A POSSIBLE ROLE FOR RHODANESE: THE FORMATION OF ' LABILE ' SULFUR FROM THIOSULFATE

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1. Introduction

Rhodanese (thiosulfate: cyanide sulfurtransferase, EC 2.8.1.1) is a well-known mitochondrial protein [1, 2]. In spite of its widespread occurrence and abundance, its physiological role is very uncertain. *In vitro* it catalyzes the transport of sulfur from thiosulfate to a nucleophilic acceptor (cyanide, reduced lipoate) [3, 4] by a double displacement reaction with the formation of an intermediate sulfur-enzyme complex [5, 6].

Although some cyanide may be formed *in vivo*, this seems insufficient to explain the ubiquity and abundance of rhodanese. The possible significance of its action on reduced lipoate is also unclear. In the present paper a new role for rhodanese is outlined, namely the possibility that the enzyme may contribute to the formation of 'labile sulfur' in non-heme iron proteins from thiosulfate.

2. Materials and methods

All chemicals used were reagent grade. Rhodanese was purified from beef kidney according to Cannella, Pecci and Federici [7]. Ferredoxin was obtained from parsley leaves by the method of Plesnicar and Bendall [8] with slight modifications in running the DEAE cellulose column. The protein concentration was determined by absorption measurements at 280 and 422 nm, using an extinction coefficient of E_{422} = 9200 and a molecular weight of 10,700 [9].

Apoferredoxin was made and ferredoxin reconstituted according to Fee and Palmer [9], except that $Fe(NO_3)_3$ was used instead of $FeCl_3$. The rate of reconstitution was followed at 422 nm using a Beckman DK 2A Ratio recording spectrophotometer equipped with a constant temperature cell-holder. Biological activity was tested by the method of San Pietro [10] using spinach chloroplasts isolated according to Arnon, Allen and Whatley [11]. The activity of rhodanese was measured by the rate of thiocyanate formation from thiosulfate and cyanide by the method of Sörbo [12].

3. Results

When apoferrodoxin is treated with Na_2S and $Fe(NO_3)_3$ in the presence of diothiothreitol there is a relatively fast increase in absorption at 422 nm (fig. 1a). The protein obtained, according to Fee and Palmer [9], is indistinguishable from native ferrodoxin. The Na_2S may be substituted by thiosulfate and rhodanese (fig. 1b, c). The rate is proportional to enzyme concentration. The kinetic study of this recombination is, however, very difficult because the reaction mixture incubated in the absence of rhodanese (fig. 1d) and/or thiosulfate turns brown at an appreciable rate, due to the formation of a complex involving iron, dithiothreitol and buffer. Although this brown substance has an absorption spectrum very different from that of ferredoxin, there is no wavelength where it is possible to follow

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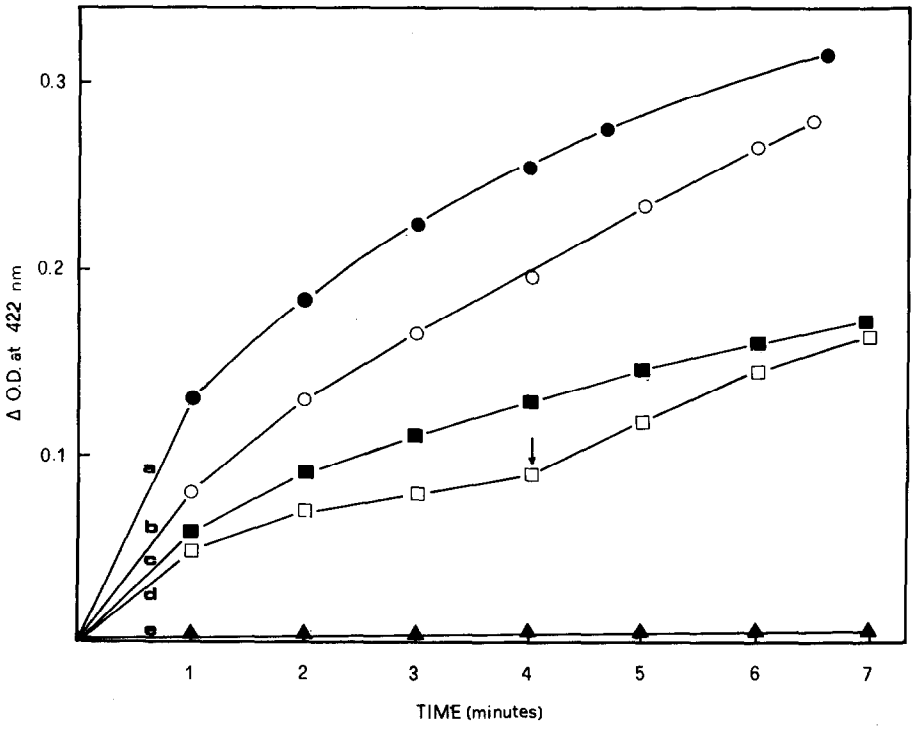

