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The Absorption Spectra, Magnetic Moments and the Binding of Iron in some Haemoproteins

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In the iron porphyrin complexes four co-ordination positions in a plane around the iron atom are occupied by the nitrogen atoms of a porphyrin, and the other two positions are filled by simple monodentate ligands such as water and ammonia. We symbolize the iron porphyrin complexes as FePXY, where P is the porphyrin and X and Y are the co-ordinated groups in the fifth and sixth positions. The relationships between the properties of the groups X and Y and the properties they induce both in porphyrin and simpler iron complexes have been outlined in earlier papers (Williams, 1956, 1959*a*). In this paper we extend and systematize these relationships, and are then able to set up diagnostic rules permitting the recognition of the nature of unknown ligands X and Y from an examination of the properties of their iron porphyrin complex. We use these rules to discuss the co-ordination of the iron atom in a number of haemoproteins. The latter are iron porphyrin complexes in which the fifth co-ordination position at least, and possibly the sixth position also, is occupied by a donor group which is also bound to the protein directly. In the following paper we go on to consider the primary compounds of peroxides with catalase and peroxidase.

Ferric porphyrin complexes

It is generally recognized that there are two main classes of ferric porphyrin complexes (Pauling, 1948). The members of one class have spin moments approximately that of five unpaired electrons, spin 5/2, and magnetic moment 5·92β (Bohr magneton), whereas the members of the other class have magnetic moments approaching that of one unpaired electron, spin 1/2, and magnetic moment 1·73β. Complexes in the first group will be called high-spin complexes, and those in the second group will be called low-spin complexes. In earlier literature the classification 'ionic' and 'covalent' has been used (Pauling, 1931) as well as the classification 'weak field' and 'strong field' (van Vleck, 1935). As both the older classifications imply something about the character of the binding of the iron in the complexes which is inferred without certainty from the magnetic characteristics, it appears preferable to classify the complexes on the basis of the experimental magnetic data. In some $Fe^{III}PXY$ complexes, equilibria exist between the two spin forms (Williams, 1956, 1959*b*; George, Beetlestone & Griffith, 1959). There is a suggestion that a third form of spin 3/2 (three unpaired electrons) is found in a very limited number of cases, e.g. in phthalocyanines (Griffith, 1958). Unfortunately, except for the purely high-spin and purely low-spin $Fe^{III}PXY$ complexes, the magnetic moment arrived at from susceptibility measurements does not uniquely distinguish among the possible configurations. Paramagnetic resonance data, which are generally less ambiguous, are not always obtainable because of experimental difficulties. It would be useful then if the absorption spectra of ferric porphyrin complexes, which are easily measured on small quantities of material, could be analysed to determine the spin state of the Fe^{3+} ion. We examine this possibility below.

Classification of absorption bands of ferric porphyrin complexes. High-spin ferric porphyrin complexes have distinctly different spectra from other metal porphyrin complexes. The particular differentiating characteristics of $Fe^{III}PXY$ spectra are two new absorption bands, one at about 600–650 mμ and a second at 450–500 mμ. Similarly situated bands appear in simpler high-spin ferric complexes with unsaturated anions, e.g. those of 8-hydroxyquinoline and acetylacetone. On this basis we have attributed these bands to properties of the high-spin Fe^{3+} ion in combination with unsaturated ligands (Williams, 1956; Tomkinson & Williams, 1958). We have further suggested that the two bands are charge-transfer bands. The reasons for this diagnosis are as follows.

