

Conformational Stability in Dinucleoside Phosphate Crystals. Semiempirical Potential Energy Calculations for Uridyl-3'-5'-Adenosine Monophosphate (UpA) and Guanylyl-3',5'-Cytidine Monophosphate (GpC)

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Synopsis

Classical potential energy calculations were performed for the dinucleoside phosphates UpA and GpC. Two widely accessible low-energy regions of conformation space were found for the ω' , ω pair. That of lowest energy contains conformations similar to helical RNA, with ω' and ω in the vicinity of 300° and 280° , respectively. All five experimental observations of crystalline GpC, two of ApU, and the helical fragment of ApApA fall in this range. The second lowest region has ω' and ω at about 20° and 80° , respectively, which is in the general region of one experimentally observed crystalline conformer of UpA, and the nonhelical region of ApApA.

It is concluded that GpC and ApU, which were crystallized as either sodium or calcium salts, are shielded from each other in the crystal by the water of hydration and are therefore free to adopt their predicted *in vacuo* minimum energy helical conformations. By contrast, crystalline UpA had only 1/2 water per molecule, and was forced into higher energy conformations in order to maximize intermolecular hydrogen bonding.

INTRODUCTION

The conformations of dinucleoside phosphates are presently the subject of intense studies because these molecules, as dimeric subunits of RNA, are yielding significant information towards elucidating the three-dimensional structures of the larger polymers, especially transfer RNA. tRNA is of particular interest because it is the largest crystalline species of a nucleic acid for which atomic resolution X-ray data are available. The crystal structures of three naturally occurring dinucleoside phosphates have been solved to date: uridylyl-3',5'-adenosine monophosphate (UpA),^{1,2} adenylyl-3',5'-uridine monophosphate (ApU),³ and guanylyl-3',5'-cytidine monophosphate (GpC),^{4,5} the latter in two crystalline modifications. Of these

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three molecular species, GpC and ApU were found to have conformations similar to double-helical RNA and to be base paired dimerically in the standard Watson-Crick scheme.⁶ By contrast, UpA crystallized with two different molecular conformations in the asymmetric unit, neither of which was like helical RNA.

Because bond lengths and bond angles in these molecules do not differ significantly from analogous smaller molecules, such as nucleosides and nucleotides, attention centers mainly on the eight flexible torsion angles that define the molecular conformation of a dinucleoside phosphate. Both potential energy calculations and observations have been leading to the inference that there is considerable rigidity in these conformational angles.⁷⁻⁹ Except for ω' and ω , which give the molecule its basic conformational flexibility, the dihedral angles are restricted to lie within about 30° of a mean value. From a consideration of these findings, Kim et al.⁹ have proposed seven possible structures for dinucleoside phosphates, using UpA as a model. These basic units could then be useful as guides for construction of models of transfer RNA consistent with X-ray diffraction data.

We have recently extended our potential energy analysis to include minimization of the energy of an entire dinucleoside phosphate molecule, GpC,⁵ with respect to all the dihedral angles. Previous studies¹⁰⁻¹³ had considered the effects of varying at most two angles simultaneously, and involved smaller fragments of RNA. Our calculation for the isolated GpC molecule¹⁴ (*in vacuo*) yielded a conformation like helical RNA as the lowest energy form. This conformation was then packed in the unit cell and used successfully as a trial structure in the crystallographic refinement of the X-ray data. The results of the X-ray study¹⁵ showed four similar but not identical GpC molecules in the crystal, all also conformationally similar to the predicted helical structure.

With the hope of gaining some further insight into the structural regions accessible to dinucleoside phosphates, we have made a number of semi-empirical potential energy calculations for UpA, as well as some additional calculations for GpC, minimizing the energy with respect to the dihedral angles. The calculations are of three types. First, the energy is minimized with respect to all eight dihedral angles, with starting conformations from observed ranges of nucleosides and nucleotides, for the purpose of determining the most stable conformations. Second, the energy is minimized as a function of ω' and ω alone, with three of the remaining angles fixed at values suggested by the global minimization and the other three as variable parameters. In this way, we explore the shape of conformation space and analyze the conformational freedom of the molecule within its minima. Third, observed conformations of crystalline UpA and GpC are taken as the starting parameters for an energy minimization, to see how close the actual conformations are to the calculated local minima. With the same view, we calculate energies for the seven conformations proposed by Kim et al.,⁹ and minimize these energies with respect to the conformational angles.

We find two widely accessible low-energy regions of conformation space for the ω' , ω angle pair. The region of lower energy contains conformations similar to helical RNA,^{7,16} with ω' and ω in the vicinity of 300° and 280° , respectively. The second lowest energy conformation has ω' and ω at about 20° and 80° , respectively, which is in the general region of one of the observed crystalline conformers of UpA.^{1,2}

METHOD

The method employed here was similar to that described earlier by us.⁵ Coordinates of the molecules were generated via the linked atom algorithm of Scott and Scheraga,^{17,18} using as bond distances and bond angles those given by Arnott et al.¹⁹ for the uracil, adenosine, guanosine, cytidine, ribose, and phosphate moieties. For the purpose of potential energy calculations, these quantities do not differ significantly from those reported in the actual crystal structures of UpA and GpC, and were not permitted to vary. The Scott-Scheraga method allows direct calculation of Cartesian coordinates, needed in the later energy calculations, from the dihedral angles themselves, which are our main independent variables.

