Copper–Purine Complexes Studied by Proton and Nitrogen-15 Nuclear Magnetic Resonance

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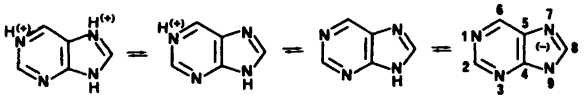
The ^{1}H and ^{15}N spin–lattice and spin–spin relaxation rates of purine were measured at the natural-abundance level as a function of added copper salt. In the anionic form of the purine ligand, with all four nitrogen atoms deprotonated, N7 and N9 resonances are selectively broadened by copper ions, while N1 and N3 are not significantly affected. Proton relaxation data also show that the metal ions bind exclusively to the imidazole part of the negatively charged purine ligand. The neutral ligand is found to bind at both N7/N9 and N1. Chemical shift data indicate appreciable stacking of neutral purine molecules, while the degree of aggregation is considerably reduced for the charged species.

The nature of the interactions between unsubstituted purine and metal ions has been studied both in the solid state[1] and in solution.[2] In the X-ray structure of a copper(II) complex of the neutral purine ligand the metal ion is found to bind at N7 and N9.[1] The exchangeable H atom has been shifted from the imidazole N7/N9 nitrogens to the pyrimidine N1 nitrogen by metal coordination. An unusual feature in this structure is the relatively large difference in Cu–N7 and Cu–N9 bond lengths; 1.90 and 2.04 Å, respectively. The short distance corresponds to a normal Cu–imidazolate bond, while the long distance is typical for interaction between a neutral imidazole ligand and Cu(II).



Proton nuclear magnetic resonance is the spectroscopic technique used most often for studies of metal complexation in solutions. However, an unambigous interpretation of such data is not straightforward, as pointed out by several authors.[3] The expression for paramagnetic metal-induced relaxation is composed of a dipolar term and a scalar term.[4] At present, the theoretical basis is not developed to a level which assures determination of reliable coordination geometries from proton NMR data. Clearly, a more sensitive method to probe metal–ligand binding is to observe the respective nuclei being directly involved in binding. For studies of biomolecule–metal interactions the most relevant nuclei are oxygen-17 and nitrogen-15. The major problem involved in observing these nuclei at natural-abundance level is the inherent low sensitivity. In addition, oxygen-17 resonances are affected by quadrupolar line-broadening.

In earlier studies Kuntz *et al.* used ^{17}O NMR to determine Co(II) binding to mononucleosides.[5] Buchanan and Bell[6] studied diamagnetic metal ion – nucleoside interactions by observing metal-induced ^{15}N chemical shifts. Levy and Dechter[7] measured spin–lattice and spin–spin relaxation of ^{15}N to investigate Mn(II)–AMP complexation. In this latter study the authors concluded that the use of T_1 rather than T_2 relaxation should be preferred in order to obtain reliable geometric information. However, as we will outline in this paper, we do not support this conclusion.

In a recent paper[1] we presented a binding study of the purine–Cu(II) interaction both in the solid state and in solution at low pH. The X-ray structure determined at this low pH showed the purine ligand to be protonated at N1, N7 and N9, with only N3 available for copper binding. However, the ^{15}N spectra recorded at the same pH clearly indicated metal binding at N7/N9 and no interaction at N1 or N3. This shift in metal binding site between the solid state and solution is difficult to rationalize. To probe the metal binding properties of purine further we present a series of proton and nitrogen-15 NMR studies at basic pH.

In order to obtain reasonable *S/N* ratios in natural-abundance ^{15}N spectra a relatively high purine concentration is required. It is known that purines tend to form aggregates (stacks) in neutral solution.[8] Interactions in solution between metal ions and purine monomers may be different from those found between metal ions and aggregates. Thus the extent of stacking at different pH values has also been investigated.

