CONFORMATIONS OF CYCLIC 3',5'-NUCLEOTIDES. EFFECT OF THE BASE ON THE SYN-ANTI CONFORMER DISTRIBUTION.*

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ABSTRACT

The furanose and the phosphate rings of cyclic 3',5'-nucleotides are locked in the $_4T^3$ and chair conformations respectively. The only variable which shows major conformational flexibility in these molecules is the rotation about the glycosyl bond which describes the orientation of the base relative to the sugar-phosphate bicyclic system. The glycosyl torsion angle has been analyzed for cyclic nucleotides with different purine and pyrimidine bases by use of conformational energy calculations. The results indicate that all the pyrimidine bases, U, T and C show a very strong energetic preference for the anti range of conformations. The calculations predict that among cyclic 3',5'-purine nucleotides cyclic GMP and cyclic IMP favor the syn conformation to the anti by 95:5 and 70:30 respectively, while cyclic AMP shows a preference for the anti conformation to syn by 70:30. Thus the purines show a greater probability for the syn conformation than the pyrimidines in cyclic 3',5'-nucleotides.

INTRODUCTION

Cyclic 3',5'-nucleotides such as cyclic AMP and cyclic GMP play the role of "messenger" molecules in the regulation of hormonal processes and thus have attracted considerable attention.[1] Recent work has implicated that cyclic pyrimidine nucleotides may also have similar biological properties.[2] In this paper we have analyzed the effect of the base on the syn-anti equilibrium by theoretical conformational energy calculations on the various cyclic 3',5'-nucleotides and have compared the results with the available solid state (x-ray) and solution (nmr) data.

It has been shown[3-7] that the ribose rings in cyclic 3',5'-nucleotides are constrained to the characteristic $_4T^3$ [C(4')-exo, C(3')-endo] conformation in contrast to the sugars in mononucleotides and nucleosides.[8-10]

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The rigidity of the sugar moiety in this class of molecules has also been confirmed by nmr studies.[11-14] The phase angle of pseudorotation[10] of the furanose ring in the known cyclic 3',5'-nucleotides is confined to the very narrow range of values of 36 to 48°.[5] It is noteworthy that there is an increase of about 20° in the phase angle compared to the standard $^{3}T_{2}$ conformation found in nucleotides and nucleosides.[10] It will be shown that this shift in the pseudorotation path influences the barrier to rotation about the glycosyl bond and consequently the syn⇄anti equilibrium.

METHODS

The schematic representation of a typical purine and a pyrimidine cyclic 3',5'-nucleotide is shown in Fig. 1. Calculations have been

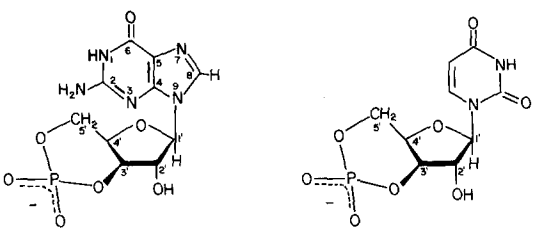

Fig. 1. Schematic representations of a typical purine (a) and a pyrimidine (b) cyclic 3',5'-nucleotide.

performed for the cyclic nucleotides 3',5'-UMP, 3',5'-CMP, 3',5'-TMP, 3',5'-AMP, 3',5'-GMP and 3',5'-IMP. The nature of the potential functions and the parameters used in the estimation of the conformational energy are the same as described earlier.[15] The structural parameters consisting of bond lengths and bond angles used in the present study come mainly from the x-ray studies.[3-7] However, all the ring hydrogen atoms of the sugar have been refixed at a distance of 1.1 in a tetrahedral direction, the trigonal hydrogens are fixed at a distance of 1.0 Å and at bond angles obtained from the corresponding internal angle of the ring.

