IRON-SULFIDE CHELATES OF SOME SULFUR-CONTAINING PEPTIDES AS MODEL COMPLEX OF NON-HEME IRON PROTEINS

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Summary : Similar absorption spectra to the spectrum of ferredoxin isolated from green plants were obtained by mixing ferric ion with an equal concentration of sodium sulfide and an excess of 2-mercaptopropionic acid, N-acetylcysteine, glutathione or pentapeptide (H·Ser-Cys-Val-Ser-Cys·OH) at pH 8.6.

The release of sulfur from some sulfur-containing peptides such as glutathione and 1-mercaptopropionyl glycine and incorporation of released sulfur to iron chelate of these peptides to form peptide-iron-sulfide chelates which show ferredoxin-like absorption spectrum were confirmed by the change of the absorption spectra and the determination of released sulfur.

Non-heme iron proteins have been studied actively in the field of biochemistry because of their importance in various biological processes(1-3). In addition, novelty in their structures and spectroscopic properties have attracted keen interest in the field of coordination chemistry. Several iron complexes, such as dithiocarbamates(4), thioxanthates(5), dithiophosphates(6) and dithiocarboxylates(7) have been investigated as possible model complexes to interpret the physical properties of non-heme iron proteins. Recently, Yang and Huennekens (8) have reported that a complex formed from 2-mercaptoethanol, ferric ion and sodium sulfide affords an absorption spectrum which is sufficiently similar to that of ferredoxin isolated from green plant, and this complex was proposed as a possible model of chromophore of non-heme iron proteins. We attempted to find the model complexes formed from the ligands which have physiological significance instead of the above-mentioned ligands which are not related to the physiological significance.

Materials and Methods

Glutathione, 1-mercaptopropionyl glycine, N-acetylcysteine, 2-mercaptopropionic acid, mercaptoacetic acid, 1-mercaptopropionic acid, 2,3-dimercapto-1-propanol, 2,3-dimercaptosuccinic acid, 1,2-ethanedithiol, 2-mercaptoethylamine, cysteine methyl ester, penicillamine and 2-ethanethiol were commercially available reagent grade materials. Purity of these reagents was checked by iodometric titrations. Pentapeptide (H·Ser-Cys-Val-Ser-Cys·OH) was synthesized by Dr.

Yajima as a peptide whose amino acid sequence is similar to that from position 7 to 11 in Clostridium ferredoxin, and its purity was checked by elemental analysis and amino acid analysis. Solutions of ferric chloride and sodium sulfide were freshly prepared with deionized water and were standardized with EDTA and iodine respectively.

Model complexes were formed by mixing the sulfur-containing ligands, sodium sulfide and ferric chloride at pH 8.6. Sodium borate (0.05 M) and NaOH (0.1 N) were used for the adjustment of pH. Absorption spectra were measured with a Shimadzu recording spectrophotometer, model Double-40R and pH measurements were made with a Hitachi-Horiba pH meter, model F-5.

Sulfide ion released from the sulfur-containing peptide iron chelate with time was determined by Rahim's method(9), which based on the green color formed by the reaction of sulfide ion with iron(III) and an excess of nitrilotriacetic acid (NTA) in ammoniacal solution. Concentrated ammonia solution (4 ml) and iron (III)-NTA reagent solution (1 ml) were added to the sample solution (5 ml), and the absorbance was measured at 635 nm after 15 minutes.

Results and Discussion

Spectral data of the iron complexes formed from various sulfur-containing ligands and sodium sulfide are summarized in Table I. Among the ligands examined, some mercaptocarboxylic acids and sulfur-containing peptides such as 2-mercaptopropionic acid, N-acetylcysteine, glutathione and pentapeptide (H·Ser-Cys-Val-Ser-Cys·OH), which are expected to form six membered chelate ring, exhibit very similar absorption spectra to the spectrum of ferredoxin isolated from green plants. On the contrary, some mercaptocarboxylic acids such as mercaptoacetic acid and 1-mercaptopropionic acid which are expected to form five membered chelate ring, form very stable chelate with ferric ion and do not afford any spectral change with the addition of sodium sulfide. In addition, the ligands of sulfur-sulfur and sulfur-nitrogen coordination types did not show ferredoxin-like absorption spectrum. The ferredoxin-like absorption spectra were observed in glutathione iron chelate with the addition of elemental sulfur, hydrogen sulfide and 2-ethanethiol in stead of sodium sulfide as the source of the inorganic sulfur. A spectral datum obtained when elemental selenium was added instead of sulfur to glutathione iron chelate is shown in Table I. Some blue shift of the absorption maxima in ferredoxin-like absorption spectrum on replacement of sulfur by selenium may suggest the possibility of charge transfer from metal to ligand, in consideration that π back-donation, namely lowering the energy of dπ-orbitals occurs with selenium to a larger extent than with sulfur (10). The effect of the replacement of labile sulfur by selenium in putidaredoxin, which is a typical non-heme protein, has been studied on the electronic and electron spin resonance spectra and the biological activity (11, 12). In the selenium analogue of putidaredoxin, a red shift of 5 - 20 nm on the principal visible absorption maxima of native putidaredoxin was observed(11). For more reliable interpretation of the spectral change caused by the replacement of sulfur by selenium, further detailed study on the various types of native

