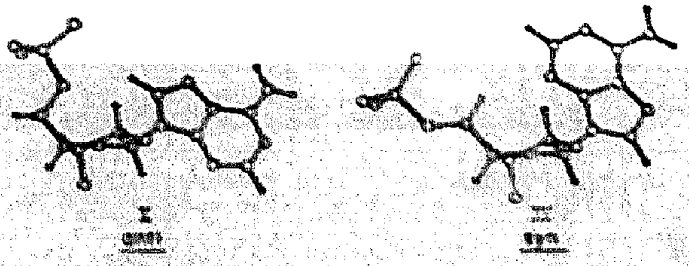
A NEW METHOD TO DETERMINE SUGAR-BASE TORSION IN PURINE NUCLEOSIDES AND NUCLEOTIDES

Frederick E. EVANS and Ramaswamy H. SARMA
Department of Chemistry, The State University of New York at Albany, Albany, New York 12222 USA

Received 25 February 1974

1. Introduction

It is well recognized that information about the glycosidic torsion in nucleosides and nucleotides is fundamental towards unraveling the molecular geometry of polynucleotides as well as of coenzymes such as DPN, FAD, ADPG, etc. The standard and unequivocal method which uses the effect of phosphate ionization on the base proton chemical shifts is not applicable to study the sugar-base torsional preference in nucleosides and 3′ nucleotides. In this paper we present a simple direct ^{1}H NMR method, based on the relative magnitudes of the base proton chemical shifts in purine compounds, to determine the torsional preference of the base in purine nucleosides, 3′ nucleotides as well as 5′ nucleotides. Using this method it is shown that adenosine, 3′AMP, 5′AMP and ATP are preferentially *anti*, with 5′AMP likely being rigid in the *anti* conformation (I). Further, it is shown that pH ionization studies cannot detect *syn* (II) conformations in purine 5′ nucleotides.



I
anti

II
syn

2. Materials and methods

Spectra of adenine, adenosine, 3′AMP, 5′AMP, ATP, 8-Br-adenine, 8-Br-adenosine, and 8-Br-5′AMP (commercial products) were taken at pD 8 or pD 6, 30°C, in D_2O, and at 100 MHz using a fast Fourier transform system. The chemical shift of the base protons was obtained at infinite dilution with the aid of a concentration study, the lowest concentration employed being 0.001 M. Mn(II) ion binding studies were performed on 5′AMP at 0.4 M and 0.04 M, on adenosine at 0.02 M, and on a mixture of 5′AMP at 0.005 M plus adenosine at 0.02 M.

3. Results and discussion

It is generally accepted that the sugar base torsion of nucleosides and nucleotides has energy minima in two ranges [1,2] termed *anti* (I) and *syn* (II) conformation. It has been shown that ionization of the phosphate group [3] or the binding of Mn(II) ion to the phosphate group [4] causes a selective perturbation of the base resonances and the data has been interpreted as showing that 5′AMP preferentially exists in the *anti* conformation. These techniques can not determine the glycosidic torsional preference in nucleosides or 3′ nucleotides. The chemical shifts of the base protons of the purine ring provide a useful probe for this detection.

The variation of the C(2)H and C(8)H chemical shifts as a function of concentration for adenine, adenosine and 5′AMP is shown in fig. 1. The chemical shifts at which there is no longer a dependance for these and several related compounds are given in table 1, and the data can be used to extract information about the intramolecular conformation. The observation that the C(2)H has identical chemical shift in the base (adenine) as well as in the nucleoside (adenosine) and the nucleotide (5′AMP) can be

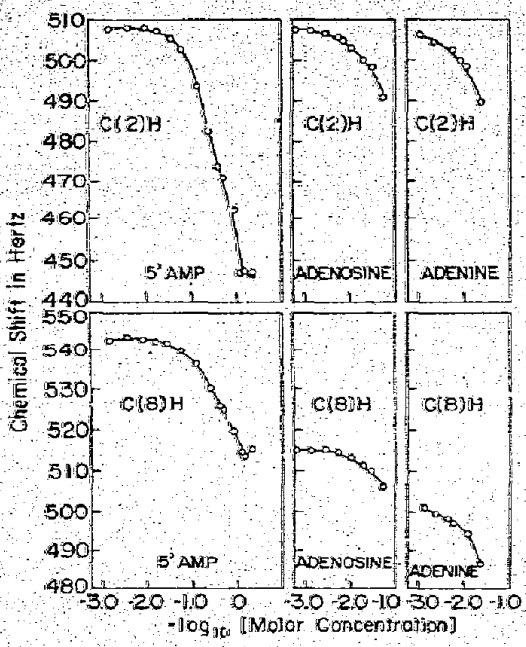


Fig. 1. Variation of C(2)H and C(8)H chemical shifts of 5'AMP, adenosine and adenine as a function of concentration, pD 8.0, temperature 30.5° C.

