^{1}H NMR STUDY OF THE MOLECULAR CONFORMATION OF THE EXOCYCLIC LINKAGE 5'-β-NUCLEOTIDES IN SOLUTION – CORRELATION BETWEEN C(4')–C(5') AND C(5')–O(5') CONFORMER POPULATIONS AND THE INFLUENCE OF THE BASE

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1. Introduction

The mononucleo*tide* is the fundamental building block of a polynucleotide [1] and information regarding the backbone conformational behaviour of a polynucleotide can be obtained by investigating the backbone conformational features of a monomer. In the present paper we have examined the conformation of fourteen different 5' mononucleotides in D_2O and have shown (i) the nature of the base has a profound influence on the conformation of the exocyclic linkage; (ii) a definite correlation exists between the population distribution of the conformers confined to the C(4')–C(5') and C(5')–O(5') bonds; and (iii) the conformer having the major population in solution is the one shown to be favored by the molecule in crystals.

2. Materials and methods

Spectra of the nucleotides (commercial products) 0.1 M, pD 8.0, 30°, in D_2O were obtained using either a 220 MHz, continuous wave or a 100 MHz, fast Fourier transform system. Details for obtaining Fourier transformed spectra are discussed by Sarma and Mynott [2]. Spectra were recorded using 16K transform both with coupling to the ^{31}P of the phosphate and with ^{31}P decoupling and were analysed using the computer program LAME. Computer simulated spectra were generated as a final test of the derived data.

3. Results and discussion

We have shown earlier [3,4] that the favored orientation about C(4')–C(5') and C(5')–O(5') bonds (fig. 1) of a nucleotide can be computed by eqs. * (1) and (2).

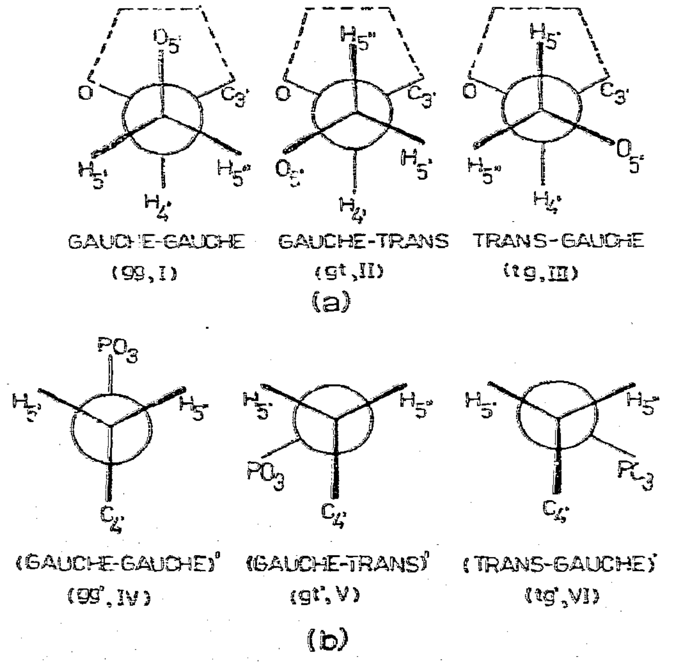

Fig. 1. (a) The classical rotamers constrained to C(4')–C(5') bond. (b) The rotamers constrained to C(5')–O(5') bond.

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* Equation (1) is modified slightly from [2,3].

$$\mathrm{Pgg} \simeq \frac{13-\Sigma}{10} \quad (1); \qquad \mathrm{Pg'g'} \simeq \frac{24-\Sigma'}{18} \quad (2)$$

where Pgg and Pg'g' are respectively the fractional populations of the gg (I) and g'g' rotamers (IV); Σ and Σ' are respectively $J_{4'5'} + J_{4'5''}$ and $J_{P-H(5')} + J_{P-H(5'')}$.

