COMPLEMENTARY STUDIES ON THE RIGIDITY-FLEXIBILITY OF NUCLEOTIDES*

Hélène BERTHOD and Bernard PULLMAN
Institut de Biologie Physico-Chimique, Laboratoire de Biochimie Théorique, associé au C.N.R.S., 13, rue P. et M. Curie, Paris 5è, France

Received 5 April 1973

1. Introduction

In view of investigating Sundaralingam's proposal [1, 2] of a "rigid" nucleotide conformation (*anti* about the quantum mechanical procedure PCILO the confor- cyclic C(4′)–C(5′) bond) we have studied recently by the quantum mechanical procedure PCILO the conformational properties of guanosine-5′- and -2′-phosphates [3]. This work has shown that in the isolated state these nucleotides should exhibit an intrinsic preference for the *syn* conformation. In fact, studies by nuclear Overhauser effect indicate that these compounds exist in solution predominantly in the *syn* conformation [4].

Calculations carried out with the use of empirical (partitioned potential functions) procedures by Yathindra and Sundaralingam [5], while in agreement with our results on guanosine-5′-phosphate, appear to suggest that this may, however, be an exceptional case and that other common 5′-nucleotides should obey the rigidity criterion of Sundaralingam. Because of the importance of this problem for the structural theory of polynucleotides and nucleic acids we have therefore extended our quantum-mechanical computations to other representatives of this class of molecules.

2. Method

The method of computation is again the PCILO (perturbative configuration interaction using localized orbitals) method utilized previously in our study on guanosine phosphates [3]. The definitions of the torsion angles and the details of the procedure are the same as in [3]. We have limited, however, our computation to the C(2′)-endo sugars, because this type of pucker is, in principle, the most favorable for *syn* conformations [5, 6]. So that if the *anti* form can be shown to predominate in nucleotides containing this pucker of the sugar, it will *a fortiori* predominate in nucleotides with the C(3′)-endo sugars. Exceptionally the pucker of the sugar for cytidine-5′-phosphate was taken to be C(3′)-exo because this conformation is the crystalline one [7]. The geometrical input data for inosine and uridine-5′-phosphates come directly from crystal data of [8] and [9], respectively. For adenosine-5′-phosphate [10] the geometry of the ribose was considered the same as for the inosine compound [8].

As in our previous study on guanosine phosphates [3], the conformational energy curves have been calculated as a function of 4 degrees of freedom: the rotation χ_{CN} about the glycosyl bond and the rotations $\Phi_{C(5')-O(5')}$, $\Phi_{O(5')-P}$ and $\Phi_{P-O_{III}}$ of the phosphate group, the rotation $\Phi_{C(4')-C(5')}$ being prefixed in the privileged *gg* positions [11]. Similarly $\Phi_{C(2')-O(2')}$ has been uniformly put equal to 180°, as it has been shown that the influence of the rotation about C(2′)–O(2′) is negligible [6].

For each compound studied, the conformational energy curves presented in this paper are representative of a large set of such curves computed in our laboratory.

3. Results and discussion

3.1. Adenosine-5′-phosphate

Fig.1 presents the variation of the conformational

* This work was supported by the R.C.P. 173 and the A.T.P. A655-2303 of the C.N.R.S.

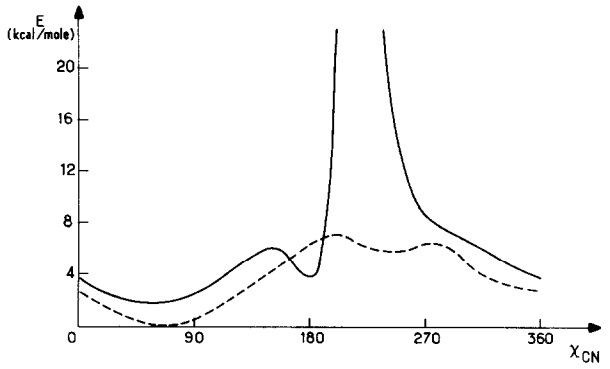

Fig.1. Conformational energy map for rotation about the glycosyl bond in adenosine-5′-phosphate, C(2′)-endo, *gg*. Full curve: results for $\Phi_{P-OIII} = -60°$, $\Phi_{O(5')-P} = -60°$, $\Phi_{C(5')-O(5')} = 150°$. Dashed curve: results for $\Phi_{P-OIII} = 90°$, $\Phi_{O(5')-P} = -60°$, $\Phi_{C(5')-O(5')} = 180°$.