rationalized on the ground that in adenosine and in 5'AMP, the C(2)H proton resides in an environment far away from the ribofuranose system as is true only in an *anti* conformation (I). Should adenosine or 5'AMP exist in the *syn* conformation (II), the C(2)H would reside above the ribose moiety and as such should be expected to show a significant difference in chemical shifts of C(2)H between adenine and adenosine or 5'AMP. This conclusion is further substantiated by the 13 Hz downfield shift of C(8)H in adenosine and 41 Hz downfield shift of C(8)H in 5'AMP compared to adenine. We grant, part of this shift may indeed originate from pure electronegativity effects; however, the magnitude and the direction of the change in chemical shift of C(8)H as one goes from adenine to adenosine and then to 5'AMP are best rationalized on the ground that both adenosine and 5'AMP preferentially exist in the *anti* conformation; the large effect on the C(8)H chemical shift of 5'AMP is due to the proximity of the phosphate group [3,5].

Since 5'AMP and adenosine at pH 8 are prefcrentially *anti* conformation, one may anticipate that similar derivatives such as 3'AMP and ATP at pH 8, as well as 5'AMP at pH 6, will also be *anti*. The data in table 1 show the C(2)H chemical shifts are identical for each case, while the C(8)H protons show the perturbation from the nearby ribose moiety, thus indicating *anti* conformation.

Additional data showing that proximity to the ribofuranose system will affect a base resonance position should be provided by a ribo-purine which is known to be in the *syn* conformation, since in this case C(2)H will be located near the ribofuranose system (II) and this chemical shift should now be affected. The data on 8-Br-adenine, 8-Br-adenosine and 8-Br-5'AMP confirm this (table 1). Mn(II) ion binding studies and theoretical calculations clearly show this nucleoside and nucleotide exist *syn* [6].

table 1
A comparison of chemical shifts of C(2)H and C(8)H in adenine nucleosides and nucleotides[a]

Adenine compound	C(2)H	C(8)H	Sugar-base torsion preference[c]
Adenine	509	503	—
Adenosine	508	516	*anti*
5'AMP	508	544	*anti*
5'AMP (pD 6.4)	50[illegible]	534	*anti*
3'AMP	5[illegible]8	519	*anti*
ATP	508	53[illegible]	*anti*
8-Br-Adenine[b]	491	—	—
8-Br-Adenosine	502	—	*syn*
8-Br-5'AMP	506	—	*syn*
8-Br-5'AMP (pD 6.0)[d]	505	—	*syn*

[a] The chemical shift of C(2)H and C(8)H expressed in hertz downfield from tetramethylammonium chloride (100 MHz) at infinite dilution (± 1 Hz) except adenine and 8-Br-adenine which are ± 2 Hz. Because of this uncertainty in chemical shift, the data can only be used to show torsional preference. Temperature 30.5° C and pD 8.0 unless otherwise indicated.
[b] The limited solubility of 8-Br-adenine required 17 000 pulses to obtain a clear C(2)H resonance.
[c] This has been determined in our laboratory for each compound by the method described in the text. It may be noted that for 5'AMP other NMR methods have also shown *anti* conformation [3,4].
[d] It is clear the phosphate group has changed ionization state since the $C(5')H_2$ chemical shift shows a gradual deshielding totaling 10 Hz in going from pD 8.0 to pD 6.0. This is about the same as the $C(5')H_2$ deshielding in 5'AMP in going from pD 8.0 to pD 6.4. The C(2)H chemical shift of 8-Br-5'AMP remains constant, ± 1 Hz, throughout this pH range.