The energy E of a molecule was calculated by the equation

$$E = E_{\text{nb}} + E_{\text{el}} + E_t \quad (1)$$

where E_{nb} , E_{el} , and E_t are the nonbonded, electrostatic, and torsional contributions to the total energy, respectively. These quantities are computed as follows:

$$E_{\text{nb}} = \sum_{i < j} (a_{ij} r_{ij}^{-6} + b_{ij} r_{ij}^{-12}) \quad (2)$$

$$E_{\text{el}} = \sum_{i < j} 332 q_i q_j r_{ij}^{-1} \epsilon^{-1} \quad (3)$$

$$E_t = \sum_{k=1}^8 \frac{V_{o,k}}{2} (1 + \cos 3\theta_k). \quad (4)$$

In Eqs. (2)–(4), r_{ij} is the distance in angstroms between atoms i and j , q_i is the partial charge assigned to atom i , ϵ is the dielectric constant, $V_{o,k}$ is the barrier to internal rotation for the k th dihedral angle, and θ_k is the value of that angle. All energy units are in kilocalories per mole. The double sums extend over all atom pairs in the dinucleoside phosphate, with the following exceptions: 1) atoms bonded to each other are omitted; 2) atoms bonded to a common atom (1–3 interactions) are omitted; 3) interactions that are invariant as a function of the dihedral angles, such as those within a structurally rigid unit like a planar base are omitted, as they serve only to shift the zero base of the energy but not the relative energies themselves. Values for the nonbonded parameters a_{ij} and b_{ij} , Coulombic charges q_i , and torsional barriers V_o were taken from the literature.* The dielectric constant was taken as 4.0.^{10,11}

* See Ref. 10–12. We are grateful to one of the referees for pointing out typographical errors for the charges on guanine C5 and ribotide C3', and report results here in which the correct values of +0.024 and +0.103, respectively, were used.

Minimization of the energy, with the dihedral angles as variable parameters, was by a modified version of the Powell algorithm,²⁰ obtained from the Courant Institute of New York University; the minimization was carried to an accuracy of 1° in each dihedral angle at the minimum, with no angle permitted to vary by more than 100° at any step.

Figures 1 and 2 give the structure and atomic numbering scheme for UpA and GpC. The dihedral angle conventions, which follow Sussman et al.,¹ are given in Table I.

A PDP-10/LDS-1 interactive computer graphics system was most useful in displaying the calculated conformations.

RESULTS AND DISCUSSION

In vacuo Minimum Energy Conformation for UpA and GpC

As in the calculations for GpC,⁵ starting conformations for UpA energy minimizations were chosen from a set of dihedral angle values representative of observed ranges for nucleotides.^{7,8,16} Starting values for the seven

TABLE I
Definition of Dihedral Angles for GpC and UpA

| 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' | O1'—C1'—N9'—C8 (GpC) |
| χ' | O1'—C1'—N1—C6 (UpA) |
| χ | C6—N1—C1'—O1' (GpC) |
| χ | C8—N9—C1'—O1' (UpA) |

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

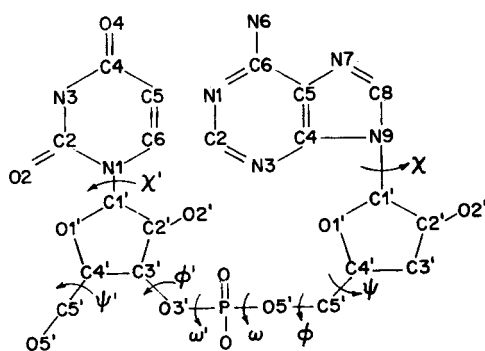


Fig. 1. Structure, numbering convention, and conformational angles for UpA.

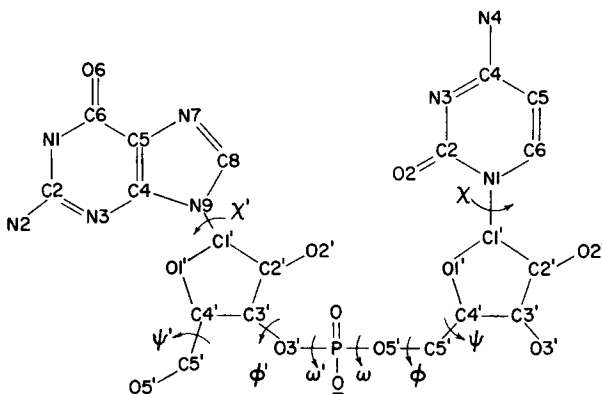


Fig. 2. Structure, numbering convention, and conformational angles for GpC.

angles that were permitted to vary were $\chi' = 15^\circ$ (anti); $\phi' = 225^\circ$; $\omega' = 60^\circ, 180^\circ, 290^\circ$; $\omega = 60^\circ, 290^\circ$; $\phi = 180^\circ$; $\psi = 60^\circ$; and $\chi = 15^\circ$ (anti). In addition, in some cases the conformational angle ψ' was fixed at 60° , as experimental results for GpC showed that ψ' was close to this value.

The ribose pucker was kept at C3'-endo, for a number of reasons. For one thing, our previous calculations for GpC¹⁴ showed C3'-endo to be lower in energy than C2'-endo. Furthermore, this is the only sugar conformation that has been observed in dinucleoside phosphates, and is believed to be the predominant conformation in RNA.¹⁶

The base conformations were initially both anti. Calculations by Yathindra and Sundaralingam⁸ have shown this to be preferred for 5'-nucleotides, especially with C3'-endo puckering. Furthermore, a calculation made here of an energy map without minimization, for UpA, for χ' versus χ showed a single broad, deep well at anti-anti. In this calculation, the dihedral angles were set as follows: $\psi' = 61^\circ$; $\phi' = 215^\circ$; $\omega' = 291^\circ$; $\omega = 284^\circ$; $\phi = 180^\circ$; and $\psi = 60^\circ$. The energy was calculated at 20° intervals of χ' and χ . A plot of energy contours for χ' versus χ is shown in Figure 3. The minimum is at $\chi' = 0^\circ$, $\chi = 60^\circ$. Evidently, the 5' base is stabilized at *anti* by the C3'-endo sugar puckering. The *anti* conformation of the 5' base in turn destabilizes the *syn* region for the 3' base by nonbonded and electrostatic interactions. Further analysis of our results shows these two components to be of approximately the same magnitude.