A charge-transfer band is associated with an electron excitation in which the electron is partially or completely transferred from the vicinity of one atom or group of atoms to another such group. In the excitation there is a considerable change of dipole moment and the transition is therefore allowed and will have a high intensity. For

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example, in ferric thiocyanate the excitation process is thought to be

$$[Fe^{3+}\text{–}S\bar{C}N] \rightarrow [Fe^{2+}\text{–}S\dot{C}N].$$

If this description is extended to ferric complexes with aromatic ligands, then the introduction of substituents in the aromatic rings should have predictable effects upon the absorption spectrum. Electron-donor substituents should facilitate electron transfer and thus cause the band to move to longer wavelengths, whereas electron acceptors should shift the absorption band to shorter wavelengths. This has been observed in the intense bands of ferric 8-hydroxyquinoline complex (Tomkinson & Williams, 1958). On the other hand, the absorption bands in the visible spectrum of ferric complexes with saturated ligands (other than those co-ordinating through sulphur) such as fluoride, acetate or water are very weak. These bands are not charge-transfer bands. Ferric porphyrin complexes $Fe^{III}PXY$ can be prepared with a variety of such simple co-ordinating groups X and Y as fluoride and acetate. The charge-transfer bands which are observed in these ferric porphyrin complexes must be due to electron transfer to the Fe^{3+} ion from the porphyrin dianion and not from the small anions X and Y.

Both high- and low-spin ferric porphyrin complexes also show the group of bands γ (Soret), β and α typical of all metal porphyrins. The bands are due to transition of the π-electrons of the porphyrin (Longuet-Higgins, Rector & Platt, 1950). However, the intensity of the α- and β-bands is low in high-spin ferric porphyrin complexes and the bands are often obscured by the charge-transfer bands. The position of these bands is also affected, although not greatly, by the spin state of the central cation. The low-spin ferric porphyrin complexes do not show charge-transfer bands, and these bands are absent too in low-spin ferric complexes with simpler unsaturated ligands. Equilibrium mixtures of high-spin and low-spin states have spectra which are additive mixtures of two components (Williams, 1956, 1959*b*; George *et al.* 1959).

Positions of the absorption bands of ferric porphyrin complexes. Table 1, which gives the positions and intensities of the bands of a large number of ferric haemoprotein complexes, shows the effect of changes of ligand in the sixth co-ordination position. The fifth position is occupied by a co-ordinating group which is attached to the peptide chain of the protein. In metmyoglobin and methaemoglobin the fifth position is occupied by a nitrogen atom of the imidazole group of a histidine residue. In catalase and peroxidase the groups are not known. The first and fourth bands, the charge-transfer bands, move to shorter wavelength in the order water $\geqslant$ azide $>$ formate $\geqslant$ acetate $>$ fluoride $>$ hydroxide. With the exception of azide this order is closely related to the complex-forming ability of these ligands in simple ferric complexes, shown in Table 2. Charge transfer, in which an electron is transferred from the porphyrin in a complex $Fe^{III}PXY$ to the ferric cation, will be the more difficult energetically the greater the donation to the iron of electrons from X and Y and the greater their negative charge/anion-radius ratio, i.e. the greater the electron density already in the vicinity of the ferric ion. These two factors largely dominate the stability of ferric complexes. Thus the correlation between stability and the position of the charge-transfer bands is hardly surprising. Azide does not conform to the pattern and it is notably different from the other anions in that it is unsaturated. The binding of unsaturated ligands to cations is different from that of saturated ligands. The charge-transfer bands in thiocyanate and cyanate methaemoglobin are also at longer wavelengths than those of the complexes of saturated anions.

The charge-transfer bands of $Fe^{III}PXY$ in which both X and Y are neutral molecules are also at longer wavelengths than when X or Y is a saturated anion. This is shown by simple methaemoglobin and metmyoglobin in which the ligands are water and histidine, and by the hydroxylamine complex of methaemoglobin for which the charge transfer bands are at $\sim 625\,m\mu$ and $490\,m\mu$ (inflexion) (Banaschak & Jung, 1956).

The α-, β- and Soret bands move much less with ligand than do the charge-transfer bands, unless there is a change of spin state. Upon such a change, the Soret band moves to longer wavelengths the lower the spin. In fact, for a series of anions, there is a linear relation between the paramagnetic susceptibility and the wavelength of the peak of the Soret band (Scheler, Schoffa & Jung, 1957). The complexes $Fe^{III}PXY$, in which both X and Y are neutral molecules, do not fit the relationship, the shift being in the same direction but of only half the size. The α- and β-bands move to shorter wavelengths with neutral bases, e.g. imidazole and ammonia, than with anions, e.g. hydroxide and cyanide.