In fig. 2 the Σ, Σ' coupling constant sums and the derived Pgg and Pg'g' values are plotted in the form of a two dimensional map. Examination of fig. 2 shows that in the case of 5' nucleotides there is a tendency for the simultaneous decrease in Pgg and Pg'g' values, strongly indicating that the rotational preferences about C(4')–C(5') and C(5')–O(5') are not independent. *The data suggest that g't' and t'g' rotamers are virtually excluded when the molecule assumes the gg orientation* and that when it assumes the gt, or tg, conformation occupation of g'g' is no longer necessary and the molecule is allowed to populate the g't' and t'g' rotamers. The data further indicate that even when the molecule has rotated out of the gg form entirely, the g'g' rotamer is still significantly populated (see the position of 5'UMP, and that of 6-aza5'UMP in fig. 2). Examination of space-filling models indicate that when any 5' nucleotide is rotated into its gg form, unfavorable close contact interactions between the phosphoryl oxygens and the furanose and the base are introduced by rotation into the g't' or t'g' conformers. The crowding of atoms becomes particularly severe in the case of 5'-OMP which has Σ and Σ' values of 13±2 and 16±2 indicating a gg and g'g' population of 100 and 45%, respectively **. If one rotates a 5'-nucleotide into either the gt or tg form potential hindrance from the base to the rotation about C(5')–O(5') is removed and that by the furanose substituants becomes less severe. However, it should be noted that the $\Sigma\Sigma'$ map indicates that when the nucleotide assumes an exclusive tg or gt conformation, the dominant conformer constrained to C(5')–O(5') is still g'g'. If the molecule is g't' or t'g' coupled with gt or tg various combinations of unfavorable close contacts involving the phosphate and H(4'), O(1'), H(3') and OH(3') are introduced during the course of the librational motion about C(5')–O(5').

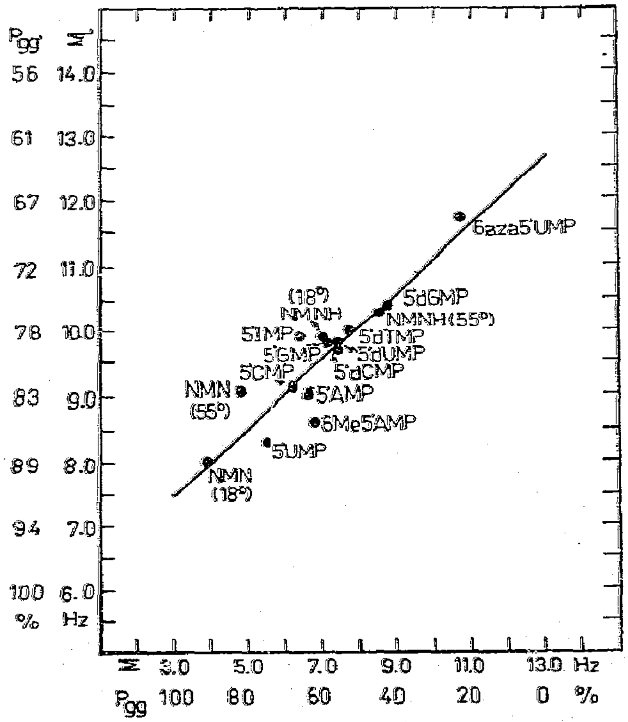

Fig. 2. Plot of Σ vs Σ'; also shown are the gg and g'g' populations. We have not included the Σ and Σ' values for 5' OMP in the figure because the experimentally observed values (13±2 and 16±2) do not have the accuracy of ± 0.1 Hz reported for the collection of molecules in the figure.

These correlations which we have derived from solution NMR data and the $\Sigma\Sigma'$ map are in eminent agreement with the X-ray studies on *common* 5' nucleotides, UpA, RNA and DNA, all which show an exclusive preference for gg and g'g' orientation [5–10]. Our conclusion from the solution $\Sigma\Sigma'$ map that when the nucleotide occupies a gt or tg conformation, the dominant conformer about C(5')–O(5') is still g'g' is also borne out by X-ray data [10].

