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Studies on the Reactions of Ferric Iron with Glutathione and Some Related Thiols. Part IV. A Study of the Reaction of Glutathione with Protoporphyrin IX Iron(III)

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Abstract

Solutions containing only protoporphyrin IX iron(III) and glutathione (the latter in 100-fold excess) show no evidence for the reduction of the iron(III) when examined using Mössbauer spectroscopy and electronic absorption spectra. This result is discussed in the light of known iron/glutathione chemistry, and iron/protoporphyrin IX chemistry. If carbon monoxide is passed into a solution of protoporphyrin IX iron(III) containing pyridine and glutathione (in excess) for twenty minutes evidence for a characteristic absorption band at 445 nm like that typical for cytochrome P450 is confirmed. This observation is discussed.

Introduction

An understanding of the chemistry of glutathione (GSH) and its role in iron metabolism is of general interest, as GSH is considered an essential constituent of living cells [1, 2]. It is worth noting that iron is always associated with cysteine and glutathione [3–8] in biological systems. We have previously reported studies on the aqueous coordination chemistry of GSH with iron [9–12]. It was demonstrated that: (a) under anaerobic conditions ferric salts react with GSH, the final products contain iron(II) [10], and (b) that in the pH range 3–7 the iron(II) binds to GSH via the carboxylate groups [11]. We have proposed a mechanism based on experimental data for the autocatalytic oxidation of iron–GSH complexes with molecular oxygen in the pH range 8–9 [12].

The ability of GSH to reduce [13] or react with [14–16] haem proteins has been the subject of many studies [13–16]. However, in many haem proteins the haem is buried so that the GSH molecule cannot bind directly to the protoporphyrin iron moiety. Thus, if reduction does occur then the electron is probably injected into the protein rather than directly onto the iron (as may be possible if the GSH could bind directly to the iron in the protoporphyrin). Or, as has been inferred, the GSH reduced the haem protein in the presence of oxidised GSH products in a complex mechanism [17].

Although there have been several studies on the reaction of protoporphyrin IX iron(III) chloride (PPIXFe(III)Cl), with GSH and other thiols as models for cytochrome P450 and related systems [18–21], these have not been primarily interested in the chemistry of PPIXFe and GSH, but have concentrated on the use as potential models.

It has been concluded from the studies of Granick [22, 23] that the terminal step in haem biosynthesis is the chelation of iron by protoporphyrin. Goldberg *et al.* [24] found that the haem was formed from Fe(II) and porphyrin. Haem formation from Fe(III) was activated by the presence of GSH, but its formation from Fe(II) was inhibited by the presence of GSH [25].

We have been interested in the aqueous chemistry of PPIXFe(II), PPIXFe(III), and related porphyrins as models for haemproteins [26–36], and were intrigued by their possible reactions with GHS. We report here Mössbauer and electronic spectroscopic studies on the reactions of GSH with PPIXFe(III).

Results and Discussion

Mössbauer Spectra

The Mössbauer spectrum of a solution PPIXFe(III) and GSH is presented in Fig. 1 (Table I). In this spectrum the envelope gives evidence of several different iron environments. However there is no evidence for a high spin iron(II) site and little evidence even for low spin iron(II). So it appears from this Mössbauer spectrum (which was typical of several we obtained) that there is no evidence for PPIXFe(III) being reduced by excess GSH.

The Mössbauer spectra however are quite different to those we recorded for frozen solutions of PPIXFe(III) in the absence of GSH [28], and thus provide evidence for PPIXFe(III) binding GSH. From the

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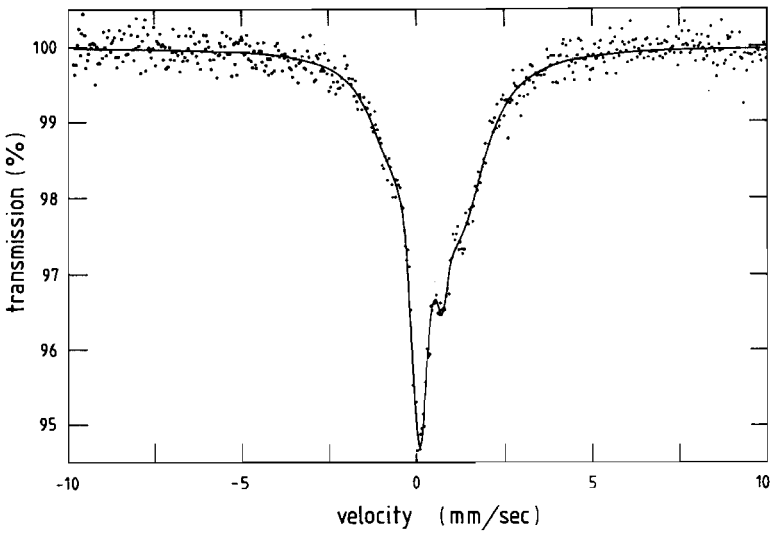


Fig. 1. The Mössbauer spectrum at 80 K of PPIXFe(III) in the presence of 100 fold excess GSH in a solution frozen at pH 8.1.

