DEPENDANCE OF SULFITE REDUCTION ON A CRYSTALLIZED FERREDOXIN FROM *DESULFOVIBRIO GIGAS*

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The isolation from *Desulfovibrio desulfuricans* of a ferredoxin has been shortly mentionned by Tagawa *et al.* (1962), and Akagi (1965) has presented some evidence suggesting that reduction of sulfite by *Clostridium nigrificans* requires ferredoxin. It has also been shown that a particulate fraction from *D. gigas* was able to reduce sulfite if some soluble proteins were added to the preparation. This soluble protein could be separated into two protein fractions by treatment with DEAE cellulose. The acidic protein fraction was thought to contain ferredoxin (Peck, 1966). Such data were strongly in favour of a ferredoxin requirement for the reduction of sulfite by *D. gigas*.

In the present paper, the purification of a ferredoxin from *D. gigas* and its role in sulfite reduction are reported.

METHODS

D. gigas was grown in a lactate-sulfate medium and harvested as already published (Le Gall *et al.*, 1965). Extracts for sulfite reduction assay were obtained using a French pressure cell and the particulate proteins prepared according to Peck (1966).

Sulfite reduction was assayed under H_2 atmosphere by conventionnal manometric method.

Proteins were estimated by a modification of the Lowry phenol reagent method (Rabinowitz *et al.*, 1962). Iron and "labile" hydrogen sulfide were respectively assayed by the methods of Harvey (1945) and Fogo *et al.* (1949), with the modifications proposed by Lowenberg *et al.* (1963).

PURIFICATION AND CRYSTALLIZATION OF FERREDOXIN

The bacteria (160 g, wet weight) from which the soluble cytochrome c_3 has been previously extracted (Le Gall *et al.*, 1965) were resuspended in 200 ml of 0.005 M tris-buffer (pH 7.6), extracted with a French Pressure cell and centrifuged for 20 mn in a Servall centrifuge at 30 000 x g. The resulting brownish extract was treated twice with 85 ml of DEAE cellulose in batch. The adsorbed proteins were eluted from DEAE cellulose by treatment with 1.0 M tris-buffer at pH 7.6, giving 80 ml of a deep brown solution which was then passed through a Sephadex G-50 column (3 cm in diameter, 160 cm in height) equilibrated with 0.005 M tris-buffer at pH 7.6.

The adsorbed proteins were eluted from the column by a 0.05 to 0.45 M gradient of tris-buffer at same pH. First fractions contained a pink colored protein, the spectrum of which was found similar to the spectrum of the rubredoxin isolated from *Clostridium pasteurianum* by Lovenberg *et al.* (1965). The ferredoxin was collected in a 83 ml

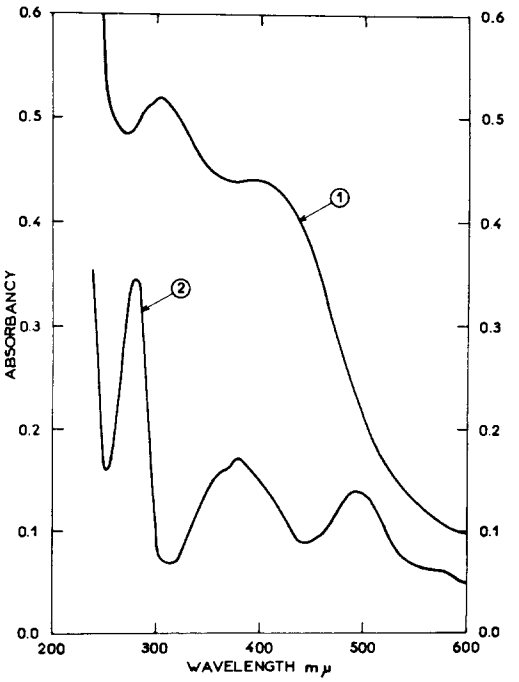

Fig. 1. Absorption spectra of ferredoxin and rubredoxin from *D. gigas*. Curve (1) : 86 µg/ml of three times recrystallized ferredoxin in 0.1 M tris-buffer, pH 7.6 - Curve (2) : purified rubredoxin in 0.1 M tris-buffer, pH 7.6. - Spectra were recorded in 1 cm light path cuvettes using a PMQII type Zeiss spectrophotometer.

volume, dialyzed against 0.05 M tris-buffer, adsorbed on a DEAE cellulose column and eluted the same way as before giving a 62 ml eluate. At that stage of purification, the ferredoxin fraction contained a material showing a strong absorption at 260 mμ. This was removed by passing the dialyzed extract through a calcinated alumina column equilibrated with 0.05 M tris-buffer pH 7.6. After this treatment, the ferredoxin was dialysed against saturated ammonium sulfate. Crystals were formed within a few hours and the protein was recrystallized using the same method (Fig. 2).

