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Preliminary X-ray Crystallographic Study of Quinoprotein Ethanol Dehydrogenase from *Pseudomonas aeruginosa*

Methanol-oxidizing bacteria possess a dye-dependent alcohol dehydrogenase of the quinoprotein type. Quinoproteins are a novel class of oxidoreductases with pyrroloquinoline quinone as prosthetic group (Duine *et al.*, 1979). The prosthetic group is also called methoxatin (Salisbury *et al.*, 1979). The usual function of this alcohol dehydrogenase is to catalyze the oxidation of methanol, although it oxidizes a wide range of primary alcohols also. Secondary alcohols are not accepted as substrate. The K_m values for methanol are in the range 10 to 20 μM and the pH optima are around pH 9. The methanol dehydrogenases need ammonia or methylamine as an activator and phenazine methosulfate is used as an electron acceptor. Usually the methanol dehydrogenases are dimers of identical subunits with molecular mass of approximately 60,000 daltons (Anthony, 1982). Lim *et al.* (1986) and Parker *et al.* (1987) have reported preliminary crystallographic data for methanol dehydrogenases from *Methylophilus methylotrophus* and *Methylosinus trichosporium* OB3b, respectively.

We have recently purified and crystallized a quinoprotein ethanol dehydrogenase from *Pseudomonas aeruginosa* (Rupp & Görisch, 1988). *P. aeruginosa* produces high levels of the enzyme when grown on ethanol. In its catalytic properties, the quinoprotein ethanol dehydrogenase from *P. aeruginosa* ATCC 17933 is very similar to the enzyme described for *P. aeruginosa* LMD 80.53 (Groen *et al.*, 1984). Both enzymes show an absorption spectrum similar to the one for methanol dehydrogenases. The quinoprotein ethanol dehydrogenase shows a high pH optimum and also requires ammonia or amines as activators. However, in contrast to the general quinoprotein methanol dehydrogenases it has a low affinity for methanol and in addition to primary alcohols also oxidizes secondary alcohols.

The enzyme from *P. aeruginosa* ATCC 17933 is dimeric, with identical subunits of molecular weight of 120,000 and shows a high degree of similarity with the dye-linked alcohol dehydrogenase isolated from *Rhodopseudomonas acidophila* (Bamforth & Quayle, 1978).

Crystals suitable for X-ray diffraction studies were prepared by the hanging-drop method. A solution of quinoprotein ethanol dehydrogenase (5 μl) with 2 mg protein/ml in 4·5 mM-glycine/NaOH buffer (pH 8), 1·5 mM-$CaCl_2$, 0·02% (w/v) NaCN was mixed with 1 μl of a solution containing 22·5% (v/v) polyethylene glycol 1550, 0·01% NaCN and 3 mM-$CaCl_2$ in 50 mM-glycine/NaOH buffer (pH 8). Crystals of up to at least 0·25 mm in the smallest dimension were obtained at room temperature. The crystals, mounted with mother liquor in thin-walled glass capillaries, diffract to beyond 2·5 Å (1 Å = 0·1 nm) resolution. They have been assigned to space group R_3 with $a = b = 160$ Å and $c = 124$ Å (hexagonal indexing). Assuming a dimer of molecular mass 120,000 per asymmetric unit, the volume per unit protein molecular mass is 2·5 Å^3/dalton.

Knowledge of the structure of this quinoprotein ethanol dehydrogenase will help us to understand not only the mechanism by which the unusual pyrroloquinoline quinone cofactor functions, but also, by comparison with the related methanol dehydrogenases and the quinoprotein methylamine dehydrogenase (Vallieux *et al.*, 1986), it should provide important insight into the substrate specificity of quinoproteins. The knowledge gained from these studies is expected to be very useful for the design of new proteins of potential industrial value.

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