Observation of Large Solvent Effects on the ^{31}P Shielding Tensor of a Cyclic Nucleotide

Ioannis P. Gerothanassis,*[a] Patrick J. Barrie*[b] and C. Tsanaktsidis[a]

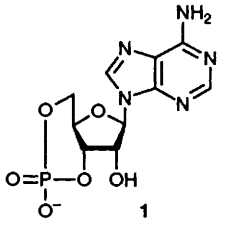
[a] *Department of Chemistry, Section of Organic Chemistry and Biochemistry, University of Ioannina, Ioannina GR-451 10, Greece*

[b] *Department of Chemistry, University College London, 20 Gordon Street, London, UK WC1H OAJ*

High-resolution ^{31}P solid-state NMR measurements on frozen solutions of a cyclic nucleotide (adenosine 3',5'-cyclic monophosphate, sodium salt) provide the ^{31}P shielding tensor and reveal that the chemical shift has a significant dependence upon hydrogen bonding.

^{31}P NMR spectroscopy has been extensively used to probe the structure and dynamics of nucleic acids and nucleic acid complexes in solution.[1-3] Although no single factor can rationalize the range of isotropic chemical shifts, experimental and theoretical studies indicate that the chemical shift is dependent upon both the P–O–P bond angles and the torsional angles which describe the orientation of the R–O bond relative to the plane of the O–P–O group.[1-4] This has prompted many workers to interpret ^{31}P chemical shifts of various polynucleotide systems in solution almost entirely in terms of the conformation of the phosphodiester group.[1-6] Although there are suggestions in the literature that ^{31}P isotropic chemical shifts are sensitive to solvent effects,[7,8] it is surprising that hydrogen bond donors are normally considered to cause only minor changes in the ^{31}P chemical shift other than those explained by a shift in pK.[9,10] It has furthermore been claimed that variations in one of the shielding tensor components will be compensated by an equally large variation in another tensor component with only a small net effect on the isotropic chemical shift.[3,4] In this communication, we report ^{31}P shielding tensor measurements on a phosphodiester nucleotide as a powder and in frozen solutions of water and dimethyl sulfoxide (DMSO). The results suggest for the first time a significant influence of hydrogen bonding on ^{31}P shielding tensor and isotropic chemical shift. The nucleotide chosen was the sodium salt of adenosine 3',5'-cyclic monophosphate (3',5'-cAMP, **1**) in which the phosphodiester group is part of a six-membered ring in a fixed chair conformation,[8,11-13] and thus is expected to have negligible change in conformation with solvent. This nucleotide is of biological importance as it has a role in many metabolic processes and can influence the activity of certain enzymes.

It was found that it was necessary to cool the water and DMSO solutions of 3',5'-cAMP significantly below the freezing point of the pure solvent in order to obtain solid-state NMR spectra of the rigid complex as interesting dynamic effects are observed at intermediate temperatures. Spectra of these solutions as a function of temperature are shown in Fig. 1. Below about 243K the spectra are essentially independent of temperature. At higher temperatures, there is peak broadening and eventual collapse of the spinning sideband pattern to give a single narrow peak with a linewidth indicative of rapid isotropic motion. The spectra imply that a reorientational process with a frequency comparable to the magnitude of the chemical shift anisotropy (approx. 23 kHz) is occurring around 253 K for both the aqueous and DMSO solutions. Good quality spectra were also obtained on the sodium salt of 3',5'-cAMP as a lyophilized powder at room temp. and 203 K to compare with those recorded on the frozen solutions.



1

Simulation of the spinning sideband intensities using an iterative fit programme based on the method of Herzfeld and Berger allows the shielding tensor components to be measured.[14,15] Excellent agreement between simulated and experimental intensities was obtained and the results are reported in Table 1. Despite the dynamic process observed above, the analysis of the spectra at 203 and 233 K give identical results thus indicating that the slow-motion regime has been reached.

