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Mössbauer Studies on Oxygen Binding in Protoporphyrin IX Iron(II) Solutions in the Presence of Other Ligands

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Abstract

Mössbauer parameters of frozen solutions of protoporphyrin IX iron(II) (containing either 2-methyl-piperidine or mercaptoethanol as the fifth iron ligand) that were exposed to oxygen before freezing are similar to those of oxyhaemoglobin. These results are discussed in relation to known porphyrin iron(II) chemistry.

Introduction

The mode of action of dioxygen with protoporphyrin IX iron(II) [PPIXFe(II)] is a subject of great interest to chemists and biochemists [1–5]. Synthetic oxygen carriers have been known for over four decades. They have aroused the attention of the scientific community because of the role of such centres in naturally occurring oxygen-storage (*e.g.* myoglobin) and -transport (haemoglobin) systems. The most ubiquitous active site found is the iron porphyrin moiety (a haem unit). Such units are the prosthetic groups in myoglobin, haemoglobin, P450 monooxygenase tryptophan dioxygenase, and cytochrome *c* oxidase. Catalase and peroxidases also contain haem centres and their utilization of hydrogen peroxide is clearly related to the dioxygen systems. Hence studies on oxygen-containing metalloporphyrins outside of a protein environment are of obvious importance for an increased understanding of the structure and function of biological systems that use oxygen, and for the development of catalysts that mimic the enzymes.

Studies on model complexes for myoglobin, and haemoglobin have concentrated on picket-fence porphyrins [7] or capped, bi-capped and pocket porphyrins [8–13]. All these models contain haem units that are (i) not naturally occurring, and (ii) depend on clever architectural design of the atomic arrangement to bind oxygen.

Stabilization of porphyrin iron(II) (O_2) species has previously been achieved:

(1) By decreasing the rate constant k for the formation of the μ-oxo-oligomer** by the use of sub-zero temperatures [1, 4–6, 15–17].

(2) By utilizing rigid surfaces, such as attaching the haem to the surface in a manner that prevents dimerization [1, 5].

The concensus of opinion is now that in addition to suppressing the irreversible oxidation of the iron(II) porphyrin complex, two additional properties are desired in constructing a model for the natural haem oxygen carriers. These are: (i) a five-coordinate geometry [*e.g.* Fe^{II}(Por)(B), {where B = nitrogen base}], and (ii) a non-polar hydrophobic environment for the haem.

In our continuing studies of the aqueous chemistry of PPIXFe(II) and PPIXFe(III) we have characterized the iron(II) and iron(III) electron environments using Mössbauer spectroscopy for a wide variety of conditions [18–25]. We have demonstrated the versatility of PPIXFe(II) stereochemistry, by comparison of Mössbauer and other spectroscopic data to other porphyrin complexes where the X-ray structures have been established.

We chose PPIXFe chemistry as a model for haem proteins for two reasons: (a) as set out above, it is the most commonly used haem in natural systems, and (b) it has been demonstrated that Mössbauer parameters of iron in haems vary with the substituents on the periphery of the porphyrin ring [26]. This shows that the iron electronic environment in the haem is sensitive to changes on the extremity of the porphyrin moiety. From this it would appear that the best model for the study of PPIXFe chemistry is itself notwithstanding the difficulties this entails.

We wish to report here Mössbauer studies on dilute methanol/tetramethylammonium hydroxide frozen

*This work was first presented at the 25th Meeting of the Mössbauer Spectroscopy discussion group of the Royal Society of Chemistry at the University of Oxford 2nd-4th July, 1984.

**The Fe(III) μ-oxo-oligomer (often described as μ-oxo-dimeric haematin) phase is accredited to J. L. Hoard, see ref. 14.

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solutions of PPIXFe(II) containing oxygen either alone or containing mercaptoethanol or 2-methyl-piperidine.

Experimental

$PPIXNa_2$, mercaptoethanol, 2-methyl-piperidine and tetramethylammonium hydroxide were supplied by SIGMA.

