Volume 258, number 1, 175-176 FEB 07834 November 1989

Crystallization and preliminary X-ray crystallographic study of the quinoprotein methanol dehydrogenase from bacterium W3A1

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Received 29 September 1989

Methanol dehydrogenase from bacterium W3A1 has been crystallized by the macroseeding method to give single crystals suitable for three-dimensional structural study at resolution greater than 3 Å. The crystals belong to the space group $P2_1$, and have unit cell dimensions $a=124.13$ Å, $b=62.87$ Å, $c=84.71$ Å, and $\beta=92.89°$. There is one dimeric molecule of 114600 Da per asymmetric unit.

Crystallization; Methanol dehydrogenase; Pyrroloquinoline quinone; Quinoprotein

1. INTRODUCTION

A new class of oxidoreductases has been recently characterized which possess as a prosthetic group pyrroloquinoline quinone (PQQ) [1]. Some quinoproteins, such as the bacterial methylamine dehydrogenases and the eukaryotic copper-containing amine oxidases, possess covalently bound PQQ. Other quinoproteins, such as bacterial methanol and glucose dehydrogenases, possess non-covalently associated PQQ. Thus far, very little is known of the structure of PQQ-containing enzymes. A recent description of the structure of the covalent quinoprotein, methylamine dehydrogenase from *Thiobacillus versutus* [2], is the first such report. Preliminary crystallographic studies of methanol dehydrogenases from *Methylophillus methylotrophus* [3] and *Methylosinus trichosporium* OB3b [4] have been reported, but no crystal structure of this enzyme or of any enzyme with non-covalently associated PQQ has yet been determined.

The restricted facultative methylotroph, bacterium W3A1, has become an important source of some novel and potentially interesting enzymes and redox proteins which participate in the oxidation of C-1 compounds. Our laboratories have been studying the structure and function of several of the enzymes involved in the utilization of C-1 compounds. As part of this continuing effort we present this X-ray crystallographic study of methanol dehydrogenase.

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2. EXPERIMENTAL

Growth of bacterium W3A1 (NCIB 11348) [5] and purification of methanol dehydrogenase [6] were according to published methods. The protein solution used for crystallization experiments contained 10 mg/ml of methanol dehydrogenase in 5 mM phosphate, pH 6.8, with 10 mM methanol. Crystals were grown by the macroseeding method from the seeding solution which contained 50 mM Tris-HCl, pH 8.25, 2.5 mg/ml of protein, and 12.0% (w/v) polyethylene glycol (PEG) 8000. The seeds were washed with a solution identical to that described above but which did not contain protein. One or two washed seeds were then put into 50 μl of fresh protein-containing solution. In order to get initial seeds the protein solution was mixed with the precipitant and the buffer to give a solution with the composition described above, except that it contained 13% PEG 8000. 25 μl of the mixed solution yielded, within one or two days, several seeds with the largest dimension less than 0.1 mm. Each of the crystallization experiments was carried out at 20°C in small, siliconized, round-bottom wells. A layer of paraffin oil was used to cover the crystallization solution in order to avoid evaporation during the crystallization. In most cases, the seeds grew in a few weeks to final size of $0.4\times0.4\times0.1$ mm or $0.3\times0.2\times0.2$ mm (fig.1). To test the quality of the crystals, a crystal of $0.3\times0.2\times0.2$ mm was used to collect the partial data of X-ray diffraction for about 12 h to a maximum resolution of 2.68 Å at the Argonne area detector facility. This crystal was suitable for structural study at resolution greater than 3 Å.

3. RESULTS AND DISCUSSION

The crystals of methanol dehydrogenase diffract to about 2.5 Å resolution. The precession photographs (fig.2) show that they belong to space group $P2_1$, and have unit cell parameters: $a=124.13$ Å, $b=62.87$ Å,

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

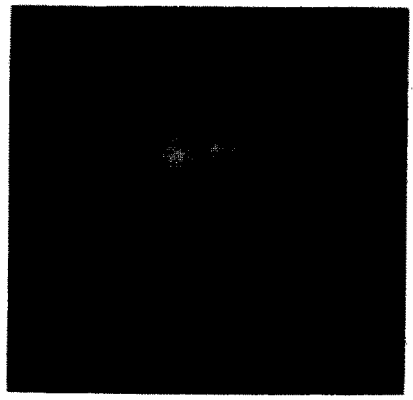

Fig.1. Crystal of methanol dehydrogenase from bacterium W3A1.

$c = 84.71$ Å, and $\beta = 92.89°$. The volume per unit protein molecular mass is 2.88 Å^3/Da for one dimeric molecule of Mr = 114 600 per asymmetric unit. This corresponds to a solvent content of about 57%. These values are comparable to the values usually found for other proteins [7].

The unit cell dimensions of methanol dehydrogenase from bacterium W3A1 are very similar to those of the enzyme from *M. methylotrophus*, except that the space group of the latter is C2 rather than P2$_1$ [3]. The conditions for crystallization of the enzymes from these two species are also similar except that seeds of the latter were obtained by the vapour diffusion method. Conversely, the enzyme from *M. trichosporium* OB3b exhibited quite different unit cell parameters [4].

Our laboratories have been interested in the structures of enzymes involved in the oxidation of C-1 compounds and of the proteins in the subsequent electron transfer chains. We have solved the structure of the iron-sulfur flavoprotein, trimethylamine dehydrogenase, from bacterium W3A1 [8], and have reported the crystallization of the copper protein amicyanin [9] and the quinoprotein methylamine dehydrogenase [10] from the facultative methylotroph *Paracoccus denitrificans*. We have also characterized many of the physical properties of the methanol dehydrogenase from bacterium W3A1 [5]. Resolution of the structure of this methanol dehydrogenase will help us to understand the mechanisms by which the PQQ cofactor interacts with the structural protein and participates in electron transfer.

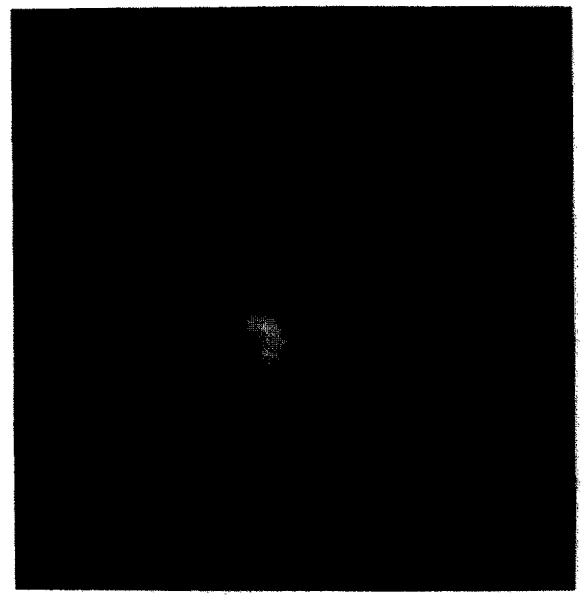

Fig.2. Precession photograph of methanol dehydrogenase from bacterium W3A1. The 8° precession photograph (hol) of methanol dehydrogenase was taken at a crystal-to-film distance of 75 mm with a 26 h exposure, using an Elliot rotating anode X-ray generator operated at 40 kV and 40 mA. The a* and c* directions are indicated.

Acknowledgements: This work was supported by National Institutes of Health Grants GM41574 and GM20530.

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