NUCLEOTIDES: RIGID OR FLEXIBLE?

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

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1. Introduction

We have studied recently with quantum-mechanical methods the conformational properties of the constituents of the nucleic acids. The studies involved in particular the problem of the torsion about the glycosyl bond linking the base with the sugar [1–3] and about the exocyclic C(4′)–C(5′) bond of the sugar [4]. Although carried out in general for nucleosides, the results were considered as representative of the situation in both nucleosides and nucleotides. Their comparison with the experimental data, in crystals and in solution, confirmed the overall validity of the calculations.

The present note is devoted to a more explicit investigation of the conformational properties of nucleotides. The interest for such an exploration stems at least in part from a recent proposal of Sundaralingam [5] that the nucleotides are conformationally far more "rigid" than the nucleosides. In particular, following this author, in contrast to nucleosides which may exist in the *syn* and *anti* conformations about the glycosyl bond, nucleotides exhibit only the *anti* conformation. Also, whereas the three possible staggered conformations, *gauche–gauche* (*gg*), *gauche–trans* (*gt*) and *trans–gauche* (*tg*) about the C(4′)–C(5′) bond are observed for nucleosides, the 5′-nucleotides exhibit only the *gg* conformer.

Sundaralingam's concept is based entirely on the survey of X-ray crystallographic results. It apparently is valid for the situation in crystals although we may quote the interesting recent finding that 6-azauridine-5′-phosphoric acid exists in the *gt* conformation in the crystal [6]. Anyway, the question may be raised whether this rigidity of the crystal conformations of nucleotides represents their genuine, intrinsic property, which would then imply a fundamental difference with respect to nucleosides, or whether it is brought about by a particular influence of the packing forces. The theoretical results obtained previously for nucleosides and which indicated the predominance for all of them of the *gg* conformation (with the *gt* and *tg*, however, sometimes not much less stable) but a large possibility of occurrence of both the *anti* and *syn* conformations and the rapid examination of nucleotide models suggest that it is this last possibility which is probable. The present calculations were undertaken in view of a deeper investigation of this question.

2. Method and definitions

The method utilized is, as in our previous studies on nucleosides, the all-valence electrons molecular orbital PCILO (perturbative configuration interaction using localized orbitals) method. The conventions and notations are essentially those of Sundaralingam [7]. The different torsion angles to be considered in purine nucleotides are:

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$\chi_{CN} = O(1')-C(1')-N(9)-C(8)$
$\Phi_{C(2')-O(2')} = C(1')-C(2')-O(2')-H(O2')$
$\Phi_{C(3')-O(3')} = C(2')-C(3')-O(3')-H(O3')$
$\Psi_{C(4')-C(5')} = C(3')-C(4')-C(5')-O(5')$
$\Phi_{C(5')-O(5')} = C(4')-C(5')-O(5')-H(O5')$

and for the phosphate group in the 5′ position:

$\Phi_{C(5')-O(5')} = C(4')-C(5')-O(5')-P$
$\Phi_{C(5')-P} = C(5')-O(5')-P-O_{III}$
$\Phi_{P-O_{III}} = O(5')-P-O_{III}-H(O_{III})$

with obvious analogous notations for the phosphates in positions 2′ and 3′.

The zero values of the angles correspond to the *cis*-planar arrangements of the terminal bonds and the torsions are counted positively in the clockwise direction. The *anti* and *syn* regions are defined by $\chi_{CN} = 0° \pm 90°$ and $\chi_{CN} = 180° \pm 90°$, respectively.

For reasons which will become obvious from the discussion we have performed our computations on the examples of guanosine-5′ and 2′ monophosphates. For guanosine-5′-phosphate we have utilized as input data the geometry of the crystal structure, involving a C(3′)-endo sugar [8]. For its C(2′)-endo variety we have adopted for the ribose the geometry from the crystal of inosine-5′-phosphate [9]. For guanosine-2′-phosphate the geometry of the ribose and phosphate groups were taken from the structure of adenosine-2′-phosphate [10].

