Purification and properties of dimethyl sulfoxide reductase from Rhodobacter capsulatus

A periplasmic molybdoenzyme

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Dimethyl sulfoxide reductase was purified from the photosynthetic bacterium Rhodobacter capsulatus. The enzyme is composed of a single polypeptide of Mr 82000 and contains a pterin-type molybdenum cofactor as the only detectable prosthetic group. The oxidized molybdenum cofactor of dimethyl sulfoxide 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 sulfoxide (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 Mr 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, 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, (NH₄)₂SO₄ 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% (NH₄)₂SO₄ in 50 mm-Tris/HCl buffer, pH 8. A linear gradient of 15-0% (NH₄)₂SO₄ in the Tris/HCl buffer was applied and peak fractions of TMAO/DMSO reductase were eluted at approx. 6% (NH₄)₂SO₄. 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.

Mr and cofactor composition of DMSO reductase

The polypeptide composition of the purified DMSO reductase

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periplasm</td>
<td>148</td>
<td>3330</td>
<td>22.5</td>
</tr>
<tr>
<td>DEAE-Sepharose</td>
<td>50</td>
<td>2885</td>
<td>57.7</td>
</tr>
<tr>
<td>Phenyl-Sepharose</td>
<td>22.4</td>
<td>3230</td>
<td>140.2</td>
</tr>
<tr>
<td>Sephacryl S200</td>
<td>14.4</td>
<td>1717</td>
<td>119.3</td>
</tr>
</tbody>
</table>

Abbreviations used: TMAO, trimethylamine N-oxide; DMSO, dimethyl sulfoxide.
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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 \( \mu \)g of heat-denatured enzyme; lane 2, 10 \( \mu \)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...
Rhodobacter capsulatus dimethyl sulphoxide reductase

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 14 of the properties molybdenum cofactor dinucleotide guanine possibility enzymes. has allowed enzyme reductase f. native capsulatus have retained its molybdenum these it of phoresis enzyme the native subunits of denitrificans is Mr 82 600 was determined since absorption coefficient absorption coefficients of the latter enzyme and the DMSO reductase are quite similar; the molar absorption coefficients of the absorption bands are less than 5000 m⁻¹·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 o xo 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 o xo group transfer and electron transport.

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REFERENCES


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