energy as a function of χ_{CN} for two typical conformations of the phosphate group, representing as said a whole set obtained by a simultaneous variations of the involved torsion angles. The full curve corresponds to the possibility of formation of an H-bond between the O-H of the phosphate and N_3 of the base, the dashed curve to the case when this possibility is precluded. It can be seen that on both curves the global energy minimum corresponds to an *anti* conformation with, however, a secondary minimum for the *syn* conformation, in circumstances in which an intramolecular H-bond is feasible, 4 kcal/mole above the global minimum.

3.2. *Inosine-5′-phosphate*

Similar representative curves for inosine-5′-phosphate are presented in fig.2. They, as all the other curves which we have constructed for this nucleotide, indicate

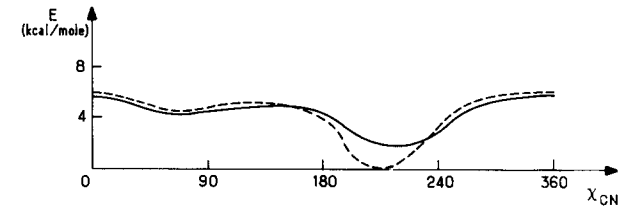

Fig.2. Conformational energy map for rotation about the glycosyl bond in inosine-5′-phosphate, C(2′)-endo, *gg*. Full curve: results for $\Phi_{P-OIII} = -30°$, $\Phi_{O(5')-P} = 180°$, $\Phi_{C(5')-O(5')} = 200°$. Dashed curve: results for $\Phi_{P-OIII} = 180°$, $\Phi_{O(5')-P} = 180°$, $\Phi_{C(5')-O(5')} = 180°$.

that the most stable conformation is a *syn* one, with $\chi_{CN} \approx 240°$, whether an intramolecular H-bond is or is not feasible. In fact, it may be observed that the global minimum is associated with an absence of such an H-bond. This minimum at $\chi_{CN} = 240°$ may be related to the lowering of the energy starting at $\chi_{CN} = 180°$ in guanosine-5′-phosphate [3] but interrupted beyond this angle because of the steric hindrance due to the NH_2 group of guanosine. Altogether, inosine-5′-phosphate shows thus, as did guanosine-5′-phosphate although less acutely, an intrinsic preference in its free state for the *syn* conformation about its glycosyl bond.

3.3. *Uridine-5′-phosphate*

Representative conformational energy curves for this compound are given in fig.3. They indicate a clear-cut preference for the *anti* conformation centered around $\chi_{CN} = 60°$.

3.4. *Deoxycytidine-5′-phosphate*

A typical conformational energy curve is presented in fig.4. The search for an intramolecular H-bond was in this case unsuccessful. There is again an evident preference for the *anti* conformation centered about $\chi_{CN} = 0°$. There is, however, a secondary minimum, 3 kcal/mole above the global one, at the onset of the *syn* region ($\chi_{CN} \approx 100°$).