We have performed our computations by taking into account in each case simultaneously four degrees of freedom: the rotation χ_{CN} about the glycosyl bond and the rotations $\Phi_{C(5')-O(5')}$, $\Phi_{O(5')-P}$ and $\Phi_{P-O_{II}}$ in the phosphate group. On the other hand the $\Phi_{C(4')-C(5')}$ rotation was prefixed in the *gg* position, because previous computations including a detailed one for guanosine-5′-phosphate [4] have demonstrated this to be the preferred conformation in all cases studied. Our study will thus center essentially about the rigidity or flexibility of the conformations about the glycosyl bond.

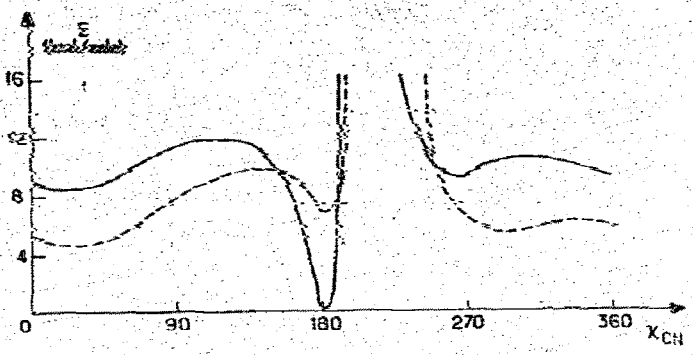

Fig. 1. Conformational energy map for rotation about the glycosyl bond in guanosine-5′-phosphate, C(3′)-endo, *gg*. Full curve: results for $\Phi_{C(2')-O(2')} = 180°$, $\Phi_{C(5')-O(5')} = 180°$, $\Phi_{O(5')-P} = -160°$ and $\Phi_{P-O_{III}} = 40°$. Dashed curve: results for $\Phi_{C(2')-O(2')} = 180°$, $\Phi_{C(5')-O(5')} = 240°$, $\Phi_{O(5')-P} = -60°$ and $\Phi_{P-O_{III}} = -60°$.

Results and discussion

3.1. *Guanosine-5′-phosphate, C(3′)-endo*

The types of energy variations obtained by the simultaneous rotations about the four above-mentioned bonds may be illustrated and summarized by the two types of conformational energy curves shown in fig. 1. The full curve corresponds to the possibility of formation of a hydrogen-bond between the O—H bond of the phosphate and the N(3) atom of the base, the dashed curve to a situation in which such a bond is impossible. It is seen that in the first case there is a deep energy minimum at $\chi_{CN} = 180°$ corresponding to the predominance of a *syn* conformation. The second case corresponds on the contrary to the preferential existence of the molecule in an *anti* conformation (minimum at $\chi_{CN} = 30°$) with nevertheless a secondary minimum at $\chi_{CN} = 180°$.

3.2. *Guanosine-5′-phosphate, C(2′)-endo*

The situation is very similar to the one described above, with small differences only in the depths and exact locations of the energy minima. We shall therefore not reproduce the figures here, but they may be obtained upon request.

On the other hand in this case, there could be a possible influence of the rotation about the C(2′)—O(2′) bond, through the interaction of the O(2′)—H bond with N(3) of the base [3]. The

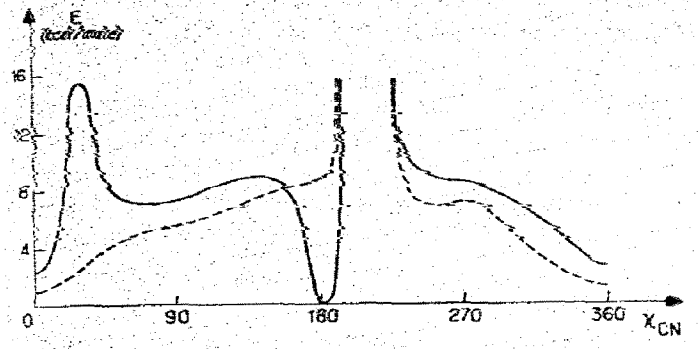

Fig. 2. Conformational energy map for rotation about the glycosyl bond in guanosine-2'-phosphate, C(3')-endo, *gg*, $\Phi_{C(2')-O(2')} = 100°$, $\Phi_{O(2')-P} = -60°$, $\Phi_{P-OIII} = 60°$. Full curve: $\Phi_{C(5')-O(5')} = 60°$, dashed curve: $\Phi_{C(5')-O(5')} = 180°$.

computations were therefore performed for the values $\Phi_{C(2')-O(2')} = 180°$, prohibiting such an interaction and $\Phi_{C(2')-O(2')} = 60°$ favoring such an interaction. The influence of this rotation is negligible for the general outlook of the results, the favorable orientation bringing about only a small stabilization of the *anti* form.

