Purification and properties of dimethyl sulphoxide reductase from *Rhodobacter capsulatus*

A periplasmic molybdoenzyme

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Dimethyl sulphoxide reductase was purified from the photosynthetic bacterium *Rhodobacter capsulatus*. The enzyme is composed of a single polypeptide of M_r 82000 and contains a pterin-type molybdenum cofactor as the only detectable prosthetic group. The oxidized molybdenum cofactor of dimethyl sulphoxide reductase is a weak chromophore and exhibits broad absorption bands in the u.v.–visible-absorption spectral region. A distinct spectrum was generated upon addition of dithionite.

INTRODUCTION

Dimethyl sulphoxide (DMSO) and trimethylamine *N*-oxide (TMAO) are used by a variety of bacteria as electron acceptors in anaerobic respiration [1]. The reduction of TMAO and DMSO by the photosynthetic bacterium *Rhodobacter capsulatus* is catalysed by a water-soluble enzyme located in the periplasmic space [2–4]. In the present paper we report the purification and characterization of DMSO reductase from *R. capsulatus*. The enzyme is shown to contain a molybdenum cofactor of the pterin type found in all molybdoenzymes other than nitrogenase. The novel properties of DMSO reductase have also provided a rare opportunity to describe the properties of the u.v.–visible-absorption spectrum of the molybdenum cofactor in an intact enzyme.

EXPERIMENTAL

R. capsulatus strain 37b4 [5] was grown phototrophically in the presence of 60 mM-DMSO. The growth medium was based on RCV medium [6] except that 40 mM-sodium propionate replaced malate as carbon source. Cells were harvested in late exponential phase and a periplasmic fraction was prepared as described previously [2]. All steps in the purification of DMSO reductase were performed at 4 °C with chromatography materials supplied by Pharmacia. DMSO reductase activities were measured as described by McEwan *et al.* [4], with dithionite-reduced methyl viologen as electron donor. Visible–u.v.-absorption spectra were recorded on a Kontron Uvikon 810 spectrophotometer. Fluorescence measurements were made with a Spex Fluorolog fluorimeter. Protein concentration was determined by the method of Markwell *et al.* [7]. The form B pterin derivative of the molybdenum cofactor was extracted from DMSO reductase as described in ref. [8]. SDS/PAGE was carried out as described previously [2], and gels were stained with Coomassie Blue. The M_r standards were supplied by Pharmacia.

RESULTS

Purification of DMSO reductase

A periplasmic fraction prepared from 40 g wet wt. of cells was

applied to a DEAE-Sepharose CL-6B column (bed volume 120 ml) that had been equilibrated with 50 mM-Tris/HCl buffer, pH 8. The column was washed with the same buffer containing 100 mM-NaCl, and this eluted cytochrome c_2 and cytochrome c' , the most abundant redox proteins in the periplasm. DMSO reductase was eluted with a linear gradient of 100–300 mM-NaCl (total volume 400 ml). Peak activities of DMSO reductase were eluted at approx. 160 mM-NaCl as reported previously [2]. The peak fractions were pooled, $(\text{NH}_4)_2\text{SO}_4$ was added to a final concentration of 15% (w/v) and the sample was applied to a phenyl-Sepharose column (bed volume 30 ml) that had been equilibrated with 15% $(\text{NH}_4)_2\text{SO}_4$ in 50 mM-Tris/HCl buffer, pH 8. A linear gradient of 15–0% $(\text{NH}_4)_2\text{SO}_4$ in the Tris/HCl buffer was applied and peak fractions of TMAO/DMSO reductase were eluted at approx. 6% $(\text{NH}_4)_2\text{SO}_4$. DMSO reductase-containing fractions were pooled, concentrated by ultrafiltration (Amicon PM10 filters), and the sample was applied to a Sephacryl S200 gel-filtration column (approx. bed volume 450 ml) equilibrated with 50 mM-Tris/HCl buffer, pH 8. The purification steps are summarized in Table 1. The enzyme was active towards TMAO, DMSO and chlorate, but not nitrate, and was stable at –70 °C for several months without significant loss of activity.

