A MOLYBDENUM-CONTAINING IRON-SULPHUR PROTEIN FROM DESULPHOVIBRIO GIGAS

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SUMMARY

A protein that contains molybdenum, iron and labile sulphide has been isolated from Desulphovibrio gigas. The molecular weight was estimated to be approx. 120,000, with 20 atoms of iron, 20 of labile sulphide, and 1 of molybdenum per molecule. The protein is acidic, with isoelectric point 4.1 ± 0.1. The iron-sulphur chro - mophores are of the 2Fe-2S type, as indicated by optical absorption and circular dichroism spectra, and EPR spectra of the protein in 80% dimethylsulphoxide. EPR spectra show the presence of two types of iron-sulphur centre in the molecule.

INTRODUCTION

The ferredoxins, which are the simplest class of iron--sulphur proteins, are known to fall into at least two categories: those that contain 2Fe-2S centres (with two atoms of iron and two of acid-labile sulphide) such as the plant ferredoxins, and those that contain 4Fe-4S centres. The majority of the bacterial ferredoxins (1,2) are of the latter type, but ferredoxins with 2Fe-2S centres have been isolated from widely differing bacterial sources (2). A number of more complex iron-sulphur proteins are known to contain molybdenum. Examples of these are xanthine oxidase from milk (3), and the molybdenum-iron protein of nitrogenase, which probably contains centres of the 4Fe-4S type (4).

The sulphate-reducing bacterium Desulphovibrio gigas is

known to contain a ferredoxin which contains 4Fe-4S centres (5,6). During the isolation of ferredoxins from this organism, we separated another protein, which proved to contain molybdenum and iron-sulphur centres of the 2Fe-2S type. In this paper we report the isolation and physicochemical properties of the protein.

EXPERIMENTAL AND RESULTS

Isolation of the Mo-Fe-S protein: A fraction containing the protein was separated during the early stages of the purification of the 4Fe-4S ferredoxins (7). The acidic protein extract (8) from 4 Kg of cells was adsorbed on a large DEAE-cellulose column and eluted with a gradient of 0.1 - 0.5 M Tris-HCl buffer, pH 7.6. An early fraction contained the Mo-Fe-S protein, together with flavoproteins, desulphoviridin and some cytochrome c_3, MW 26000, while the 4Fe-4S ferredoxins were eluted later. The Mo-Fe-S protein was purified by rechromatography on DEAE-cellulose,

Table 1: Composition of the Mo-Fe-S protein

Non-haem iron was determined by the o-phenanthroline method (17,18). Labile sulphide was determined by an adaptation of the methods of Fogo and Popowsky (19), and Lovenberg et al. (18). Molybdenum was determined by neutron activation analysis (J. J. G. Moura, C. M. Pássaro, A. V. Xavier, J. P. Cabral and J. Le Gall, unpublished).
The isoelectric point was determined by isoelectric focusing (20) on an LKB Multiphor apparatus.

Molecular weight	120,000 ± 5000
Mo atoms/molecule	1
Non-haem iron	approx. 20
Labile sulphur	approx. 20
Isoelectric point	4.1 ± 0.1

followed by columns of Sephadex G-50, silica gel and alumina. The final product had an absorbance ratio A_{278}/A_{322} of 4.5.

Molecular weight: This was estimated by gel filtration on Sephadex G-150 (9) and by electrophoresis on polyacrylamide gels containing 7.5% polyacrylamide, in the presence of 3% urea and 0.1% sodium dodecylsulphate (10), with seven proteins of known molecular weight as standards.

With both techniques the protein gave a value of approx. 120,000 daltons. No evidence was obtained for the presence of subunits. A similar result was obtained for the apo-protein produced by treatment with 3% HCl.

