THE CONFORMATIONS OF ADENOSINE MONONCULEOTIDE IN WATER AND DIMETHYLSULFOXIDE

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Received March 3, 1972

SUMMARY

The conformation of adenosine-5'-monophosphate has been determined, using the lanthanide probe technique, in DMSO. The conformation found and the one previously found in aqueous solution are compared. The two differ by a large rotation of the base about the glycosidic linkage. The results help define in precise terms the nature of solvent dependent conformational changes for this molecule and the technique may be extended to many others.

In the NMR literature many reports have appeared on the conformations of a given molecule based upon work in different solvent systems (1,2,3). Special attention has focused on water and dimethylsulfoxide. In this paper we shall describe the conformation of adenosine-5'-monophosphate (AMP) in DMSO and compare the structure with that which we have found in water (4).

Our method for determining structures depends upon the perturbation of the nuclear magnetic resonance (NMR) spectrum of a molecule in solution by added lanthanide ions. As described in the paper mentioned above the ions may shift the spectral lines according to the pseudo-contact mechanism (5). In the case of ions with singlet ground states and long electron relaxation times, isotropic broadening effects are also present. Using these perturbations a computer program seeks a self-consistent structure satisfying the NMR data and the possible Van der Waals allowed structures. Our procedure consists in observing the chemical shifts of the various nonexchangeable protons on the solute molecule in question and the changes in these shifts upon addition of lanthanides. We

extrapolate the observed shifts to zero metal ion concentration in order to eliminate misleading solvent-ion effects. In addition, the experiments reported here are performed with 0.03 M solute concentration in order to minimize molecular association effects.

We have found that anhydrous lanthanide chlorides dissolve readily in anhydrous DMSO and may therefore be used as shift probes in an analogous manner to aqueous solutions.

Anhydrous DMSO-d_6 was prepared by drying commercial material with 99.7% isotopic purity for several weeks over Linde 4x molecular sieve. Anhydrous europium chloride was prepared by mixing the wet salt with NH_4Cl and heating (6). The adenosine monophosphate used was highest purity commercial material lyophilized from H_2O at pH = 2. Handling of solvent, solute and solutions must be under dry conditions. Since the positions of the exchangeable -OH protons are very sensitive to water content we were able to monitor our samples to insure that no spurious water induced shifts were present. In addition to the europium shift probe we expanded the data set to include a broadening probe - gadolinium. In the former case the experiments produce shift ratios $R_{i,extr}$ extrapolated to zero metal ion concentration expressed as,

$$R_{i,extr} = \frac{\left\langle \frac{3\cos^2\theta_i - 1}{r_i^3} \right\rangle}{\left\langle \frac{3\cos^2\theta_o - 1}{r_o^3} \right\rangle} \qquad (1)$$

where the normalization is to any given proton (usually the most shifted one) and the angles θ_i refer to those of the ith proton from the principle symmetry axis of the ion and the distances r_i from the same ion. In the latter case

the gadolinium ion results in isotropic broadening of lines and the data are expressed in ratios

$$R_i \text{ broad, extr} = \frac{\left\langle 1/r_i^6 \right\rangle}{\left\langle 1/r_o^6 \right\rangle} \qquad (2)$$

These equations assume that a dipole-dipole mechanism is responsible for the spectral perturbations, a fact which we established experimentally in aqueous solution in our previous paper. We also established, by extensive computer search for acceptable alternatives, that the lanthanide ion interacts with the singly charged phosphate group as a 1st sphere complex. Since we found no evidence to the contrary in DMSO solutions we have assumed that the metal ion is in the same position here as in aqueous solution. In support of this

TABLE I

Shift and broadening ratios for AMP (aq.) and AMP (DMSO) from $EuCl_3$ and $GdCl_3$ experiments in D_2O and DMSO-d_6 solutions.

Shifts Ratios (Experimental) *

	H_8	$H_{2'}$	$H_{1'}$	$H_{5'}$
D_2O	0.27	- 0.03	0.05	1.00
DMSO-d_6	- 0.45	- 0.21	- 0.05	1.00

Broadening Distance Ratios (Experimental)

	r_8 / r_2	$r_8 / r_{1'}$
D_2O	1.4	1.0
DMSO-d_6	0.9	1.0

* Theoretical structures were accepted only when the theoretical R_i predicted for them agreed with each experimental R_i to within ± 0.03.

we find that the binding constant in DMSO is approximately the same as in D_2O.

