Solid-state ^{31}P N.M.R. Spectroscopy of 5′-AMP Adsorbed on Zinc-exchanged Clay

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The chemical shift tensor of the nucleotide phosphorus shows that the phosphate binds through one oxygen to the zinc ion.

Nucleotides will adsorb onto metal-exchanged bentonite clays.[1] It has been proposed that such adsorption formed an integral part of the condensation and polymerisation of nucleotides in prebiotic conditions. A study of the conditions under which adsorption will occur indicated that only dication exchanged clays can readily adsorb the nucleotides with the highest binding constant seen for a zinc ion-exchanged clay and 5′-AMP.[1,2] These observations point to the role of the metal ion in providing a binding site. Furthermore it has been noted that the presence of zinc ions can catalyse template directed polymerisation reactions of an activated nucleotide derivative.[3] Recently the mode of binding of the nucleotide to the zinc ion has been examined by INDO semi-empical M.O. calculations.[2] Three types of binding were examined. If we concern ourselves only with the co-ordination of the phosphate group these were: (i) one phosphate oxygen binds to the zinc, (ii) the phosphate bonding to the zinc is mediated through a bound water molecule, and (iii) the phosphate binds through two oxygens. These are shown in Figure 1. From these calculations it was concluded that for zinc-exchanged clay and 5′-AMP there is type (i) bonding.

We report here a preliminary study of 5′-AMP phosphate binding to a zinc-exchanged bentonite clay using solid-state ^{31}P n.m.r. spectroscopy. From an analysis of the chemical shift

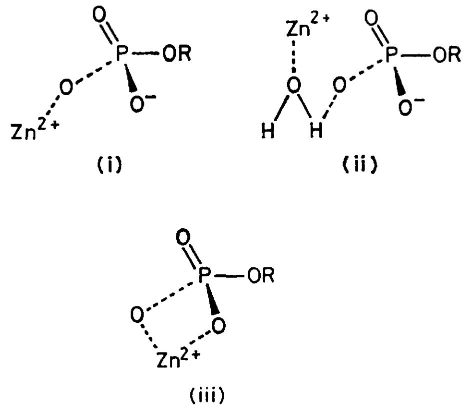

Figure 1. Types of phosphate group binding to the zinc in the metal-exchanged clay.

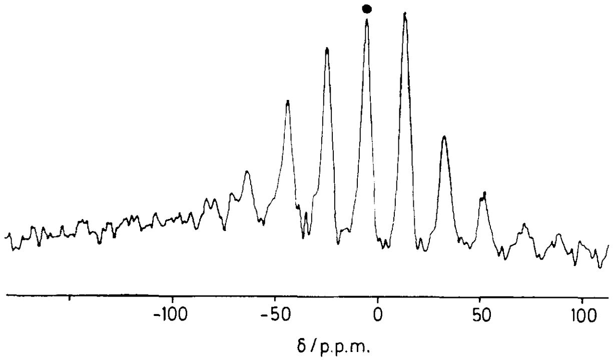

Figure 2. ^{31}P Magic-angle spinning cross-polarisation n.m.r. spectrum of 5′-AMP adsorbed onto a zinc-exchanged bentonite clay. Contact time 1.0 ms. Spinning at 1.6 kHz, 4000 transients accumulated. Chemical shifts in p.p.m. from 85% H_3PO_4 reference; ● indicates the isotropic line.

tensor anisotropy we can suggest a plausible phosphate co-ordination.

We prepared the zinc-exchanged bentonite clay by repeated washing with a 10.0 M zinc chloride solution. To adsorb 5′-AMP we mixed *ca.* 30 g zinc-exchanged hydrated bentonite clay with 20 cm^3 of a 0.07 M solution of the disodium salt of 5′-AMP. The n.m.r. sample of the 5′-AMP adsorbed clay, in an Andrew type rotor,[4] was plugged with Teflon to prevent the clay flowing whilst spinning. The experiments were done at room temperature on a home-built spectrometer operating at 68.38 MHz for ^{31}P and 168.9 MHz for ^1H. Spectra were acquired under proton dipolar decoupling with cross-polarisation and magic-angle spinning.[5] A typical spectrum is shown in Figure 2 together with the experimental conditions.

Approximate values for the principal components of the chemical shift tensor of the phosphorus in the 5′-AMP phosphate group, were derived from a moment analysis of the intensities of the spinning sidebands[6] up to fourth order: $\sigma_{11} = -67.8 \pm 10$ p.p.m., $\sigma_{22} = -10.3 \pm 10$ p.p.m., $\sigma_{33} = 61.9 \pm 5$ p.p.m., and $\bar{\sigma} = -5.4$ p.p.m. Consistent results were found from the spectra obtained at two different spinning speeds. Difficulties in the measurement of the intensities of the higher order sidebands are the most serious source of error and will cause the magnitude of the anisotropy to be underestimated. However, the pattern of the tensor components is not significantly affected by including the higher order sidebands.

Table 1. Chemical shift tensors for phosphorylethanolamine and 3′, 5′-cAMP.

	Phosphorylethanolamine	3′,5′-cAMP
σ_{11}	−67.2 p.p.m.	−88.0 p.p.m.
σ_{22}	−12.0 p.p.m.	−38.0 p.p.m.
σ_{33}	68.6 p.p.m.	124.0 p.p.m.

For example, using only up to the second order sidebands we get $\sigma_{11} = -50.4$ p.p.m., $\sigma_{22} = -10.4$ p.p.m., and $\sigma_{33} = 44.4$ p.p.m.

In order to interpret the ^{31}P chemical shift tensor we have made use of the analysis of Kohler and Klein.[7] They found that the principal axes of the chemical shift tensor in a phosphate group correlates with the ground state electron distribution. Consequently it is to be expected that similarities in the chemical shift tensor components of different phosphates will reflect similarities in the bonding in the phosphate group. The observed chemical shift tensor components closely resemble those of phosphorylethanolamine which has the phosphate environment RPO_3H^- and are quite unlike those of a diester such as 3′,5′-cAMP (Table 1).[8]

We can consider phosphorylethanolamine to be a model for a type (i) phosphate group if we assume that the action of the proton and zinc ion are analogous in involving one of the oxygens in bonding, thus preventing the electron density from participating in π-bonding. This will result in high π-density along the O–P–O axis involving the unco-ordinated oxygens. Similarly, we can treat the cyclic nucleotide as a model for a type (iii) phosphate group as it has symmetric co-ordination to two phosphate oxygens. This suggests that the 5′-AMP adsorbed on the zinc-exchanged clay is not a type (iii) phosphate. To differentiate between type (i) and (ii) bonding we examine σ_{22} and σ_{33}. In the 5′-AMP dianion we have axial symmetry, $\sigma_{22} = \sigma_{33}$.[8] As the interaction between the oxygen atom and ligand increases so electron density is no longer available for π-bonding and charge delocalisation becomes limited to two P–O bonds. This causes σ_{33} to increase. The more similar σ_{22} and σ_{33} the weaker the oxygen–ligand interaction. Thus, since we observe a large difference between σ_{22} and σ_{33} for the adsorbed 5′-AMP we can rule out type (ii) bonding. Furthermore the similarity between these components in adsorbed 5′-AMP and phosphorylethanolamine supports the interpretation of the chemical shift tensor in terms of type (i) bonding. Therefore the ^{31}P chemical shift tensor indicates type (i) bonding between the zinc ion and the 5′-AMP phosphate group in the zinc-exchanged bentonite clay.

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