Consideration of electronic properties along with steric interaction arguments enable us to rationalize the relative positions of β-NMN(β-nicotinamide-5-

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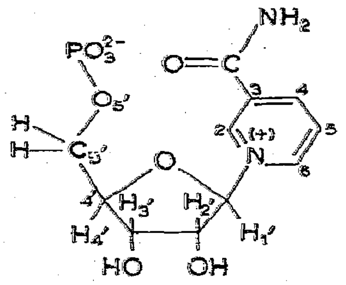
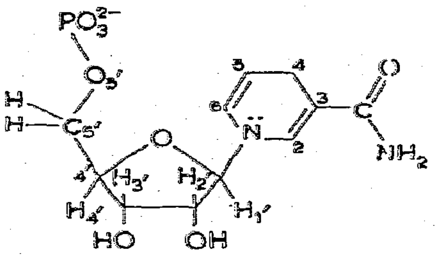
** Evidence has been presented [17] that orotidine, the parent nucleoside, favors the *syn* conformation in which the 2-keto oxygen of the base lies above the furanose in juxtaposition to the 5' position and in all likelihood this is so in 5'-OMP as well. Even if the molecules were to assume the *anti* conformations, t'g' and g't' rotamers are expected to be completely depopulated if the molecule is gg since the proximity of the large, and negatively charged carboxyl group should destabilize g't', t'g' even more effectively in a gg orientation.



β-NMN (VII)



β-NMNH (VIII)

mononucleotide, VII), β-NMNH (reduced β-NMN, VIII), and 6-aza-5′-UMP in the $\Sigma\Sigma'$ map. β-NMN carries a formal positive charge which is expected to exert an attractive force on the $CH_2OPO_3^{2-}$ group. An electrostatic base-backbone attraction stabilizes, the sterically favored, gg, g′g′ conformer and as such β-NMN appears near the gg, g′g′ extreme of the map. In fact, ^{31}P NMR [12–13] studies have confirmed the proposed electrostatic interactions in β-NMN. In the case of the reduced pyridine mononucleotide β-NMNH, VIII, the gg conformer depopulates compared to β-NMN. This depopulation is due to the loss of gg stabilization by the electrostatic attraction present in β-NMN as well as due to the repulsion interaction between the lone pair of electrons on the dihydropyridine moiety and the backbone negative charges on the exocyclic linkage. On similar grounds we find it reasonable to predict that the 7-methyl derivatives of 5′IMP and 5′GMP (formal + charge on the imidazole moiety) will show a marked increase in the gg, g′g′ population compared to their nonalkylated counterparts. It is our belief that the properties of the base account for the lowered gg, g′g′ population of 6-aza-5′-UMP and 5′UMP. Proton magnetic resonance and X-ray data [3, 4, 10] show that 6-aza-5′-UMP prefers the *anti*-conformation in which the 6-aza nitrogen of the base lies above the furanose ring. Since, theoretical calculations [14, 15] indicate that this aza nitrogen is an electron rich center, then the apparent attenuated probability of the gg, g′g′ conformation is understood if an electrostatic repulsion between base and $CH_2OPO_3^{2-}$ moieties are postulated. For 5′-OMP a similar interpretation based on repulsion of the $CH_2OPO_3^{2-}$ group by the unshared pairs on the 2-keto oxygen (as well as steric crowding) can be provided for the low gg, g′g′ populations.

The remainder of the molecules include nucleotides commonly found in DNA and RNA and fall in the mid range of $\Sigma\Sigma'$ map and show only slight variations in the gg g′g′ populations. This is not surprising since none of the bases carry formal charges or bulky substituents at the ortho position to precipitate steric crowding. Comparison of the purine and pyrimidine, the riboside and deoxyriboside, the bromo and the nonbromo-nucleotides indicate some variations in the gg g′g′ populations. Even though these variations are small, they are outside any experimental error. It may be noted that all these intermediate points are contained in an area where the observed Pgg values lies between 40–75 and that of Pg′g′ between 75–85%. The variation in these values among 5′UMP, 5′CMP on the one hand and among 5′AMP, 5′ADP, 5′IMP and 5′GMP, on the other, is very small indeed.

The ability of the base to precipitate backbone conformational aberrations may play an important role in determining the overall geometry about the loop region of tRNA. The interdependence of the populations of the conformers about C(4′)–C(5′) and C(5′)–O(5′) bonds may be able to give insight into the conformational events which accompany unwinding of polynucleotides.

Acknowledgements

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