Backbone Conformations in Secondary and Tertiary Structural Units of Nucleic Acids. Constraint in the Phosphodiester Conformation*

(energy calculation/polynucleotide backbone/constrained phosphodiester conformation/dinucleoside mono-, di-, and triphosphates)

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ABSTRACT The possible backbone phosphodiester conformations in a dinucleoside monophosphate and a dinucleoside triphosphate have been investigated by semi-empirical energy calculations. Conformational energies have been computed as a function of the rotations $\omega$ and $\omega'$ about the internucleotide P-O(3') and P-O(5') linkages, with the nucleotide residues themselves assumed to be in one of the preferred [C(3')-endo] conformations. The terminal phosphates in a dinucleoside triphosphate greatly limit the possible conformations for the backbone (in a polynucleotide) compared to a dinucleoside monophosphate. There appear to be two major types of conformations that are favored for the backbone. The phosphodiester conformation ($\omega, \omega'$) $\approx$ (390°,290°) characteristic of helical structures is one of them, indicating that the polynucleotide backbone shows an inherent tendency for the helical conformation. The other favored conformation is centered at ($\omega, \omega'$) $\approx$ (190°,300°) and results in an extended backbone structure with unstacked bases. A third possible conformation centered at ($\omega, \omega'$) $\approx$ (200°,60°) and the (190°, 300°) conformation appear to be important for the folding of a polynucleotide chain. The conformation ($\omega, \omega'$) $\approx$ (80°,80°), observed in a dinucleoside monophosphate and believed to be a candidate for producing an abrupt turn in a polynucleotide chain, is found to be stereochemically unfavorable in a dinucleoside triphosphate and a polynucleotide.

Considerable attention is currently being focused on the backbone conformations in the tertiary folds and loops of nucleic acids such as transfer RNA. It has been found that the nucleotide residues in a polynucleotide exhibit essentially two favored conformations differing in the sugar pucker, and the major flexibility in a polynucleotide backbone resides primarily in the internucleotide P-O links (1-3). Results from both X-ray and potential energy calculations have indicated that there can be several possible conformations about the P-O bonds for a dinucleoside monophosphate (1, 3-14). In continuation of our studies on the conformations of nucleic acids and their structural units, we recently reported (15) results of our semiempirical energy calculations on mononucleotides. Here we present results of similar calculations on a dinucleoside monophosphate and a dinucleoside triphosphate. It is shown that in contrast to a dinucleoside monophosphate, the presence of the terminal 3' and 5'-phosphate groups greatly restricts the number of possible phosphodiester conformations in a dinucleoside triphosphate. This distinction in the conformational properties of a dinucleoside triphosphate and a dinucleoside monophosphate has provided considerable insight into the folding of a polynucleotide chain in nucleic acids.

METHODS

Conformational energies for a dinucleoside monophosphate and a dinucleoside triphosphate have been computed as a function of the rotations $\omega$ and $\omega'$ about the P-O(3') and P-O(5') bonds (Fig. 1) by using partitioned potential functions that take into account the van der Waals, electrostatic, and torsional potentials. The van der Waals energies have been evaluated by use of the 6-12 Lennard-Jones function with the parameters reported earlier (15, 16). Electrostatic interactions have been estimated in the monopole approximation, with the charges given by Renugopalakrishnan et al.

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![Fig. 1. Section of a polynucleotide chain showing the backbone atoms. The notations and definitions used for the torsion angles are the same as given in ref. 1.](image-url)
Fig. 2. Equienergy contours for a dinucleoside monophosphate drawn as a function of the rotations \( \omega' \) and \( \omega \) about the P-O(3') and P-O(5') bonds, respectively. The conformations along the lines 60°, 180°, and 300° are referred to as gauche (+) (g'), trans (t), and gauche (−) (g−).

A 3-fold symmetric potential with a barrier height of 1 kcal/mole has been assumed for P-O bond torsions. In this paper the calculations are restricted to nucleotides with C(3')-endo sugars, and the torsion angles used for the nucleotide units are given in Table 1. These angles are similar to the values observed for nucleotide residues in di- and oligonucleotide structures (4, 5, 7-10). Calculations are also being carried out for dinucleoside phosphates comprising C(2')-endo sugars and alternate C(3')-endo and C(2')-endo sugars in addition to varying the nucleotide torsion angles over the observed range (1).