Since the X-ray crystallographic work had shown that UpA was protonated at the N1 atom of the adenine^{1,2} (due to its being crystallized at low pH), our calculations were initially made for both the protonated and unprotonated case. Table II gives the results of minimization of the six protonated and six unprotonated UpA starting conformations, as well as results of similar calculations for GpC. The global minima attained by all three molecular species were nearly identical and are like the conformation of helical RNA.^{7,16,21} For protonated and unprotonated UpA, the minima reached from all six starting conformations were virtually identical.

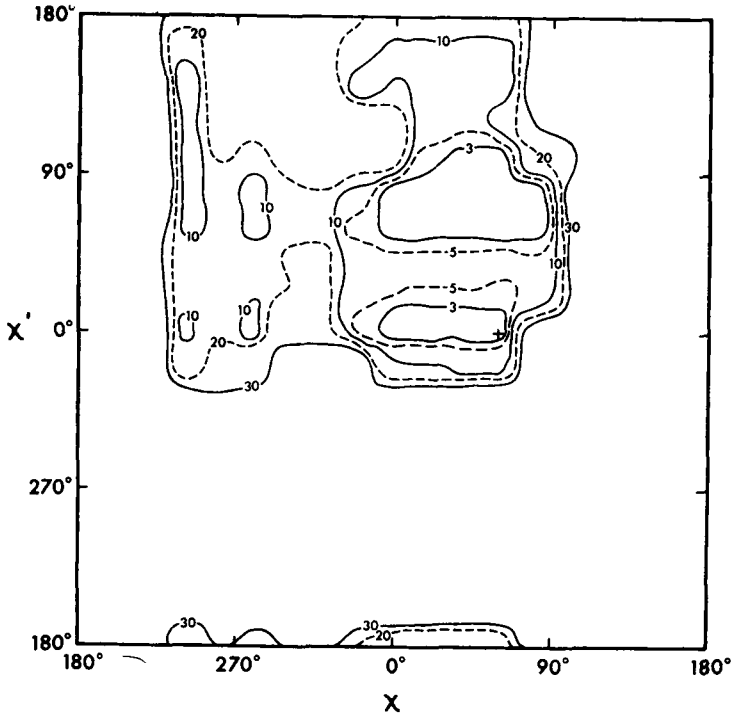


Fig. 3. Unminimized energy contour map for UpA, for the dihedral angles χ' vs. χ . Other dihedral angles are fixed at $\psi' = 61^\circ$, $\phi' = 215^\circ$, $\omega' = 291^\circ$, $\omega = 284^\circ$, $\phi = 180^\circ$, and $\psi = 60^\circ$. Global minimum indicated by +.

Furthermore, for GpC, only starting conformation 5 (Table II) led to a minimum that was different from that of UpA. The angles χ' , ψ' , ϕ' , ϕ , ψ , and χ remained within the general region of their starting conformations prior to minimization. This result is consistent with the concept of relative rigidity for these torsion angles,⁷⁻⁹ and it suggested that ω' and ω are the critical angles whose joint conformation space ought to be explored further in these studies. The ranges calculated for these other angles are χ' , -13 – $+7^\circ$; ψ' , 61 – 62° for GpC (fixed at 60° for UpA); ϕ' , 180 – 240° ; ϕ , 153 – 205° ; ψ , 54 – 76° ; χ , 5 – 45° .

Average values of the 18 results for each of these angles are $\chi' = 2^\circ$, $\psi' = 61^\circ$, $\phi' = 192^\circ$, $\phi = 178^\circ$, $\psi = 65^\circ$, $\chi = 25^\circ$.

The main variation in conformation was attributable to ω' and ω , the other angles serving to absorb somewhat the brunt of any high-energy situation created by a particular combination of ω' and ω . Average values of ω' and ω for these 18 minima, and of ΔE , the energy difference between the local and the global minimum, are given in Table III. From Tables II and III, then, it can be seen that the conformation with the lowest energy and widest accessibility has dihedral angles like helical RNA, with ω' and ω averaging 297° and 279° , respectively. Second is a region with ω' and ω centered at 20 and 81° , respectively.

