Inorganica Chimica Acta, 35 (1979) 151–160

A Quantitative Investigation of the Interaction of Copper(II) and Manganese(II) with some Purine Bases, Nucleosides and Nucleotides by Nuclear Magnetic Resonance

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Received October 30, 1978

The aqueous solution interaction between Cu(II) or Mn(II) and base coordination sites on some purine bases is examined quantitatively. For Cu(II) it was found that a relatively strong chelate is formed with adenines through coordination at N-7 and the amino group (or imine group). When the C-6 substituent is an oxygen donor a chelate structure is not found and monodentate coordination at N-7 predominates. Mn(II) interacts with hypoxanthines and inosines only through formation of a chelate at N-7 and the C-6 substituent. The competition between base and phosphate for these metal ions is examined by studying the 3':5' nucleotides. Cu(II) interacts only with the base of cyclic AMP but ca. *50% of the Cu(II) is phosphate bound with cyclic IMP and 6-chloropurine-riboside 3':5' phosphate. Mn(II) is predominantly phosphate bound with cyclic IMP and almost wholly so with the others.*

Introduction

Reactions of nucleic acids in biological systems are generally mediated by the presence of metal ions [1]. This interaction may be complex as a variety of sites (on the base and/or phosphate) must be considered. Before examining nucleotide interactions the potentially simpler bases and nucleosides have been studied. Early NMR work employing line width measurements [2–4] on the interaction of Cu(II) with adenines and adenosines was interpreted in terms of only monodentate coordination. Recently the validity of T_2 measurements with Cu(II) has been questioned [5] but a reinvestigation of these systems in dimethyl sulphoxide/water mixture [6] apparently confirmed the validity of the earlier work that the amino group is not involved in metal coordination. An NMR and ESR study was similarly interpreted [7] but with the interesting conclusion that at high pH the ribose is involved in coordination. None of these studies nor others employing a wide variety of techniques [8–12] have arrived at a satisfactory quantitative picture of Cu(II) interaction. Once the nature of the possible interactions with the base is known the competition for coordination between the base and phosphate can be studied and extended to Mn(II) interactions.

Experimental

NMR spectra were run on a Bruker WH-90DS operating in the Fourier transform mode. Acetone was used as an internal reference. Longitudinal relaxation times, T_1, were determined using a $180°-\tau-90°$ pulse sequence. T_2 was determined from line width measurements taking care to avoid the effects of the removal of spin–spin coupling on titration with the metal ion.

The substrates were dissolved in ^2H_2O (99.8%) and adjusted to the required pH with ^2HCl or NaO^2H. They were then lyophilized three times with ^2H_2O.

The internuclear distances were obtained from careful model building using Dreiding models corresponding to known bond lengths and angles from available crystal structure data.

The reported data are corrected for bulk susceptibility changes by subtracting the effect upon the reference resonance. This correction was usually very small and never exceeded 10% of the observed relaxation times.

The experimental techniques involved in the determination of $(T_{1M})^{-1}$ and $(T_{2M})^{-1}$ have been described previously [13, 14]. All experiments were carried out under conditions of fast chemical exchange as demonstrated by the change in T_{1M}^{-1} with temperature.

The metal ion sources were $Cu(NO_3)_2 \cdot 6H_2O$, a solution of a 1:1 mole ratio of $Cu(NO_3)_2 \cdot 6H_2O$ and ethylenediamine and $MnCl_2 \cdot 6H_2O$. Stock solutions 1×10^{-1} *M* and 1×10^{-2} *M* made up in ^2H_2O were adjusted to the same pH as the ligand to be studied. Incremental additions were made with microlitre syringes. Depending upon the pH at which experiments were carried out the metal ion concentrations were in the range 0.1–4 m*M* for Cu(II) titrations and 0.02–1 m*M* for Mn(II) titrations. The observed pH was corrected for isotope effects.

Results and Discussion

Cu(II) markedly affected the relaxation times (T_1 and T_2) of bound ligands. In principle the strong local fields produced by unpaired electrons can be coupled to a nucleus by simple dipole–dipole interactions or by a scalar or hyperfine coupling transmitted through chemical bonds. The Solomon [15] and Bloembergen [16] equations for the electronic contributions are,

$$\frac{1}{T_{2M}} = \frac{1}{15}\frac{g^2\gamma_I^2\beta^2S(S+1)}{r^6}\left[4\tau_c + \frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{13\tau_c}{1+\omega_s^2\tau_c^2}\right] + \frac{1S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left[\frac{\tau_e}{1+\omega_s^2\tau_e^2} + \tau_e\right] \quad (1)$$

