Synthesis and X-ray Study of the Structure of $[Fe(5'\text{-}GMP)(H_2O)_5]\cdot 3H_2O$ and $[Mn(5'\text{-}IMP)(H_2O)_5]\cdot 2H_2O$, and their Relevance to Nucleotide Sugar Conformations

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There is continuing interest [1] in the determination by X-ray methods of the ways in which metal ions bind to nucleotides and how such binding influences such factors as the conformation of the sugar units and the hydrogen-bonding patterns in the vicinity of the metal ions. With purine ribonucleotides and deoxyribonucleotides (nucl) the binding of divalent transition, and other heavy, metal ions, M^{2+}, generally results in the formation of compounds of formula $[M(nucl)(H_2O)_5]\cdot nH_2O$ in which the metal binds to N(7) of the purine ring. In nearly all cases, the sugar adopts C3'-*endo* puckering in the solid compound. The only exception to this, hitherto, is the complex $[Fe(5'\text{-}IMP)(H_2O)_5]\cdot 2H_2O$ which was found [2] to contain three crystallographically independent molecules within the unit cell, one of them having the ribose ring C2'-*endo*, while the other two are C3'-*endo*.

As, to our knowledge, the Fe^{II}–5'-IMP compound was the only iron–nucleotide complex for which X-ray structural information had been reported, we have sought to extend our studies to iron(II) complexes with other purine nucleotides to see if that combination of sugar conformations was shown by those as well. We have isolated crystals of $[Fe(5'\text{-}GMP)(H_2O)_5]\cdot 3H_2O$ and we report here the results of an X-ray study on them and also on crystals of $[Mn(5'\text{-}IMP)(H_2O)_5]\cdot 2H_2O$.

Experimental

Synthesis

$[Fe(5'\text{-}GMP)(H_2O)_5]\cdot 3H_2O$

The preparation was carried out under an atmosphere of nitrogen and the water used was deoxygenated by refluxing for 2 h with passage of nitrogen through the liquid, followed by cooling and storage under nitrogen.

A solution of iron(II) sulphate heptahydrate (60 mM) in water (7.5 cm^3) was brought to pH 5.7 with nitric acid (2 M) and then added to a solution of the disodium salt of guanosine 5'-monophosphate (Sigma) (60 mM in 7.5 cm^3). The mixture was stored under nitrogen for 1 month. The very pale green crystals which formed were collected under nitrogen, but once obtained in the dry state they were reasonably stable to air oxidation. *Anal.* Found: C, 21.41; H, 3.91; N, 12.40. Calc. for $[Fe(5'\text{-}GMP)(H_2O)_5]\cdot 3H_2O$: C, 21.38; H, 4.99; N, 12.47%.

$[Mn(5'\text{-}IMP)(H_2O)_5]\cdot 2H_2O$

Crystals of this compound were obtained by three different procedures, two of which (a) and (b) gave single unit celled versions while the third (c) gave a tripled unit cell form.

(a) Manganese(II) nitrate hexahydrate (60 mM) in water (7.5 cm^3) was added to an aqueous solution of the disodium salt of inosine 5'-monophosphate (Sigma) (60 mM in 7.5 cm^3). The solution obtained (pH 6.8) was allowed to stand at room temperature (*ca.* 20 °C) for 24 h after which the crystals which formed were collected, washed with a little water and air dried.

(b) Method (a) was followed but the solution of reactants was gently heated. At *ca.* 27 °C a faint white precipitate formed, which redissolved on the addition of 3 drops of HNO_3 (2 M), giving a resultant solution of pH 5.5. The solution was then heated at 60 °C for 15 min. After being allowed to cool, the solution was filtered and stored at room temperature. The colourless crystals which formed were collected as in (a).

(c) Method (b) was followed but after heating at 60 °C the solution was immediately filtered and then transferred to a sealed vessel maintained at 35 °C in a water-bath. This solution gave a few colourless crystals after 2 weeks. They were collected as in (a).