Preparations of $PPIX^{57}Fe(II)$ Solutions for Mössbauer Experiments

5 mg of enriched $PPIX^{57}Fe$ prepared according to the method of Caughey [28] was dissolved in 3 ml $CH_3OH/(CH_3)_4NOH$ aqueous solution and appropriate ligands added in excess in the presence of dithionite (as the reducing agent for the iron). Oxygen was then bubbled and the solutions frozen before they could turn green.

Instrumentation

The Mössbauer spectra were recorded on an instrument previously described [29]. The source was ^{57}Co (10 mCi) in rhodium (Radiochemical Centre, Amersham), at 20 °C. The spectrometer was operated in a saw tooth mode and the spectra computer fitted. The spectrometer was calibrated with a 25 μM thick natural iron reference absorber. All isomer shifts are referred to this as zero shift.

Results and Discussion

The Mössbauer parameters obtained from the frozen solutions are presented in Table I (and Fig. 1, a typical spectrum) along with those of oxyhaemoglobin [27] for comparison. These parameters when compared to those of oxyhaemoglobin are interesting. Those obtained when 2-methyl-piperidine was used as the fifth ligand are identical to those found for oxyhaemoglobin at liquid N_2 temperature. These Mössbauer parameters are also clearly different to those found for such solutions in the absence of oxygen, *cf.* 2-methyl-piperidine in the absence of oxygen, Table II. (This table also contains Mössbauer data for other typical haemochromes [26].

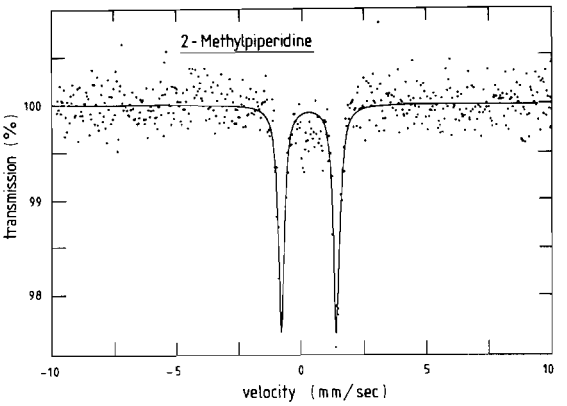

Fig. 1. Mössbauer spectrum of a frozen solution of protoporphyrin IX iron(II) with 2-methylpiperidine and oxygen as the fifth and sixth ligands.

The Mössbauer parameters (Table I) found in the presence of tetramethylammonium hydroxide alone (fifth ligand either methanol OH^- or H_2O) with O_2 as the sixth, or when mercaptoethanol was the fifth ligand had smaller quadrupole splittings and larger chemical shifts. These larger chemical shifts suggest either more 's' electron density at the nucleus or more 'p' and 'd' electrons are used in the bonding and thus there is less shielding of the 's' electrons. The

TABLE I. Frozen Solution (80 K) Mössbauer Parameters for the Oxygen Binding of Protoporphyrin IX Iron(II) in Tetramethyl Ammonium Hydroxide with Various Ligands in the Fifth Position.

Ligands	Temp (°K)	δ (mms^{-1})	Δ (mms^{-1})	Γ (mms^{-1})	% Abs
H_2O or OH or methanol	80	0.32(1) 0.41(1)[a]	2.16(2) 0.54(4)[a]	0.41(3) 0.56(7)	54(5) 46(7)
2-methylpiperidine	80	0.26(1)	2.20(1)	0.30(2)	100
mercaptoethanol	80	0.33(1)	2.02(3)	0.80(4)	100
oxyhaemoglobin[b]	195 77 1.2	0.20(5) 0.26(5) 0.24(5)	1.89(5) 2.19(5) 2.24(5)		
$FePocPiv(1,2\text{-}Me_2Im)O_2$[c]	77	0.24	2.32		

[a]Mössbauer parameters found for μ-oxo-bridged protoporphyrin IX iron(III) oligomer ref. 20. [b]Mössbauer parameters taken from ref. 26. [c]Mössbauer parameters of a pocket iron(II) porphyrin with oxygen and 1,2-methyl imidazole bound ref. 13.

TABLE II. Mössbauer Parameters for Haemocromes and other Relevant Species Reported in the Literature[a].