$$\frac{1}{T_{1M}} = \frac{2}{15}\frac{g^2\gamma_I^2\beta^2S(S+1)}{r^6}\left[\frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{\tau_e}{1+\omega_s^2\tau_c^2}\right] + \frac{2S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left[\frac{\tau_e}{1+\omega_s^2\tau_e^2}\right] \quad (2)$$

where γ_I is the nuclear magnetogyric ratio, β the Bohr magneton, ω_I and ω_s the nuclear and electronic Larmor frequencies, S the electronic spin quantum number, A the hyperfine coupling constant, τ_c and τ_e the dipolar and scalar correlation times, and r the metal-ion–proton internuclear distance.

The full equations are not easy to work with in terms of trying to determine distances. To simplify them three conditions must be met: (1) that the second term (the scalar contribution) is negligible; (2) that the condition of extreme narrowing holds ($\omega_I^2\tau_c^2 \ll 1$); (3) that τ_c in the equation

$$\frac{1}{\tau_c} = \frac{1}{\tau_s} + \frac{1}{\tau_m} + \frac{1}{\tau_r} \quad (3)$$

where τ_s and τ_r are the electronic and rotational correlation times and τ_m is the correlation time for chemical exchange, is determined by the value of τ_r.

The first condition has been found to be met for Gd(III) and Mn(II) for both T_1 and T_2 [17, 18]. Although in many studies [2–4] it has also been assumed to hold for Cu(II) it has recently been shown that T_2 is rather dominated by the scalar term while T_1 is essentially dipolar [5]. This provides a means of establishing at least the major contributions to an equilibrium involving coordination at more than one site on a ligand. The second condition is met if relatively small molecules are being studied. In this work τ_c is *ca.* 10^{-11}s and $\omega_I^2\tau_c^2$ is *ca.* 10^{-4}s. The third condition is satisfied if τ_r is the shortest correlation time. For small molecule systems and these paramagnetic ions τ_m, $\tau_s > 10^{-9}$s while τ_r *ca.* 10^{-11}s [19].

Under these conditions of fast chemical exchange the paramagnetic ion induced effect upon the relaxation times is given by [20],

$$\frac{1}{T_{ip}} = \frac{1}{T_{iobs}} - \frac{1}{T_{i(o)}} = \frac{f}{T_{iM}} \quad (4)$$

where $T_{i(o)}$ is the relaxation time in the absence of paramagnetic ion and f, the ratio of total paramagnetic ion concentration to total ligand concentration.

Thus provided the conditions above are met equations (1) and (2) become

$$\frac{1}{T_{iM}} = \frac{1}{fT_{ip}} = \frac{K}{r^6} \quad (5)$$

where K is a constant during the experiment. To obtain real values for r requires the accurate determination of τ_r which presents some difficulties. Although some information is lost it is simple to calculate the relative internuclear distances.

Interaction of Purine Bases and Nucleosides with Cu(II)

1-methyl 9-ethyl adenine

The choice of this base for initial study was dictated by the need to reduce as far as possible the number of unknowns in the experiment (which in this case is the number of possible sites of coordination) and have the maximum number of observable protons. A variety of other substituted adenines were examined but were found to have either overlapping base resonance (H-2 and H-8) or overlapping resonances of the blocking groups (*e.g.* the two methyl resonances of 1-methyl 9-methyl adenine).

The protolytic dissociation constant of the ligand has not been determined accurately. However the pK's of 1-methyl adenine and 1-methyl adenosine are 7.2 and 7.6 respectively. A pH titration of the ligand shows that its pK is *ca.* 7.4. Its exact value is not required for this study. At low pH a cationic amino species predominates while at high pH a neutral imine species predominates (Fig. 1).

Relaxation experiments were carried out at pH 4.5 and at 28 °C where all five proton resonances (H-2, H-8, N-1(CH_3), N-9(CH_2·CH_3) and N-9(CH_2·CH_3)) are resolved and measurable. The relaxation data are given in Table I. This shows preferential relaxation of H-8 by bound Cu(II). Consideration must be given to the nature of the species present during the titration.

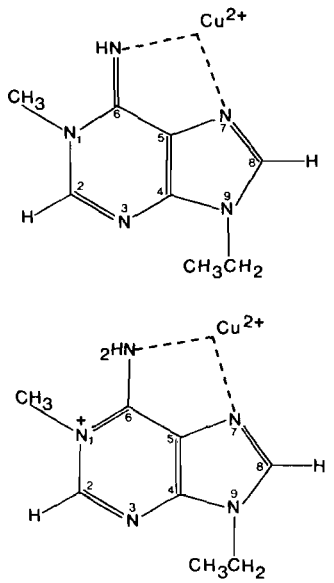

Fig. 1. Predominant species with pH.