Intensity of the absorption bands of ferric porphyrin complexes. The Soret band does not vary greatly in intensity among the $Fe^{III}PXY$ complexes. This suggests that if 'complexes' are prepared in which a large drop in the intensity of this band is observed, then, in all probability, an attack has taken place on the porphyrin ring rather than a simple change in the co-ordination to the iron (see following paper). The α- and β-bands are more intense in low-spin than in high-spin complexes (see Table 1). The intensity of the α-band in low-spin complexes is very dependent on

Table 1. *Spectroscopic and magnetic characteristics of complexes of some ferric haemoproteins*

The positions of the maxima of the absorption bands ($\lambda_{max.}$) are given in mμ and the millimolecular extinction coefficients (ϵ_{mM}) are per haematin. The columns of charge-transfer bands are headed C.T. The wavelength at which an inflexion in the spectrum occurs is indicated by placing the position in brackets and writing infl. below, or by the latter only if the position is obscure. The magnetic moments are given in Bohr magnetons, β.

Complex		Absorption bands C.T.	α	β	C.T.	γ	Magnetic moment (β)	Reference
Methaemoglobin (horse)								
Fluoride	$\lambda_{max.}$	605	—	(550)	483	403	5·76	1, 2
	ϵ_{mM}	10·9		infl.	10·3	144		
Water pH 6·4		631	(580)	(540)	500	405	5·65	1, 2
		4·4	infl.	infl.	10·0	179		
Acetate		620	575	(540)	497	404	5·44	1
		5·5	5·2	infl.	10·5	178		
Formate		620	570	(540)	496	404	5·44	1
		5·8	5·3	infl.	9·2	178		
Hydroxide		(600)	575	540	(480)	410	4·66	1, 2
		infl.	9·2	11·0	infl.	120		
Ammonia		None	(563)	535	(483)	411	2·93	3, 4
			infl.	10·8	infl.	126		
Imidazole		None	560	534	None	411	2·87	1
			12·5	14·7		105		
Cyanide		None	None	540	None	419	2·50	1, 2
			infl.	12·5		124		
Azide		(630)	575	540	None	417	2·35	1
		infl.	9·9	12·8		134		
Metmyoglobin (horse)								
Fluoride	$\lambda_{max.}$	604	(585)	(550)	487	406	5·77	1, 5
	ϵ_{mM}	9·2	infl.	infl.	9·9	146		
Water pH 6·4		630	(580)	(530)	502	408	5·73	1, 5
		3·9	infl.	infl.	10·2	188		
Hydroxide		(600)	585	539	(490)	411	5·04	1, 5
		infl.	7·8	8·8	infl.	119		
Azide		(635)	570	540	None	420	3·30	1
		infl.	8·7	11·2		123		
Cyanide		None	None	540	None	422	1·96	1
			infl.	11·3		116		
Catalase (liver)								
Fluoride	$\lambda_{max.}$	600	None	(535)	~480	407	5·89	2, 4
	ϵ_{mM}	13·9		infl.	< 20	125		
Water pH 6·0		622	585	537	490	407	5·89	2, 4
		10·5	9·5	13·2	17	138		
Azide		620	580	530	490	412	5·86	2, 4
		12	10·3	12·8	16·8	138		
Cyanide		None	580	553	None	425	4·02	2, 4
			12·2	14·6		110		
Peroxidase (horseradish) (ferric)								
Fluoride	$\lambda_{max.}$	612	560	(530)	488	404	5·90	2, 4
	ϵ_{mM}	6·9	4·8	infl.	7·6	130		
Water pH 5·6		641	~580	~530	497	403	5·48	2, 4
		2·8	~2·5	~7·5	10·0	91		
Hydroxide		~635	572	545	None	416	2·66	2, 4
		~1·5	6·9	8·6		89		
Azide		635	565	534	(495)	416	—	2
		1·7	5·5	8·2	infl.	114		
Cyanide		None	568	538	None	423	2·67	2, 4
			7·5	10·2		94		

References: 1, Scheler, Schoffa & Jung (1957); 2, Keilin & Hartree (1951); 3, Scheler, Schoffa & Jung (1958); 4, Hartree (1946); 5, Theorell & Ehrenberg (1951).