RESULTS AND DISCUSSION

Cyclic 3',5'-UMP: The energy profile was obtained as a function of the glycosyl torsion angle χ for the two slightly different sugar ring conformations found in the two symmetry independent molecules of cyclic 3',5'-UMP in the crystal structure.[7] The sugar rings in both molecules are locked in the ${}_4T^3$ conformation with pseudorotation phase angles[10] of 42.0° (molecule 1) and 47.9° (molecule 2). The energy profile for molecule 1 (Fig. 2a) exhibits a broad minimum in the anti region (χ = 40-80°) and a narrow minimum in the syn region ($\chi \simeq$ 220-240°) separated by a high energy barrier. It is of interest to note that there is a shift in the minimum towards larger values of χ in the anti region compared to that obtained for the common pyrimidine nucleotides exhibiting the usual C(3')-endo pucker (pseudorotation phase angle range 3-15°),[10] indicating the effect of the changes in the phase angle of pseudorotation on the glycosyl conformation. Both the syn and the anti conformations possess nearly the same van der Waals energy (dashed curve) (Fig. 2a). However, when the contribution form the electrostatic interactions are also included (solid line), the anti range of conformations become energetically more favorable than the syn. It may be mentioned that the syn conformation is about 3 kcal/mole higher in energy than the anti. The conformer population distribution in the anti and syn ranges computed using the Boltzman expression[15] are given in Table 1. It is seen that more than 95% of the conformer population is anti in 3',5'-cyclic UMP. The results (Fig. 2b) obtained for the sugar geometry observed in molecule 2 of cyclic 3',5'-UMP are essentially similar to the above. The only noteworthy difference is that there is a slight shift towards higher χ angle in the position of occurrence of the minimum in the syn region (Fig. 2b). It is interesting that both the molecules of cyclic 3',5'-UMP exist in the anti conformation in the crystal[7] in conformity with the theoretical predictions. Similar results have been obtained for the cyclic 3',5'-nucleotides of other common pyrimidine bases thymine and

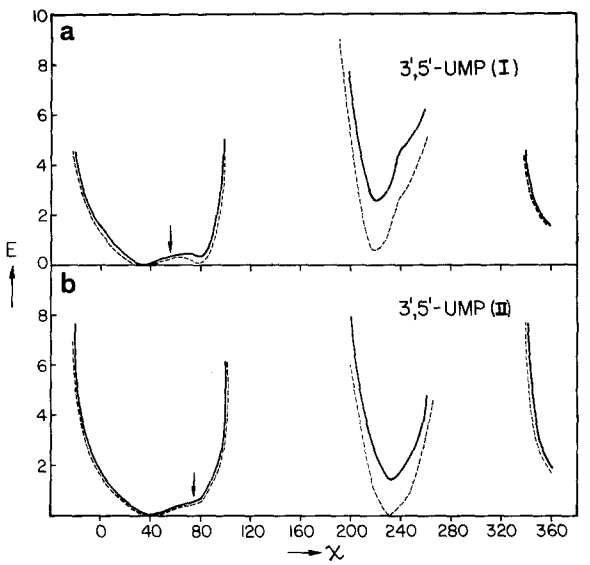

Fig. 2. Potential energy profile showing the variation of energy E (in kcal/mole) as a function of the rotation (χ) about the glycosyl bond in (a) cyclic 3',5'-UMP (molecule 1), (b) cyclic 3',5'-UMP (molecule 2). The initial or the zero conformation of χ is defined according to Sundaralingam.[9] In this and other figures the continuous curve (——) represents the variation of total energy (van der Waals + electrostatic) and the dashed curve (---) represents the variation of van der Waals energy alone. The observed crystal structural conformations are shown by (↓).

Table 1

Population of the anti and syn conformers in cyclic 3',5'-purine and pyrimidine nucleotides

	Anti	Syn
3',5'-UMP (I)	96 (69)	4 (31)
3',5'-UMP (II)	99 (89)	1 (11)
3',5'-TMP	99 (87)	1 (13)
3',5'-CMP	99 (95)	1 (5)
3',5'-AMP	69 (28)	31 (72)
3',5'-GMP	8 (27)	92 (73)
3',5'-IMP	36 (31)	64 (69)

*Values given within the parentheses correspond to those obtained by considering van der Waals interaction energy alone.

cytosine and hence they are not discussed again. It therefore appears that the pyrimidine bases show a strong preference for the anti conformation in 3',5'-cyclic nucleotides. Nmr studies[14] have also indicated that the uridine base in cyclic 3',5'-UMP takes up the anti conformation.