Potentiometric studies on the Cu(II)–adenosine system have been reported [21]. The logK for the formation of the 1:1 complex is 0.71, a low figure which is in accord with the relatively high concentrations of Cu(II) needed to induce substantial changes in the relaxation times. No figure is reported for the 1:2 metal to ligand complex. However, throughout the titration the ligand is in great excess. The final points correspond to the Cu(II) to ligand ratio of 1:40. It seems likely that the highest order complex would be present throughout the titration. A further check on this is available. If initially a 1:2 complex predominates changing to a 1:1 during the titration the apparent correlation time for the bound species might be expected to change. This would induce a gradual change in K (eqn. 5) resulting in non-linear graphs of $(T_{ip})^{-1}$ against f. However excellent straight line plots were always obtained indicating that the average stoichiometry remains essentially unaltered during the titration. The precise nature of the complexes need not be known and the results are equally valid whether 1:1, 1:2 or a mixture of species exist in solution.

In considering the position of the metal ion with respect to the ligand the potential binding sites are, (a) monodentate coordination at N-7, (b) bidentate coordination at N-7 and the amino group and (c) monodentate coordination at N-3. Calculations were carried out allowing the presence of all three and also the pairs (a) and (c), (b) and (c). The quality of the fit of the data is expressed in terms of the agreement between the values of K in eqn. 5 for each proton or set of equivalent protons observed. The programme searches for the best fit of these K values using internuclear distances measured from models based on the available crystal structure data. A fit of the values to within ±20% is considered good. This corresponds to an error of the same magnitude in the relaxation data.

The results of the calculations are shown in Table I. When the chelate site is permitted in the calculations excellent correspondence of the K's is found and only this site is observed to be occupied. If only the two monodentate sites are permitted the best fit is extremely poor. If this binding situation were to exist it would imply errors of a factor of 50 in the observed $(T_{1M})^{-1}$ data for some protons. Clearly this binding postulate must be rejected. Two metal ion species were employed, firstly in aqueous solution of copper(II) nitrate and secondly a solution of a 1:1 complex of this and ethylenediamine. The log of the formation constant for the 1:1 Cu(II)–ethylenediamine species is 10.7 [22]. Using the available formation constant data a programme ECCLES [23] was used to calculate the concentrations of the species in solution. More than 99% of the Cu(II) is present bound to ethylenediamine permitting only a 1:1 complex to be formed with the adenine base. Good correspondence of the $(T_{1M})^{-1}$ data for the copper(II) nitrate and copper(II) ethylenediamine systems was found suggesting that only 1:1 complexes are formed in the former system and coordination occurs at the same site in both cases.

The titration was repeated at 60 °C to check that the results were not complicated by intermolecular stacking. Within experimental error the ratio of the $(T_{1M})^{-1}$ data for the observed protons remains constant indicating that intermolecular stacking is negligible.

The best fit is obtained if the metal ion is placed 1.95 Å from N-7 and 2.15 Å from the nitrogen of the amino group. The latter bond distance may not be accurate as changes in the angles at C-6 from those of free adenosine may occur on complexation resulting in a shortening of the predicted bond length. However the conclusion of bidentate coordination is clear.

The observed relative internuclear distances normalified on H-2 are shown in Table I. The agreement between these and distances measured for a model is excellent. With the sixth root dependence of $(T_{1M})^{-1}$ upon r a 10% error in the relaxation time measurements results in a 1.6% error in the internuclear distances. Comparison with the measured internuclear distances for N-7 coordination illustrates clearly that this postulate must be rejected.

Although it has been observed that T_1 is essentially dipolar in origin with Cu(II) [5] the possibility exists that a second mechanism may contribute to the observed effect all be it a minor way. A combination of mechanisms has been recently proposed for Ni(II) interactions [24]. For Cu(II) this would be a dominant dipolar mechanism with a minor scalar interaction in T_1, exactly the reverse order of contributions to proton T_2's in Cu(II) complexes.

TABLE I. Metal Ion Induced Changes in the Relaxation Times Corresponding to the Bound Environment $(T_{1M})^{-1}$ at 28 °C for Coordination postulates.

