# Iron (III) tris(monohydrogen phosphito) chloride – a novel complex

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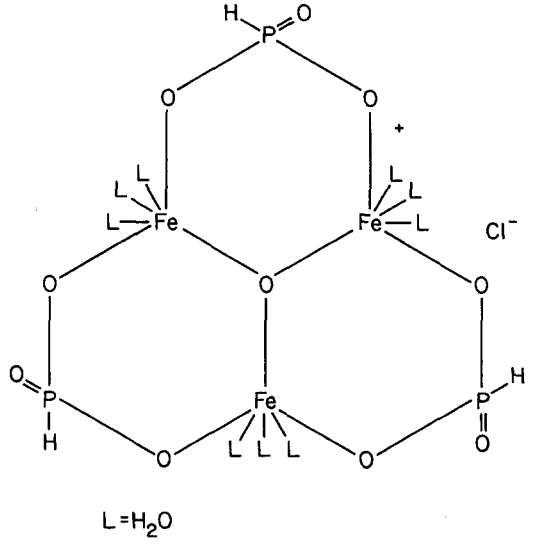
*Summary.* This paper describes the spectral and magnetic properties of a novel complex, iron(III) tris(monohydrogen phosphito) chloride, obtained unexpectedly by the reaction of ferric chloride, L-valine, and phosphorous acid.

Previous reports concerning the complexes of iron(III)-L-amino acid perchlorates[2-4] and iron(III)-L-amino acid nitrates[5] containing the trimeric species $[Fe_3O]^{7+}$ have indicated that they are strong contenders as model compounds for the ferritin iron core. During an effort to prepare iron(III)-L-valine tris(dihydrogen phosphito) chloride by refluxing a mixture of an aqueous solution of L-valine, ferric chloride, and phosphorous acid under the conditions employed for the preparation of trinuclear oxobridged iron(III)-L-valine tris(dihydrogen phosphito) nitrate[6], it was observed no incorporation of L-valine into the $[FE_3O]^{7+}$ unit took place. The structure of iron(III)-L-amino acid perchlorates has been established in our laboratory by X-ray diffraction, Mössbauer and magnetic susceptibility measurements, electronic and spectral studies[2-4,7,8]. However, a detailed examination of the analytical, spectral and magnetic data of the complex in this work and comparison of these properties with those of the known iron-(III)-L-amino acid perchlorates[2-4,8] and nitrates[5], and iron-(III)-carboxylates[9] containing the trinuclear oxobridged iron(III) unit, $[Fe_3O]^{7+}$ revealed the formation of a novel complex iron(III) tris(monohydrogen phosphiti) chloride, $[Fe_3O(HPO_3)_3(H_2O)]Cl \cdot 3\ H_2O$ to which the molecular structure shown in the figure has been assigned. The same trinuclear oxobridged iron(III) complex was obtained when the reaction was carried out with glycine, L-alanine, L-proline, and L-leucine under the conditions employed for L-valine.

The observed analytical values for the elements iron and chlorine (found: Fe, 23.59; Cl, 5.48 and calculated: Fe, 24.88; Cl, 5.26) for the postulated formulation $[Fe_3O(HPO_3)_3(H_2O)_9]Cl \cdot 3H_2O$ are in agreement with the calculated values. However, in our hands it was not possible to obtain correct phosphorus analysis. This observation agrees with our experience with other trinuclear iron(III)-L-amino acid complexes containing phosphorus[6] and the experience of other investigators[10]. The solid state (nujol mull) electronic spectrum of the complex (fig.) exhibits bands at 10,416 and 16,393 $cm^{-1}$; these may be assigned to the transitions $^6A_1 \rightarrow {}^4T_1$ and $^6A_1 \rightarrow {}^4T_2$ respectively and result from the presence of $[Fe(III)O_6]_{oct}$ unit in molecular complexes containing trinuclear oxobridged iron(III) nucleus, $[Fe_3O]^{7+}$. Similar absorption bands have been observed in the spectra of the known complexes[2-6,8] containing the $[Fe_3O]^{7+}$ unit. The infrared spectrum of the complex in this work shows a broad and strong absorption in the region 3100–3600 $cm^{-1}$ attributed to asymmetric and symmetric O–H stretching modes of water present as coordinated ligand and/or as lattice water[11,12]. The broad absorption band at 1640 $cm^{-1}$ in the spectrum has been assigned to the water of crystallization[13]. The spectrum shows a broad and weak absorption at 2420 $cm^{-1}$ characteristic of a P–H stretching mode[14]. One very important absorption band in the IR-spectrum of the trinuclear complex in question is the presence of broad and intense absorption in the region 1070–1200 $cm^{-1}$; this is attributed to the P=O stretching mode[15], and further confirms the conclusion that phosphorous acid in this complex coordinates to iron(III) in a bidentate fashion, transferring 2 units of negative charge to the metal ion, different from that of its bidentate coordination in the iron(III)-L-amino acid tris(dihydrogen phosphito) nitrates[6] where it coordinates to the metal ion in a manner analogous to that of the carboxyl function of the amino acids and serves to transfer only 1 unit of negative charge to the metal ion. The 2 different modes of ligation of phosphorous acid to iron(III) confer different charges on the resulting cationic complexes as evidenced by the analytical values. The spectrum also exhibits broad and weak absorption bands in the region 300–600 $cm^{-1}$; these are attributed to Fe–O stretches[3,4]. The symmetric and asymmetric stretch frequencies of the carboxylate function of L-valine are noticeably absent from the IR-spectrum of the complex showing that the amino acid residue is not ligated to the irons in this complex. The magnetic susceptibility per iron atom and magnetic moment per iron atom at 294 °K were found to be 5993 cgsu and 3.77 BM respectively. The corresponding value for the exchange integral, $-J$, calculated by the method of Earnshaw et al.[16], was found to be 18.2 $cm^{-1}$. The value of the magnetic moment and the exchange integral suggest that complex in the figure is antiferromagnetic in character. However, a value of 3.77 BM for the tris(monohydrogen phosphito) complex although very suggestive of the presence of $[Fe_3O]^{7+}$ unit in the molecular structure, is slightly higher than the values of similarly constituted known complexes[2-6,8] containing the $[Fe_3O]^{7+}$ unit. This may be due to spin-orbit coupling effect but the precise reasons must await detailed examination of its 3-dimensional structure.

