A FERREDOXIN FROM HALOBACTERIA

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1. Introduction

Ferredoxins have been isolated from many different kinds of organisms [1,2]. They are structurally well characterised iron-sulfur proteins, which contain by definition an equal number of iron and sulfide ions [3]. There are two types, which are clearly distinguished by the structure of their iron-sulfur centres [1,2,4]. A number of soluble ferredoxins containing one or two 4 Fe-4S-clusters per molecule have been isolated from various species of bacteria. Ferredoxins containing one 2Fe-2S-cluster are present in all algae and green plants, but few of them have been isolated from bacterial and mammalian sources. This paper describes the isolation and some properties of a 2Fe-2S-ferredoxin of still unknown function which was obtained in homogenous form from Halobacterium halobium.

2. Material and methods

2.1. *Isolation and purification of the ferredoxin*

Halobacterium halobium NRL R_1 M_1 cells were grown as described in [7]. 200 g of cell paste were stirred in 2 l of water containing 5 mg of DNAse (Roth oHG, Karlsruhe) until a homogenous suspension was obtained. After centrifugation for 3 h at 55 000 *g*, the supernatant was 3-fold diluted and poured on to a column of Whatman DE 52-cellulose (4 × 10 cm). Stepwise elution with 0.06 M, 0.1 M, 0.13 M and 0.17 M NaCl in 0.15 M Tris–HCl (pH 7.0), followed, until the brown band reached the end of the column. Elution with 0.4 M NaCl in buffer and concentration by dialysis against 20% polyethyleneglycol gave about 10 ml of crude ferredoxin solution. The next step was chromatography on DE 52-cellulose with 0.18 M NaCl in 0.15 M Tris–HCl (pH 7.0). All fractions showing a distinct brown colour and the characteristic ferredoxin absorption spectrum were combined, diluted twice, absorbed on a 2 × 2 cm cellulose bed and eluted with 0.5 M NaCl in 0.15 M Tris–HCl (pH 8.0). Chromatography on a Sephadex G-75 column (2 × 100 cm) with 0.12 M NaCl in 0.1 M Tris–HCl (pH 8.0), gave a product which was homogeneous in polyacrylamide disc-gel electrophoresis (fig.1). The yield was up to 80 mg from 200 g cell paste.

2.2. *Analyses*

The amino acid analysis was carried out using the Biotronic LC 6 000 amino acid analyzer. Hydrolysis

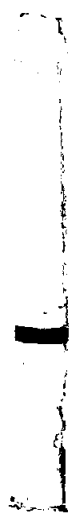

Fig.1. Polyacrylamide-disc-gel of H. halobium ferredoxin purified as described under Material and methods. 15 μg of ferredoxin were applied to a gel of 5 mm diameter. Electrophoresis was carried out according to [6] but 18% acrylamide containing gels prepared.

was carried out with 6 N HCl at 100°C for 24, 48 or 72 h or with 4 N methane sulfonic acid at 105°C for 48 h (determination of tryptophane). A performic acid oxidized sample was prepared according to Weber et al. [8] and used for cysteine determination.

Iron was determined as described by Lovenberg et al. [9], acid-labile sulfur according to Brumby et al. [10].

Optical absorption spectra were recorded on a Perkin Elmer Spectrophotometer 124; samples were reduced by adding solid sodium dithionite.

The redox potential was determined as described by Knoell and Knappe [11] using benzyl viologen (E'_0 = −359 mV) as the indicator dye. All chemicals and materials were commercially available and of analytical grade.

Table 1
Amino acid composition of halobacterial ferredoxin

Arg	3	Ala	15
His	1	Gly	10
Lys	5	Pro	4
Phe	2	Glx	22
Tyr	6	Ser	3–4
Leu	9	Thr	3–4
Ile	6	Asx	25–26
Met	3–4	Cys	5
Val	8	Trp	6

Total residues: 132–136

Calculated molecular weight from amino acid composition 14 600 ±200. Various samples of the ferredoxin were hydrolysed as described under Material and methods. The results are corrected for degradation or resistance to hydrolysis. The table shows the nearest integers to the corrected values.