Ligand		Metal Ion Source		Proposed Coordination Schemes and Populations
Adenine Model				Cu(II) bound at N-1 rÅ Cu(II) bound at N-7 rÅ Cu(II) bound at N-3 rÅ Cu(II) chelate site rÅ
1-Methyl 9-Ethyl Adenine	pH 4.5	Cu(II)	$(T_{1M})^{-1}$Hz	
			K[a]	Chelate C-6(NH_2), N-7 100% Model rÅ Observed rÅ (Based on Cu-H.2)
			K	N-3 74.4%, N-7 25.6%
		Cu(II) 60 °C	$(T_{1M})^{-1}$Hz	
	pH 9.5	Cu(II) (en)[b]	$(T_{1M})^{-1}$Hz	
			K	Chelate C-6(NH), N-7 93% N-3 7%
			K	N-3 83%, N-7 17%
9-Methyl Adenine	pH 2.0	Cu(II)	$(T_{1M})^{-1}$Hz	
			K	Chelate C-6(NH_2), N-7 95% N-3 4%, N-1 1%
	pH 7.1	Cu(II)(en)	$(T_{1M})^{-1}$Hz	
			K	Chelate C-6(NH_2), N-7 85.5% N-1 14.5%
1-Methyl Adenosine	pH 9.0	Cu(II)(en)	$(T_{1M})^{-1}$Hz	
			K	Chelate C-6(NH_2), N-7 93% N-3 7%
			K	N-7 11%, N-3 89%
Tubercidin	pH 7.0	Cu(II)(en)	$(T_{1M})^{-1}$Hz	
			K	N-1 56%, N-3 44%
1-Methyl Inosine	pH 5.5	Cu(II)	$(T_{1M})^{-1}$Hz	
			K	N-7 100% Chelate C-6(O), N-7 98%
			K	N-3 2%
	pH 6.0	Mn(II)	$(T_{2M})^{-1}$Hz	
			K	Chelate C-6(O), N-7 100%
9-Methyl Hypoxanthine	pH 5.5	Cu(II)	$(T_{1M})^{-1}$Hz	
			K	N-7 77%, N-3 3%, N-1 20%
	pH 6.0	Mn(II)	$(T_{2M})^{-1}$Hz	
			K	Chelate C-6(O), N-7 100%
1-Methyl Hypoxanthine	pH 6.3	Mn(II)	$(T_{2M})^{-1}$Hz	
			K	Chelate C-6(O), N-7 100%
2',3'-O-Isopropylidine Inosine	pH 6.0	Mn(II)	$(T_{2M})^{-1}$Hz	
			K	Chelate C-6(O), N-7 100%

[a] Ratios of the constant in equation 5 corresponding to the lifetimes and sites given. [b] en = ethylenediamine.

However the excellent fit in the case of 1-methyl 9-ethyl adenine suggests that in this case the scalar interaction is negligible. We have studied model systems such as substituted pyridines where no ambiguity in terms of the position of the metal ion exists and found very good agreement between observed and calculated distance ratios. In the systems studied below the calculated fit requires a very small (<4%) contribution from a particular coordination site these figures should be treated with caution

Purine Bases and Nucleosides (20 m*M*) Titrated with Cu(11) and Mn(II) and the Best Calculated Fit of the Data for Different

Observed Protons							
H-2	H-7	H-8	H-1′	N-1(CH_3)	N-9(CH_3)	N-9(CH_2CH_3)	N-9(CH_2CH_3)
2.9	5.8	7.35			7.3		
6.65		3.2		6.2	6.3	6.3	5.8
3.0	6.2	5.5		5.6	3.0		
5.7		3.7		4.95	5.7	5.7	6.7
39.4		553		92		38.8	13.8
1.00		1.05		1.01		0.99	0.92
5.7		3.7		4.95		5.7	6.7
5.70		3.71		4.97		5.74	6.82
1.00		52.0		80.5		7.85	4.50
42		61		11		8	v.small
1130		1400		426		580	104
1.00		1.06		0.88		0.99	0.96
1.00		18.1		28.1		1.91	1.38
348		1070			146		
1.00		1.00			1.00		
751		896			54		
1.00		1.11			0.87		
650		1670	2580	630			
1.00		0.99	1.06	1.08			
1.00		22.6	0.78	37.9			
1360	24	24	47				
1.00	1.32	1.40	1.77				
24		1400	76	45			
1.00		0.73	1.65	1.35			
1.00		12.6	3.82	1.62			
236		1605	426	158			
1.00		1.10	0.95	1.00			
857		1340			130		
1.00		1.00			1.00		
245		1510			175		
1.00		1.00			0.99		
214		1330		354			
1.00		0.95		1.10			
195		1920	152				
1.00		1.05	0.92				

as it is not possible to check in each case whether a minor scalar contribution may be present. Even this is unlikely to disturb the overall conclusions to any greater extent as it has been observed that in these systems T_1 and T_2 fortuitously tend to follow the same relative order [5].

