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Crystallization and preliminary X-ray diffraction studies of a cobalt-substituted derivative of the iron-dependent alcohol dehydrogenase from *Zymomonas mobilis*

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Abstract

The iron-dependent alcohol dehydrogenase from *Zymomonas mobilis* has been crystallized in a form suitable for X-ray diffraction studies. The crystals grew in hanging drops by vapor diffusion, equilibrating with a solution comprising 25–27% methoxypolyethylene glycol 5000 and 1 m*M* Co^{2+} in a 0.2 *M* succinic acid/potassium hydroxide buffer at pH 5.5–5.7 at 281 K. Crystals are tetragonal, $P4_122$ (or $P4_322$), with unit-cell dimensions $a = b = 125.7$, $c = 248.1$ Å. Four molecules comprise the asymmetric unit, and a self-rotation function indicates twofold local symmetry perpendicular to the unique axis and 15° from a crystallographic twofold axis. Diffraction data to 3.0 Å have been collected.

Alcohol dehydrogenases (ADH's) are widely distributed in Nature, where, possibly *inter alia*, they reversibly catalyze the oxidation of alcohols to aldehydes (Jörnvall, von Bahr-Lindström & Höög, 1989; Reid & Fewson, 1994). There are at least three major categories: the classical $NAD(P)^+$-dependent ADH's, the $NAD(P)^+$-independent ADH's and members of a keto-aldose oxidoreductase superfamily for which ADH activity is essentially irreversible. Within the group of $NAD(P)^+$-dependent ADH's three distinct families are known. (i) The zinc(II)-dependent ADH's, comprised of about 350 residues, formerly referred to as 'long chain' or classical alcohol dehydrogenases (Jörnvall *et al.*, 1989), but now termed 'medium chain' (Persson, Krook & Jörnvall, 1991; see below) and crystallographically well characterized; (ii) the 'short chain' zinc(II)-independent family, comprising about 250 residues and also crystallographically characterized; and a family whose shorter chain representatives are comprised of about 380 residues and for which the best characterized member is an iron(II)-dependent ADH from *Zymomonas mobilis* (Scopes, 1983).

This last family of $NAD(P)^+$-dependent ADH's, which remains crystallographically uncharacterized, bears no homology to the other two families of $NAD(P)^+$-dependent ADH's, or to the other major groups of ADH's. In addition to the iron(II)-dependent ADH, denoted ZADH-2, the bacterium *Zymomonas mobilis* contains also a classical zinc(II)-dependent 'medium chain' ADH (Wills, Kratofil, Londo & Martin, 1981). ZADH-2 appears to be induced by heat and ethanol stress (An, Scopes, Rodriguez, Keshav & Ingram, 1991). The activity of ZADH-2, when metal(II) substituted, is complementary to that of the zinc family (Bertini & Luchinat, 1994), remaining active when cobalt(II) substituted but becoming inactive when zinc(II) substituted (Scopes, 1983). The protein sequence is known from the DNA sequence (Conway, Sewell, Osman & Ingram, 1987; Yoon & Pack, 1990). From a variety of spectroscopic techniques on both native and metal-substituted species, it is established that, in the active form of the enzyme ZADH-2, the iron is divalent high spin and octahedrally coordinated by probably four nitrogen ligands (histidines) and two oxygen ligands (Tse, Scopes & Wedd, 1988, 1989; Bakshi, Tse, Murray, Hanson, Scopes & Wedd, 1989). The iron-coordination environment bears no resemblance to that of other alcohol dehydrogenases, for example, to the $Zn(S)_2(N)(O)$ site of horse liver alcohol dehydrogenase (Eklund *et al.*, 1976); rather it bears resemblance to the iron sites of catechol-2,3-dioxygenase, 3,4-dioxygenase and soybean lipoxygenase, proteins for which there appears to be no sequence homology and for which the iron is redox active (Bakshi *et al.*, 1989).

