DETECTION OF LABILE SULFIDE IN SERUM ALBUMIN*

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Summary: An artificial non-heme iron protein was synthesized from serum albumin in the presence of ferrous ammonium sulfate and mercaptoethanol. The absorption spectrum of the synthesized protein displays maxima at 279 mμ and 386 mμ with two broad peaks at 327 mμ and 630 mμ. By chemical analyses, the protein contains non-heme iron and labile sulfide in approximately equimolar amounts. It is concluded that labile sulfide originates from an iron-coordinared complex.

There are contradictory interpretations of the chemical nature of so-called labile sulfide liberated as H_2S upon acidification from bacterial and plant ferredoxins, animal non-heme iron proteins (adrenodoxin, testodoxin), and some iron-flavoproteins (succinic dehydrogenase, etc.).

In clostridial ferredoxins, Bayer, Parr, and Kazmaier (1964) proposed that labile sulfide originates from cysteinyl residue by a β-elimination reaction in the presence of iron salt. Contrary to this report, Malkin and Rabinowitz (1966a) have stated that no significant amount of dehydroalanine was detected in samples of apoferredoxin, assuming that if the evolved H_2S originates from cysteine residue, a quantitative conversion of cysteine to dehydroalanine should occur. From this observation, they concluded that the β-elimination is not the case and that labile sulfide attests to the presence of "inorganic sulfur" in ligands of the iron complex of ferredoxin (Malkin and Rabinowitz 1966b).

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Later, Bayer's group (1966) refuted the objection made by Rabinowitz's group, providing further evidence of stoichiometric formation of alanine by chemical reduction of dehydroalanine. Recently, Keresztes-Nagy and Margoliash (1966), working on alfalfa ferredoxin, have supported the finding proposed by Rabinowitz's group on the basis of results which indicate that the amounts of pyruvate formed from dehydroalanine by hydration and elimination of ammonia in acid hydrolysates of the protein can not account for the sulfide.

We have investigated whether labile sulfide originates from cysteine residue in consequence of a β-elimination reaction or not. To avoid possible confusion, we have chosen to study bovine serum albumin which has no iron atom in the protein. In this paper we shall offer evidence that bovine serum albumin can yield H_2S upon acidification in the presence of iron, but not in the absence of it. Our results support the finding presented by Bayer et al (1964, 1966).

The analytical determinations of non-heme iron and labile sulfide have been described previously (Kimura and Suzuki, 1967). Synthetic non-heme iron protein from bovine serum albumin (Fraction V) was prepared as follows: 3 mg of the protein was dissolved in 3 ml of 0.01 M phosphate buffer(pH 7.4). To this solution, 1100 µmoles of mercaptoethanol and 885 mµmoles of ferrous ammonium sulfate were added. The total volume was made up to 3.225 ml by the addition of water. The reaction mixture was allowed to stand at 37° for 2 hrs with occasional stirring. Then, after vigorous shaking, the reaction mixture was stored at 0° overnight. The reaction mixture was next passed through a Sephadex G-25 column which had been equilibrated with 0.01 M phosphate buffer (pH 7.4) in order to remove the excess amounts of mercaptoethanol and ferrous ammonium sulfate. By gel filtration, the protein fractions were collected. As shown in Fig. 1, the absorption spectrum of the synthetic non-heme iron protein which had previously been treated with both mercaptoethanol and ferrous ammonium sulfate displays maxima at 279 mµ and 386 mµ with two broad peaks at 327 mµ and 630 mµ. The ratio of absorbance at 327 mµ to that at

386 mμ was calculated to be 0.87 - 0.95. The samples which contained, in addition to serum albumin, either only mercaptoethanol or only ferrous ammonium sulfate did not reveal the maximum at 386 mμ nor the two broad peaks.

The quantitative determinations of iron and labile sulfide contents in the three samples are presented in Table 1. The sample with mercaptoethanol contains neither iron nor labile sulfide. The sample with ferrous ammonium sulfate contains a considerable amount of iron but no labile sulfide is present. The third sample which is treated with both mercaptoethanol and ferrous ammonium sulfate reveals the existence of non-heme iron and labile sulfide. The spectrum of the colored product derived from the sulfide in the presence of p-aminodimethylaniline and $FeCl_3$ was completely identical with that of methylene blue. The molar extinction coefficient at 386 mμ expressed per iron atom was calculated to be 7,000. It can be assumed that the visible absorbance in the artificial non-heme iron protein is due to the formation of iron-coordinated complex. The ratio of iron to labile sulfide is about one, although the values slightly vary, depending on preparations of the synthetic non-heme iron protein.

Table 1. Iron and Labile Sulfide Analysis of Samples Treated with Ferrous Ammonium Sulfate and Mercaptoethanol

Samples	Iron	Labile Sulfide
	mμatoms/mg protein	mμmoles/mg protein
Serum Albumin + Mercaptoethanol	0	0
Serum Albumin + Ferrous Ammonium Sulfate	267	0
Serum Albumin + Mercaptoethanol + Ferrous Ammonium Sulfate	81	82

(See details in the text.)

A similar experiment done with egg albumin indicated that the spectrum of the synthetic non-heme iron protein from egg albumin has an identical spectrum with that of the artificial protein from serum albumin. However, the content of labile sulfide was much lower than that in the protein derived from serum albumin.

Serum albumin contains 34 cysteinyl residues in a molecular weight of 69,000. The synthetic non-heme iron protein contains approximately 6 atoms of iron and 6 moles of labile sulfide in the molecule. Since serum albumin per se does not contain any labile sulfide, the presence of iron atoms and mercaptoethanol can make a coordinated compound with ligands (possibly sulfhydryl groups) of protein; and this iron-coordinated structure can further provide labile sulfide upon acidification. Although interpretations of these observations must be made with caution, it would be reasonable to conclude that we should no longer consider labile sulfide to be a constituent restricted to ferredoxins and iron-flavoproteins.

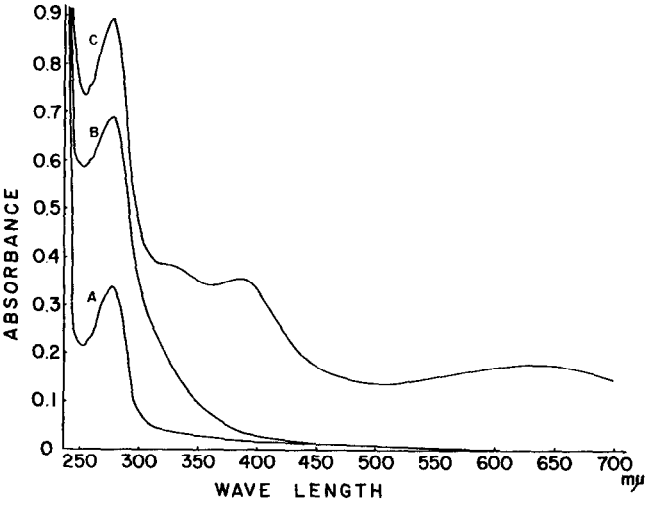

Fig. 1. Absorption Spectra of Samples Treated with Ferrous Ammonium Sulfate and Mercaptoethanol.
Curve A: mercaptoethanol-treated sample. Curve B: ferrous ammonium sulfate-treated sample. Curve C: synthesized non-heme iron protein from serum albumin, treated with both mercaptoethanol and ferrous ammonium sulfate.
(See details in the text.)

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