Titration of the ligand at pH 9.5 where it exists predominantly in the imine form (Fig. 1) with Cu(II)–ethylenediamine gives fairly similar results to the low pH experiment. At this pH the formation of the complex is considerably stronger as shown by the higher $(T_{1M})^{-1}$ data and the data for the ethyl side

chain are higher in proportion to H-8. Two attempts were made to fit the data, (a) allowing coordination at approximately the same position found at low pH and at N-3 and (b) monodentate coordination at N-7 and N-3. Table I shows that a good fit is found with (a) but an unacceptably poor one with (b). However the best fit requires that 7% of the complexed species have Cu(II) bound at N-3. This might seem a surprising result in view of the fact that N-3 is partially sterically hindered by the ethyl group. However coordination at N-3 is definitely observed in other cases (see below). The best fit is found by placing the metal ion 1.9 Å from N-7 and 1.95 Å from the nitrogen of the imine group. Allowing for an error of ±20% in the observed relation times the contribution from coordination at N-3 is 7 ± 3%. A zero contribution from this species would require an unacceptable error in the observed data. We have found that, contrary to all previous reports, the chelate site dominates coordination of Cu(II) to adenines substituted at N-1 and N-9. That only this site is found to be occupied when an amino group is present for coordination but a combination of sites when an imine is involved might be rationalized in terms of ring strain. The formation of such a 5 membered ring does not yield perfect overlap of orbitals and some distortion to accommodate this by bending of the C-6-NH_2 bond may well occur. Such an effect is less likely to be observed when the C-6 substituent is in the imine form.

9-methyl adenine

At pH 2.0 the ligand exists predominantly in the protonated form. Titration with $Cu(NO_3)_2$ shows, Table I, that H-8 is preferentially relaxed by the paramagnetic ion. As there are only three protons observed calculations can only be carried out limiting the number of possible sites of coordination also to three. As the chelate site is unambiguously observed in the disubstituted adenine there is no reason to expect a change in coordination with this molecule. A perfect fit of this data is found corresponding to species coordinated at the chelate site (with bond lengths corresponding to the amino form of 1-methyl 9-ethyl adenine) 95%, at N-3 4% and N-1 1%. If we allow a ±10% error in the relaxation of H-2 the species coordinated at N-1 can disappear and may not be genuine but the N-3 bound species is definitely required.

Titration of the same ligand at pH 7.1 where the predominant species is the neutral ligand with Cu(II)–ethylenediamine shows, Table I, a nearer equal effect upon H-2 and H-8. Calculations employing the same postulates as at low pH reveal that the chelate complex contributes 86% and the N-1 complex 14% with no coordination at N-3. The relative values of the relaxation data for H-2 and H-8 in 9-methyl adenine are similar to those previously reported for adenosine for the protonated and neutral ligands [2, 6]. For the neutral ligand the near equal effect upon H-2 and H-8 has been assumed to arise from approximately equal contribution from N-1 and N-7 coordination. This appears reasonable if the internuclear distances are considered, *viz.* Cu(N-1) ... H-2 = 2.9 Å and Cu(N-7) ... H-8 = 3.2 Å. For the reasons given above it is not possible to solve the problem of the relative importance of the various sites of coordination for this ligand or adenosine without firstly making an assumption. For both ligands there are 3 useful observable protons but 4 possible sites of coordination: N-1, N-3, N-7 and the chelate site. Previously the chelate site was assumed to be absent but our results on the di-substituted adenines prove this to be incorrect. Rather it is the monodentate N-7 site which should be eliminated from the calculations. In view of these observations all the previous conclusions reached with respect to copper coordination in aqueous solution to substituted adenines need to be re-evaluated.

1-methyl adenosine

The H-2 and H-8 resonances of the protonated species are coincident and prohibit useful studies. However at pH 9.0 they are clearly resolved. In more alkaline solution (at pH 11) the molecule undergoes a Dimroth rearrangement [25] but at pH 9.0 no change was observed in the NMR spectrum over 4 hours. With N-1 blocked and four useful observable protons there is again enough information to discriminate between possible sites of coordination. The observed relaxation is similar to those found with 9-methyl adenine at low pH although relatively H-1′ is higher. Allowing coordination at the chelate site and N-3 produces a very good fit of the data corresponding to 93.5% at the former site and 6.5% at the latter. Replacing the chelate by monodentate coordination almost reverses the concentrations of the complexed species and a best fit is found, Table I, when 89% of complexation occurs at N-3 and 11% at N-7. Such a result in itself seems unlikely but the fit is in any case totally unacceptable.