It is seen that there is a significant difference in the overall anisotropy (manifested in the $\Delta\sigma$ and Ω parameters) between the three different states. The span varies from 226 ppm for 3',5'-cAMP in DMSO, which is expected to have no hydrogen bonding to the phosphodiester group and to reduce any intermolecular hydrogen bonding interactions which might persist in the solid, to 200 ppm for 3',5'-cAMP in water. It is interesting to observe that the lyophilized powder has an intermediate anisotropy which probably reflects the presence of a single intermolecular hydrogen bond to the phosphate

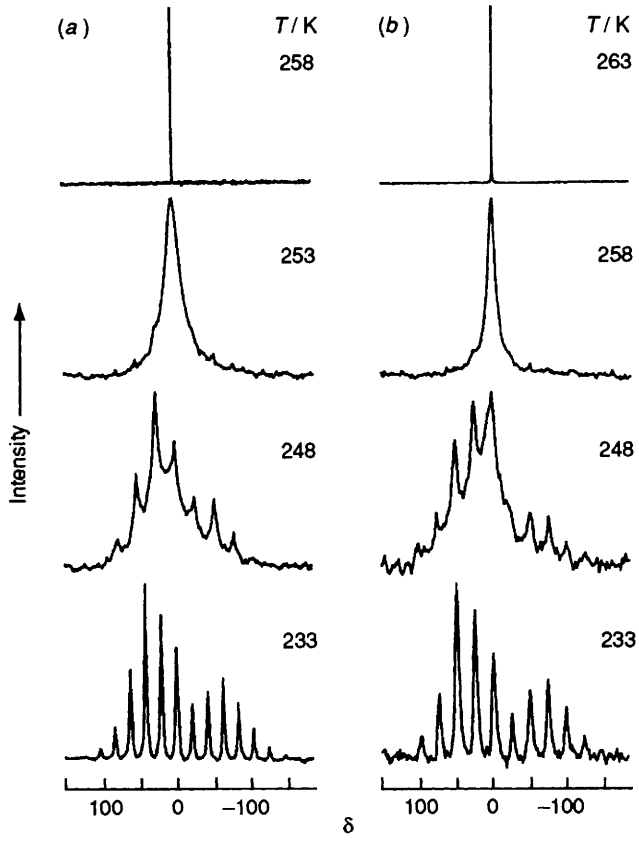


Fig. 1 ^{31}P MAS NMR spectra of 1 mol dm^{-3} solutions of 3',5'-cAMP in water (*a*) and DMSO (*b*) as a function of temperature. The spectra were recorded on a Bruker MSL-300 spectrometer without cross-polarization at spinning speeds of about 3000 Hz (with the exception of the aqueous solution spectrum at 233 K which was recorded using 2525 Hz spinning speed).

Table 1 ^{31}P Shielding tensor results on 3′,5′-cAMP[a]

State	T/K	δ_{iso}	δ_{11}	δ_{22}	δ_{33}	$\Delta\sigma$/ppm	η	Ω/ppm	κ
Powder	295	−1.1	91	33	−127	189	0.46	218	0.47
	203	−1.5	89	33	−126	187	0.45	215	0.48
H_2O (frozen	258	−1.7							
solution)	233	−1.5	83	31	−118	175	0.45	201	0.48
	203	−1.5	83	28	−116	172	0.48	199	0.44
DMSO (frozen	263	−3.0							
solution)	233	−2.8	90	37	−135	199	0.40	225	0.53
	203	−2.9	92	34	−135	198	0.44	227	0.49