3.3. *Guanosine-2'-phospahte, C(3')-endo*

For this nucleotide we have to investigate besides the four torsions χ_{CN}, $\Phi_{C(2')-O(2')}$, $\Phi_{O(2')-P}$ and Φ_{P-OIII} the torsion about C(5')–O(5') because of the possibility of hydrogen-bonding between O(5')–H

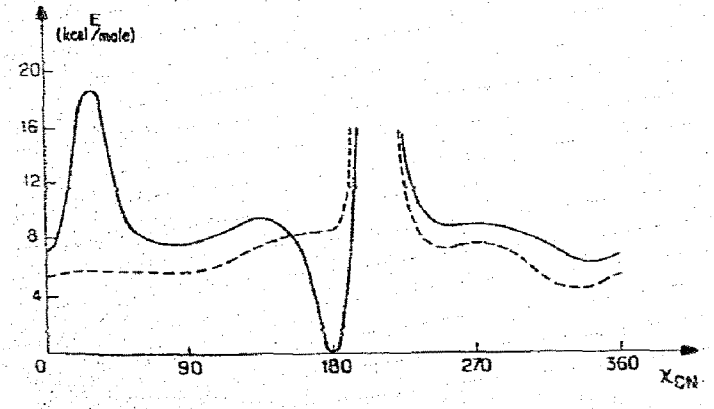

Fig. 3. Conformational energy map for rotation about the glycosyl bond in guanosine-2'-phosphate, C(3')-endo, *gg*, $\Phi_{C(2')-O(2')} = 80°$, $\Phi_{O(2')-P} = 0°$, $\Phi_{P-OIII} = 60°$. Full curve: $\Phi_{C(5')-O(5')} = 60°$, dashed curve: $\Phi_{C(5')-O(5')} = 180°$.

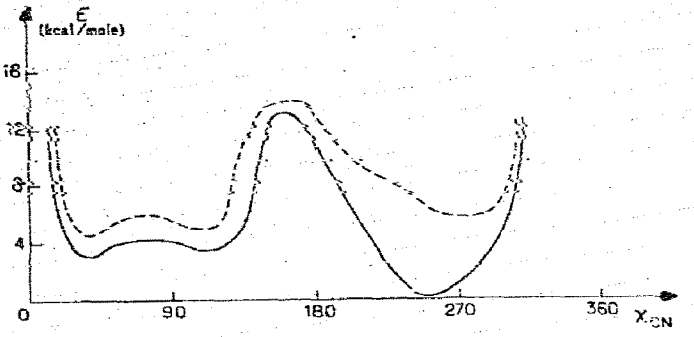

Fig. 4. Conformational energy map for rotation about the glycosyl bond in guanosine-2'-phosphate, C(2')-endo, *gg*, $\Phi_{C(2')-O(2')} = 120°$, $\Phi_{O(2')-P} = -120°$, $\Phi_{P-OIII} = 60°$. Full curve: $\Phi_{C(5')-O(5')} = 60°$; dashed curve: $\Phi_{C(5')-O(5')} = 180°$.

and N(3) of the base [3]. This was done by computing the quadruple rotations for the two preselected values: $\Phi_{C(5')-O(5')} = 180°$ prohibiting such a bond and $\Phi_{C(5')-O(5')} = 60°$ favoring such a bond.

Figs. 2 and 3 are representative of the results obtained. Fig. 2 corresponds to the possibility of interaction between the phosphate group through its O_{III}–H bond and N_3 of the base and fig. 3 to the absence of such a possibility. Moreover on each figure the full curve corresponds to the possibility of hydrogen bonding between O(5')–H and N_3 of the base and the dashed curve to the absence of this possibility.

The close similarity of the two figures is a clear indication of the negligible role played by the possibility of interactions between the phosphate and the base. On the other hand the interaction between the O(5')–H bond and the base has a decisive influence on χ_{CN}. When this interaction is possible, the results indicate a global energy minimum at $\chi_{CN} = 180°$, thus associated with a *syn* conformation, followed by a secondary energy minimum at $\chi_{CN} = 0°$ (*anti*). When this interaction is prevented the global energy minimum occurs at $\chi_{CN} = 0°$ and the whole *syn* region is highly unstable.