Analysis of constituents of the protein: A high content of iron and labile sulphide (Table 1) was found, but only one molybdenum

Table 2: Amino acid composition

Amino acid analyses were carried out on a Beckman Multichrom amino acid analyser. Protein samples were hydrolysed in 6M HCl at 110° C for 20 h (21). The values for threonine, serine and tyrosine were corrected for decomposition during hydrolysis. Cysteine and methionine were analysed after performic acid oxidation as cysteic acid and methionine sulphone respectively (22).
The results are expressed per 12ρ00 molecular weight.

Lys	7	Ala	13
His	4	Cys	3/4
Arg	4	Val	8
Asp	12	Met	3
Thr	7	Ile	5
Ser	5	Leu	9
Glu	12	Tyr	4
Pro	5	Phe	4
Gly	14	Trp	ND

atom. The amino acid composition (Table 2) is typical of ferredoxine, with a high proportion of acidic residues and approx. 2 cysteine residues per iron atom. The low isoelectric point of 4.1 (Table 1) also shows the acidic nature of the protein. However the 4Fe-4S ferredoxins are even more acidic, being eluted from DEAE-cellulose by a higher concentration of Tris.

N-terminal amino acid sequence: Only a single type of N-terminal acid was found. The sequence near the N-terminal was determined on a Socosi PS 100 Sequenator to be:

Gly-Leu-Gln-Lys-Val-(Leu) - (Thr) - Val - Asn - Gly
5 10

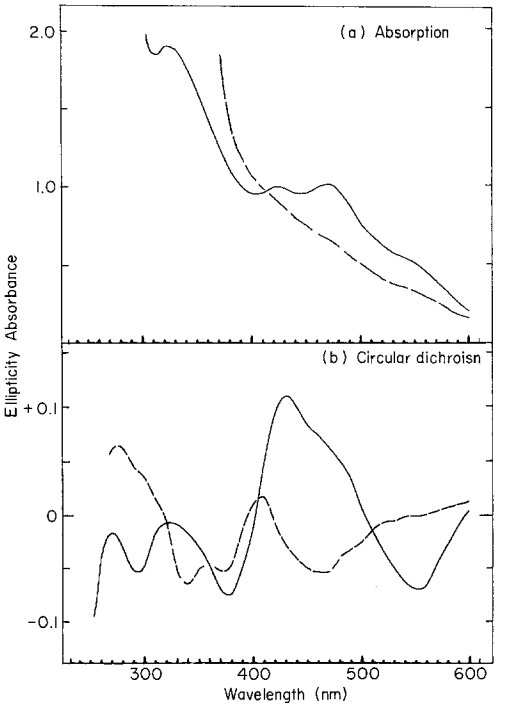

Fig. 1 (a) Optical absorption and (b) CD spectra of D. gigas iron-sulphur protein; the dotted curves are for the reduced form. The optical absorption spectra were recorded on a Cary 14 spectrophometer (Varian Assiciates, Palo Alto, California). C. D. spectrawere recorded on a FICA spectropolarimeter (SOFICA, St. Denis, France).

Optical Spectra: The optical absorption and circular dichroism (CD) spectra of the protein in the oxidized and reduced states are shown in Fig. 1. In general features, the spectra are all typical of proteins with 2Fe-2S centres (2), although some minor differences can be seen. The intensity of the absorption band at 467 nm in the oxidized protein is somewhat larger than usual; the ratio A_{425}/A_{467} is 0.98, compared with 1.19 for Pseudomonas putida ferredoxin (11) for example. There is a corresponding shoulder in the CD spectrum at 460 nm. The circular dichroism spectra are particularly strong evidence for the presence of 2Fe-2S centres, being very similar to the characteristic spectra of plant ferredoxins and xanthine oxidase (12).

EPR spectra: Spectra of the dithionite-reduced protein at relatively high temperatures (60-77 K) (Fig. 2 a) can be resolved as a rhombic signal with g-values 2.02, 1.94, 1.93 typical of an iron-sulphur centre, and a complex signal around g = 1.98, presumably due to Mo(V) On reducing the temperature below 55 K (Fig. 2 b) a further iron-sulphur signal is observed. This is most clearly seen in the feature at g = 2.06. The two types of iron-sulphur centre will be referred to as Fe-S_I and Fe-S_{II}, by analogy with the somewhat similar signals observed in milk xanthine oxidase (13).