X-ray Studies

The iron(II)/5'-GMP complex is monoclinic, with $a = 27.750(3)$, $b = 11.172(2)$, $c = 6.740(1)$ Å, $\beta = 93.43(1)^\circ$ (at 19 °C), space group is $C2$ and $Z = 4$ for a formula unit of $[Fe(5'\text{-}GMP)(H_2O)_5]\cdot 3H_2O$. The manganese(II)/5'-IMP complex obtained by method (c) is orthorhombic with $a = 20.420(3)$ (*i.e.* 3×6.807 Å), $b = 11.324(2)$, $c = 25.775(4)$ Å (at 20 °C), space group is $P2_12_12_1$ and $Z = 12$ for a formula unit $[Mn(5'\text{-}IMP)(H_2O)_5]\cdot 2H_2O$. X-ray intensity data were collected on a Nicolet R3m/Eclipse S140 diffractometer system with graphite monochromated Cu Kα radiation. A total

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of 1498 (Fe compound) and 4501 (Mn compound) independent reflections were measured to $\theta = 57°$. Both structures were refined using the blocked-cascade least-squares method [3] to final values of R of 0.030 and 0.038 respectively. Full details of the crystal structure analysis will be published elsewhere.

When the Mn/5′-IMP compound is crystallized at *ca.* 20 °C (methods (a) and (b)) a different, monoclinic form is obtained, with $a = 6.859(1)$, $b = 11.188(3)$, $c = 26.332(8)$ Å, $\beta = 93.12(2)°$, probable space-group $P2_1$ and $Z = 4$. These crystals are unstable under X-rays and the short 6.86 Å axis quickly becomes tripled on exposure. The similarity of cell dimensions suggests that the basic molecular structure for this form is of the $[M(nucl)(H_2O)_5]$ type, but a full structure determination has, as yet, not been carried out.

Results and Discussion

Figure 1 shows the molecular structure of $[Fe(5'\text{-}GMP)(H_2O)_5]$ in which the iron atom is octahedrally coordinated to five water molecules and to the N(7) position on the guanine moiety. The Fe–N bond length is 2.191(3) Å, while the Fe–O(water) distances are in the range 2.089(3)–2.199(3) Å, with a mean of 2.147 Å. The Fe–N is some 0.08 Å shorter than the mean of three distances (2.269 Å) found in $[Fe(5'\text{-}IMP)(H_2O)_5]$ [2]. The conformation of the ribose ring is C3′-*endo*.

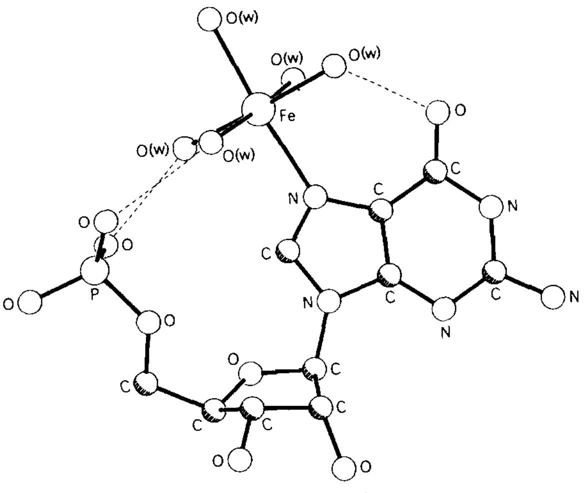

Fig. 1. Molecular structure of $[Fe(5'\text{-}GMP)(H_2O)_5]\cdot3H_2O$. Dashed lines indicate intramolecular hydrogen bonds.

The crystal structure of $[Mn(5'\text{-}IMP)(H_2O)_5]\cdot2H_2O$ shows that it is a second example of the ‘tripled’ cell found for the iron(II) analogue. There are three independent molecules in this structure, two of which have the ribose ring C3′-*endo* and one C2′-*endo*. The molecular structures are very similar to those shown in Fig. 1 of ref. 2. Mn–N distances are in the range 2.318(4)–2.366(4) Å (mean 2.343 Å). This is close to the 2.3 Å reported for Mn(II) bonded to yeast phenylalanine t-RNA [4]. This mean of 2.343 Å is some 0.08 Å longer than Mn–N distances found in several Mn–imidazole compounds [5, 6]. The range of Mn–O distances is 2.130(4)–2.258(4) Å, with a mean of 2.166 Å, typical of Mn–O(water) distances [7, 8].