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Experimental

Purine was purchased from Sigma and dissolved without further purification in D_2O (99.7 %) to a concentration of 1 M. A stock solution of 0.1 M $CuCl_2$ made up in D_2O was adjusted to the appropriate pH using DCl and NaOD obtained from Stohler Isotope Chemicals. A Radiometer PMQ-64 pH-meter equipped with an Ingold combination electrode was used; pH values are not corrected for isotope effects.

^{15}N spectra were recorded on a Bruker AM-400 MHz wide-bore spectrometer at 40.55 MHz using 10 mm sample tubes. The typical spectral width was 8000 Hz with 16 K data points. For proton decoupled ^{15}N spectra a reasonable signal-to-noise (*S/N*) ratio was obtained after collecting about 1000 transients. In order to improve *S/N* an exponential multiplication was applied to the FID, adding 4 Hz to the linewidth. Spin–lattice relaxation rates were measured by the conventional inversion–recovery technique (IRFT), and in some cases by the recently published super-fast inversion–recovery (SUFIR) technique.[9] While in IRFT experiments 12–15 τ-values were used, a SUFIR-run requires only 2 τ-values. In cases where the T_1-values can be predicted within a narrow range the latter method has been shown to produce acceptable results within an appreciably shorter time period than by using conventional IRFT. ^1H spectra for shift measurements and T_1 relaxation were recorded as described previously.[2]

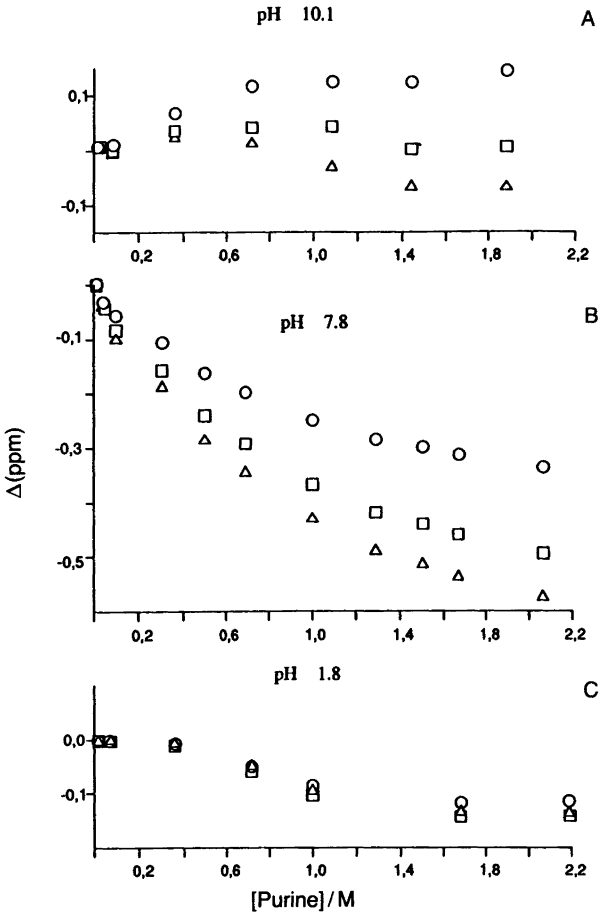

Fig. 1. Concentration dependence of the proton chemical shifts for purine in aqueous solution at 303 K: (□) H2; (△) H6; (○) H8; (A) pH 10.1, (B) pH 7.8, (C) pH 1.8.

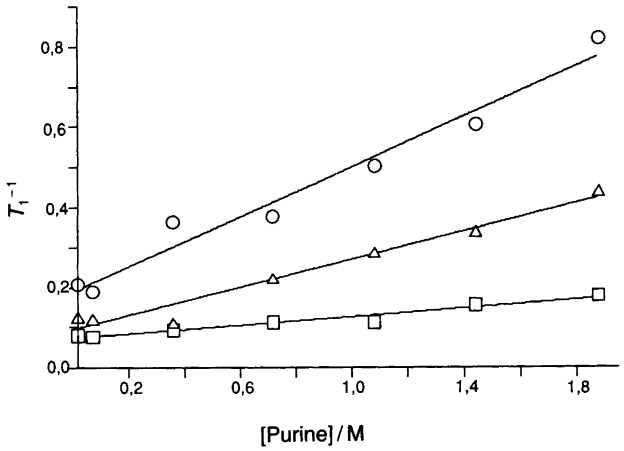

Fig. 2. Proton spin–lattice relaxation rates vs. purine concentration at 303 K, pH 10.1: (□) H2; (△) H6; (○) H8.