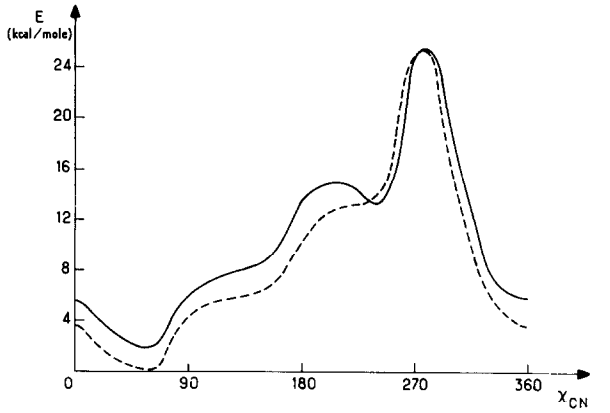

Fig.3. Conformational energy map for rotation about the glycosyl bond in uridine-5′-phosphate, C(2′)-endo, *gg*. Full curve: results for $\Phi_{P-OIII} = 60°$, $\Phi_{O(5')-P} = 180°$, $\Phi_{C(5')-O(5')} = 180°$. Dashed curve: results for $\Phi_{P-OIII} = 90°$, $\Phi_{O(5')-P} = 270°$, $\Phi_{C(5')-O(5')} = 180°$.

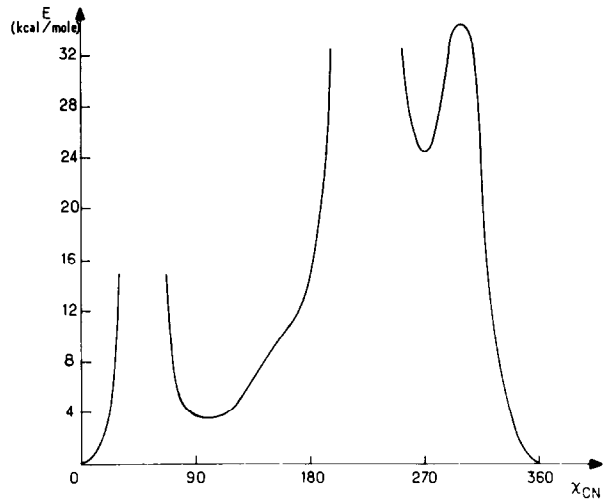

Fig.4. Conformational energy map for rotation about the glycosyl bond in deoxycytidine-5′-phosphate, C(3′)-exo, *gg*. $\Phi_{P-OIII} = -90°$, $\Phi_{O(5')-P} = 300°$, $\Phi_{C(5')-O(5')} = 160°$.

4. Conclusions

Altogether, our results indicate thus, in agreement with those of Yathindra and Sundaralingam, that guanosine-5′-phosphate is different from the corresponding nucleotides of *the other nucleic acid bases* in preferring intrinsically the *syn* conformation about the glycosyl bond, while adenosine-, uridine- and cytidine-5′-nucleotides show a preference for the *anti* conformation. These last three nucleotides may thus be considered as more rigid than the corresponding nucleosides which manifest a more diversified behavior [6].

On the other hand from a broader point of view guanosine-5′-phosphate is not the only nucleotide to manifest an intrinsic preference for the *syn* conformation. Inosine-5′-nucleotide is expected to behave similarly. In the crystal this compound is in the *anti* form [8] and it would therefore be particularly interesting to study its conformational properties in solution. In connection with the results presented in fig.4 it may perhaps also be useful to quote a recent suggestion following which cytidine-5′-nucleotide exists in solution at room temperature as a *syn–anti* mixture, with *anti* the lower conformation [12].