A problem does arise in postulating the position of the metal ion when bound at N-3. Clearly coordination can only occur there when the ribose is anti with respect to the base and the position used to calculate the results is one where the metal ion lies as close as possible to the bisector of the internal angle at N-3 and such that all atoms are separated by the sum of their van der Waals radii. The Cu–N bond distance is 2.1 Å. Changing this position slightly has a significant effect upon the calculated relaxation of H-1′ and hence upon the predicted contribution from the N-3 coordinated species. The precise contribution from this species lies in the range 4–10%.

It has recently been suggested from ESR data that the interaction between Cu(II) and adenosine in the region around pH 9.5 gives a Cu(II) bridged diamag-

netic complex with the metal ion bound to the ribose [7]. At much higher pH a paramagnetic ribose bound complex is suggested. As two of the coordination sites on Cu(II) are blocked in our experiments by ethylenediamine the same type of diamagnetic complexes are unlikely to be formed. However ribose binding is still possible. This complex would be expected to be paramagnetic as there is little likelihood of Cu–Cu interaction.

As the $(T_{1M})^{-1}$ results are similar to those found for 1-methyl 9-ethyl adenine so too must be the overall formation constant for coordination at base sites. Our results do not unambiguously prove or disprove ribose coordination. A slightly better fit is found if the contribution from N-3 bound species is slightly reduced in favour of ribose coordination. Removing N-3 from the calculation and replacing it by the ribose site gives a poor fit of the data.

Tubercidin (7-deaza-adenosine)

At pH 7.0 tubercidin coordinates to the Cu(II)–ethylenediamine species showing strong preferential relaxation of H-2 and H-1′. This pH is well below the pK of the ribose hydroxyls (*ca.* 12) and the strong effect upon H-1′ must arise through coordination at N-3. Only N-1 and N-3 need be considered for coordination and the calculation gives the surprising result of near equal coordination at each site, N-1 56%, N-3 44%. The fit is not as good as in the previous studies but this arises again through the sensitivity of $(T_{1M})^{-1}$ for H-1′ to very small changes in the metal position. The calculated populations are very sensitive to the exact location of the metal ion when bound at N-3 and are certainly less accurate than those in other systems.

1-methyl inosine

Titration with Cu(II) at pH 5.5 shows that H-8 is strongly preferentially relaxed by the metal ion. An attempt was made to fit the observed data to the two possible coordination schemes, (a) N-3 and chelate formed between the C-6(O) and N-7, (b) N-3 and N-7 monodentate coordination. The former postulated gives a very poor best fit to the data and must be rejected. Monodentate coordination is in accord with the data with the metal ion bound only at N-7. Under the conditions of the experiment the protons H-2 and H-8 are only 5Hz apart. This makes accurate determinations of T_1 values difficult and the data are an average of three independent experiments. The uncertainty in the relaxation data is not so great as to cast doubt on the conclusion of monodentate coordination.

9-methyl hypoxanthine

The relaxation data differs markedly from 1-methyl inosine. The relative increase in the effect upon H-2 indicates that N-1 must be involved in coordination. Calculations based upon possible coordination at N-1, N-3 and N-7 show, Table I, 20% coordination at N-1, 77% at N-7 and 3% at N-3 although this latter figure must be treated with caution for the reasons discussed above.

Interaction of Pusine Bases and Nucleosides with Mn(II)

Mn(II) interacts very weakly with adenines and adenosines, the log of the formation constant for the 1:1 species with the latter being –0.82 [26]. By contrast coordination to bases with an oxygen donor in place of the amino group is strong, the corresponding formation constant for hypoxanthine is 2.4 [27]. Attempts to carry out titrations with adenine type ligands showed that the effect upon the relaxation times of the base protons was only about double that upon the reference protons and such results are not considered reliable. Thus monodentate coordination at base nitrogen donors can be expected to contribute negligibly to the overall relaxation induced by Mn(II) in hypoxanthines and inosines. The only unknown is the precise position of the coordinated metal ion.

As $(T_{2M})^{-1}$ for Mn(II) interaction is dominated by the dipolar term it was found easier and more accurate to make line width measurements. All the Mn(II) titrations were carried out at pH 6.0.