Table I

Spectral Data of Some Iron Complexes and Non-heme Iron Proteins

			Absorption Maxima, nm(Extinction Coefficients, x10^3)[d]			
Non-heme Proteins	Ferredoxin[a]	Spinach	332	422	465	
		Chlorella pyrenoidosa	330	420	460	
		Chromatium	300	390		
		Desulphovibrio gigas	305	390		
	Rubredoxin[a]	Clostridium pasteurianum		380	490	
		Clostridium sticklandii	355	375	490	565
	Putidaredoxin[b]	Pseudomonas putida	325	415	455	
		Selenium analogue	330	433	477	
Iron Complexes[c]	Glutathione-Fe		352(5.5)	482(3.5)	502(3.4)	
	Glutathione-Fe-S		330(6.5)	415(4.0)	452(3.6)	
	Glutathione-Fe-Se		328(6.7)	398(4.2)	440(3.8)	
	Pentapeptide-Fe-S		330(7.0)	415(4.0)	450(3.7)	
	N-Acetylcysteine-Fe		345(4.7)	480(3.2)		
	N-Acetylcysteine-Fe-S		333(5.9)	416(4.0)	453(3.7)	
	2-Mercaptopropionic acid-Fe		350(5.7)		505(3.3)	
	2-Mercaptopropionic acid-Fe-S		317(6.0)	412(3.8)	450(3.3)	

a) Buchanan, B. B. and Arnan, D. I. in Advances in Enzymology, Vol. 33, F. F. Nord, ed., Interscience Publishers, New York, 1970, pp. 119-176.
b) Tsibris, J. C. M., Namtvedt, M. J. and Gunsalus, I. C., Biochem. Biophys. Res. Commnm., 30, 323 (1968).
c) All data were obtained by mixing sulfur-containing ligand(100 mM) and $FeCl_3$(0.2 mM), or sulfur-containing ligand(100 mM), Na_2S(0.2 mM) and $FeCl_3$(0.2 mM) at pH 8.6.
d) Extinction coefficients were expressed per gram atom of iron.

non-heme protein and the model complexes may be necessary. The effect of the concentration of sulfide on glutathione iron chelate is shown in Fig. 1. When the molar ratio of sulfide to iron is 1 to 1, ferredoxin-like absorption spectrum was observed, whereas when the molar ratio comes up to 8 to 1, an absorp-

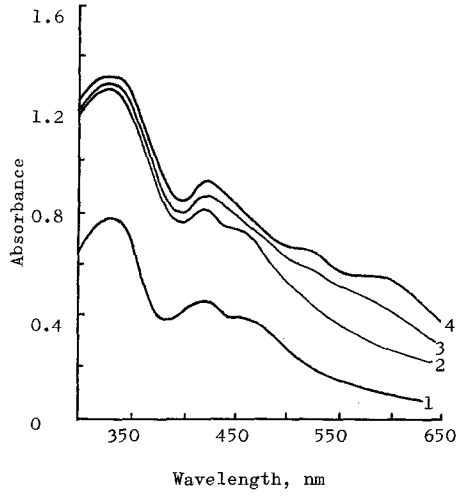

Fig. 1 Effect of Na_2S concentration on the formation of glutathione-iron-sulfide chelate at pH 8.6.
Curve 1, $FeCl_3$(0.2 mM), glutathione(100 mM) and Na_2S(0.1 mM) ; Curve 2, $FeCl_3$(0.2 mM), glutathione(100 mM) and Na_2S(0.2 mM) ; Curv 3, $FeCl_3$(0.2 mM), glutathione(100 mM) and Na_2S(0.4 mM) ; and Curve 4, $FeCl_3$(0.2 mM), glutathione(100 mM) and Na_2S(1.6 mM).

tion spectrum which resembles to that of rubredoxin was observed. In rubredoxin, tetrahedral coordination of iron by four sulfur atoms of cysteine residues was proposed by x-ray studies(13, 14). Therefore, the spectral change induced by the increase of the ratio of sulfur to iron may be attributed to a geometric alteration from distorted octahedral coordination to tetrahedral coordination in glutathione iron chelate. The spectral characteristics of the glutathione-iron-sulfide chelate which differ entirely from those of the simple iron complexes may be considered as a reflection of unusual environment around iron atom. A dimeric structure has been suggested for ferredoxin isolated from green plants(15) and also sulfur-bridged dimeric structures have been proposed for some iron complexes(5, 16, 17). From the observations and discussions mentioned above, a highly distorted binuclear octahedral structure involving inorganic sulfur as bridging atom may possibly be assumed for the glutathione-iron-sulfide chelate as a model of ferredoxin, (Fig. 2).