Table 2. *The logarithm of the stability constants,* log K, *of some ferric complexes*

Ligand	H_2O	HCO_2^-	$CH_3CO_2^-$	F^-	OH^-	N_3^-
log K	0·0	3·1	3·2	5·2	11·5	4·5
Reference	—	1	1	2	2	2

References: 1, Perrin (1959); 2, Bjerrum, Schwarzenbach & Sillen (1958).

the character of the ligand, but the β-band intensity is almost independent of the fifth and sixth ligands. For the mainly low-spin ferric porphyrin complexes in Table 1 the intensity ratio α/β increases in the order cyanide < imidazole = ammonia < hydroxide. This is the order of increase of π-electron donor character of the ligands.

Rules for the identification of groups attached in ferric porphyrin complexes. This survey of the spectra of the ferric porphyrin complexes shows that three main correlations between spectra and magnetic moment exist.

(i) The intensity of the charge-transfer bands is high in high-spin complexes and very low (or zero) in low-spin complexes.

(ii) The intensity of the β-band is greater in low-spin complexes.

(iii) The Soret band is at longer wavelengths for low-spin as opposed to high-spin complexes. The change from high to low spin and the movement of the Soret band follows the ligand sequence fluoride > acetate = formate > hydroxide > cyanide for anions, and water > imidazole for neutral bases. There is also the possibility of obtaining information about the groups bound to the ferric ion in the fifth and sixth positions from the following correlations.

(iv) The charge-transfer bands move to shorter wavelengths the greater the electron density in the vicinity of the Fe^{3+} ion, owing to ligands in the fifth and sixth positions.

(v) The intensity ratio α/β increases the stronger the π-donor character of these ligands.

Before attempting to use these observations we will describe other properties of iron porphyrin complexes which are dependent upon the fifth and sixth ligands.

Ferrous complexes and redox potentials

A similar set of comparisons between absorption spectra and magnetic properties in the complexes of ferrous porphyrins, $Fe^{II}PXY$, has permitted diagnostic rules to be established.

(i) Strong π-acceptor ligands such as nitric oxide, cyanide, oxygen and carbon monoxide have a different effect upon the absorption spectra from all other ligands (Williams, 1956).

(ii) With the simple bases, such as ammonia, the intensity of the α-band is greater the stronger the π-donor character of the ligand.

(iii) The Soret band and the α- and β-bands move to shorter wavelengths the greater the percentage of low-spin form for all typical haemoproteins.

(iv) The α- and β-bands of purely low-spin ferrous porphyrin complexes move to longer wavelengths the stronger the donor character of the ligand.

(v) In the spectra of low-spin ferrous porphyrin complexes there is a band at about 500 mμ. This band also appears in simple low-spin ferrous complexes of unsaturated nitrogen bases, e.g. Fe^{II}(*o*-phenanthroline)$_3$.

(vi) The order of change of magnetic moment from high-spin to low-spin ferrous complexes is nitrogen bases > oxygen bases in all model complexes. Here oxygen base refers to a neutral group such as water or alcohol which acts as an electron donor through the oxygen atom.

The oxidation–reduction potentials of iron complexes can also be used diagnostically. The order of decreasing redox potentials of model complexes, Fe^{II}(dimethylglyoxime) (base)$_2$ as a function of the bases is pyridine > imidazole > water > ammonia > hydroxide (Williams, 1959*a*). (*N.B.* The imidazole group of a histidine residue of the protein can play the same role as imidazole in this connexion, and in general for the properties discussed in this paper.) There is no reason to suppose that the iron porphyrin complexes will differ in their behaviour from the iron dimethylglyoxime complexes with respect to the effect of the fifth and sixth ligands perpendicular to the porphyrin or dimethylglyoxime ring.