TABLE II
Minimum Energy Conformations of UpA and GpC

| Starting Conformation | | | | | | Final Conformation | | | | | | | | | | Energy, ΔE^b | |
|-----------------------|---------|---------|-----------|--------|--------|-----------------------|-----------------------|-------------|---------|------|---------|---------|-----------|--------|--------|-------------------------|------|
| Dihedral Angles (deg) | | | | | | Dihedral Angles (deg) | | | | | | | | | | | |
| χ' | ψ' | ϕ' | ω' | ϕ | ψ | χ | Region | | χ' | | ψ' | ϕ' | ω' | ϕ | ψ | χ | |
| | | | | | | | Molecule ^a | Designation | | | | | | | | | |
| 15 | 50 | 225 | 60 | 180 | 60 | 15 | G | 2 | 2 | 62 | 180 | 18 | 80 | 194 | 57 | 23 | 1.1 |
| | | | | | | | U | 2 | 5 | (60) | 180 | 20 | 81 | 193 | 69 | 7 | 0.5 |
| | | | | | | | U ⁺ | 2 | 7 | (60) | 181 | 20 | 81 | 193 | 71 | 6 | 0.3 |
| 15 | 50 | 225 | 60 | 290 | 180 | 15 | G | 4 | -13 | 62 | 182 | 24 | 250 | 153 | 70 | 45 | 5.9 |
| | | | | | | | U | 4 | -2 | (60) | 181 | 25 | 248 | 155 | 69 | 48 | 1.9 |
| | | | | | | | U ⁺ | 4 | 2 | (60) | 186 | 7 | 243 | 174 | 64 | 40 | 2.2 |
| 15 | 50 | 225 | 180 | 180 | 60 | 15 | G | 5 | -3 | 62 | 186 | 214 | 59 | 171 | 54 | 37 | 14.5 |
| | | | | | | | U | 5 | 4 | (60) | 188 | 213 | 58 | 175 | 60 | 23 | 10.8 |
| | | | | | | | U ⁺ | 5 | 5 | (60) | 188 | 213 | 58 | 174 | 60 | 23 | 10.8 |
| 15 | 50 | 225 | 180 | 290 | 180 | 15 | G | 1 | 4 | 61 | 205 | 296 | 279 | 182 | 57 | 24 | 0.0 |
| | | | | | | | U | 1 | 4 | (60) | 201 | 303 | 275 | 177 | 63 | 23 | 0.0 |
| | | | | | | | U ⁺ | 1 | 7 | (60) | 200 | 296 | 278 | 173 | 65 | 30 | 0.0 |
| 15 | 50 | 225 | 290 | 180 | 60 | 15 | G | 3 | -6 | 62 | 240 | 314 | 106 | 205 | 76 | 38 | 1.7 |
| | | | | | | | U | 2 | 5 | (60) | 180 | 19 | 81 | 193 | 69 | 6 | 0.5 |
| | | | | | | | U ⁺ | 2 | 7 | (60) | 181 | 20 | 81 | 193 | 70 | 5 | 0.3 |
| 15 | 50 | 225 | 290 | 290 | 180 | 15 | G | 1 | 2 | 62 | 205 | 296 | 279 | 182 | 57 | 24 | 0.0 |
| | | | | | | | U | 1 | 5 | (60) | 203 | 298 | 279 | 173 | 63 | 26 | 0.0 |
| | | | | | | | U ⁺ | 1 | 7 | (60) | 202 | 295 | 281 | 171 | 63 | 30 | 0.0 |

^a G = GpC, U = UpA, U⁺ = UpA with N1-protonated adenine.

^b kcal/mol. ΔE is the difference in energy between global minimum and local minima. Global minima are -40.7, 3.0, and 2.6 kcal/mol for G, U, and U⁺, respectively.

TABLE III
Five Major Global Minimum Energy ω', ω Regions for UpA and GpC

| Region | Number of Observations | Average angle (Deg) $\omega' \quad \omega$ | Average ^a energy: $\overline{\Delta E}$ (kcal/mol) |
|--------|------------------------|---|--|
| 1 | 6 | 297 279 | 0.0 |
| 2 | 5 | 20 81 | 0.5 |
| 3 | 1 | 314 106 | 1.7 |
| 4 | 3 | 18 247 | 3.0 |
| 5 | 3 | 213 58 | 12.0 |

^a Boltzmann averages at $T = 298^\circ\text{K}$.

Three other local regions of minimum energy were also noted and have been tabulated in Tables II and III. In Figure 4, we show models of the UpA molecule in each of the five minimum energy regions. Region 1, the global minimum, corresponds to the helical RNA conformation. Careful observation of region 2, the $(20^\circ, 80^\circ)$ conformer, via computer graphics, showed that while the $\omega' = 20^\circ$ angle generates a mild eclipsing situation, there are no bad intramolecular contacts present, and favorable van der Waals and electrostatic interactions between the U and A bases and between the U and A ribose groups compensate. The other local minima likewise appear to be free of repulsive contacts.

The seven conformations proposed by Kim et al.⁹ as models for tRNA secondary structure were also used as starting conformations in a minimization for both UpA and GpC. Energies prior to and after minimization were calculated. The results are given in Table IV. Since these conformations were delineated as likely for dinucleoside phosphates, little change in the dihedral angles was expected upon minimization. This turned out to be the case in most instances, but not all. Table V shows the ω' and ω values before and after minimizing. Comparing these with the average ω', ω regions and their energies given in Table III, a number of observations are possible. 1) Conformations designated by Kim et al. as P1, P3, S3, A1, and A2 stay in the same general regions on minimizing; 2) P2 remains in its starting conformation only for UpA, and with anomalous ϕ and χ of 122° and 95° , respectively, while for GpC the P2 conformation becomes like helical RNA; 3) conformation S1 is shifted, going over to what is here designated as region 4; 4) at the minimum, P1, S3, and A1 are higher energy "neighbors" of regions 4, 3, and 2, respectively; 5) conformation A2 goes over to angles that are the same as region 5. P3, the helical range, is like our region 1.

Energies of Observed Conformations of UpA and GpC

Energies for the conformations of the two UpA molecules actually occurring in the crystal^{1,2} were calculated to see how these compared with the five minimum energy ω', ω regions discussed above. A similar calculation was made for the four GpC molecules found in space group P2₁ by