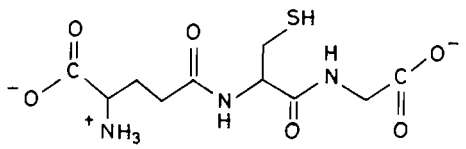
TABLE I. Mössbauer Parameters of Frozen Solutions Containing Protoporphyrin IX Iron(III).

Solution	γ/mm s^{-1}	Δ/mm s^{-1}	Γ/mm s^{-1}	
PPIXFe(III) + GSH at pH 8.1	0.21(8)	2.15(8)	1.43(20)	1.71(14)
	0.40(2)	0.65(2)	0.62(4)	0.42(10)
PPIXFe(III) at pH 8.0	0.39(1)	0.60(1)	0.26(1)	
PPIXFe(II) at pH 7.7	0.52(2)	1.43(2)	0.44(2)	

overall shape of the spectrum it is likely that the iron to GS′ bonding is via a variety of the glutathione coordinating sites.

The predominant glutathione isomer (I) at physiological pH values possesses eight potential binding sites: two carboxylic acid groups, an amino group, a sulfhydryl group and two amide functions. As all these eight potential binding sites may bind to the iron(III) in PPIXFe(III) it is not yet possible for us to actually say which do.

The Mössbauer spectrum (Fig. 1) could easily conceal the presence of low as well as high spin Fe(III) electronic environments.



(I)

Electronic Spectra

The electronic spectra obtained from solutions containing PPIXFe(III) and excess GSH are presented in Table II, Fig. 2. These data are different to those we obtained from PPIXFe(III) solutions in the pH

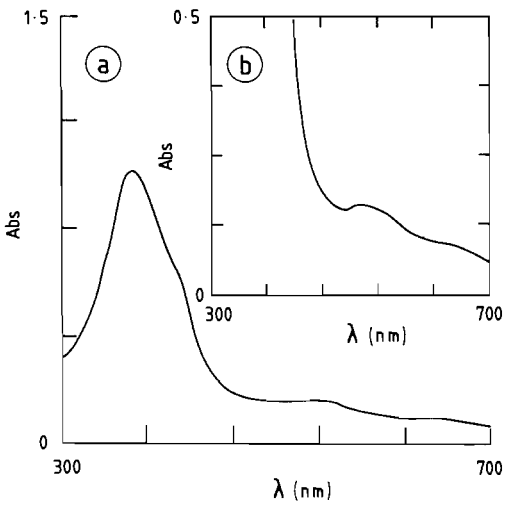


Fig. 2. (a) The electronic absorption spectrum of PPIXFe(III) and GSH solution at pH 8.2. (b) As in (a) but enlarged.

range 7 to 9 in the absence of GSH, showing that the latter binds to the iron(III) [28]. This confirms the results of the Mössbauer study. If the electronic spectra for the PPIXFe(III) GSH solutions are compared to those in which dithionate was present to reduce the PPIXFe(III) to PPIXFe(II), Table I, it can

TABLE II. Electronic Absorption Spectra of PPIXFe(III)/Fe(II) Solutions Containing GSH or GSSG.

Solution	pH	λ_1 (Soret) (nm)	λ_2 (nm)			λ_3 (nm)
PPIXFe(III) + GSH	6.8	367		515		620
	8.2	367		515		620
	9.4	367		515		620
	10.5†	367	382	515	610	
	11.5†	367	386	515	610	
	12.2†	367	388	519	609	
PPIXFe(III) + GSSG	7.0†	370	386	515	608	
	9.0†	374	386	515	608	
	11.2†	375	385	515	608	
			λ_3	λ_4		
PPIXFe(II) + GSH	7.0*		553	580		
	8.0*		552	577		
	11.0*		551	574		

† These spectra were similar to those reported in ref. [28] taken in the absence of GSH. *These spectra were identical to those reported in ref. [29] taken in the absence of GSH.

be seen that the resulting spectra are different. The spectra of PPIXFe(II)–GSH solutions (Table I) are identical to those of PPIXFe(II) without GSH present [29]. Thus GSH does not bind to PPIXFe(II), nor does it reduce PPIXFe(III).