Cyclic 3',5'-AMP: Fig. 3a shows the energy profile obtained as a function of χ in cyclic 3',5'-AMP. The sugar pucker in this molecule has been taken to be the same as observed in cyclic 3',5'-GMP.[5,6] Two broad minima corresponding to the anti and syn regions are observed again with a barrier in the region near $\chi \simeq 120^{\circ}$. This barrier is mainly due to the nonbonded repulsive interactions between the atoms C(2') and H(2') of the sugar and the C(8) and H(8) of the base. Although both the anti and syn conformational ranges are stereochemically accessible, the anti conformation is found to be slightly more energetically favored than the syn. It appears therefore that the adenine base tends to prefer the anti conformation. It is found that nearly 70% of the statistical weight is associated

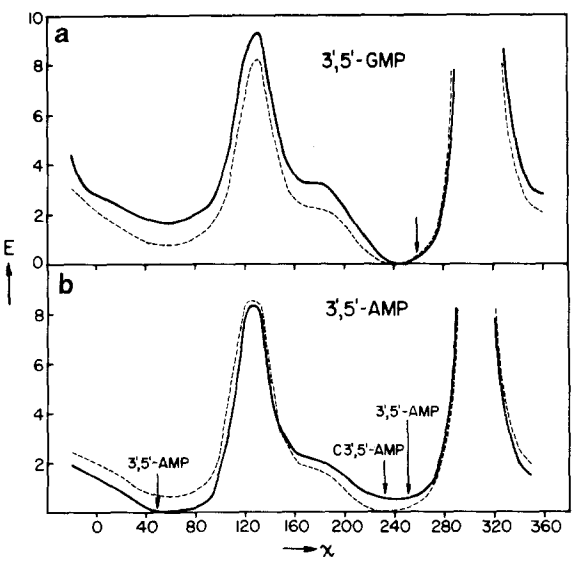

Fig. 3. Potential energy profile showing the variation of energy E (kcal/mole) as a function of χ in (a) cyclic 3',5'-AMP, (b) cyclic 3',5'-GMP.

with the anti domain while 30% is associated with the syn domain (Table 1). It is noteworthy that both the anti and syn conformations have been found in the solid state,[3,4,16] while nmr studies[14] predict that the anti conformation is more stable in aqueous media at room temperature in conformity with theory.

Cyclic 3',5'-GMP: The χ energy profile obtained for the guanine base in cyclic 3',5'-GMP is shown in Fig. 3b. It is found that the syn conformational range is energetically more favorable than the anti in contrast to the results obtained for cyclic 3',5'-AMP. This is mainly because of the additional favorable interactions provided by the amino group at the C(2) position of the guanine base in the syn conformation and the phosphate group of the bicyclic moiety. The guanine base therefore shows an intrinsic preference for the syn conformation and thus appears to behave differently compared to the other common bases. It is noteworthy that the predicted syn conformation has been found in the crystal structure of 3',5'-GMP.[5,6] Furthermore, nmr studies of Schweizer and Robins[14] have also found the predominance of syn conformation in solution.

Cyclic IMP: We have carried out similar calculations on cyclic 3',5'-IMP. The results indicate that the ionsine base tends to show a preference for the syn conformation although not as pronounced as cyclic 3',5'-GMP. Nmr results[14] however predict the anti conformation for cyclic 3',5'-IMP (Table 1). It will be of interest to compare these results with the x-ray structure which is currently being analyzed in our laboratory.

CONCLUSIONS

It is found that in general, there are two sterically accessible ranges of conformations for both the purine and pyrimidine cyclic 3',5'-nucleotides in the familiar anti and syn regions. Consideration of van der Waals energy alone suggests that both these regions are favored. But, it is found that the electrostatic interactions between the base

and the sugar-phosphate moiety tend to stabilize strongly the anti conformation in all the pyrimidine cyclic 3',5'-nucleotides. This emphasizes the importance of the electrostatic interactions in deciding the preferred conformations in these systems besides van der Waals interactions which play a major role in determining the sterically accessible conformations about the glycosyl bond. Among, the purine cyclic 3',5'-nucleotides, adenine favors the anti conformation while guanine shows a distinctive preference for the syn conformation. The theory predicts a syn conformation for ionsine. In general, the purine cyclic 3',5'-nucleotides show a greater probability for the syn conformation than the pyrimidine cyclic 3',5'-nucleotides. It is seen that the theoretical results are in good agreement with the available x-ray and nmr data.

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