[a] The shielding tensor components are given as chemical shifts, δ, rather than shieldings, σ, which have the opposite sign, using the convention $\delta_{11} > \delta_{22} > \delta_{33}$. The other parameters given are the anisotropy, $\Delta\sigma$, defined as $0.5\ (\delta_{11} + \delta_{22}) - \delta_{33}$; the asymmetry parameter, η, defined as $(\delta_{11} - \delta_{22})/(\delta_{33} - \delta_{iso})$; the span, Ω, defined as $\delta_{11} - \delta_{33}$; and the skew, κ, defined as $3\ (\delta_{22} - \delta_{iso})/\Omega$.[16] The isotropic chemical shift is accurate within 0.5 ppm, while the estimated error in the individual shielding tensor components (95% confidence intervals but ignoring the effect of spectral noise[15]) is less than 2 ppm.

group in the solid which is known from the single crystal X-ray structure.[11] This change in anisotropy is due principally to a large variation in the δ_{33} component to high frequency with increased hydrogen bonding (by *ca.* 18 ppm), which is only partially compensated by a smaller shift to low frequency in the δ_{11} component (by *ca.* 8 ppm). The orientation of the δ_{33} component is expected to be in the plane of the P–O(terminal) bonds, and perpendicular to the plane containing the P–O-(bridging) bonds.[17] Hence any change in electron density of the P–O(terminal) bonds due to hydrogen bonding might be expected to affect principally the δ_{33} component, as is observed here. In the case of the nucleotide studied here this results in a change greater than 1 ppm in the isotropic shift which must be due to hydrogen bonding considerations alone. Bearing in mind that the spread of ^{31}P chemical shifts in solution for duplex DNA fragments is generally less than 0.7 ppm,[3] this shows that hydrogen bonding to the phosphate group is a key factor, together with the detailed conformational geometry, in determining ^{31}P isotropic chemical shifts.

We are grateful for financial support from the Greek General Secretary for Research and Technology. The NMR spectra were obtained on the ULIRS solid-state NMR facilities at University College London.

Received, 5th September 1994; Com. 4/05413J

References

1 D. G. Gorenstein, in *Phosphorus-31 NMR: Principles and Applications*, ed. D. G. Gorenstein, Academic, 1984, pp 1–53.
2 D. G. Gorenstein, *Chem. Rev.* 1987, **87**, 1047.
3 D. G. Gorenstein, *Chem. Rev.* 1994, **94**, 1315.
4 D. G. Gorenstein, *Methods Enzymol.*, 1989, **117**, 295.
5 D. G. Gorenstein, E. M. Goldfield, R. Chen, K. Kovar and B. A. Luxon, *Biochemistry*, 1981, **20**, 2141.
6 S. A. Schroeder, J. M. Fu, C. R. Jones and D. G. Gorenstein, *Biochemistry*, 1987, **26**, 3812.
7 G. E. Maciel and R. V. James, *Inorg. Chem.*, 1964, **3**, 1650.
8 D. B. Lerner and D. R. Kearns, *J. Am. Chem. Soc.*, 1980, **102**, 7611.
9 D. G. Gorenstein and A. M. Wyrwicz, *Biochem. Biophys. Res. Commun.*, 1973, **54**, 976.
10 W. Haar, J. C. Thompson, W. Mauer and H. Ruterjans, *Eur. J. Biochem.*, 1973, **40**, 259.
11 K. Watenpaugh, J. Dow, L. H. Jensen and S. Furberg, *Science*, 1968, **159**, 207.
12 B. J. Blackburn, R. D. Lapper and I. C. P. Smith, *J. Am. Chem. Soc.*, 1973, **95**, 2973.
13 W. Saenger, in *Principles of Nucleic Acid Structure*, Springer-Verlag, New York, 1984, ch. 7.
14 J. Herzfeld and A. E. Berger, *J. Chem. Phys.*, 1980, **73**, 6021.
15 G. E. Hawkes, K. D. Sales, L. Y. Lian and R. Gobetto, *Proc. R. Soc. London A*, 1989, **424**, 93.
16 J. Mason, *Solid State Nucl. Magn. Reson.*, 1993, **2**, 285.
17 A. Olivieri, *J. Magn. Reson.*, 1990, **88**, 1.