In the ferrous form, ZADH-2 has a half-life of 5–10 h, aerobically oxidizing to the inactive ferric form (Bakshi *et al.*, 1989), concomitant with oxidation of a histidine residue in the absence of NAD^+ (Cabiscol, Aguilar & Ros, 1994). Thus, crystallization studies have been made on the more stable, but enzymatically still competent, cobalt(II) derivative. The cobalt(II)-substituted derivative bears close resemblance in its electronic spectrum to cobalt(II) glyoxylase I, and in its EPR spectrum to cobalt(II) forms of glyoxylase I, alkaline

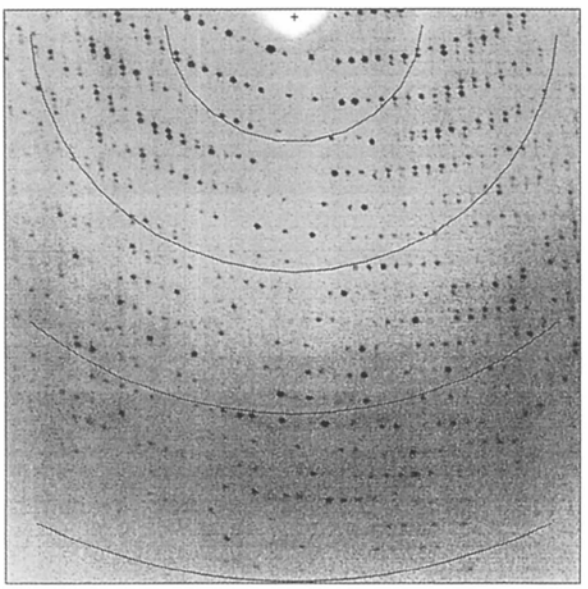

Fig. 1. Part of a representative frame of data for ZADH-2, captured by means of program *IPDISP* (Collaborative Computational Project, Number 4, 1994). Resolution contours are drawn at 10.8, 5.4, 3.6 and 2.7 Å.

phosphatase, phospholipase C and enolase (Bakshi *et al.*, 1989). In particular, glyoxylase I is proposed to have an $(N)_4(O)_2$ suite of ligands. Thus, the coordination spheres of the native and of the cobalt(II)-substituted derivative appear to be very similar.

The protein was purified as described earlier (Bakshi *et al.*, 1989). The initial experiment to determine crystallization conditions was based upon an orthogonal array (Kingston, Baker & Baker, 1995). Crystals of size suitable for X-ray diffraction experiments were prepared at about 281 K by the hanging-drop vapour-diffusion method (McPherson, 1982): a 3 µl aliquot of protein solution [24 mg protein ml^{-1}, 1 m*M* NAD^+, 10 m*M* Hepes buffer at pH 7.0, 1 m*M* $Co^{2+}(CoCl_2)$] was mixed with a 3 µl aliquot of the reservoir solution [0.2 *M* succinic acid/KOH buffer at pH 5.5–5.8 and 27–29%(*w*/*v*) of monomethylpolyethylene glycol 5000], and left to equilibrate over 0.5 ml of well solution. Prismatic needles up to 0.2 × 0.2 × 1.0 mm in size crystallized after one to two months out of initially precipitated material. With macro-seeding good-sized crystals grew in 10 d. On handling, the crystals appeared rather sensitive to small changes in temperature and pH of the reservoir solution. Crystals were tetragonal, $P4_122$ (or $P4_322$), with unit-cell dimensions $a = b = 125.7$ and $c = 248.1$ Å. The search for heavy-atom derivatives is underway. Four molecules, 383 amino acids, molar mass 40.1 kDa per molecule, probably comprise the asymmetric unit, giving a value for V_M of 3.1 Å^3 Da^{-1}, which is within the range normal for protein crystals (Matthews, 1968). Diffraction data to 3.0 Å have been collected on a Rigaku RU200/R-AXIS II system (Molecular Structure Corporation). A total of 103 302 reflections to 3.5 Å resolution have been processed by means of *MOSFLM*, yielding, after scaling and averaging by means of *ROTAVATA* and *AGROVATA* (Collaborative Computational Project, Number 4, 1994) a unique data set of 23 737 reflections (98.6% complete in $P4_122$), for which $R_{\rm merge} = 0.085$ for data to 4.2 Å; for data to 3.5 Å, $R_{\rm merge} = 0.150$, but in the shell from 3.7 to 3.5 Å the signal-to-noise ratio $[I/\sigma(I)]$ is only 1.4. A representative frame is shown in Fig. 1. A self-rotation function (Fig. 2) indicates twofold local symmetry that is perpendicular to the unique axis. Assuming from other work that the protein associates in tetramers (Tse *et al.*, 1989), the local twofold axis relates two halves of the tetramer; or a crystallographic twofold axis relates two halves of a tetramer, and half-tetramers are related by a local twofold axis; other possibilities exist for other degrees of oligomerization. However, the possibility of association into decamers, as reported for the partially homologous methanol dehydrogenase from *Bacillus methanolicus* (Vonck *et al.*, 1991), appears to be incompatible with the rotation function.