Diagnosis of co-ordination of iron porphyrin complexes

Adequate information on magnetic moments, spectra and oxidation–reduction potentials in the two oxidation states should allow us to predict the way in which the iron in a porphyrin complex is bound. For example, we can consider the series of proteins which have the same porphyrin group: catalase, peroxidase, myoglobin and haemoglobin. This is the order of increasing redox potential (Table 3). We are assuming here that the catalase potential is very low as it is not known to have been reduced to the ferrous state. The magnetic moment of these haemoproteins in the ferric state increases in the sequence peroxidase < methaemoglobin < metmyoglobin < catalase. If we con-

sider only the three ligands most probably available on the proteins (amino groups, imidazoles of histidine residues and carboxylate anions) and relate the above two sequences to our diagnostic rules on redox potentials and paramagnetism, we conclude that (*a*) haemoglobin and myoglobin are imidazole–water complexes with the imidazole more tightly (but still not very tightly) bound in haemoglobin, (*b*) peroxidase is an amino–carboxylate or less probably a water–amino complex, and that (*c*) catalase is a dicarboxylate or less probably a carboxylate–water complex.

This conclusion is substantiated in Table 3 where all the other diagnostic properties are considered. For each property the proteins are ordered according to the magnitude observed, as are, for comparison, model complexes with known pertinent ligands in the fifth and sixth positions. For example, the study of the intensity of the charge-transfer bands in the series of methaemoglobin complexes $Fe^{III}P$(histidine)Y, where the histidine is a residue of the protein, shows that the intensity falls in the sequence of ligands Y as follows: fluoride > carboxylate > water > hydroxide > imidazole = amines. This order is also observed for a series of haemin complexes (Scheler, 1960). Amongst the haemoproteins the order of intensity is catalase > methaemoglobin > metmyoglobin > peroxidase. We conclude that the groups which bind iron in catalase come earlier in the above sequence of ligands than those which bind iron in methaemoglobin and metmyoglobin. Similarly, the groups which bind to iron in peroxidase come later in the sequence. Table 3 shows that an account of all the properties of haemoproteins can be given if catalase is a carboxylate complex and peroxidase is an amino complex. Some chemical properties provide additional confirmation of this analysis.

Chemical properties of iron complexes

Ferric complexes. In Table 4 we list the pH at which certain ferric complexes are 50 % hydrolysed to give hydroxy compounds. It is particularly noticeable that complexes with zero or negative charge are difficult to hydrolyse. In general the higher the negative charge on the groups which bind the ferric ion the greater the pH of hydrolysis. Peroxidase, methaemoglobin and metmyoglobin hydrolyse at similar pH values. This suggests that the groups binding the iron carry the same or very similar numbers of negative charges. Thus, in the fifth position, the group binding iron in peroxidase is probably a nitrogen base. Catalase is not hydrolysed even in strong alkali. It seems likely that one if not both of the groups binding the iron in this haemoprotein is a carboxylate group.

Ferrous complexes. The model complexes Fe^{II}(dimethylglyoxime)$_2$(imidazole)$_2$ and Fe^{II}(dimethylglyoxime)$_2$(histidine)$_2$ can absorb both oxygen and carbon monoxide reversibly (Williams, 1959*b*). The complexes Fe^{II}(dimethylglyoxime)$_2$(amine)$_2$ ab-

Table 3. *Observed effects of ligands on the properties of model complexes compared with the observed properties of haemoproteins*

The model systems referred to are: A, iron dimethylglyoxime complexes; B, methaemoglobin with a variety of ligands in the sixth position, see Table 1; C, haemin complexes. Haemo, haemoglobin; Myo, myoglobin; Per, peroxidase; Cat, catalase; Imid, imidazole.