At this stage, it is interesting to analyse the reason for the difference in behavior between guanosine- and inosine-5′-nucleotides on the one hand (preference for a *syn* conformer) and adenosine-5′-nucleotide on the other hand (preference for an *anti* conformer). The analysis of the different terms of the 2nd and 3rd order perturbation energy of our PCILO calculations, indicates that this difference is due essentially to the delocalization term obtained through the configuration interaction between the zeroth order determinant and the monoexcited configurations built upon the promotion of an electron from a bonding to an antibonding orbital. This delocalization energy is of the order of 14 kcal/mole in guanosine-5′-phosphate, of 5 kcal/mole in inosine-5′-phosphate and of only 0.3 kcal/mole in adenosine-5′-phosphate. A further analysis enables to determine the components of the delocalization energy, whose *variation* is dominant when passing from the *anti* to the *syn* conformation and which are thus responsible for the difference of behavior of the different nucleotides. The results are indicated in table 1. Three such terms are visible. In the first place, the proximity of the phosphate and of the NH_2 group of the base in the *syn* conformation creates in the case of the guanosine-5′-phosphate a strong perturbation which is represented by a component of delocalization energy of about 6 kcal/mole due to a jump of an electron of the lone pair of the NH_2 group into the antibonding $\pi^*_{C(2)-N(3)}$ orbital. A similar delocalization term does not exist in adenosine-5′-phosphate in which the NH_2 group is further away from the phosphate. Secondly, there is in guanosine and inosine-5′-phosphate a delocalization energy component of about 3 kcal/mole due to the jump of an electron of the lone pair of N_1 into the antibonding $\pi^*_{C(2)-N(3)}$ and $\pi^*_{C(6)-O(10)}$ orbitals, which is absent, of course, in adenosine-5′-phosphate. Finally guanosine-5′-phosphate also shows a slightly greater value of the delocalization energy component due to the jump of an electron of the lone pair of an oxygen of the phosphate group upon the antibonding $\pi^*_{C(2)-N(3)}$ orbital than the other two nucleotides. The summation of these factors accounts for the strong stabilization of the *syn* form in guanosine-5′-phosphate, a somewhat weaker stabilization of this form in inosine and, on the contrary, the predominance of the *anti* form in adenosine-5′-phosphate.

Acknowledgement

The authors wish to thank Dr. Sundaralingam for communication of data prior to publication.

Table 1

Principal contributions to the variations of the delocalization energy between the *anti* and *syn* conformations (kcal/mole).

Electron jump	Guanosine-5'-phosphate	Inosine-5'-phosphate	Adenosine-5'-phosphate
Lone pair $\pi_{NH_2} \rightarrow \pi^*_{C(2)-N(3)}$	−6.0		
Lone pair $\pi_{N_1} \rightarrow \pi^*_{C(2)-N(3)}$, $\pi^*_{C(6)-O(10)}$	−2.9	−2.7	
Lone pair O (phosphate group) $\rightarrow \pi^*_{C(2)-N(3)}$	−0.5	−0.1	−0.1

References

[1] M. Sundaralingam, in: Conformation of biological molecules and polymers, 5th Jerusalem Symposium on Quantum Chemistry and Biochemistry, eds. E.D. Bergmann and B. Pullman (Academic Press, New York) in press.

[2] J. Rubin, T. Brennan and M. Sundaralingam, Biochemistry 11 (1972) 3112–3128.

[3] H. Berthod and B. Pullman, FEBS Letters 30 (1973) 231–235.

[4] T.D. Son, W. Guschlbauer and M. Guéron, J. Am. Chem. Soc. 94 (1972) 7903–7911.

[5] N. Yathindra and M. Sundaralingam, Biopolymers, in press.

[6] B. Pullman and H. Berthod, in: Conformation of biological molecules and polymers, 5th Jerusalem Symposium on Quantum Chemistry and Biochemistry, eds. E.D. Bergmann and B. Pullman (Academic Press, New York) in press.

[7] M.A. Viswamitra, B.S. Reddy, G.H.Y. Lin and M. Sundaralingam, J. Am. Chem. Soc. 93 (1971) 4565–4573.

[8] S.T. Rao and M. Sundaralingam, J. Am. Chem. Soc. 91 (1969) 1210–1217.

[9] E. Shefter and K.N. Trueblood, Acta, Cryst. 18 (1965) 1067–1077.

[10] J. Kraut and L.H. Jensen, Acta Cryst. 16 (1963) 79–88.

[11] A. Saran, B. Pullman and D. Perahia, Biochim. Biophys. Acta 287 (1972) 211–231.

[12] D.K. Lavallee and C.L. Coulter, J. Am. Chem. Soc. 95 (1973) 576–581.

[13] A. Pullman, Ann. New York Acad. Sci. 158 (1969) 65–85.

[14] A. Pullman, in: Quantum aspects of Heterocyclic compounds in chemistry and biochemistry, Proc. 2nd Jerusalem Symposium, eds. E.D. Bergmann and B. Pullman (Academic Press, New York, 1970) pp. 9–31.