M_r and cofactor composition of DMSO reductase

The polypeptide composition of the purified DMSO reductase

Table 1. Purification of DMSO reductase from *R. capsulatus*

For experimental details see the text. One unit of activity is defined as the oxidation of 1 μmol of methyl viologen/min.

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)
Periplasm	148	3330	22.5
DEAE-Sepharose	50	2885	57.7
Phenyl-Sepharose	22.4	3230	140.2
Sephacryl S200	14.4	1717	119.3

Abbreviations used: TMAO, trimethylamine *N*-oxide; DMSO, dimethyl sulphoxide.

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was examined by SDS/PAGE (Fig. 1). When the sample was boiled for 5 min in the presence of 2-mercaptoethanol before electrophoresis, a single polypeptide of M_r 82000 was observed (Fig. 1, lane 1). However, when the boiling step was omitted the electrophoretic behaviour of DMSO reductase was quite different and the apparent M_r of the polypeptide was 46000 (Fig. 1, lane 2). The native M_r of DMSO reductase was also determined by gel filtration on a calibrated Superose 12 f.p.l.c. column. An M_r value of 93000 was calculated (results not shown). This value was not affected when the DMSO reductase was first incubated and then chromatographed in the presence of 10 mM-dithiothreitol.

In previous work we had noted that DMSO reductase activity was inhibited by the presence of tungstate in the growth medium, and this suggested the enzyme contained molybdenum [2]. The presence of molybdenum in the purified DMSO reductase was confirmed by detection of a characteristic e.p.r. spectrum (N. A. Turner & R. C. Bray, personal communication). The organic component of the common molybdenum cofactor comprises a pterin moiety, which can be released from molybdoenzymes. Degradative products of the pterin have highly characteristic fluorescence spectra [8]. Fig. 2 shows fluorescence spectra of material extracted from 2 mg of DMSO reductase. Excitation maxima at 375 nm and emission maxima at 480 nm were observed. These spectra are very similar to those published for the form B of the molybdenum cofactor, which has been prepared from a number of molybdoenzymes [8]. Analysis by atomic absorption spectroscopy detected less than 0.15 mol of Fe/mol of DMSO reductase (results not shown).

Fig. 1. SDS/PAGE of purified DMSO reductase

Lane 1, 10 μ g of heat-denatured enzyme; lane 2, 10 μ g of a non-denatured form of enzyme that retains its molybdenum cofactor.

Visible-u.v.-absorption spectra of DMSO reductase

A visible-u.v.-absorption spectrum of DMSO reductase shows (Fig. 3a) that the enzyme contained no strongly absorbing chromophores other than those attributable to aromatic amino acids. Increasing the sensitivity of the spectrophotometer revealed the presence of broad absorption bands (Fig. 3b) between 380 nm and 520 nm and at 700 nm in the air-oxidized form. The addition of DMSO to the sample did not alter the spectrum of air-oxidized DMSO reductase (results not shown), indicating that the prepared enzyme was fully oxidized. Dithionite caused the bleaching of these absorption bands and an absorbance increase at around 640 nm (Fig. 3b). The weak electronic spectra described in Fig. 3 may be attributed to the molybdenum cofactor. The visible-absorption spectrum confirmed the absence of iron-sulphur centres, which was indicated by iron analysis.

DISCUSSION

We have described a three-step purification for the DMSO reductase of *R. capsulatus*. The enzyme is composed of a single polypeptide of M_r 82000, and is likely to be very similar to the DMSO reductase that was purified from the photosynthetic bacterium *Rhodobacter sphaeroides* f. sp. *denitrificans* [9]. DMSO reductases and TMAO reductases have also been purified from

Fig. 3. U.v.-visible-absorption spectrum of purified DMSO reductase

The protein concentration was 1.5 mg/ml in 50 mM-Tris/HCl buffer, pH 8. —, Air-oxidized sample; -----, dithionite-reduced sample.