As a check on the type of iron-sulphur centre present in the protein, the spectrum of the reduced protein in 80% dimethylsulphoxide (DMSO) was measured. The rationale of this experiment is that, whereas in the native state the EPR spectrum is influenced by protein conformation, in DMSO solution the protein is unfolded and the spectrum is more characteristic of the iron-sulphur centre only (14, 15). In this case, the spectrum of the protein in DMSO (Fig. 2C) is readily detected at 77 K as observed with other 2Fe - 2S proteins. In fact, the spectrum is very similar in its lineshape to that of xanthine oxidase in 80% DMSO (14). By contrast, 4Fe - 4S centres give an axial signal that is only observed below 30K. No signals of this type were

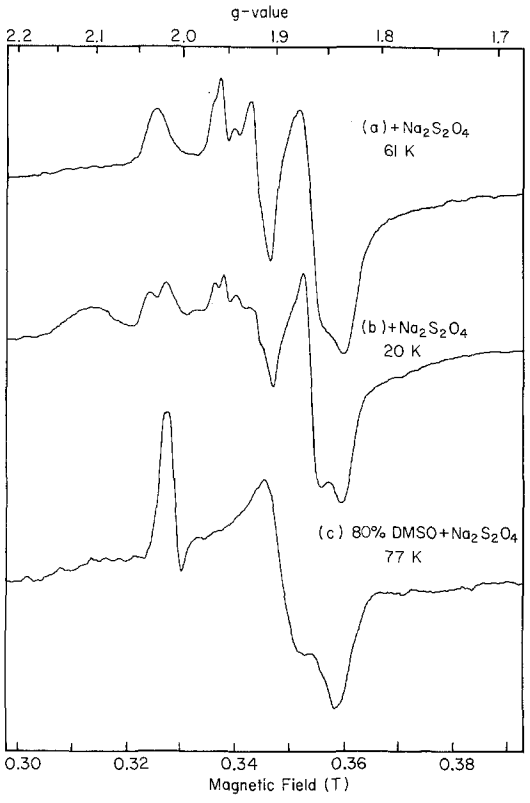

Fig. 2 EPR spectra of (a) D. gigas Mo-Fe-S protein, reduced with dithionite measured at 61K, microwave power, 20mW; (b) reduced protein at 20K, power 1mW; (c) the protein in 80% DMSO solution, reduced with dithionite measured at 77K, power 20mW. The sample for (c) was prepared as described by Cammack and Evans (15). Spectra were recorded on a Varian E4 X-band EPR spectrometer.

detected. It seems likely therefore that all the centres in the molecule are of the 2Fe - 2S type.

Physiological role: The purified Mo-Fe-S protein was tested for activity in mediating electron transport between hydrogenase and sulphite reductase (desulphoviridin) . No activity was detected. The formate dehydrogenase activity (16) was also tested; although this activity is found in the crude acidic protein fraction, it could not be detected in the purified Mo-Fe-S protein. The function of the protein therefore remains unknown.

DISCUSSION

The spectroscopic properties of the protein show that it contains 2Fe-2S centres similar to those found in plant ferredoxins. The similarity extends to the amino acid composition of the protein. Analysis shows the presence of 2 gram atoms of iron and 2 of labile sulphide per 12,000 g of protein, a value typical of ferredoxins. However the protein appears to have a particularly high molecular weight, (120,000) and one Mo atom per molecule. Other complex iron-sulphur proteins which have been isolated, such as xanthine dehydrogenase, have high molecular weights, but not such a high content of iron-sulphur centres. The Mo-Fe-S protein from *D. gigas* therefore appears to be of a new type.

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