3. Results and discussion

Halobacterium halobium cells were found to contain large amounts of a ferredoxin with an optical absorption spectrum (fig.2) typical of a 2Fe-2S-protein [4]: absorption maxima are at 329, 421 and 467 nm with millimolar absorptions of 13.0, 10.0 and 8.9 mM^{-1}. On reduction with dithionite the absorption decreases by 51% at 421 nm and by 54% at 467 nm. No influence of the salt concentration on these spectra was found up to 3 M KCl.

Amino acid analysis (table 1) showed in agreement with the protein's highly anionic character a large number of acidic residues (although the amide content is not known) and only few basic residues. The minimum molecular weight calculated from amino acid analysis is 14 800 ± 200 for the ferredoxin. Sephadex-chromatography showed a molecular weight of 16 500. 2.05 mol of iron and 1.7 mol of acid-labile sulfur per 14 800 g protein were found.

The redox potential of the ferredoxin was determined according to Knoell and Knappe [11]. The log $\frac{Fd_{ox}}{Fd_{red}}$ plotted against the potential gives a straight line (fig.3), which intersects the potential axis at a midpoint potential of −340 mV. The slope of the line indicates a single electron transfer as typically found for 2Fe-2S-ferredoxins. Table 2 summarizes the measured properties of the H halobium ferredoxin.

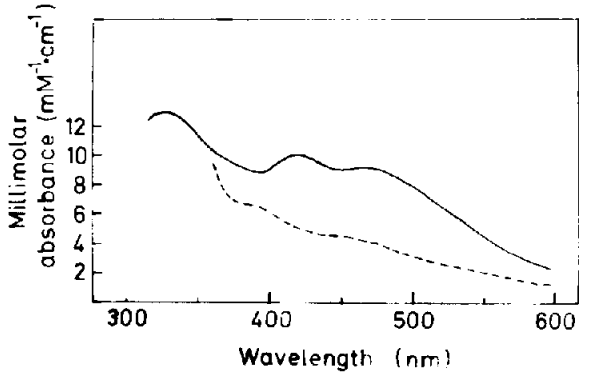

Fig.2. Optical absorption spectra of oxidized (solid line) and dithionite-reduced (dotted line) H. halobium ferredoxin.

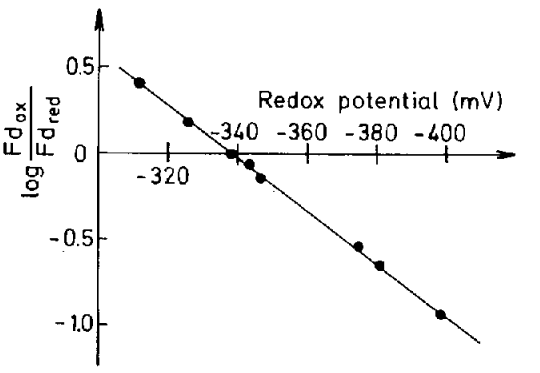

Fig.3. Determination of the redox potential of H. halobium ferredoxin by the benzyl viologen method [11].

Table 2
Properties of H. halobium ferredoxin

Molecular weight determined by:			
(a)	Amino-acid composition		14 800 ± 200
(b)	Sephadex-chromatography		16 500
Atoms iron found per molecule			2.05
Sulfide-ions found per molecule			1.7
Redox potential (E_0')			- 340 mV
Absorption maxima of the oxidised protein:			
(nm)	:	329 421	467
($mM^{-1} \cdot cm^{-1}$)	:	13,0 10.0	8.9

The strains *Halobacterium cutirubrum, H. salinarium* and *H. trapanicum* (obtained from the Canadian National Research Council) were found to contain similar amounts of ferredoxins showing the same optical absorption spectra and the same behaviour during chromatography on DEAE-cellulose. The halobacterial ferredoxin ressembles in its optical absorption spectrum more the plant ferredoxins than other bacterial 2Fe-2S-ferredoxins. Although its redox potential and its molecular weight do not allow to link this new ferredoxin with any of the known ferredoxins further spectroscopic measurements demonstrated a close relationship to plant ferredoxins [5].

Acknowledgements

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