Results

Changes in proton chemical shift versus purine concentration are plotted in Fig. 1 at acidic, neutral and basic pH. At pH 7.8 H2, H6 and H8 are shifted upfield with increasing purine concentration. At acidic pH (1.8) a much smaller upfield shift is observed. A more complex pattern is found at pH 10.1, where H2, H6 and H8 exhibit both upfield and downfield shifts within a narrow range.

The proton spin–lattice relaxation rates, T_1^{-1}, are plotted versus purine concentration in Fig. 2 at pH 10.1. A pronounced increase in relaxation rates is observed for all three protons in the order H8 > H6 > H2. No significant variation in T_1^{-1} versus concentration was observed in acidic and neutral solutions. The relaxation rates were found to increase linearly with temperature (data not shown).

A series of proton-decoupled natural-abundance ^{15}N spectra of purine titrated with a solution of $CuCl_2$ (pH 9.2) are displayed in Fig. 3. The assignments of the resonances are based on an analysis of long-range coupling patterns and are in agreement with those published by Schumacher and Gunther.[10] Addition of $CuCl_2$ produces selective line-broadening of N7 and N9, while N1 and N3 are left unaffected. The corresponding T_1 data (Fig. 4) give, qualitatively, a similar interaction pattern, with an appreciable effect on N9, slightly less on N7 and no significant effect on N1 and N3 at a maximum metal/ligand ratio of 5.6×10^{-5}.

In successive experiments at pH 9.2 and 3.1, respectively, ^1H spin–spin and spin–lattice relaxation rates were measured as a function of added copper salt. The maximum

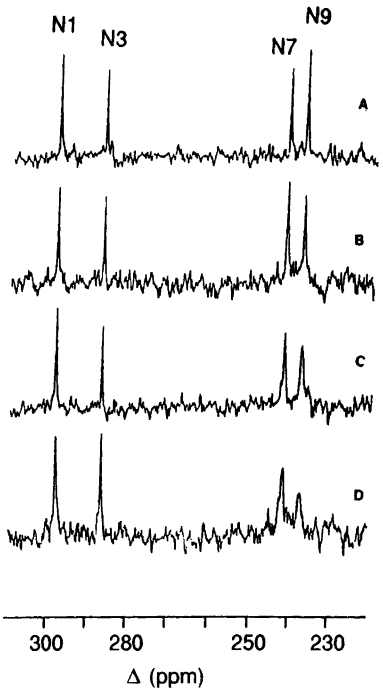

Fig. 3. Natural abundance 40.55 MHz ^{15}N spectra of 1 M purine titrated by a solution of $CuCl_2$ at 303 K, pH 10.1. Copper concentration (A) 0 M, (B) 5.33×10^{-7} M, (C) 1.60×10^{-6} M, (D) 2.93×10^{-6} M.