### Possible backbone conformations for a dinucleoside monophosphate

The conformational energy map obtained for the rotations about the adjacent P-O(3') (\( \omega' \)) and P-O(5') (\( \omega \)) bonds in a dinucleoside monophosphate is shown in Fig. 2. The global minimum at \( (\omega',\omega) \simeq (60°,170°) \) corresponding to the (g+,t) conformation and the minimum at \( (\omega',\omega) \simeq (80°,80°) = (g^+,g^-) \) have nearly the same energies. The latter conformation has been observed in the x-ray structures of the dinucleoside monophosphate, uridylyl (3',5')-adenosine, UpA (4,5), and in the A'pA2 fragment of the dinucleoside diphosphate, adenyl-(3',5')-adenyl-(3',5')-adenosine A'pA2pA2 (9). The low energy domain at \( (\omega',\omega) \simeq (290°,290°) = (g^-,-g^-) \) is characteristic of helical structures and has been observed in the dinucleoside monophosphates, adenyl-(3',5')-uridine ApU (8), guanylyl-(3',5')-cytidine GpC (7), and in the A'pApA2 fragment of the dinucleoside diphosphate, A'pApA2pA2 (9). The only other conformation (t,g−) that has been observed corresponds to the domain \( (\omega',\omega) = (190°,300°) \) and is exhibited by the second molecule of UpA (4, 5) and thymidyl-(5',3')-thymidylylate-5' (pTpT) (10). It is worthwhile to

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**Table 1. Conformational angles for the nucleotides**

<table>
<thead>
<tr>
<th>Sugar puckers</th>
<th>C(3')-endo</th>
</tr>
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<tbody>
<tr>
<td>( \psi )</td>
<td>60°</td>
</tr>
<tr>
<td>( \phi )</td>
<td>180°</td>
</tr>
<tr>
<td>( \phi' )</td>
<td>210°</td>
</tr>
</tbody>
</table>

*These angles show a range of 15–30°.*

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Fig. 3. Equienergy contours for a dinucleoside triphosphate drawn as a function of the rotations \( \omega' \) and \( \omega \) about the P-O(3') and P-O(5') bonds, respectively.

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Fig. 4. Perspective view of the conformation \( (\omega',\omega) = (80°,80°) = (g^+,g^-) \), which produces the sharpest turn in the backbone of a dinucleoside monophosphate, but appears to be unfavorable for a polynucleotide (see text), as illustrated with the molecule UpA.
would render the situation quite difficult. It is because of the lone orbitals (1, 3) that the terminal phosphates in the polydeoxyribonucleotide are not accounted for, and consideration of these orbitals may reduce the importance of the (t,t) conformation. It is interesting that ab initio calculations on dimethyl phosphate (although not an exact model of a deoxyribonucleotide) show that the fully extended conformation (t,t) turns out to be a high energy conformation (18, 19).

Possible backbone conformations for a dinucleoside triphosphate and a polynucleotide. Role of the phosphates

The above studies on dinucleoside monophosphates (see also refs. 1, 3, 6, and 11–14) show that the rotations about the ester P–O bonds are quite flexible and suggest that there can be several backbone conformations for a polynucleotide. This situation would have made the conformational analysis of polynucleotides quite difficult. But in a polynucleotide, unlike the situation in a dinucleoside monophosphate, the 3'- and 5'-hydroxyl groups are phosphorylated. It was anticipated that the terminal phosphates of a dinucleoside triphosphate would render some of the folded conformations for the backbone sterically unfavorable. Consequently, calculations were carried out for a dinucleoside triphosphate similar to those performed for the dinucleoside monophosphate. The (ω',ω) map thus obtained is shown in Fig. 3. It is seen that the number of possible conformations for the dinucleoside triphosphate is strikingly limited, in sharp contrast to the dinucleoside monophosphate (Fig. 2). The minima corresponding to the folded backbone conformations (ω',ω) ≈ (80°,80°) (g+, g*) and (60°,170°) (g*, t) are conspicuously absent in Fig. 3 due to steric and electrostatic interactions introduced by the terminal phosphates. These interactions also render the region near (ω',ω) ≈ (80°,240°) a higher energy conformation. Thus, the conformations for the range ω' ≈ 0° to 80° are expected to be energetically unfavorable in dinucleoside triphosphates and polynucleotides. As mentioned above, the fully extended conformation (18°,180°) (t,t) is also not likely to be favored (1, 3).