Fig. 1. Rate of reconstitution of ferredoxin. The reconstitution of ferredoxin was followed by measuring the increase of optical density at 422 nm. The standard incubation mixture contained 0.17 M Tris HCl, pH 7.6; 1.25×10^{-4} M apoferredoxin; 3×10^{-4} M $Fe(NO_3)_3$; 2×10^{-3} M dithiothreitol; 1.25×10^{-3} M $Na_2S_2O_3$ and 6.0×10^{-6} M rhodanese. The reaction was started by adding dithiothreitol. Curve a: standard incubation mixture except that rhodanese and thiosulfate were substituted by 4×10^{-4} M Na_2S. Curve b: standard incubation mixture but with rhodanese 1.2×10^{-5} M. Curve c: standard incubation mixture. Curve d: without rhodanese. At the arrow 6×10^{-6} M rhodanese was added. Curve e: without dithiothreitol.

only the reconstitution of ferredoxin. When dithiothreitol is omitted there is no change in optical density (fig. 1e).

Fig. 2 shows the visible absorption spectra of apoprotein reconstituted with rhodanese-thiosulfate and of native ferredoxin. It is evident that the spectrum of the reconstituted material is identical to that of native ferredoxin. Biological activities determined according to San Pietro [10] show that reconstituted ferredoxin is as active as the native one, whereas the apoenzyme is not active at all. Usual tests of rhodanese activity [12] show that it is fully active in the experimental conditions used.

4. Discussion

The non-heme iron, labile sulfide-containing proteins are ubiquitous and essential in living organisms. They are functionally linked to the electron transport system, which is located in organized structures such as chloroplasts or mitochondria. The discovery of mitochondrial DNA has suggested to many authors that specific proteins may be built inside mitochondria [13]. However, the non-protein component of conjugated proteins most probably comes from the outside. In the case of non-heme iron proteins, this relates to the formation of 'labile' sulfide. As shown by Fee and Palmer [9] the *in vitro* recombination of iron with the apoferredoxin takes place only in the presence of sulfide and a reducing agent. However, it seems unlikely that inorganic sulfide is the source of labile sulfide *in vivo*. Thiosulfate which is known to be metabolized in mitochondria to form sulfate may be a more reasonable candidate [14]. This suggests a possible role

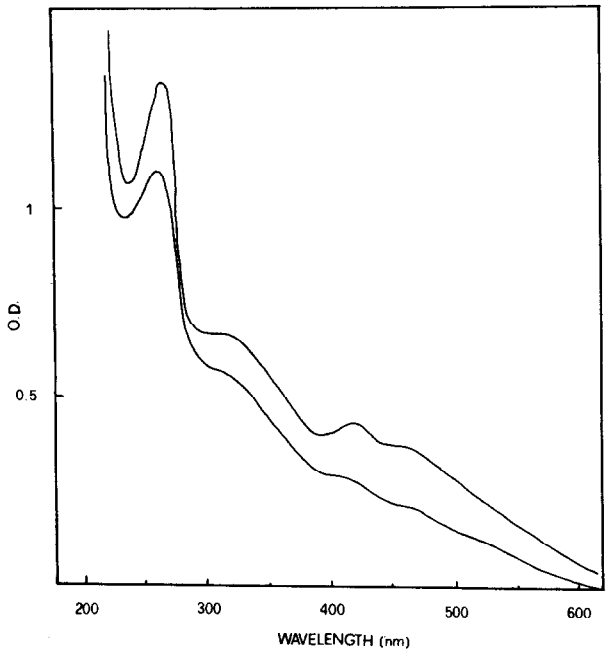

Fig. 2. Absorption spectra of native ferredoxin and the rhodanese-thiosulfate reconstituted protein. 3×10^{-5} M native ferredoxin (lower curve) and 5×10^{-5} M ferredoxin (upper curve) reconstituted by the rhodanese-thiosulfate method. The reconstituted protein was concentrated and separated from excess reagents by passage through a DEAE cellulose column. Both samples were dissolved in 0.5 M Tris HCl pH 7.6.

for rhodanese which, as shown above, catalyzes the transport of sulfur from thiosulfate to apoferredoxin. The enzyme could be responsible also for sulfite formation instead of glutathione, as previously suggested [14]; the latter may participate in the reaction either as a nucleophilic acceptor of sulfide or as the reducing agent. This last function is fulfilled *in vitro*, in our experiments, by dithiothreitol.

The presence of a reducing agent is essential for incorporation of the sulfur into apoprotein when the donor is sulfide or thiosulfate. The formation of artificial non-heme iron proteins from bovine serum albumin also requires the presence of a reducing agent [15].

The rhodanese located in mitochondria may employ thiosulfate, which is freely diffusible as the sulfide donor, in order to terminate the synthesis of non-heme iron proteins in the interior of mitochondria.

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