These results demonstrate that the presence of the two ribose conformations previously found for $[Fe(5'\text{-}IMP)(H_2O)_5]\cdot2H_2O$ [2] cannot be uniquely attributed to the iron atom, as $[Fe(5'\text{-}GMP)(H_2O)_5]\cdot3H_2O$ has the ‘normal’ structure with all ribose rings C3′-*endo*, and also $[Mn(5'\text{-}IMP)(H_2O)_5]\cdot2H_2O$ crystallized at 35 °C has the tripled unit cell, with both

TABLE I. Crystal Data for $[M(nucleotide)(H_2O)_5]\cdot nH_2O$ Compounds

M	Nucleotide	n	Space group	a (Å)	b (Å)	c (Å)	β (°)	Volume (Å^3)		Reference
Mn	5′-GMP	3	$C2$	27.809(3)	11.230(1)	6.757(1)	93.11(1)	2107.1		10
Fe	5′-GMP	3	$C2$	27.750(3)	11.172(2)	6.740(1)	93.43(1)	2085.8	b	this work
Co	5′-GMP	3	$C2$	27.647(5)	11.133(3)	6.724(2)	93.62(2)	2065.5		11
Ni	5′-GMP	3	$C2$	27.604(4)	11.087(2)	6.715(2)	93.94(1)	2050.2	b	12
Cd	5′-GMP	3	$C2$	27.849(7)	11.361(5)	6.774(3)	92.78(3)	2140.7	b	13
Co	5′-dGMP	3	$C2$	27.574(6)	11.140(3)	6.750(2)	92.22(2)	2071.9	b	14
Ni	5′-dGMP	3	$C2$	27.433(30)	11.034(20)	6.713(6)	92.53(5)	2030.0	b	14
Ni	5′-AMP	1	$C2$	25.736(3)	10.815(1)	6.925(2)	90.50(3)	1927.4		15
Mn	5′-IMP	(?)	(?)	26.332(8)^a	11.188(3)	6.859(1)^a	93.12(2)	2017.7		this work
Mn	5′-IMP	2	$P2_12_12_1$	25.775(4)^a	11.324(2)	20.420(3)^a		5960.1	b	this work
Fe	5′-IMP	2	$P2_12_12_1$	25.686(3)^a	11.277(1)	20.395(2)^a		5907.6	b	2
Co	5′-IMP	2	$P2_12_12_1$	26.011(4)	10.879(1)	6.861(1)		1941.6		11
		2	$P2_12_12_1$	25.987(5)	10.859(5)	6.845(5)		1924.9	b	16
Ni	5′-IMP	2	$P2_12_12_1$	25.898(5)	10.812(5)	6.845(5)		1916.7	b	16
		2	$P2_12_12_1$	25.925(3)	10.812(2)	6.853(1)		1920.9	b	17
Co	5′-dIMP	2	$P2_12_12_1$	25.421(6)	10.904(2)	6.877(3)		1907.1	b	1

^{a}The values of a and c are interchanged. ^{b}A full structure determination was carried out.

sugar conformations (C3′-*endo* and C2′-*endo*) present.

This provides further support for our suggestion [2] that in at least some of the $[M(nucl)(H_2O)_5]$ systems there may be little energy difference between the two conformations and both may coexist in solution. If that is so, the dominance of the C3′-*endo* conformation deduced from the accumulated X-ray studies (Table I) may derive from the crystallization conditions, and especially the temperature (rarely stated), employed. It has been noted previously [9] that a modest increase in temperature to 34 °C is sufficient to cause a change in the geometry around the metal ion in the Co(II)/5′-AMP system from octahedral to tetrahedral in water. It would appear that the influence of temperature changes, within a biologically relevant range, on the structural consequences of the binding of metal ions to nucleic acids and their components would merit more detailed investigation.

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