Fig. 2. Fluorescence excitation and emission spectra of form B of the pterin component released from DMSO reductase

Excitation was at 380 nm for the emission spectrum, and emission was measured at 480 nm for the excitation spectrum.

a variety of enteric bacteria (see Weiner *et al.* [10]). *Escherichia coli* possesses a water-soluble TMAO reductase of M_r 200 000 as judged by gel filtration [11] and a membrane-bound DMSO reductase that was composed of three subunits [10]. In the latter enzyme the polypeptide containing the molybdenum cofactor (*dmsA*-gene product) was found to have M_r 82 600 as determined by SDS/PAGE [10]. However, anti-(*E. coli* DMSO reductase) antibodies did not appear to react with the *R. capsulatus* enzyme in Western-blot experiments (results not shown), and this is taken to indicate that the two enzymes are unrelated immunologically.

We have previously reported that a single polypeptide of M_r 46 000 stained for TMAO reductase activity after electrophoresis of a partially purified enzyme in the presence of SDS [2]. This apparent M_r is identical with that of the unheated sample of the purified DMSO reductase (Fig. 1). Our interpretation of these data is that the DMSO reductase must have a relatively stable tertiary structure that is not fully disrupted by SDS. Since it possessed activity under these conditions the enzyme must have retained its molybdenum cofactor and a degree of its tertiary structure. It follows that the enzyme is not fully denatured if boiling is omitted, and therefore the probable M_r of the DMSO reductase of *R. capsulatus* is 82 000. However, the apparent M_r of the native enzyme was 93 000, and this raised the possibility that the native form of the enzyme was a dimer composed of two subunits of M_r 46 000 linked through a cystine bridge. This possibility has been excluded, since gel filtration in the presence of dithiothreitol did not affect the apparent M_r of the enzyme. The results indicate that the DMSO reductase is a monomer. The failure of a periplasmic molybdenum-containing enzyme to unfold completely, thus retaining activity, when solubilized in SDS at room temperature has recently been described in the case of the nitrate reductase of *R. capsulatus* [12].

The presence of a molybdenum cofactor in DMSO reductase was confirmed by the release of a degradation product of this cofactor with characteristic fluorescence properties (Fig. 2). The molybdenum cofactor in the DMSO reductase of *R. sphaeroides* f. sp. *denitrificans* has recently been identified as a molybdopterin guanine dinucleotide [13], and it will be of interest to determine whether this modified molybdenum cofactor is present in the *R. capsulatus* enzyme.

The absence of strongly absorbing prosthetic groups in the enzyme has allowed an examination of the optical spectroscopic properties of the molybdenum cofactor in an intact enzyme. The only previous description of such properties for the molybdenum cofactor is in the tryptic fragment of rat liver sulphite oxidase [14]. The spectral features of the latter enzyme and the DMSO reductase are quite similar; the molar absorption coefficients of the absorption bands are less than $5000 \text{ M}^{-1} \cdot \text{cm}^{-1}$ in both enzymes. The broad absorption bands in the oxidized form of

DMSO reductase most probably represent the interaction of the molybdenum centre with sulphur and oxo ligands [15]. These bands are attributable to the Mo(VI) form of the enzyme, since they were bleached upon reduction with dithionite. The absorption band at 640 nm that was generated upon reduction of dithionite is assigned to the Mo(IV) form of the DMSO reductase. Although DMSO reductase of *R. sphaeroides* f. sp. *denitrificans* was originally reported to lack any chromophores in the visible-absorption spectrum [9], absorption bands similar to those in the *R. capsulatus* enzyme have now been noted [16]. The DMSO reductases of *R. capsulatus* and *R. sphaeroides* f. sp. *denitrificans* appear to be the simplest molybdenum enzymes in terms of redox centres so far isolated. Their properties will allow optical spectroscopy to be used to investigate the role of the molybdenum cofactor in oxo group transfer and electron transport.

This work was supported by a grant from the Science and Engineering Research Council (U.K.) to S.J.F. and J.B.J. We thank Dave Richardson for helpful discussions, Dr. Joel Weiner (University of Alberta) for the provision of antibodies to the *E. coli* DMSO reductase, and Faye Monkera for help with iron analysis.

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