In 1-methyl inosine four proton resonances were observed and the relaxation times, Table I, show strong preferential relaxation of H-8. Knowing that coordination must involve the oxygen donor a unique site was searched for and the apparent bond lengths examined to see if they were reasonable. A very good fit was found with the metal ion placed in a chelate position bound to the oxygen and N-7. The observed bond lengths are 2.25 Å to N-7 and 2.15 Å to oxygen. Studies on 1-methyl hypoxanthine and 9-methyl hypoxanthine give very similar results, Table I, indicating that coordination to other N donors is negligible. Mn(II) would be expected to have a higher affinity for coordination at the ribose than Cu(II). To check that at pH 6.0 the results for 1-methyl inosine are not complicated by ribose coordination 2′:3′-O-isopropylidine inosine in which two of the hydroxyl groups are blocked was studied. The results parallel closely those for 1-methyl inosine indicating no ribose interaction. If any contribution is present from coordination at other than this chelate site its effect is negligible.

Interaction of Some Purine 3′:5′ Nucleotides with Cu(II) and Mn(II)

It is of interest to compare the relative affinity for coordination at the base and the phosphate with these nucleotides as models for DNA interaction. As phosphodiesters they more closely parallel the poly-

TABLE II. Metal Ion Induced Changes in the Relaxation Times Corresponding to the Bound Environment $(T_{1M})^{-1}$ at 28 °C for Some Purine 3':5' Nucleotides (20 m*M*) with Cu(II) and Mn(II) and the Best Fit of Observed Times Averaged Internuclear Distance Rates (r_{Hi}/r_{H_2}) with Calculated Ratios.

Ligand		Metal Ion Source	Internuclear Distance Ratios[a]	Proposed Coordination Schemes and Populations	Observed Protons							
					H-2	H-8	H-1'	H-2'	H-3'	H-4'	H_u-5'	H_d-5'
Cyclic AMP	pH 5.9	Cu(II) $(T_{1M})^{-1}$Hz			1870	1080	81	55	56	-	24	-
			r_{obsd}	Chelate C-6 (NH_2), N-7 70%, N-1 30%'	1.00	1.08	1.68	1.80	1.80		2.06	
			r_{calc}		1.00	1.12	1.73	1.76	1.81		2.07	
	pH 5.9	Mn(II) $(T_{1M})^{-1}$Hz			1390	1390	310	1130	7710		7700	
			r_{obsd}	PO_4 100%	1.00	1.00	1.29	1.04	0.75		0.75	
			r_{calc}		1.00	0.97	1.11	0.90	0.63		0.63	
Cyclic IMP	pH 5.9	Cu(II) $(T_{1M})^{-1}$Hz			1170	1200	50	65	100	176	61	176
			r_{obsd}	N-7 34.5%, N-1 14.5% PO_4 51%	1.00	0.99	1.70	1.62	1.51	1.37	1.63	1.37
			r_{calc}		1.00	0.84	1.51	1.37	1.06	1.35	1.05	1.30
	pH 5.9	Mn(II) $(T_{1M})^{-1}$Hz			224	1550	214	332	2400	550	2130	600
			r_{obsd}	Chelate C-6(O), N-7 30%, PO_4 70%	1.00	0.72	1.01	0.94	0.67	0.86	0.69	0.85
			r_{calc}		1.00	0.74	1.06	0.92	0.70	0,90	0.70	0.85
6-Chloro-Purine-Riboside 3':5' Phosphate	pH 4.0	Cu(II) $(T_{1M})^{-1}$Hz			1100	2510	31	313	175	125	128	157
			r_{obsd}	N-1 9%, N-7 33% PO_4 58%	1.00	0.87	1.82	1.24	1.37	1.45	1.45	1.39
			r_{calc}		1.00	0.90	1.52	1.39	1.06	1.34	1.06	1.30
	pH 4.0	Mn(II) $(T_{1M})^{-1}$Hz			1320	1210	550	1550	10.250	-	15.900	-
			r_{obsd}	PO_4 100%	1.00	1.01	1.16	0.97	0.71		0.66	
			r_{calc}		1.00	1.03	1.15	0.90	0.66		0.66	

[a] r_{calc} assumes the *syn:anti* populations found previously [14].

nucleotide case than do the purine 5′ monophosphates. With the additional binding site it is necessary to observe as many protons as possible and this was achieved by shifting the ribose resonances downfield by the addition of praseodymium nitrate (100 m*M*). A further variable must be brougth into the calculations in that the conformation about the glycosyl torsion angle must be known to predict distances between the metal bound on the base (or phosphate) and protons on the ribose (or base). We have shown previously [14] that these nucleotides exist in a rapid equilibrium between *syn* and *anti* forms with varying contributions from each depending upon the nature of the C-6 substituent. Those results are incorporated into the calculations below. Further it has been suggested [28] that the 5′ nucleotides form metal bridged dimers. However the steric requirements of the 3′:5′ nucleotides prohibit the formation of such dimers and the Mn(II) results are certainly consistent with this.