It can be concluded that under different reaction conditions, phosphorous acid can ligate to metal ions in at least 2 different bidentate modes. It is interesting to note that under identical experimental conditions iron(III) providing reagents ferric nitrate and ferric chloride give rise to 2 different types of cationic complexes with L-amino acids:




Schematic representation of the molecular structure of the trinuclear oxobridged iron(III) tris(monohydrogen phosphito) chloride.

with the former reagent there is an incorporation of the L-amino acid in the $[Fe_3O]^{7+}$ unit while with the latter no incorporation of L-amino acid in the $[Fe_3O]^{7+}$ unit is observed.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## RNA polymerase activities in the isolated perfused rat liver


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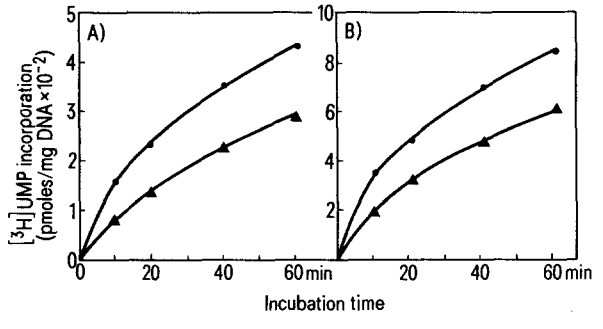
*Summary.* The routine procedure for the isolation of rat liver induces a significant fall in RNA polymerase I and II activities which are rapidly restored to the control levels during perfusion.


The technique of isolated liver perfusion offers a convenient model of an integrated metabolic system where cell and tissue organization is preserved, and regulatory mechanisms are still operating under physiological conditions, but without the interference of other organs[1]. In addition, the perfusion of liver with a recirculated medium offers the advantage over in vivo studies that serial sampling of the perfusion fluid can be made and portions of the liver can be removed at any stage during the perfusion for biochemical determinations and for ultrastructural analysis[1,2].

Recently it has been demonstrated that the isolated perfused liver technique is suitable for studying hepatic RNA synthesis as evaluated by the incorporation of orotic acid into different species of RNA[3-5]. In this paper we report studies on the levels of nuclear RNA polymerase I and II activities (the enzymes responsible for rRNA and mRNA synthesis, respectively) during perfusion of the isolated rat liver.

Our results demonstrate that at the start of perfusion the levels of RNA polymerase I and II activities are significantly below control values and are rapidly restored during perfusion.

*Materials and methods.* Male Wistar rats (160–200 g), fasted overnight, were anesthetized with Pentothal (50 mg/kg b.wt) and opened for liver isolation and perfusion[5]. The perfusion medium was Krebs-Ringer bicarbonate buffer, pH 7.4, containing bovine serum albumin (3%, w/v), and washed rat erythrocytes to give a haematocrit of 20%. The perfusate also contained: glucose 50 mg, heparin 5000 units, penicillin 1500 units, streptomycin 1.5 mg, lactate 1 mM, pyruvate 0.1 mM and an amino acid mixture corresponding to the normal rat plasma concentration in a final volume of 50 ml[6]. In addition, from the start of perfusion a concentrated amino acid solution was continuously infused into the perfusate reservoir to avoid a decrease in the perfusate amino acid concentration during




Time-course of nuclear RNA polymerase I (A) and II (B) activities in nuclei isolated from the liver portion removed at the start of perfusion (▲) and from the remaining liver perfused for 4 h (●). RNA polymerase activities are expressed as [$^3$H] UMP pmoles/mg DNA/20 min. The values represent the arithmetical mean of 8 experiments.

Nuclear RNA polymerase I and II activities in nuclei isolated from perfused rat livers and controls

| Experimental conditions | RNA polymerase I | RNA polymerase II |
|---|---|---|
| Control (4) | 218±36 | 421±71 |
| Liver sample removed at the start of perfusion (15) | 118±14 | 288±37 |
| Lobectomized liver perfused for 2 h (3) | 193±38 | 383±49 |
| Lobectomized liver perfused for 4 h (13) | 210±25 | 432±46 |
| Intact liver perfused for 4 h (8) | 196±32 | 454±51 |

RNA polymerase activities are expressed as [$^3$H] UMP pmoles/mg DNA/20 min. The values represent the arithmetic mean ± SD of the number of experiments given in parentheses. Control values are from livers rapidly removed from rats stunned and killed by decapitation.