Property	Model	Order of property in model complex	Order of property in haemoproteins	Reference
Redox potential	A	Imid $\geqslant$ H_2O > NH_3 > OH^-	Haemo > Myo > Per > Cat	1
Magnetic moment (ferric)	B	NH_3 $\leqslant$ Imid < H_2O < CO_2^-	Per < Haemo $\leqslant$ Myo < Cat	2, 3
Intensity of charge-transfer bands (ferric)	A, B, C	CO_2^- > H_2O > Imid $\geqslant$ NH_3	Cat > Haemo $\geqslant$ Myo > Per	1, 2, 4
Position of Soret band (ferric)	B, C	NH_3 = Imid > H_2O = CO_2^-	Per $\simeq$ Haemo $\simeq$ Myo $\simeq$ Cat (very small differences)	2, 4
Position of charge-transfer bands (ferric)	B	H_2O > CO_2^-	Per > Myo = Haemo > Cat	2
Intensity ratio β: charge-transfer (ferric)	B, C	NH_3 $\geqslant$ Imid > H_2O = CO_2^-	Per > Haemo $\geqslant$ Myo > Cat	2, 4
Intensity ratio α:β (ferric)	B, C	CO_2^- > NH_3 = Imid	Per < Haemo $\leqslant$ Myo < Cat	2, 4
Magnetic moment (ferrous)	A, B	Imid = NH_3 > H_2O = CO_2^-	Not known but spectra suggest Myo = Haemo > Per	1
Intensity ratio α:β (ferrous)	B	Imid = NH_3 > H_2O	Not known	1

References: 1, Williams (1959*b*); 2, Scheler, Schoffa & Jung (1957); 3, Hartree (1946); 4, Scheler (1960).

sorb carbon monoxide, but the absorption of oxygen which takes place in these complexes results in oxidation, i.e. is irreversible. Fe^{III}(dimethylglyoxime)$_2$(water)$_2$ does not absorb carbon monoxide to form Fe^{II}(dimethylglyoxime)$_2$(water)(carbon monoxide), and is immediately oxidized by oxygen. The iron porphyrin complexes Fe^{II}P(pyridine)$_2$ absorb oxygen reversibly but only in the absence of water. (This evidence strongly suggests that oxygen is only absorbed reversibly if the atom bound to iron does not carry active hydrogen.)

Haemoglobin and myoglobin absorb oxygen and carbon monoxide reversibly. In these iron porphyrin complexes histidine is bound to the iron. Peroxidase with iron in the bivalent state absorbs carbon monoxide but is oxidized by molecular oxygen. This behaviour, in comparison with that of the model complexes above, indicates that the iron in peroxidase is not complexed with imidazole but is similar in its reactions with oxygen to an amine complex.

From the above analyses of physical and chemical properties it is clear that we can explain the characteristics of the four haemoproteins on the basis of differences in the groups binding the iron. Summarizing, we think of haemoglobin and myoglobin as FeP(histidine)(water), catalase as FeP(carboxylate)$_2$ and peroxidase as FeP(amino)(carboxylate) or FeP(amino)(water).

Peroxide compounds of catalase, peroxidase, methaemoglobin and metmyoglobin

Catalase and peroxidase react with hydrogen peroxide and methyl and ethyl hydroperoxide to form four compounds, named I, II, III and IV according to the order of their appearance in the reactions. The reactions and the properties of the compounds formed have been reviewed by Keilin & Hartree (1951) and by Chance & Fergusson (1954).

Table 4. pH *at which hydroxide of ferric complex is* 50% *formed*

Ligand	Charge on complex	pH of 50% formation of hydroxide	Reference
Water $(H_2O)_6$	+3	2·5	1
(Phenanthroline)$_3$	+3	<2·0	2
(Dimethylglyoxime)$_2$	+1	4·5	3
Phthalocyanine	+1	5·5	4
8-Hydroxyquinoline	0	9·5	5
Ethylenediaminetetra-acetate	−1	7·5	5
Ethylenediaminetetra-acetate hydroxide	−2	>11·0	5
Methaemoglobin	—	8·9	6
Metmyoglobin	—	9·0	7
Peroxidase	—	~10·0	8
Catalase	—	No hydroxide formed	8

References: 1, Bjerrum, Schwarzenbach & Sillen (1958); 2, Gaines, Hammett & Walden (1936) (the hydroxy complex is the stable form even in strong acid); 3, Jillot & Williams (1958); 4, A. Riley & R. J. P. Williams (unpublished results); 5, Tomkinson & Williams (1958); 6, George & Hanania (1953); 7, George & Hanania (1952); 8, Keilin & Hartree (1951).