Cyclic AMP (pH 5.9)

The Mn(II) titration gave results, Table II, which parallel very closely those obtained previously with Gd(III) [14]. The result is expected as coordination at the adenine base is known to be very weak but it does show that no bridging with the Mn(II) coordinated to N-7 directly or through a bridging water molecule occurs. In either case the relaxation of H-8 relative to other protons would be enhanced. The similarity of the results shows that Mn(II) is entirely phosphate bound and that the conformation of the complex is the same as that observed through interaction with Gd(III).

Titration with Cu(II) yields data, Table II, that parallels neither the above experiment nor very closely that on 9-methyl adenine. H-2 is found to be the most strongly relaxed nucleus which can only be explained in terms of a greater contribution from coordination at N-1. The very small induced change in the relaxation of H_u-5′ indicates that coordination at the phosphate group is negligible. This could arise from effective blocking of this site by the lanthanide but repeating the experiment in the absence of the lanthanide where only H-2, H-8 and H-1′ can be observed shows no change within experimental error in the absolute magnitudes of $(T_{1M})^{-1}$ for these protons. A change of at least their relative magnitudes would have been expected if significant coordination at the phosphate did occur. The apparent average metal-ion–proton distances are shown in Table II together with those calculated for the best fit of the data permitting coordination at the sites previously found in the studies on adenines and also the phosphate group. The time average syn-anti equilibrium was also taken into account. The increased contribution from coordination at N-1 may arise through electronic changes in the purine ring on substitution of the ribose for the methyl group at N-9. A recent study [29] of the Cu(II)–ATP system did show phosphate coordination at neutral pH and we have observed a similar interaction with Cu(II) and ADP [30]. However the adenine base (but not others, see below) coordinates to Cu(II) much more strongly than the phosphate.

Cyclic IMP (pH 5.9)

The Mn(II) titrations do not follow closely those observed with Gd(III) [14]. The induced effect upon H-8 is very much greater in comparison to H-2 and H_u-5′ indicating that the chelate site on the purine ring as well as the phosphate is involved in coordination. Control experiments in the absence of the lanthanide show again no significant change in the relative effect upon H-2, H-8 and H-1′. The fit of the apparent internuclear distances with those from averaging 70% coordination at phosphate and 30% at the chelate site is very good.

As with cyclic AMP Cu(II) affects the relaxation of the base protons preferentially but relatively the ribose protons are more strongly affected than in cyclic AMP. In the absence of the base chelate site which has been shown to dominate coordination with adenines coordination at the phosphate could become more important. The best fit of the data supports this with approximately 50% of the metal ion phosphate bound. However the agreement between calculated and experimental results is not as good as with cyclic AMP and it is possible that the results overemphasize the phosphate site but the conclusion of significant coordination there is clear.

6-chloropurine-riboside 3′:5′ monophosphate

At pH 4.0 the Mn(II) data follow closely those found previously with Gd(III) [14]. In the absence of an oxygen donor on the base no significant contribution from base binding would be expected and none is found.

At the same pH Cu(II) induces the largest changes in the relaxation times of H-2 and H-8 but as with cyclic IMP the ribose protons are more strongly affected than coordination at the base alone would predict. Coordination at N-3 is clearly negligible from the observation that H-1′ is the least influenced proton. The best fit of the data gives contributions from N-1 and N-7 but with phosphate as the predominant (58%) site. As above this figure may be somewhat exaggerated as the fit of the data on H_u-5′ is poor.

Conclusion

We have observed that Cu(II) does bind to the amino group in adenines in aqueous solution forming a chelate with N-7. This site dominates coordination

for the adenine bases, nucleosides and for cyclic AMP. With other than an amine (or imine) substituent at C-6, N-7 is the preferred site of coordination for bases and nucleosides but the phosphate group is preferred with the nucleotides studied. Mn(II) has similar chelate structures with hypoxanthine bases and nucleotides but with all the nucleotides studies phosphate binding predominates.

Acknowledgements

This work was supported by grants from the University of Cape Town and the Council for Scientific and Industrial Research.

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