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The site of metal binding in an iron(II) derivative of inosine 5′-monophosphate

An X-ray and spectral study

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An iron(II) derivative of inosine-5′-monophosphate has been prepared and fully characterized by X-ray structure analysis. Its composition is $[Fe(5'\text{-IMP})(H_2O)_5]\cdot 2H_2O$, and the metal atom binds at the N(7) position on the purine moiety. Two different conformations coexist within the structure, so that of the 3 crystallographically independent molecules, one has the ribose ring C2′-*endo*, while the other two are C3′-*endo*.

1. INTRODUCTION

During the past few years there has been appreciable interest in the sites of metal ion binding to nucleic acids and their component units. As well as the well-established range of metal ions involved in natural nucleic acid processes [1], there are also metals, e.g., platinum [2], whose binding to DNA leads to chemotherapeutic behaviour. A significant development in this area has been a rapid growth in the number of X-ray structural determinations of metal ion binding sites [3–5]. The majority of these studies have involved the metal derivatives of units of nucleic acids rather than the nucleic acids themselves, but such information has been valuable in providing detailed information about potential binding sites. This is useful for checking deductions made, e.g., from NMR spectroscopy and for aiding in X-ray studies on more complex systems such as tRNA [6].

One surprising omission from the list of metal ions for which such X-ray information is available is iron. This metal ion plays an important role in natural processes in which its relative ease of undergoing changes in oxidation state, and attendant bonding changes, is a key factor [7]. It was recognized very early that iron was a component of tobacco mosaic virus DNA and that it was strongly bonded [8]. Although there have been reports of studies of the binding between iron and nucleic acids and component nucleotides, using techniques such as NMR, Mössbauer spectroscopy etc. [9], firm X-ray evidence is, apparently, lacking.

We have been able to isolate a crystalline sample of a hydrated iron(II) derivative of inosine 5′-monophosphate and have determined its structure by X-ray methods. We have also measured the optical spectrum of the solid so that the spectral data of this sample of known geometry can be used for comparison with those of related iron(II) derivatives in solution or in solids not amenable to X-ray study. We here report the results of this work.

2. MATERIALS AND METHODS

2.1. *Synthesis*

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.

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A solution of iron(II) sulphate heptahydrate (60 mM) in water (7.5 cm^3) was added to one of the disodium salt of inosine 5′-monophosphate (Sigma) (60 mM, in 7.5 cm^3). An immediate, flocculent off-white precipitate was obtained. Over a period of 2–3 days at room temperature the supernatant liquid gave some very pale green crystals on the walls of the container. These crystals were filtered off under nitrogen, washed with deoxygenated water and dried under nitrogen. Once obtained in dry state, the crystals are apparently reasonably stable to air oxidation. Analytical results (Microanalytical Laboratory, Imperial College) were consistent with the formulation: $Fe(C_{10}H_{11}N_4O_8P)\cdot 7H_2O$; Calc. C, 22.74; H, 4.77; N, 10.61. Found, C, 22.69; H, 4.10; N, 10.54%.

Samples can also be obtained using hydrated iron(II) perchlorate instead of iron(II) sulphate. (Anal. Found. C, 22.75; H, 4.35; N, 10.39%).

2.2. *Physical measurements*

The magnetic moment at room temperature (5.38 B.M.) was measured on a conventional Gouy balance with field calibration by $HgCo(NCS)_4$. The reflectance spectrum in the visible-near i.r. region was obtained on a Cary 14 spectrometer.

2.3. *X-ray studies*

The most common type of metal–purine nucleotide complexes of formula $[ML(H_2O)_5]\cdot nH_2O$ (where L = 5′-GMP, 5′-IMP, and 5′-AMP) have unit cells which are orthorhombic of about 7 × 11 × 26 Å, or similarly sized monoclinic with β close to 90° [10]. The iron(II) complex has an orthorhombic unit cell where the sub-cell is similar, but the true unit cell has one of the sides tripled, with a = 20.395(3) (i.e., 3 × 6.798), b = 11.277(1), c = 25.686(3) Å (at 18°C). Space group is $P2_12_12_1$ and $Z = 12$ for a formula unit of $[Fe(5'\text{-}IMP)(H_2O)_5]\cdot 2H_2O$. X-Ray intensity data were collected on a Nicolet R3m/Eclipse S140 diffractometer system with graphite monochromated Cu-Kα radiation. A total of 4451 independent reflections were measured to $\theta = 57°$.

The difficulty in solving this structure lay in the fact that the metal atoms with their coordinated waters, the purine bases, the phosphate groups and some of the solvated waters, all nearly conform to the simple untripled cell, and the only major difference is in the conformations of the ribose rings and the positions of 3 solvated water molecules. Refinement based on the untripled cell, and then careful use of Fourier methods and the block cascade least-squares procedure [11] on the true cell led to a solution of the structure, and R is now 0.059. Details of the X-ray analysis will be published elsewhere.

3. RESULTS AND DISCUSSION

Within the structure all 3 independent metal-nucleotide molecules have the iron atom octahedrally coordinated to 5 water molecules and to the N(7) position on the purine moiety (fig.1). The Fe–N distances are in the range 2.240(8)–2.311(8) Å (mean 2.269 Å), while the Fe–O distances are in the range 2.072(8)–2.228(8) Å (mean 2.124 Å).