TABLE IV
Minimization of Conformations Proposed by Kim et al.^a

| Name | Mole- cule | Starting Conformation | | | | | | | | Final Conformation | | | | | | | | ΔE (kcal/mol) | |
|------|---------------|-----------------------|---------|---------|-----------|----------|--------|--------|--------|----------------------|---------|---------|-----------|----------|--------|--------|--------|--------------------------|------|
| | | Dihedral Angle (deg) | | | | | | | | Dihedral Angle (deg) | | | | | | | | | |
| | | χ' | ψ' | ϕ' | ω' | ω | ϕ | ψ | χ | χ' | ψ' | ϕ' | ω' | ω | ϕ | ψ | χ | | |
| P1 | U | 15 | 60 | 215 | 90 | 270 | 200 | 55 | 40 | 14.2 | 5 | 61 | 199 | 91 | 306 | 183 | 61 | 35 | 11.1 |
| | G | | | | | | | | | 18.8 | -2 | 61 | 196 | 91 | 308 | 183 | 61 | 43 | 14.8 |
| P2 | U | 15 | 60 | 215 | 170 | 270 | 200 | 55 | 40 | 15.1 | 5 | 61 | 187 | 195 | 278 | 122 | 53 | 95 | 6.0 |
| | G | | | | | | | | | 20.1 | 4 | 61 | 205 | 297 | 278 | 182 | 57 | 26 | 0.0 |
| P3 | U | 15 | 60 | 215 | 290 | 280 | 200 | 55 | 40 | 12.3 | 3 | 61 | 204 | 320 | 268 | 181 | 59 | 17 | 0.0 |
| | G | | | | | | | | | 15.5 | 4 | 61 | 205 | 297 | 278 | 182 | 57 | 26 | 0.0 |
| S1 | U | 15 | 60 | 215 | 110 | 200 | 200 | 55 | 40 | 14.8 | -2 | 62 | 181 | 25 | 248 | 155 | 69 | 49 | 1.9 |
| | G | | | | | | | | | 19.9 | -13 | 62 | 182 | 23 | 250 | 153 | 70 | 45 | 5.0 |
| S3 | U | 15 | 60 | 215 | 250 | 160 | 200 | 55 | 40 | 13.1 | 3 | 61 | 187 | 292 | 163 | 181 | 59 | -1 | 6.2 |
| | G | | | | | | | | | 17.7 | 4 | 61 | 192 | 301 | 159 | 169 | 53 | 23 | 7.7 |
| A1 | U | 15 | 60 | 215 | 80 | 80 | 200 | 55 | 40 | 33.5 | 7 | 60 | 180 | 76 | 90 | 234 | 57 | 32 | 4.2 |
| | G | | | | | | | | | 37.8 | 4 | 61 | 184 | 74 | 85 | 222 | 63 | 41 | 7.7 |
| A2 | U | 15 | 60 | 215 | 180 | 80 | 200 | 55 | 40 | 14.1 | 5 | 61 | 188 | 213 | 58 | 175 | 60 | 24 | 10.7 |
| | G | | | | | | | | | 19.3 | -2 | 62 | 186 | 214 | 59 | 171 | 54 | 37 | 14.5 |

^a kcal/mol. Energy above global minimum prior to minimization.

Hingerty et al.¹⁵ and for the GpC molecule observed in space group C2 by Day et al.⁴ These conformations were then used as initial parameters in a minimization. The results are shown in Table VI. The energies, ΔE_0 for UpA, are high, namely, 17.9 and 15.8 kcal/mol above the global minimum for this molecule. Upon minimization, however, relatively minor adjustments in the angles of one of the UpA conformers produced a much

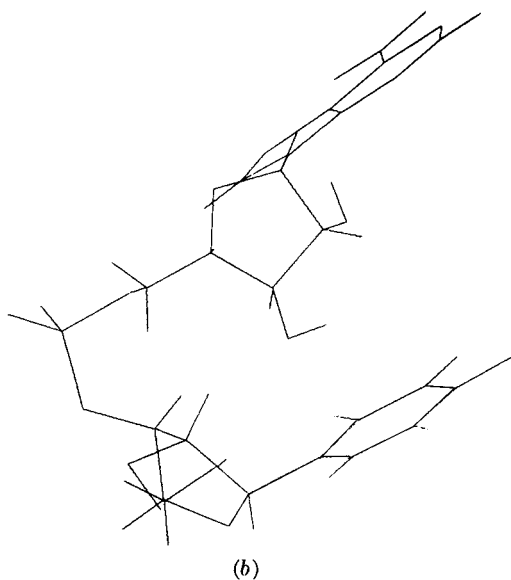
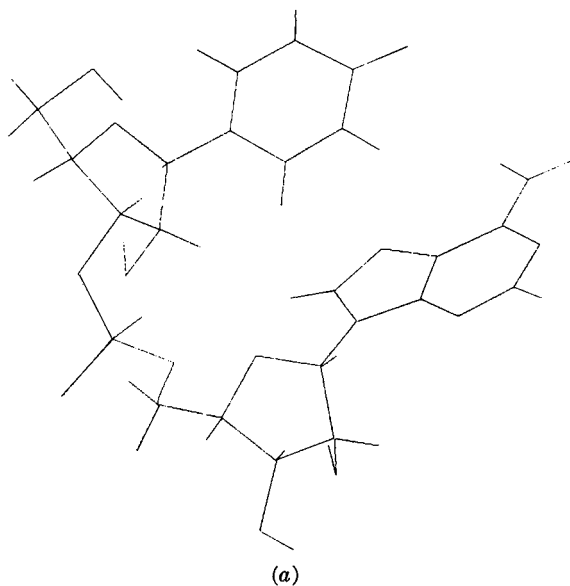


Fig. 4 (continued)