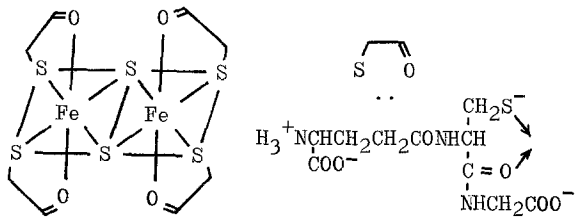

Fig. 2 Probable structure of glutathione-iron-sulfide chelate

In an attempt to find out a model reaction of incorporation of sulfur to form non-heme iron proteins, we investigated the release of sulfur from some sulfur-containing peptides such as glutathione and 1-mercaptopropionyl glycine and incorporation of released sulfur without the addition of inorganic sulfur into the iron chelate of these peptides to form peptide-iron-sulfide chelate. It was observed that the spectrum of glutathione iron chelate changes with time gradually and becomes to be identical to ferredoxin-like spectrum, after the time course of about 300 minutes. In the case of 1-mercaptopropionyl glycine iron chelate, the similar spectral change was observed distinctly, as shown in Fig. 3. The spectrum, which is similar to that of 1-mercaptopropionic acid iron chelate at the beginning stage of the time course, gradually changes to become identical to that of 1-mercaptopropionyl glycine-iron-sulfide chelate, which is formed by the addition of sodium sulfide to 1-mercaptopropionyl glycine iron chelate. These results indicate the incorporation of sulfur released from the iron chelate of these sulfur-containing peptides into the iron chelate to form iron-sulfide chelates which show ferredoxin-like spectra. The quantitative study of the release of sulfur from 1-mercaptopropionyl glycine in the

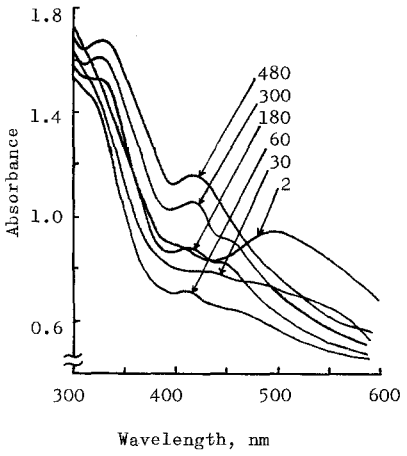

Fig. 3 Spectral change with time of l-mercaptopropionyl glycine iron chelate at pH 8.6.
The sample solution contains l-mercaptopropionyl glycine(150 mM) and $FeCl_3$(0.3 mM). The numbers on each spectrum indicate minutes after mixing the reagents.

presence or absence of ferric ion was carried out by the determination of sulfide ion with Rahim's method(9). As seen in Table II, sulfur is released from l-mercaptopropionyl glycine in the presence of ferric ion but not in the absence of it. The results of the determination of sulfide ion and the spectral change of the formation of peptide-iron-sulfide chelate comparably correspond each other in the quanity of sulfide ion required to form peptide-iron-sulfide chelate. This result may afford a strong support to consider that the labile sulfur of iron-sulfide chelate of l-mercaptopropionyl glycine comes from l-mercaptopropionyl glycine itself. On the contrary, in the case of 2-mercaptopropionic acid, ferredoxin-like spectrum was observed only when sodium sulfide was added to its iron chelate. Judging from these results, peptide bond seems to play very important role in release and incorporation of sulfur to form iron-sulfide chelate. The importance of the existence of peptide bond in the elimination of sulfur from some derivatives of cysteine has been suggested by Bayer et al. by the qualitative study(18). The release and incorporation of sulfur of the iron chelate of the sulfur-containing peptide may be regarded as a suggestive observation for the source of labile sulfur in non-heme iron proteins.

Table II

Determination of Sulfide Ion Released from l-Mercaptopropionyl glycine(150 mM) at pH 8.6

Reaction Time (minutes)		0	90	180	300	480
Concentration of Sulfide Ion (mM)	in absence of $FeCl_3$	0	0	0	0	0
	in presence of $FeCl_3$(0.3 mM)	0	0.33	0.63	1.03	1.59

Iron-sulfide chelate of sulfur-containing peptide is considered to be significant as a model complex for the future study of the structure and the formation of non-heme iron proteins.

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