The reason that the tripling of the unit cell occurs is that two molecular conformations coexist within the structure. One of the molecules has the ribose ring C2′-*endo*, while the other two are C3′-*endo* as in all the simple untripled $[ML(H_2O)_5]\cdot nH_2O$ structures. The coexistence of these two conformations would suggest that there is little energy difference between them, and that both may exist in solution.

A feature of the molecular geometry is the existence of a pair of intramolecular hydrogen bonds between two of the phosphate oxygens and two of the coordinated water molecules. They have the effect of bringing the phosphate group into the vicinity of the iron atom. (For similar compounds of the $[ML(H_2O)_5]\cdot nH_2O$ type, this proximity originally led to the postulate, based on changes in the i.r. spectra, that the phosphate was bonding directly to the metal atom [12,13].) Within each molecule there is a further, weaker, hydrogen bond between 0(6) and another of the coordinated water molecules.

Apart from the intramolecular hydrogen bonds, there is a network of intermolecular hydrogen bonds of the type O–H...O, O–H...N, and N–H...O involving also the waters of crystallization, the OH groups of the ribose moieties, and the N(1)-H and N(3) atoms of the purine ring systems.

The magnetic measurements showed that the iron(II) atom is in the high-spin state ($\mu_{eff} = 5.38$). This is further demonstrated by its electronic spectrum (fig.2), which also shows the effects of the

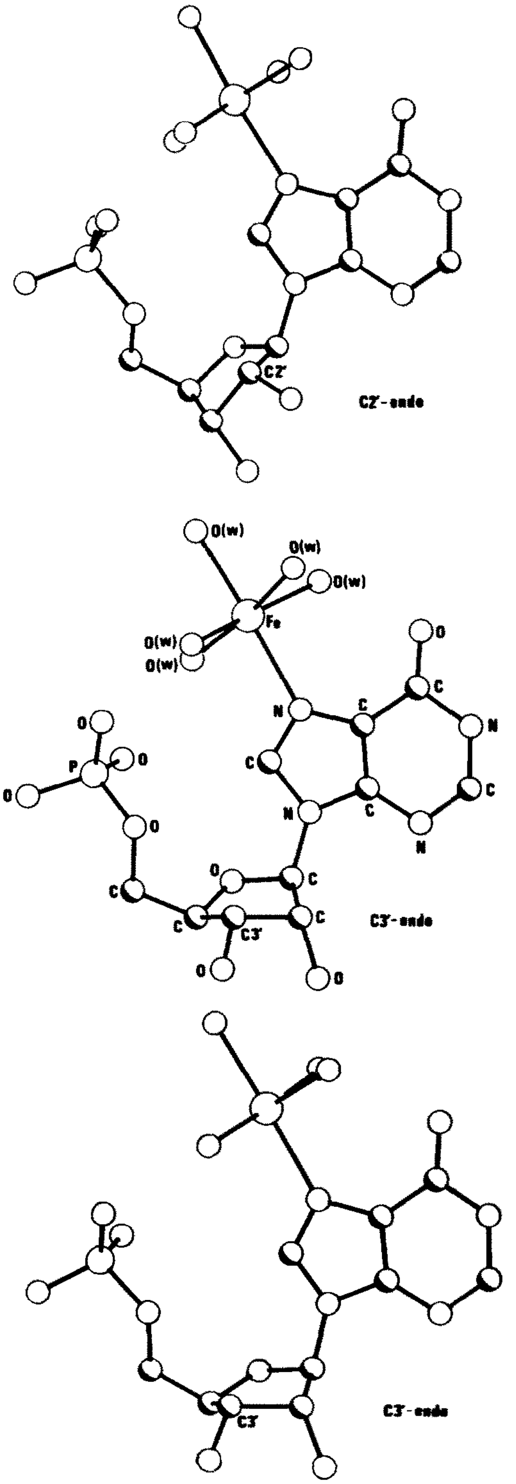

Fig.1. The 3 crystallographically independent molecules in the title compound. Each molecule is viewed normal to the plane of the purine ring system.

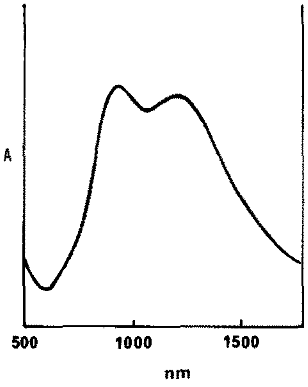

Fig.2. Reflectance spectrum of [Fe(5'-IMP)(H_2O)$_5$]·$2H_2O$.

distortion from O_h symmetry of the ligand field, in the splitting of the ${}^5E_g$ level. If iron(II) were to bind in an analogous fashion in a nucleic acid system the high-spin state would result in a kinetically labile situation. Replacement of some of the coordinated water molecules by additional nitrogen donors would result in a strengthening of the ligand field about the iron atom and could lead to a change in oxidation state. The type of geometry we have found for the title compound is thus one which could clearly be involved in a range of reaction sequences in iron/nucleic acid chemistry.

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