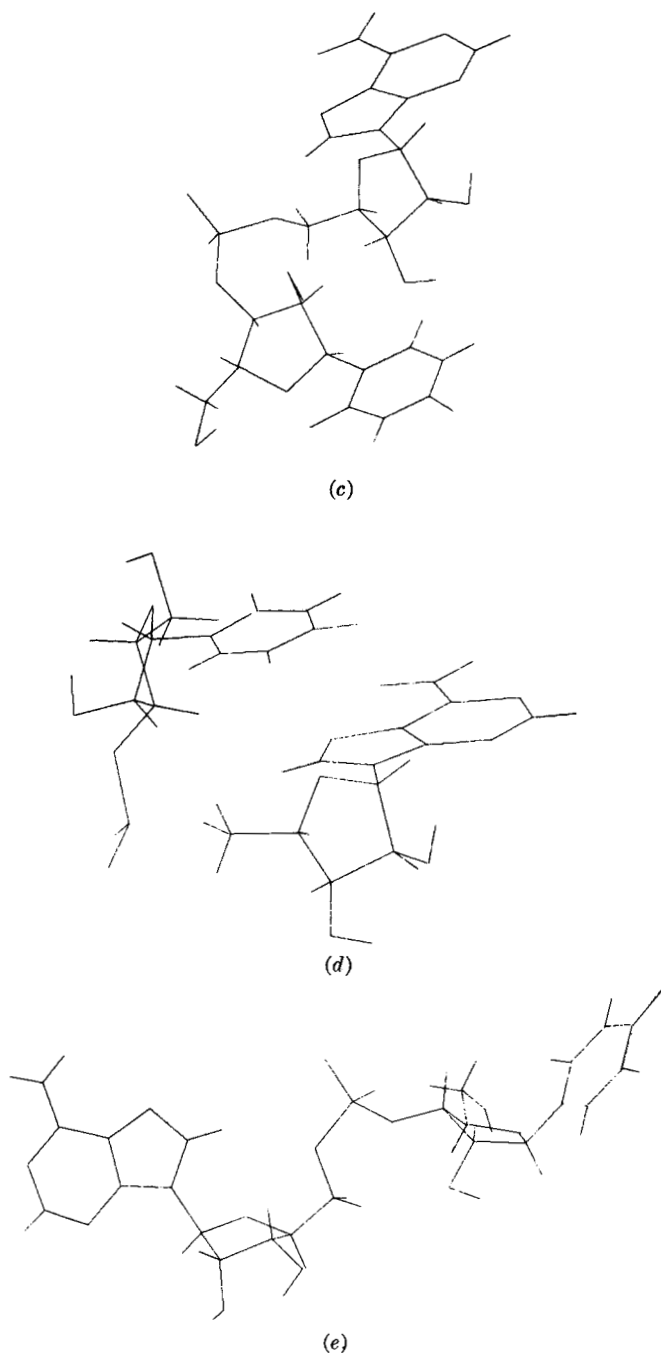


Fig. 4. UpA in five average minimum energy conformations. $\chi' = 3^\circ$, $\psi' = 61^\circ$, $\phi' = 196^\circ$, $\phi = 182^\circ$, $\psi = 64^\circ$, and $\chi = 26^\circ$. (a) Region 1: $\omega' = 297^\circ$, $\omega = 279^\circ$. (b) Region 2: $\phi' = 20^\circ$, $\omega = 81^\circ$. (c) Region 3: $\omega' = 314^\circ$, $\omega = 106^\circ$. (d) Region 4: $\omega' = 18^\circ$, $\omega = 247^\circ$. (e) Region 5: $\omega' = 213^\circ$, $\omega = 58^\circ$.

TABLE V
Behavior of Phosphodiester Angles ω' and ω in Minimizations of Table IV

| Name | Molecule | Starting Conformation (deg) | | Final Conformation (deg) | | Nearest Corresponding Minimum Energy Region (Table III) | ΔE (kcal/mol) |
|------|----------|-----------------------------|----------|--------------------------|----------|---|-----------------------|
| | | ω' | ω | ω' | ω | | |
| P1 | U | 90 | 270 | 91 | 306 | 4 | 11.1 |
| | G | | | 90 | 308 | 4 | 14.8 |
| P2 | U | 170 | 270 | 195 | 278 | — | 6.0 |
| | G | | | 297 | 278 | 1 | 0.0 |
| P3 | U | 290 | 280 | 320 | 268 | 1 | 0.0 |
| | G | | | 297 | 278 | 1 | 0.0 |
| S1 | U | 110 | 200 | 25 | 248 | 4 | 1.9 |
| | G | | | 23 | 250 | 4 | 5.0 |
| S3 | U | 250 | 160 | 292 | 163 | 3 | 6.2 |
| | G | | | 301 | 159 | 3 | 7.7 |
| A1 | U | 80 | 80 | 76 | 90 | 2 | 4.2 |
| | G | | | 74 | 85 | 2 | 7.7 |
| A2 | U | 180 | 80 | 213 | 58 | 5 | 10.7 |
| | G | | | 214 | 59 | 5 | 14.5 |

lower energy value within region 2. For the other conformer, substantial changes in the dihedral angles resulted during minimization. In particular, ω' and ω were shifted to the helical RNA range. By contrast, the observed GpC molecules are all similar to each other and close to the global minimum as well. Therefore, minimization produced only small changes in conformation.

ω' , ω Maps

The energies of UpA and GpC were minimized for a complete set of values of ω' and ω , to obtain a contour map in which every point on the map corresponds to the conformation of least energy for the given ω' and ω . For these minimum energy maps, ω' and ω were incremented in 20° intervals for GpC and 36° intervals for UpA. (The results for GpC, which was run first, had indicated that 36° intervals were adequate.) At each point the energy was minimized with respect to χ , ϕ , and ϕ' , while χ' was fixed at 4° , ψ' at 60° , and ψ at 60° (the latter three values suggested by comparison of the preceding calculations with experimental angles). Initial values for the three variable parameters were $\chi = 20^\circ$, $\phi' = 195^\circ$, and $\phi = 180^\circ$. This type of study was suggested by the observation, noted above for UpA that an otherwise plausible conformation of seemingly high energy might be relieved by minor changes in only one or two other angles.