Sakurai *et al.* in their studies on PPIXFe(III) GSH solutions as models for cytochrome P450, show a difference spectrum. This was obtained from a solution of PPIXFe(III), GSH and pyridine through which CO was passed for 20 minutes, with the same solution without CO in the reference beam. They point out that a peak at 445 nm is similar to that found in cytochrome P450 [19]. It must be remembered that this is a difference spectrum, and that this only shows the difference between the other two. So it only signifies the presence of a small amount of the total iron in this state. We obtained a similar result to Sakurai *et al.* (peak at λ = 440 nm) under the same conditions (Fig. 3), but only in the presence of pyridine. We and other workers have previously reported the ability of pyridine to reduce PPIXFe(III) in pure pyridine solution [26, 36–40]. We and others have also recently reported the ability of CO to reduce PPIXFe(III) under aqueous conditions [41, 42]. It is possible that it is either the pyridine with GSH or the CO present with GSH, or a combination of these with GSH that reduces the PPIXFe(III) under these conditions. The GSH may not be directly involved in the reduction, but then binds to form a PPIXFe(II)CO·GS complex which causes the difference spectrum observed.

To return to the difference spectrum (Fig. 3), it originates from spectra presented in Fig. 4, Table III. It must be noted that Fig. 3 shows no bands in the 350 to 430 nm range. This is because this area is negative in our method, and in that used by Sakurai

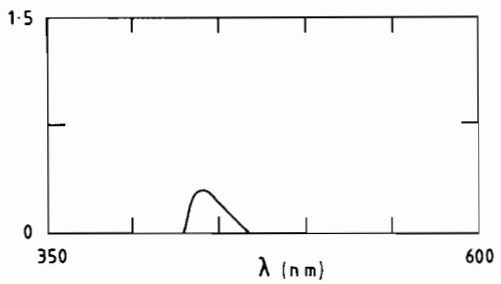


Fig. 3. Difference electronic absorption spectrum of a solution of PPIXFe(III) containing excess GSH and pyridine at pH 7.4 after passing CO for 20 minutes compared to the same solution without the CO present.

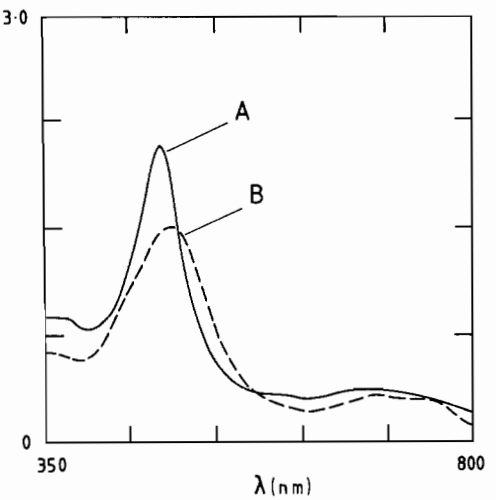


Fig. 4. Electronic absorption spectrum of A) a solution of PPIXFe(III), excess GSH and pyridine at pH 7.4, B) solution from A after passing CO for 20 minutes.

TABLE III. Electronic Absorption Spectra of PPIXFe(III) Solutions Containing Pyridine, and in Some Cases Pyridine and CO. All spectra were taken at pH 7.4.

Solution	λ_1 (Soret) (nm)	λ_2 (nm)	λ_3 (nm)
PPIXFe(III) + GSH + pyridine + CO (twenty minutes)	418	539	
PPIXFe(III) + GSH + pyridine	424	543	571
PPIXFe(III) + pyridine	406	528	556
PPIXFe + pyridine + CO (twenty minutes)	406	528	556
PPIX(III) + pyridine + CO + GSH + dithionite*	412	536	563
PPIXFe(III) + pyridine + CO + dithionite*	412	536	564

*Dithionite is present to reduce all the PPIXFe(III) to PPIXFe(II).

et al. [19], it can be seen that the spectrum is negative in the region below 430 nm. This spectrum in Fig. 3 therefore shows only part of the difference spectrum and caused Sakurai to suggest that this spectrum is similar to that of cytochrome P450 [19]. This illustrates the problems of setting up conditions correctly if difference spectra are to be used.

Table III also shows the electronic spectrum of a solution of PPIXFe(III) and pyridine at pH 7.4. This spectrum does not change on addition of CO as the latter does not bind PPIXFe(III). It is clearly modified by the presence of GSH and shows that GSH binds PPIXFe(III) in the presence of pyridine to form a complex containing both ligands, resulting in a different electronic environment to that in the absence of pyridine (Table II). The presence of CO in the solution containing GSH, PPIXFe(III) and pyridine (Fig. 4) causes changes that suggest some PPIXFe(II) has been prepared. Figure 5a shows the spectrum of a solution containing PPIXFe(III), pyridine, GSH and CO that has dithionite present to convert all the Fe(III) to Fe(II). This is quite different to that containing CO in Fig. 4 and different to the difference spectrum in Fig. 3 showing no evidence for a peak at 445 nm. This appears to indicate that under dithionite conditions GSH takes no part in the reaction, this is confirmed by Fig. 5b which is of a solution containing PPIXFe(III), CO and pyridine, (no GSH present) and dithionite and is almost identical to Fig. 5a.