Compound	Temp (°K)	δ (mms^{-1})	Δ (mms^{-1})	ref.
PPIXFE(II) 2-pyridine	77	0.46	1.21	21
PPIXFe(II) 2-imidazole	77	0.43	0.95	21
PPIXFe(II) 2-piperidine	77	0.50	1.42	26
	298	0.43	1.43	
PPIXFe(II) 2-methylpiperidine (frozen solution)	80	0.43(1)	1.17(2)	This work
PPIXFe(II) solution containing mercaptoethanol (frozen solution)	80	0.86(2)	2.39(2)	24

[a] Natural iron as standard.

ligands in the latter cases are lower in the spectrochemical series than 2-methyl-piperidine and are more likely to bond to the iron via the metal p and d orbitals and thus the larger chemical shift is due to a decrease in 's' and 'd' electron shielding of the 's' electrons at the nucleus.

The fact that the Mössbauer spectrum obtained when tetramethylammonium hydroxide was present indicated a large amount of a second PPIXFe species was instructive. The parameters of this species show it to be the iron(III) species we have previously assigned as the μ-oxo-oligomer [21]. This species was often found when these experiments were attempted in the absence of strong competitive ligands for PPIXFe(III) such as unhindered nitrogen ligands and is the result of oxidation of the PPIXFe(II) by oxygen due to prolonged exposure at room temperature.

Prolonged exposure of any of these solutions to air gave rise to only one set of Mössbauer parameters, namely those of the μ-oxo-oligomer. This clearly supports our assignment of the initial quick frozen solutions as (Ligand) PPIXFe(II)O_2 species.

Conclusions

Although in the light of the introduction it is not too surprising that we were able to isolate the PPIXFe(II)O_2 2-methyl-piperidine complex, we were not able to do this using imidazole as a ligand. This might suggest that a slightly hindered nitrogenous ligand is necessary in the fifth position when 'naked' haems are used as models for haemoglobin. Such a large ligand as 2-methyl-piperidine in the fifth position may cause the propionate and allyl groups of PPIXFe(II) to take up a conformation on the side of the porphyrin plane opposite itself by steric crowding effects, thus effectively causing the PPIXFe(II) to become a 'picket fence' type haem, and thus oxygen uptake might be expected.

However, such an argument cannot be used to explain the presence of a PPIXFe(II)O_2 species when tetramethyl ammonium hydroxide is present (OH^-, H_2O or methanol must be the fifth ligand). Nor is it likely that the PPIXFe(II)O_2 mercaptoethanol complex could be explained in a similar manner as the mercaptoethanol molecule is much smaller than 2-methyl-piperidine.

If the oxygen binding is not linked to PPIXFe(II) acting as a 'picket fence' type haem then the bonding considerations are of importance. Previously the view has been widely held that the oxygen binding (without oxidation) to the PPIXFe(II) moiety in haemoglobin and capped haems was aided by steric factors restricting the position of the O_2 molecule. From our results and those of others where 'naked' haems bind O_2 it appears that the O_2 molecule binds iron(II) porphyrins without oxidizing them as an initial first step, and that the oxidation is then a relatively slow step. The initial O_2 binding from our Mössbauer parameters generates very similar data to those found for oxyhaemoglobin, suggesting the O_2 molecule binds in a similar manner (Fig. 2). It may be that in addition to the binding of the Fe(II) by the O_2 molecule, there is also a partial bonding interaction between the π-orbitals of the O_2 molecules and those of the porphyrin ring, and it is the latter interaction that stabilizes the initial structure.

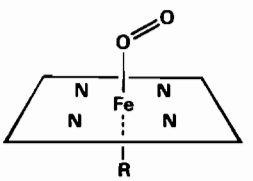

Fig. 2. Proposed geometry of oxygen bound protoporphyrin IX iron(II) complex. Where R = mercaptoethanol, 2-methyl-piperidine or one of OH, H_2O, MeOH as the fifth ligand.

To our knowledge this is the first time that evidence has been presented for O_2 binding to iron(II) porphyrins where the fifth ligand is not a nitrogenous one.

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