A comparison of the C(8)H chemical shifts between the *anti* compounds adenosine and 5'AMP (table 1) shows that the presence of the nearby phosphate group (I) affects the resonance position. It is interesting that for the *syn* compounds 8-Br-adenosine and 8-Br-5'AMP, the data (table 1) indicate the phosphate group has no significant effect on the chemical shift of C(2)H even though C(2)H is positioned near the ribose moiety (II) for these two compounds. Consistent with this observation is that ionization of the phosphate group in 8-Br-5'AMP has little effect on C(2)H (table 1). We have argued elsewhere [7] that the charge on the phosphate group has minimal perturbation on the chemical shifts of the base protons when the torsional diastereomer constrained to the C(4')-C(5')bond is *gauche-trans*, and indeed this is the case for 8-Br-5'AMP [6]; 5'AMP is preferentially *gauche-gauche* about this bond [8]. It may be a general rule in purine nucleotides that the spatial relationship of P(5') relative to C(2)H is such that phosphate ionization can not detect the presence of *syn* conformation, this being contrary to what has usually been expected.

In the case of 5'AMP, Mn(II) ion binding studies may be used to distinguish between exclusive versus prefered glycosidic orientations. Since Mn(II)-induced line broadening is distant dependant [4], one anticipates that intermolecular broadening in base stacked 5'AMP may be present along with the intramolecular broadening. To test this prediction Mn(II) ion was added to adenosine, and since adenosine has no strong binding site, little broadening was observed (fig. 2a). However when 5'AMP was added to this solution, the resonances of both moved upfield indicating base stacking between the two, and the resonances of adenosine were then broadened (fig. 2a). Thus Mn(II) bound to the phosphate of 5'AMP will broaden resonances of base stacked molecules. In fig. 2b Mn(II) ion was added to 5'AMP at 0.4 M and 0.04 M concentration levels; in the latter case little stacking occurs (fig. 1) and we observed C(8)H and C(2)H to be broadened by about 10 Hz and 0.4 Hz respectively. The tiny amount of broadening of C(2)H may be experimental error; however even with 5'AMP rigid in the *anti* conformation, a little C(2)H broadening may result due to non-specific broadening and the small amount of base stacking at this concentration. The data indicates 5'AMP is probably rigid in the *anti* conformation in solution.

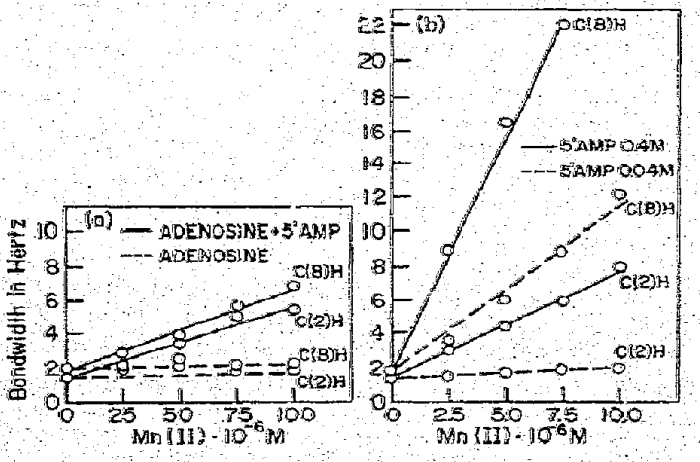


Fig. 2(a). Effect of Mn(II) ion on the line widths of C(2)H and C(8)H resonances of 0.02 M adenosine in the absence and presence of 5'AMP. The concentration of 5'AMP employed was 0.005 M, temperature 30.5°C, pD 8.0. (b) Effect of Mn(II) ion on the line widths of C(8)H and C(2)H of 5'AMP at 0.4 M and 0.04 M concentrations. At 0.04 M, C(2)H is broadened about 0.4 Hz while C(8)H about 10 Hz.

Acknowledgements

This work was supported by grants from the National Science Foundation (GB28015 and BC28015-001) and the National Cancer Institute of the National Institutes of Health (CA12462-03).

References

[1] Donohue, J. and Trueblood, K. N. (1960) J. Mol. Biol. 2, 363.
[2] Sundaralingam, M. (1969) Biopolymers 7, 821.
[3] Schweizer, M. P., Broom, A. D., Ts'o, P. O. P. and Hollis, D. P. (1968) J. Amer. Chem. Soc. 90, 1042
[4] Chan, S. I. and Nelson, J. H. (1969) J. Amer. Chem. Soc. 91, 168.
[5] Danyluk, S. S. and Hruska, F. E. (1968) Biochemistry 7, 1038.
[6] Sarma, R. H., Lee, C. H., Yathindra, N. and Sundaralingam, M., submitted to J. Amer. Chem. Soc.
[7] Sarma, R. H. and Mynott, R. J. (1973) J. Amer. Chem. Soc. 95, 1641.
[8] Evans, F. E. and Sarma, R. H., J. b. C., in press.