Contour plots of the results are shown in Figures 5 and 6. Two major broad and deep favorable regions result, corresponding to our major regions 1 and 2 of Table III. Minima for GpC were at $\omega' = 300^\circ$, $\omega = 280^\circ$, $\Delta E = 1.3$ kcal/mol for region 1, and at $\omega' = 20^\circ$, $\omega = 80^\circ$, $\Delta E =$

TABLE VI
Calculation of Energy and Minimization of Conformations Observed in Crystalline UpA and GpC

| Mole- cule | Ref. | Starting Conformation | | | | | | | | | | ΔE_0 (kcal/mol) | Final Conformation | | | | | | | | | | ΔE (kcal/mol) |
|---------------|------|-----------------------|--------------|---------|-----------|--------|--------|--------|----------|--------|--------|----------------------------|--------------------|----------|--------|--------|--------|----------|--------|--------|--------|--|--------------------------|
| | | χ' | ψ' | ϕ' | ω' | ϕ | ψ | χ | ω | ϕ | ψ | | χ | ω | ϕ | ψ | χ | ω | ϕ | ψ | χ | | |
| UpA-1 | 1, 2 | 12 | 53 | 206 | 81 | 82 | 203 | 55 | 37 | 17.9 | 7 | 60 | 180 | 78 | 88 | 233 | 59 | 31 | 4.0 | | | | |
| UpA-2 | 1, 2 | 20 | 58 | 224 | 164 | 271 | 192 | 54 | 44 | 15.8 | -1 | 61 | 177 | 256 | 283 | 164 | 53 | 103 | 4.6 | | | | |
| GpC-1 | 15 | 13 | 55 | 226 | 284 | 290 | 173 | 49 | 42 | 9.2 | 3 | 62 | 204 | 297 | 278 | 184 | 56 | 25 | 0.1 | | | | |
| GpC-2 | 15 | 3 | 49 | 219 | 287 | 295 | 172 | 57 | 13 | 6.5 | 3 | 62 | 204 | 297 | 278 | 184 | 56 | 25 | 0.1 | | | | |
| GpC-3 | 15 | -2 | 47 | 225 | 290 | 291 | 164 | 56 | 31 | 3.8 | 3 | 62 | 204 | 297 | 278 | 184 | 56 | 25 | 0.1 | | | | |
| GpC-4 | 15 | 2 | 59 | 215 | 291 | 277 | 183 | 57 | 32 | 4.9 | 4 | 61 | 207 | 294 | 283 | 179 | 55 | 25 | 0.1 | | | | |
| GpC | 4 | 13 | ^a | 209 | 291 | 284 | 186 | 51 | 25 | 7.4 | 3 | 61 | 204 | 297 | 277 | 185 | 56 | 25 | 0.1 | | | | |

^a 61° assumed.

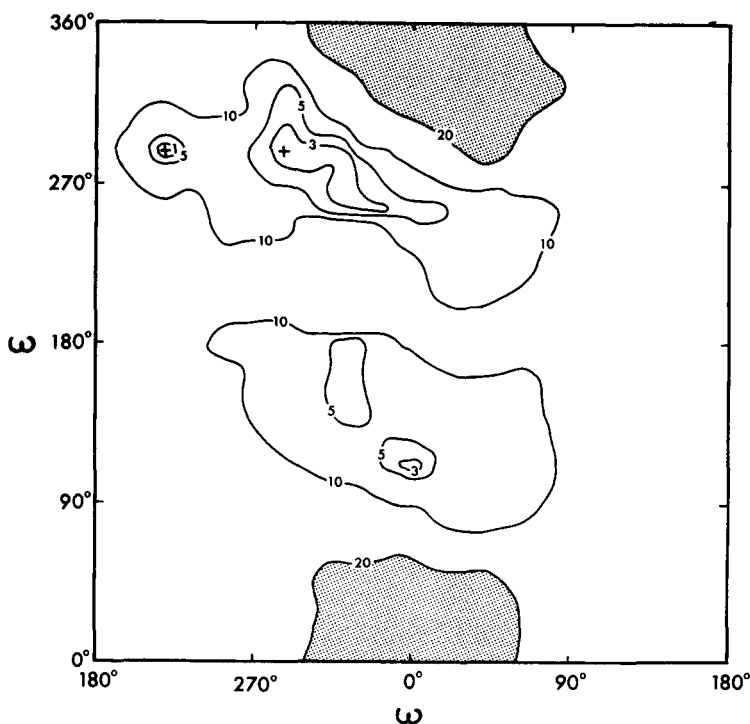


Fig. 5. Minimized energy contour map for UpA, for the dihedral angles ω' vs. ω . χ' , ψ' , and ψ were set at 4° , 60° , and 60° respectively, and the energy was minimized with respect to χ , ϕ' , and ϕ , at each point. Two global minima are indicated by +. Stippling indicates regions more than 20 kcal/mol above global minimum.

1.4 kcal/mol for region 2. For the UpA molecule a very narrow minimum within region 1 is at $\omega' = 216^\circ$, $\omega = 288^\circ$, and a broader one at $\omega' = 288^\circ$, $\omega = 288^\circ$, $\Delta E = 0.3$ kcal/mol. For region 2 of the UpA map, the minimum is $\omega' = 0^\circ$, $\omega = 108^\circ$, with $\Delta E = 1.6$ kcal/mol. These minima are at different locations and energies than the corresponding ones for regions 1 and 2 in Table III, because only three parameters were permitted to vary.

For both molecules, region 1 is of lower energy than region 2. Also, there are forbidden regions of very high energy (greater than 20 kcal/mol), which are shaded on the contour maps. In addition, a broad central plateau exists whose energy ΔE is about 10–13 kcal/mol above the global minimum for UpA, and about 15–20 kcal/mol for GpC. Thus, particularly for UpA, these regions could become accessible for the crystal if sufficient lattice energy were available to compensate.