There appear to be two predominant conformations for the dinucleoside triphosphate pXpXp. The conformation centered at (ω',ω) ≈ (290°,290°) (g', g-) (Fig. 5a) corresponding to the global minimum is characteristic of all known double helical nucleic acids and polynucleotides (1, 20) and appears to be the most favored conformation for the helical backbone. It is interesting that despite the omission of both the base stacking and base pairing energies, the helical conformation turns out to be a favored conformation. This result substantiates an earlier observation (1) that the polynucleotide backbone shows an inherent tendency for the helical conformation and that base stacking and base pairing interactions provide additional stabilization. The other favored conformation is centered at (ω',ω) ≈ (190°,300°) (t,g-) and corresponds to an unstacked conformation with an extended backbone (Fig. 5b). The above results indicate that in solution, polyenu-

Fig. 5. Perspective views of the three favored backbone conformations for a dinucleoside triphosphate and a polynucleotide. (a) (ω',ω) ≈ (290°,290°) (g', g-) is the most favored backbone conformation for a helix and it can also be a part of loop structures; (b) (ω',ω) ≈ (190°,300°) (t,g-) generates extended helix-like conformation for the backbone and appears to be important in single-stranded regions of nucleic acids; (c) (ω',ω) ≈ (200°,60°) (t,g+) appears to be the conformation favored for the folding of a polynucleotide chain in hairpin loops. Note that the terminal phosphates are not shown in the above figures.
tides are predominantly composed of a mixture of the stacked (g+,g−) and unstacked (t,g−) conformations. The relative populations of these two conformations will be expected to depend on the nature of the bases and their sequence. The conformation centered at (ω',ω) ∼ (200°,60°) (t,g+) (Fig. 5c), which is considerably less favored compared to the above two, turns out to be a possible candidate for effecting a hairpin turn in a polynucleotide chain. In addition to the above three conformations, the (290°,190°) (g−,t) and (100°,270°)(g+,g−) conformations may also be important in regulating the backbone conformations of single-stranded regions of nucleic acids.

The (g+,g+) conformation

Although the conformation around (ω',ω) = (80°,80°) (g+,g+) is energetically unfavorable within a polynucleotide chain, it can be entertained by the terminal dinucleoside diphosphate residues in a nucleic acid when stabilized by an intramolecular hydrogen bond involving the free hydroxyl group O(3')-H or O(5')-H and the corresponding penultimate phosphate at the 3'-or 5'-end, respectively. In fact, this conformation has been observed in the trinucleoside diphosphate A+pAP+pA (9), which exhibits an intramolecular hydrogen bond between the hydroxyl group O(3')-H and the distal phosphate group. By analogy, a similar intramolecular hydrogen bond can stabilize the (g+,g+) conformation in a dinucleoside diphosphate. Therefore, the (g+,g+) backbone conformation seems to be restricted to the dinucleoside monophosphates, dinucleoside diphosphates, and the terminal dinucleoside diphosphate moieties of nucleic acids (Fig 4).

CONCLUSIONS

The main result that has emerged from this work is that the rotations about the internucleotide P-O bonds in a dinucleoside triphosphate and a polynucleotide are considerably more restricted than in a dinucleoside monophosphate. It must be stressed that the P-O bonds still show greater rotational flexibility (besides the sugar pucker) than the other backbone bonds C(4')-C(5'), C(5')-O(5'), and C(3')-O(3'). It is found that certain phosphodiester conformations that are likely in dinucleoside monophosphates turn out to be energetically unfavorable in a polynucleotide, suggesting that extrapolation of the results derived from dinucleoside monophosphates for the analysis of polynucleotide conformations can be misleading. The conformation (ω',ω) ∼ (80°,80°) (g+,g+), which produces the sharpest turn in a dinucleoside monophosphate, is found to be stereochemically unfavorable for a dinucleoside triphosphate and a polynucleotide. It appears that the phosphodiester conformations (t,g+) and (g−,t) are the most probable candidates for the folding of a polynucleotide chain. The distinction between the conformational properties of a dinucleoside triphosphate and a dinucleoside monophosphate is reminiscent of the conformational rigidity found in a nucleotide in contrast to a nucleoside (3). The presence of the phosphate group in a nucleotide strikingly restricts the conformations about the backbone C(4')-C(5'), C(5')-O(5'), and C(3')-O(3') bonds and the side-chain glycosyl C(1')-N bond (15). Therefore, the phosphate groups play a major role in limiting not only the conformations of the nucleotide building blocks themselves, but also their relative orientations about the internucleotide P-O linkages in a polynucleotide. The observations that the terminal phosphates in a dinucleoside triphosphate greatly limit the possible conformations for the phosphodiester group, taken in conjunction with the rigid nucleotide concept, further simplifies the conformational analysis of polynucleotides.

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