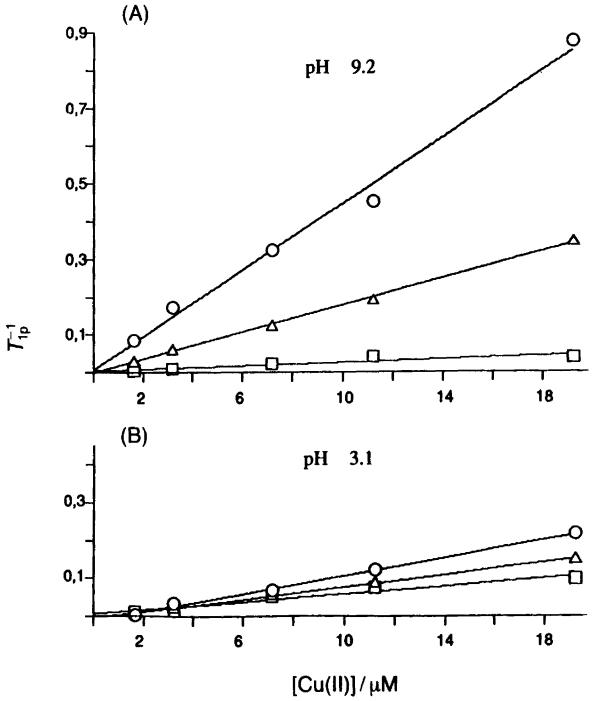

Fig. 5. Paramagnetic, induced ^1H spin–lattice relaxation rates vs. copper ion concentration ([purine] = 1 M, T = 303 K): (A) pH 9.2, (B) pH 3.1; (□) H2, (△) H6, (○) H8.

metal/ligand ratio used in the titration was less than one third that used for ^{15}N. The induced paramagnetic effect was found to decrease in the order H8 > H6 > H2 at pH 3.1 (Fig. 5B). At high pH, H6 and H8 exhibited a corresponding relaxation pattern, while H2 was left unaffected by the added metal ions (Fig. 5A).

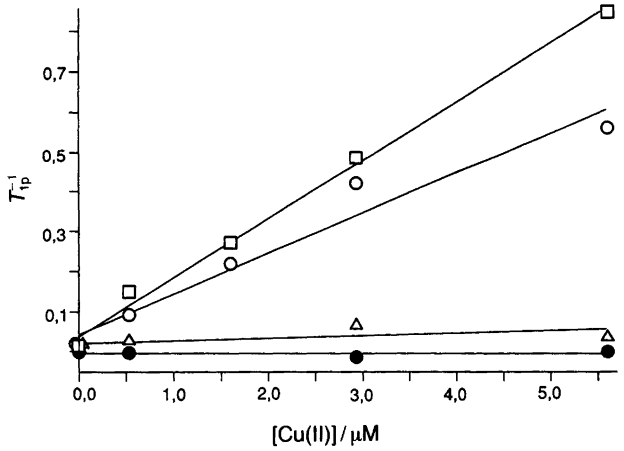

Fig. 4. Paramagnetic, induced ^{15}N spin–lattice relaxation rates in purine vs. copper ion concentration ([purine] = 1 M, T = 303 K, pH 10.1): (□) N9, (○) N7, (●) N3, (△) N1.

Discussion

Paramagnetic line-broadening is always observed for a nucleus directly engaged in binding to a paramagnetic metal ion. The effect is due to scalar 'through-bond' relaxation and dipolar 'through-space' coupling between the nucleus and the paramagnetic centre. It is possible to estimate qualitatively the metal distribution between the N1/N3/N7/N9 sites by comparing changes in relaxation rates as a function of added metal salt. At the low metal/ligand ratio used, only 1:1 metal complexes are expected to be present in solution. Furthermore, since the concentration of metal-bonded species is very low, it is not likely that inter-complex relaxation plays a significant role. However, a large number of purine molecules will be stacked in aggregates at the concentration used, and metal bonding is expected to alter the stacking pattern.[11,12] Thus the dipolar interaction between protons in these aggregates may produce relaxation not directly related to specific metal binding sites.

Shifts toward higher fields with increasing concentration are well known for aromatic systems and are generally attributed to the magnetic anisotropy associated with the ring currents in neighbouring molecules. The influence of hydrogen bonding is expected to produce shifts in the opposite direction. In Fig. 1 the chemical shift data plotted versus purine concentration at acidic, neutral and basic solutions may be interpreted as follows. The most pronounced upfield shift is observed at pH 7.8, indicating

appreciable stacking of neutral purine molecules at 1 M concentration. As expected, the aggregation of charged molecules is much less pronounced, as indicated by shift variations at acidic and basic pH. The complex shift pattern at basic pH may be rationalized in terms of hydrogen-bonding and a certain amount of Na^+-mediated stacking interaction.[12]

