The Interaction between Haem-iron and Thiols

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ALTHOUGH it has been known for many years that haemoglobin will form complexes with hydrogen sulphide,[1,2] model iron–haem thiol complexes have not been examined in any detail. In part this is due to the rapid reduction of the Fe^{III}–haem complexes by thiols and in part to the catalysis of the oxidation of thiols by oxygen in the presence of haem-iron. We have now found that the thiol complexes of haemoglobin and myoglobin are sufficiently stable to permit a detailed study of their physical properties and, using this information, we have been able to characterise the more transient model complexes.

On adding a thiol to haemin chloride in dimethylformamide we observed the signals shown in Table 2. The intensity of the signals for a given amount of iron was much less than in the protein complexes. Absorption spectral measurements showed that a large percentage of the iron was in the reduced Fe^{II} state. Evidently in these model systems the e.s.r. signal is a measurement of the amount of Fe^{III}-haem thiol complex in the reaction mixture. The e.s.r. signals show a change as the nature of the thiol is changed (Table 2) which will be discussed in a more extensive publication.[8]

TABLE 1. *The physical properties of some Fe^{III}-haem proteins*

Protein complex	E.s.r. g_1	g_2	g_3	Ref.	Absorption max. (nm.)				Ref.
Mb-OH	2·61	2·19	1·85	3		580	540	415	6
Mb-N_3	2·8	2·25	1·7	3	653	580	540	420	6
Hb-OH	2·6	2·3	1·7	4		575	540	415	
Hb-N_3	2·82	2·2	1·70	4	630	575	540	420	
Mb/H_2S	2·4	2·3	1·91		625	570	545	425	
	2·6	2·24	1·85*						
Mb/CH_3SH	2·46	2·24	1·94		780	570	545	425	
Hb/H_2S	2·46	2·25	1·92		625	570	545	425	1,2
	2·56	2·25	1·86*						
Hb/CH_2SH	2·33	2·24	1·95		780	570	545	425	
	2·46	2·24	1·93*						
P-450	2·41	2·26	1·91	5	650	570	535	418	7

Hb and Mb refer to haemoglobin and myoglobin, respectively, and the figures with an asterisk indicate where two sets of signals have been observed depending upon the solution conditions.[8] E.s.r. measurements were made at 77° K, frozen aqueous solutions, using a JEOL X-band spectrometer, model JES-3BX, calibrated in the conventional manner.

The haemoglobin and myoglobin complexes of the thiols with iron in the (III) oxidation state were prepared by direct reaction with hydrogen sulphide or methanethiol at pH 6·0. These complexes were quite stable even in the presence of oxygen. Their e.s.r. and absorption spectra are reported in Table 1 together with corresponding data for the known low-spin hydroxide and azide complexes of the same haem-proteins. The thiol spectra are typical of low-spin Fe^{III}–haem complexes but the three g values in the e.s.r. spectrum are closer to 2·0 than those of other low-spin complexes. It is possible that this is due to a change in geometry of the protein around the haem reducing the tetragonality relative to, say, the azide complexes. A more probable explanation is that the unpaired electron of the Fe^{III} is more strongly associated with the thiol ligand than in previously measured haem complexes in keeping with the strong electron-donor properties of the sulphur anion.

Given a knowledge of the e.s.r. spectra of Fe^{III} haem-protein complexes with thiols, we have been able to interpret the interaction between free Fe^{III} haem and thiols.

In Table 1 we also give the absorption and e.s.r. spectra of the microsomal oxidase haem-enzyme, P-450. It will be seen that the physical properties of the enzyme suggest that it could well be an iron–thiol complex in the oxidised state.

TABLE 2. *The physical properties of model Fe^{III}-haem thiol complexes in dimethylformamide*

Thiol	E.s.r. spectrum (77° K) g_1	g_2	g_3
$C_{10}H_{21}SH$	2·40	2·24	1·95
$PhCH_2SH$	2·35	2·24	1·94
o-$NH_2{\cdot}C_6H_4{\cdot}SH$	2·46	2·31	1·93
PhSH	2·46	2·27	1·91
AcSH	2·52	2·34	1·89

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