global minimum.

Favorable sugar–base interactions appear to stabilize the g^-t conformations of GpG and UpU. For ApA and CpC the base–base interactions apparently dominate and yield the g^-g^- conformation.

The ω',ω maps show, in all cases except GpG, two low-energy regions. One is g^-g^-, and the second encompasses both g^-t and g^+g^+. That g^-t and g^+g^+ are enclosed in the same low-energy contour indicates flexibility between these conformations in dinucleoside monophosphates. Recent calculations[22] on a dinucleoside triphosphate, without bases, indicate g^+g^+ is sterically inhibited. This implies that only g^-g^- and g^-t are favorable conformations for polynucleotides. The energy paths at 10–13 kcal/mole connecting the g^-g^- region with the g^-t–g^+g^+ regions suggest the activation energy needed for conformational changes between these regions. For GpG with both bases *syn*, the ω',ω map shows a restricted low-energy region at g^-t. Since glycosidic angles were fixed in calculating the ω',ω energy maps, it was not possible to determine a low-energy pathway between the global minimum region, *syn*-g^-t, and *anti*-g^-g^-, the helical RNA conformation. A minimum energy of 8.5 kcal/mole separates these conformations *in vacuo*.

Comparison of Conformational Calculations with Experimental Observations

The conformations which are calculated for the dinucleoside phosphates can be compared with the results of X-ray crystallographic analyses. An earlier work[2] has compared the predicted minimum energy conformations for GpC and UpA with their crystal structures. Table VII summarizes parts of these earlier findings. Also shown is a comparison of calculated conformations for ApA to the crystalline structure of ApApA. This table shows the extent of agreement between calculated and observed conformations. The predicted global minimum for GpC is in excellent agreement with the crystal structures of this molecule. Although the predicted global minimum has not been observed for crystalline UpA, low-energy conformations at 0.5 kcal/mole and 4 kcal/mole above the global minimum are in good agreement with one of two UpA conformers. The difference between the calculated and observed conformations of UpA can be accounted for by the large number of inter-

molecular bonds in the crystal. A full discussion of this has been given.[2] For ApA, our calculations find the global minimum at g^-g^-. g^+g^+ is one kcal/mole higher. In crystalline ApApA, which has two ω',ω angle pairs, one pair was g^-g^- and the other g^+g^+; again indicating good agreement.

Finally a comparison of our findings with conformational studies of dinucleoside phosphates and single-stranded homopolynucleotides in solutions is of interest. The calculated results for UpU and GpG predict that these sequences are unlike helical RNA in their lowest energy conformations. This is consistent with the conclusion that UpU[23] and poly(rU)[24] have little propensity for forming single-stranded stacked structures at pH 7.0. Poly(rG)[25] is known to have solution properties unlike single-stranded A-form RNA polymers. ApA[23] and poly(rA),[26] and CpC[23] and poly(rC),[27] on the other hand, form stacked structures in solution, in agreement with the helical RNA global minima calculated for ApA and CpC. Recent laser Raman studies[28] also indicate that both UpA and GpC occur in conformations similar to that of helical RNA in solution.

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