In the crystalline state the neutral purine molecule is found in the N7H tautomeric form.[13] Quantum-mechanical calculations[14] on the relative stabilities of the N7H and N9H tautomers indicate practically indistinguishable stabilities, and this is also in accordance with ^{15}N shift measurements in neutral water.[15] It is evident from analysis of the ^{15}N spectra of the ionic ligand (Fig. 3) that N9 rather than the N7 site is preferred. In the crystal structure of the copper complex of neutral purine, N7 is found to form a stronger Cu bond than N9.[1] These observations indicate that the tautomeric equilibrium and charge distribution in purine may be different in solution and in the solid state owing to packing effects.

At the maximum metal/ligand ratio used the ^{15}N relaxation rates (Figs. 3 and 4) show no indication of interaction between metal ions and N1 for the ionic ligand. This observation is corroborated by the proton T_1 rate data (Fig. 5A) which show that H2 is unaffected by paramagnetic ions while H8 and H6 rates reflect the effect of neighbouring N7/N9 coordination. However, at pH 3.1, where purine is in its neutral form, paramagnetic induced relaxation is of comparable magnitude for all three protons, but appreciably less than for the ionic ligand. In unsubstituted purine the intrinsic donor properties of the ligating atoms seem to determine the overall coordination geometry. In this respect metal ions can be regarded as 'fat' protons. For substituted purines like adenine and guanine, on the other hand, the coordination pattern is determined not only by the basicity of the binding site, but also by steric factors. Formation of intra-ligand hydrogen bonds and steric crowding have been shown to favour certain bonding schemes.[16,17] Thus the distribution of metal ions between the N1/N7 sites in nucleotides depends to a large degree on the preferred coordination geometry of the metal ion in question. An octahedrally coordinated metal ion will only fit into the N7 site, while an ion with preferred tetrahedral geometry, e.g. zinc, may equally well be positioned at the N1 site.

Whenever paramagnetic-induced proton relaxation is used to obtain information about metal complexation, certain assumptions are made concerning spin diffusion, scalar interaction and intermolecular outer-sphere contacts. It is generally accepted that geometric information derived from ^1H T_1 measurements is more reliable than that obtained from line-broadening (T_2) data. T_1 effects are determined mainly by a dipolar 'through space' mechanism, so that interpretations based on an r^{-6}-dependence are valid in most cases (r is the distance between the paramagnetic ion and the measured nucleus). Selective proton broadening (T_2 effects), on the other hand, originates predominantly from scalar 'through bond' interactions and does not follow a simple r^{-6}-dependence. For directly bonded nuclei, e.g. nitrogen, the situation is reversed. Scalar 'through bond' paramagnetic induced line-broadening (T_2 effect) is always present when ^{15}N nuclei are bonded to the metal ion. If no such effect is observed one may safely conclude that there is no direct metal bond even if significant paramagnetic T_1 relaxation is present. As mentioned above, this latter effect may be caused by outer-sphere interactions and is no final proof of a direct metal–nitrogen interaction.

In a ^{15}N NMR study of Mn–ATP complexation,[7] N7 and N1 were found to exhibit large paramagnetic T_2 effects, while the amino substituent, N6, showed no such effect. T_1 data, on the other hand, showed almost equally large paramagnetic induced relaxation rates for N1, N7 and N6. In a parallel ^{15}N study[18] on the Cu–AMP system, we have observed similar T_1 and T_2 effects as for Mn–AMP. These results may be interpreted in terms of direct metal–N1/N7 binding, with the amino group hydrogen-bonded to a water molecule in the coordination sphere. Such a bonding scheme is observed in a number of crystal structure determinations of metal complexes of adenine derivatives.[16] Direct coordination between the amino group and the metal ion should produce appreciable scalar T_2 broadening on ^{15}N. Consequently there is no NMR spectroscopic evidence supporting the proposed[7] direct chelate formation involving the amino group and N7.

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