Comparison of Calculated and Observed Conformations

Both conformers of UpA crystallized in high energy forms, with one of these having conformations of ω' and ω that are in the high energy plateau

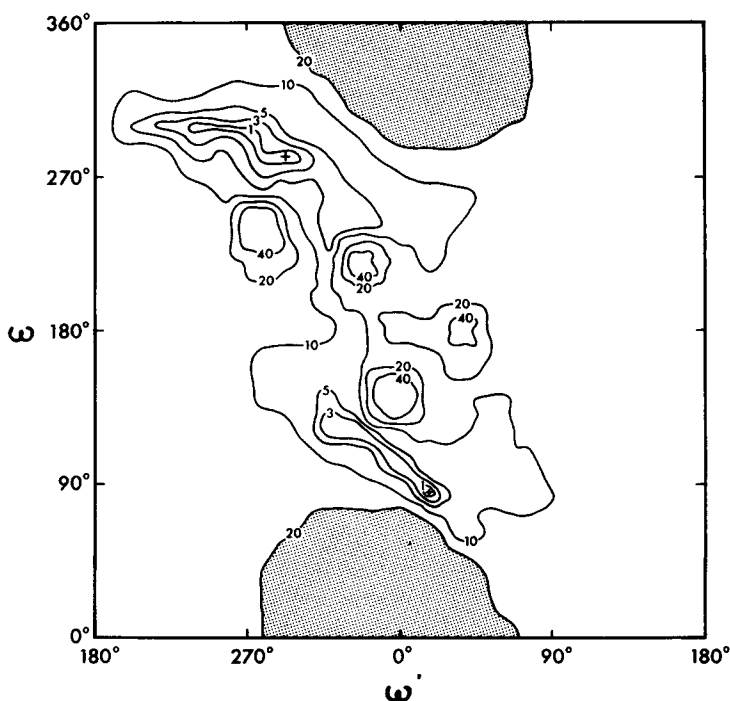


Fig. 6. Minimized energy contour map for GpC, similar to Fig. 5.

of the contour map. It is interesting to consider why this happened for UpA but not for crystalline GpC or ApU, which occur in the crystal in the lowest energy helical RNA conformation. Crystalline ApApA,²² interestingly enough, possesses both a helical and nonhelical part.

The differences in degree of hydration probably account for this observation. Crystalline GpC has $\frac{1}{2}$ Ca^{+2} or one Na^+ and nine waters of hydration per molecule, the cations balancing the charge on the singly ionized phosphate group. ApU has one Na^+ and six waters of hydration per molecule.³ ApApA²² has six waters per molecule, with the helical part being the most hydrated. As has been noted by Day et al.,⁴ the water has the effect of isolating each GpC from interactions with its neighbors; thus in crystalline GpC, each molecule participates in only two intermolecular hydrogen bonds, besides the six involved in Watson-Crick base pairing. Thus, there are four such bonds per pair of molecules, and with 3–5 kcal/mol yielded per hydrogen bond, enough energy is supplied to compensate for the distortion from the lowest energy conformation shown in Table VI. In UpA, however, molecule 1 is involved in 21 intermolecular hydrogen bonds and molecule 2 in 20 such bonds.² With virtually no waters to shield the individual UpA molecules from their neighbors ($\frac{1}{2}$ water per UpA molecule^{1,2}), the conformation in the crystal is the one that maximizes the number of intermolecular hydrogen bonds. Ten such bonds per molecule (20 per pair) will bring the energy of UpA (Table IV) in the crystal

below that of the lowest energy helical RNA form; this form, by analogy with ApU, would participate in only four hydrogen bonds for base pairing, and probably two additional intermolecular bonds (like GpC), for each pair of molecules.

The extensive hydration of GpC and ApU results at least in part from inclusion of the cation in the crystal structure, since most of the water is located in the first or second coordination sphere of the metal.¹⁵ With the charge on UpA balanced by protonation of the adenine (UpA is a zwitterion), no metallic cation, with its attendant waters, is incorporated in the crystal. Thus, when the individual molecules are shielded by water in the crystal, calculation of the lowest energy *in vacuo* conformation is more likely to lead to the correct crystal structure.

CONCLUSION

Our calculations show two widely accessible low energy regions of conformation space for dinucleoside phosphates. The lowest energy conformation is like that found for double-helical RNA. The second region is only about 0.5 kcal/mol higher in energy and is like one of the observed conformers of crystalline UpA. It has been observed by Sussman et al.¹ and by Rubin et al.² that a conformation like this form of UpA is likely to occur in the loop structure of tRNA, since this conformation allows for a sharp bend in a single strand. Our finding that such a conformation is of low energy and high probability is consistent with this view. An additional conformation, shown in Figure 4d and having $\omega' = 18^\circ$, $\omega = 247^\circ$, is similar to it, but with ω flipped by about 180° . This conformation also permits a loop, is of reasonably low energy, and therefore may well be observed in crystalline tRNA.

Of possibly greater significance, these results give a theoretical justification for the helical conformation of nucleic acids first deduced experimentally through X-ray analysis over 20 years ago.⁶ The depth and breadth of the helical RNA region of the maps demonstrate that helicity is an intrinsically favorable conformation for the single-stranded polymer,⁷ and that base pairing by hydrogen bonds is simply a further stabilizing factor for double-stranded species (RNA, DNA, and RNA-DNA hybrids). We may surmise that the size of the helical region, corresponding to its statistical weight or entropy, shows furthermore that each individual chain probably possesses sufficient flexibility to permit localized unwinding of the chain during the replication process, and also enough flexibility to permit a limited amount of mismatching of complementary base pairs in a double-stranded nucleic acid (hence, mutations), without engendering a prohibitive amount of potential energy in the backbone.

The authors thank Russell Massey for the photographic work. The molecular modeling was performed at the Princeton University Computer Graphics Laboratory, supported by NIH Grant RR-00578; other support was received from NIH GM-16539 and NSF GB-28021.

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Received November 8, 1973

Accepted March 12, 1974