The absorption spectrum of ferredoxin, recorded after three successive crystallizations, (Fig. 1) shows two peaks at 305 mμ and 390 mμ, and a shoulder at 290 mμ. The protein was found to contain per mg 0.90 μmole of non-heme iron and 0.83 μmole of "labile" hydrogen sulfide. The redox potential was tested using an anaerobic Thunberg type cuvette containing a preparation of purified hydrogenase from *D. gigas* under an atmosphere of hydrogen, and found to be within the range of safranin (E'_o = - 310 mV).

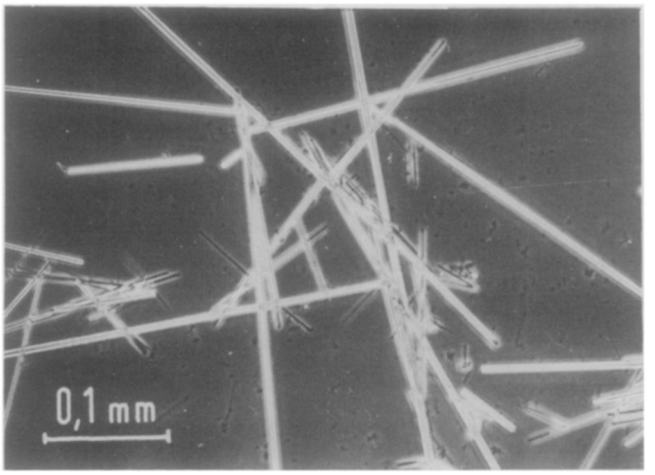

Fig. 2. Crystals of three times recrystallized ferredoxin from *D. gigas*.

ROLE IN SULFITE REDUCTION

The ferredoxin was essayed for sulfite reduction. The reaction mixture contained proteins and soluble proteins pretreated with DEAE in order to remove ferredoxin. Addition of 0.97 mg of ferredoxin completely restored the activity of untreated soluble protein extract (Fig. 3). Rubredoxin from *D. gigas* could not replace ferredoxin for sulfite reduction

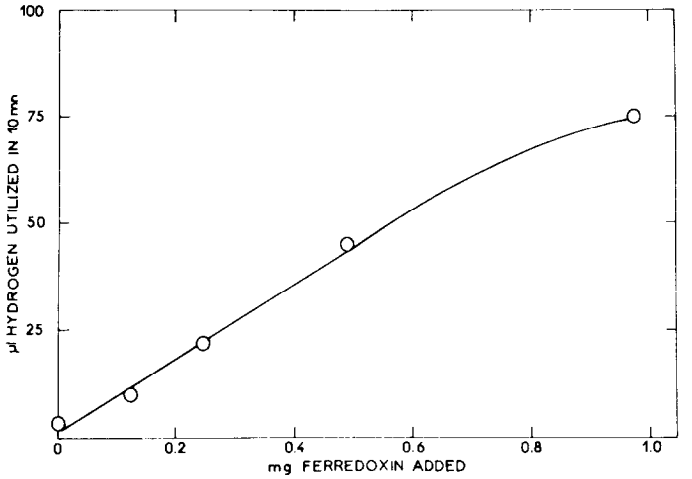

Fig. 3. Effect of ferredoxin from *D. gigas* on sulfite reduction. The reaction mixture contained in µmoles : phosphate pH 7.6 : 100 ; $MgCl_2$: 20 ; Na_2SO_3 : 20 ; particule protein : 5 mg ; DEAE treated soluble protein 27 mg, in a total volume of 3 ml. The center well contained 0.05 ml of 10 % Cd SO_4 and 0.05 ml of 20 % KOH. Temperature : 37°. Gaz phase : H_2.

The spectral properties and the iron and "labile" hydrogen sulfide content of *D. gigas* ferredoxin are very similar to those of the ferredoxins extracted by Lowenberg *et al.* (1963) from various species of *Clostridium*. The spectral differences shown by *C. nigrificans* ferredoxin, as published by Akagi (1965), are probably due to the fact that the preparations obtained by this author were contaminated by the 260 mµ absorbing material which was also found by us, and which, when present, completely masks the peaks in the 280-300 mµ region.

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