Table 5. *Spectroscopic and magnetic characteristics of peroxide compounds II and III of catalase and peroxidase, and of the peroxide compounds of methaemoglobin and metmyoglobin*

The band positions ($\lambda_{max.}$) are given in mμ and the millimolecular extinction coefficients (ϵ_{mM}) are per haematin. The magnetic moments are given in Bohr magnetons, β.

	α-Band		β-Band		γ-Band			
Compound	$\lambda_{max.}$	ϵ_{mM}	$\lambda_{max.}$	ϵ_{mM}	$\lambda_{max.}$	ϵ_{mM}	β	Reference
Catalase II	568	(13)	536	(12)	429	(70)	2·83	1, 2, 3
Catalase III	585	(11)	545	(12)	416	(84)	Unknown	3, 4
Peroxidase II	555	(7)	527	(8)	418	(87)	3·45	2, 3, 4
Peroxidase III	583	(8·5)	546	(10)	416	(97)	Unknown	3, 4
Peroxide compound of metmyoglobin	590	(8)	549	(9)	420	(100)	2·83	5, 6
Peroxide compound of methaemoglobin	585	(7·5)	545	(9)	Unknown		Unknown	3

References: 1, this paper (for experimental methods, see following paper); 2, Theorell & Ehrenberg (1952); 3, Keilin & Hartree (1951); 4, Chance (1952); 5, George & Irvine (1953); 6, Brill, Ehrenberg & Hartog (1960).

Methaemoglobin and metmyoglobin also react with these peroxides, and the resultant compounds have very much the same absorption spectrum as the compounds III of catalase and peroxidase. The optical and magnetic characteristics of compounds II and III of catalase and peroxidase and the peroxide compounds of methaemoglobin and metmyoglobin are given in Table 5, and these compounds will now be discussed. The compounds I, which are peculiar to catalase and peroxidase, we discuss in the next paper.

It has been suggested by George (1953) that compounds II of peroxidase and of catalase by analogy are quadrivalent (i.e. Fe^{IV}) iron complexes. More specifically he suggests that oxygen is bound in the sixth position so that compound II is a co-ordination complex of the hypothetical ferryl ion, $Fe^{IV}O^{2+}$. Peroxidase compound II has been shown by George to have one oxidizing equivalent above the parent haemoprotein, and its magnetic moment is that for two unpaired electrons with an orbital contribution. The magnetic moment of catalase compound II is exactly that for two unpaired electrons with no orbital moment. The only simple alternative to George's proposal is that compound II is a ferric complex and that the oxidizing equivalent is associated with the porphyrin ring as a stable free radical. The magnetic moment would then be made up of the radical's contribution plus that of the ferric ion which is then required to be in the low-spin state. Although the absence of the charge-transfer bands at 600–650 mμ and 450–500 mμ would be in agreement with this, the presence of the Soret band at its usual intensity argues against the oxidation of the porphyrin ring. It is also impossible to see how the β-bands of catalase and peroxidase compounds II, which are at 536 and 527 mμ respectively, can be at quite so short a wavelength. In their lowest-spin complexes, the cyanides, the β-band of catalase cyanide is at 553 mμ and that of peroxidase cyanide is at 538 mμ. In contrast with known ferric complexes of catalase and peroxidase, where the magnetic moments of the former always exceed those of the latter, the moment of catalase compound II is less than that of peroxidase compound II. These arguments are against the assumption that compound II is a ferric complex. There are several points which support George's postulation of ferryl iron, $Fe^{IV}O^{2+}$:

(i) Low-spin quadrivalent iron would have two unpaired electrons; the position of the Soret band, strongly displaced to the red, indicates a low-spin complex.