The spectra of the solutions of the dried material at 0.03 M concentration in DMSO-d_6 were taken as a function of added lanthanide chloride, both europium (III) chloride (as a shift probe) and gadolinium (III) chloride (as a broadening probe) being used. In the case of the broadening probe extrapolation to zero metal ion concentration it is not necessary since the concentrations causing broadenings are of the order of 10^{-5} M, in contrast to the 0.05 - 0.5 M concentrations of the shift probe ions. The maximum extrapolation is about 10% from R_i { $[Eu^{+3}] = 0$ } to R_i { $[Eu^{+3}] = 0.5$ M }. Ratios of shifts of the different protons relative to those of 5' protons were calculated as described previously and a computer study analogous to the aqueous case performed. Table I shows the extrapolated shift and broadening rations for AMP (aq.) and AMP (DMSO).

The sets of data for the aqueous and DMSO cases are clearly different and this is reflected in the structures produced by the computer analysis.

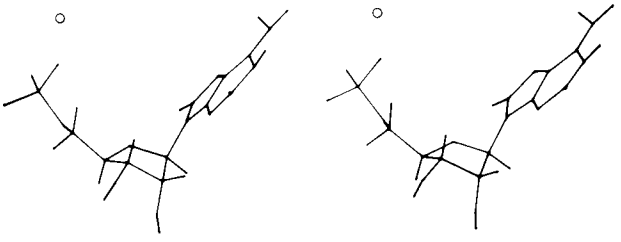

Figure 1: Computer produced stereo view of average structure of AMP in DMSO solution.

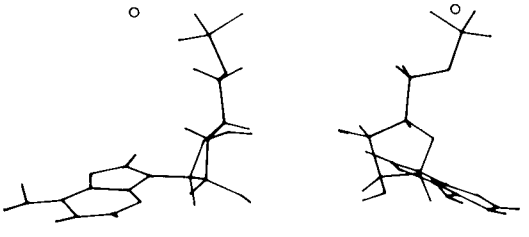

Figure 2: Computer produced orthogonal view of average structure of AMP in DMSO solution.

Figure 1 shows a stereo view of the structure of AMP in DMSO. Figure 2 shows orthogonal projections of the structure and in Figure 3 the structure in DMSO is superimposed on that previously found in water (4). The structures shown in Figure 3 differ mainly by a large angle rotation of the base about its bond to the sugar phosphate. However, the sugar-base configuration is "anti-" (7) in both cases. Without doubt this result could be rationalized by several arguments involving the relative importance of the forces involved in maintaining conformations.

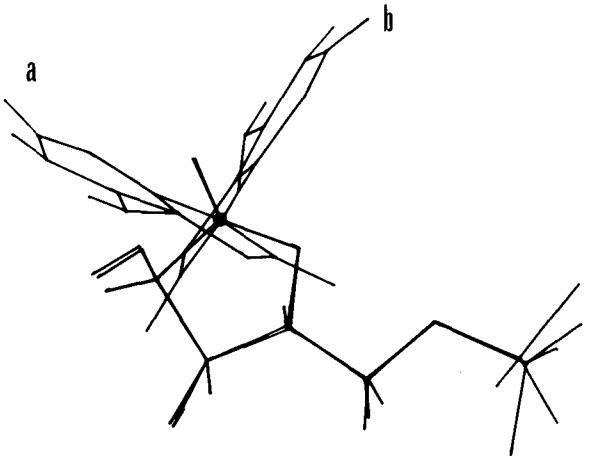

Figure 3: Computer produced super-position of structure of AMP in water (Reference 4) and in DMSO A. Water B. DMSO.

We believe that this technique is applicable to many molecules in DMSO solutions. Moreover, while there has been an awareness of the fact that conformational differences arise in these systems due to solvent effects (1), it has not previously been possible to define the differences other than to state them in general terms. Thus, the terms anti- and syn- mean conformations which may differ by arbitrary small or large amounts depending upon the investigator's limits (7). We believe that the technique described here and in the previous paper serves to define the conformational differences in geometric rather than semantic terms and will therefore enable a more precise comparison with theo-

retical treatments (8) of, for instance, mononucleotide conformation when these are able to include solvent effects.

ACKNOWLEDGEMENTS

A.V. Xavier is under the auspices of the Calouste Gulbenkian Foundation. J.A. Glasel was on leave of absence from the University of Connecticut when this work was performed. R.J.P. Williams and A.C.T. North are members of the Oxford Enzyme Group which is supported by the Science Research Council. We also wish to thank the Medical Research Council for financial assistance.

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