3.4. *Guanosine-2'-phosphate, C(2')-endo*

The overall results are similar to those obtained for the C(3')-endo pucker of the sugar, but the details are different. They are presented in figs. 4 and 5 which, by now, are self-explanatory.

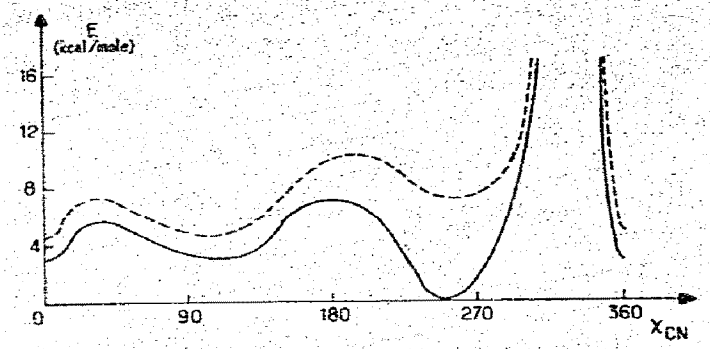

Fig. 5. Conformational energy map for guanosine-2′-phosphate, C(2′)-endo, *gg*, $\Phi_{C(2')-O(2')} = 120°$, $\Phi_{O(2')-P} = 180°$, $\Phi_{P-OH} = -60°$. Full curve: $\Phi_{C(5')-O(5')} = 60°$; dashed curve: $\Phi_{C(5')-O(5')} = 180°$.

3.5. *Guanosine-3′-phosphate*

No computations have been carried out for this isomer. It is obvious that a phosphate in the 3′ position cannot have any direct effect on the torsions considered here and that the compound will thus behave, from that point of view, similarly to the nucleoside, for which previous calculations [3] show the possibility of a *syn* conformation when the interaction of O(5′)–H with the base is permitted.

4. General discussion and conclusions

The preceding results show that considered isolated and unhindered by environmental factors, nucleotides are not expected to show the type of conformational rigidity postulated by Sundaralingam, on the basis of crystal studies, to be their constant characteristic. In particular, while the predominance of the *gg* conformation although not its exclusiveness may be admitted in their case, the predominance of the *syn* or *anti* conformations depends on the possibility or impossibility of interaction between the base and the 5′-phosphate group or, in its absence, the O(5′)–H bond of the exocyclic CH_2OH group, the interaction between the base and the 2′-phosphate group or the O(2′)–H bond being of much less significance. In the case of a complete freedom the *syn* conformation appears generally as intrinsically the most stable one so that the predominance of the *anti* conformation observed in the crystal must be attributed to the action of the packing forces which prevent the corresponding intramolecular hydrogen bond to occur.

Under these circumstances it may be expected that in different conditions, in particular when the environmental forces are less pronounced than in the crystal, conformations different from those observed in the crystal and corresponding closer to the intrinsic preferences of the free nucleotides may be observed. Some of the very recent results on the conformation of nucleotides in solution seem to confirm this viewpoint. Thus, while adenosine-, thymidine-, uridine- and cytidine-5′-monophosphates are considered by NMR techniques to exist essentially in the *anti* form in solution [11–15], studies by nuclear Overhauser effect indicate that guanosine-5′, -3′, and -2′ monophosphates exist in solution predominantly (50–80%) in the *syn* conformation [16]. A *syn* conformation is also proposed on the basis of circular dichroism studies for guanosine in guanylyl-3′-5′-uridine (GpU) [17]. A preferred *syn* conformation has also been established by NMR studies for oxidized pyridine 5′-mononucleotide in solution [18].

Thus both theoretical and experimental data seem to indicate that the intrinsic conformational rigidity (or flexibility) of a nucleotide may not be considered as fundamentally different from that of the corresponding nucleoside. This finding does not diminish the value of Sundaralingam's concept of the conformational rigidity of nucleotides in crystals and in polynucleotides (see also [15]) the interactions leading to *syn* conformations being in the later further restricted by the structure of the backbone.

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