(ii) The intensity ratio α-band/β-band is greater than unity for catalase compound II and only a little less than unity for peroxidase compound II, positive evidence that a strong electron donor ligand, such as hydroxide or oxide, is bound to the iron.

(iii) The intensity ratio α-band/β-band is greater in catalase compound II than in peroxidase compound II. This ratio is generally greater in catalase complexes than in peroxidase complexes (Table 1). The α-band and β-band of catalase compound II are displaced about 11 mμ towards the red from the α- and β-bands of peroxidase compound II. Much the same difference in α- and β-band positions is found between other complexes of catalase and peroxidase (Table 1). Thus the compounds II exhibit the differences found between other complexes of catalase and peroxidase in which only the co-ordination in the sixth position is changed. This points to the preservation in compounds II of the co-ordination in the first five positions, and a common change in position six. This is not true of compounds III of catalase and peroxidase, however, which have very similar spectra. These spectra are very like those of the peroxide compounds of methaemoglobin and metmyoglobin, which suggests that one structure is common to them all, i.e. they all have the same co-ordination in the fifth and sixth positions. An obvious possibility is $Fe^{IV}P(hydroxide)_2$.

SUMMARY

1. A classification of the absorption bands of ferric porphyrin complexes is suggested. There are two groups of absorption bands, one group of two due to charge transfer to high-spin Fe^{3+} ions, and another group of three due to the absorption bands of the porphyrin. Only the second group of bands appears in low-spin complexes. An analysis of the absorption spectrum can be used to estimate the amounts of low-spin and high-spin ferric iron present in complexes which are equilibrium mixtures.

2. The relationships between the properties of known groups in the fifth and sixth co-ordination positions and the absorption spectra of iron porphyrin complexes are described. These relationships lead to a set of diagnostic rules by which the type of group bound to the iron porphyrin can be recognized. The rules for ferric and ferrous porphyrin derivatives differ and are separately discussed.

3. Magnetic, oxidation–reduction and some chemical properties provide additional evidence upon which the diagnosis of co-ordinating groups can be based.

4. As an illustration of the diagnostic method, the nature of the groups binding the iron in the haemoproteins catalase, peroxidase, haemoglobin and myoglobin is discussed, as is the co-ordination in the peroxide compounds II and III of catalase and peroxidase.

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Primary Compounds of Catalase and Peroxidase

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The mechanisms by which many haemoproteins fulfil their roles in biological oxidation and peroxide catalysis are not yet completely defined in spite of great experimental activity in this field of research. It would be of considerable help in arriving at an understanding of those mechanisms involving the higher oxidation states of haemoproteins if we knew the structures of the higher oxidation compounds. Great effort has been put into a determination of the absorption spectra (Keilin & Hartree, 1951; Chance, 1952*a*; George, 1953) of the higher oxidation compounds for it is only through the absorption spectra that these compounds can be followed separately and quantitatively through their reactions. Other properties, such as the magnetic moments and number of oxidizing equivalents of the compounds, can only be evaluated accurately after the absorption spectra have been established. The spectra themselves are useful in that they can be related to structure (see preceding paper).

Attention in this paper is directed particularly to the green primary compounds, 'compound I', of catalase and peroxidase with peroxides. Chance (1949*a*) has stated that alkyl hydroperoxides can convert free catalase completely into compound I. Our experiments show that this is only approximately correct, and we produce an absorption spectrum different from those arrived at in the past for primary compounds. The spectrum is not similar to any known haematin-containing protein, nor to any known porphyrin. We tentatively conclude that the oxidative event which has taken place is in part an attack on the porphyrin conjugated ring system, saturating it at one methene bridge. This conclusion is reinforced by similarities between our spectrum of compound I and the spectra of certain bile pigments.

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