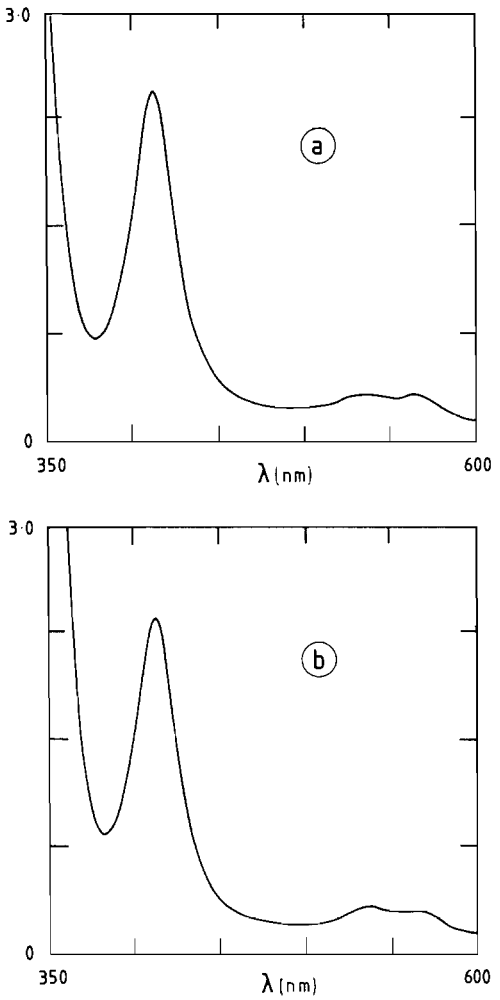


Fig. 5. Electronic absorption spectra of a) a solution of PPIXFe(III), CO, pyridine, GSH and dithionite. b) A solution of PPIXFe(III), CO, pyridine and dithionite (no GSH). The dithionite is present in these solutions to reduce all the PPIXFe(III) to PPIXFe(II). Both solutions were at pH 7.4.

Conclusions and General Discussion

From our studies the following statements can be made:

1) GSH alone is not able to reduce PPIXFe(III) in aqueous solution in the pH range 7–11.

2) In aqueous solution GSH binds PPIXFe(III) in the pH range 7–9.

3) PPIXFe(II) does not bind to GSH in the pH range 7–11 in aqueous solution.

When our studies are put in perspective with our earlier work on iron glutathione chemistry, and with the results of other workers then:

1) Fe(II) complexes with porphyrins to form haems [24].

2) Fe(III) does not complex with porphyrins to form haems, under non-reducing conditions [24].

3) GSH retards the formation of haems from porphyrins [24]. This is because GSH binds to Fe(II) at pH 8 [11, 12] and therefore competes with the reaction.

4) GSH activates the formation of haems from Fe(III) and porphyrins at pH 8 (result of ref. 24). This is because Fe(III) is reduced by GSH to Fe(II) [11, 12], and it is this Fe(II) that is incorporated into the porphyrin. The porphyrin is a competitive chelator with GSH for Fe(II).

The presence of GSH in all living cells can at least be understood for the chemistry of haems. If in the unlikely event of the iron dechelating from the haem as a consequence of oxidation to Fe(III), then GSH would be able to reduce the 'free iron' and solubilize it so that the haem could readily be reconstituted. The fact that GSH does not directly reduce PPIXFe(III) ensures that it does not interfere with the metabolic roles of haem proteins. GSH is also able to keep Fe(II) in solution in the pH range 2–8 [9–12]; its presence in living cells may also be associated with this property.

It is therefore not surprising that the 'redox reaction between haemin (PPIXFe(III)Cl) and glutathione' does not interfere with inhibition studies on yeast glyoxylase I at 25 °C [43, 44] as there is no redox reaction possible. In fact the spectral changes shown in that study [Fig. 1, ref. 43] for the reaction of PPIXFe(III) with GSH are probably due to monomer PPIXFe(III) and the μ-oxo oligomer $(PPIXFe(III))_2O$ [28], being converted by GSH to the monomeric PPIXFe(III)/GSH types of species reported in this work, and also to some residual $(PPIXFe(III))_2O$.

Experimental

Preparation of GSH-hemin Solution for Mössbauer Experiment

5 mg of enriched ^{57}Fe PPIX Cl prepared according to the usual method was dissolved in a minimum amount of 1 N NaOH solution. This solution was then diluted to 3 ml with distilled water and transferred into a two necked flask connected to N_2 atmosphere. 20 mg GSH was then dissolved into this solution and the pH was adjusted to 8 using 1 N HCl.

Electronic Spectra

The solutions used for the measurements were made up to the same concentrations as used in previous publications [27–33].

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