The structure of this protein should be of wide significance, not only as an alternative alcohol dehydrogenase, but also because of significant homology with a number of other proteins of diverse origin, oxygen sensitivity and functionality that also remain structurally uncharacterized, such as alcohol dehydrogenase, ADH4, from the yeast *Saccharomyces cerevisiae* (Williamson & Paquin, 1987), 1,3-propanediol dehydrogenase from *Citrobacter freundii*, methanol dehydrogenase from *Bacillus methanolicus*, lactaldehyde reductase/1,2-propa-

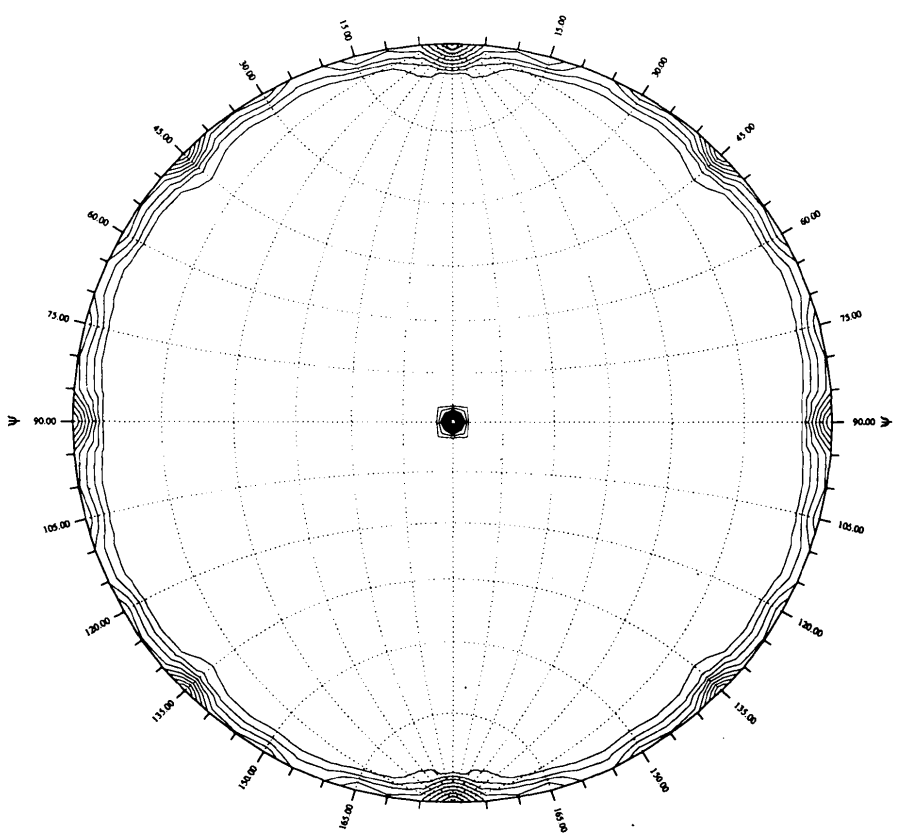

Fig. 2. Self-rotation function presented in polar coordinates. A total of 8858 reflections in the resolution range 30.0 to 5.0 Å was used in the calculation by means of program *GLRF* in the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994) with a 30.0 Å radius of integration (in the polar-angle convention *XYK*). The only significant features, which were independent of resolution range and radius of integration, were found in sections at $\kappa = 90°$ (at $\psi = 90°$, $\varphi = 0°$, corresponding to the crystallographic tetrad), and at $\kappa = 180°$ (illustrated; contours begin at the 3σ level). The peak at the center of the illustrated section corresponds to the crystallographic dyad implicit in the tetrad parallel to *c*. In addition to the peripheral peaks at $\psi = 0°$, 45°, 90°, ..., corresponding to the crystallographic dyads, there is at six times the r.m.s. noise of the section a local dyad at $\psi = 15°$, 30°, ...

nediol oxidoreductase from *Escherichia coli*, NADH- and NADPH-dependent butanol dehydrogenases from *Clostridium acetobutylicum*, and probable *trans*-dienelactoneisomerases from *Pseudomonas* and *Alcaligenes eutrophus*. A 21-residue sequence is found to be highly conserved: two histidine residues are proposed to coordinate while a third is identified as the residue susceptible to metal-catalyzed oxidation (Cabiscol *et al.*, 1994). In addition, substantial homology is enjoyed by these 380-residue proteins with the C-terminal end of the recently termed (Persson *et al.*, 1991) 'long-chain' multifunctional alcohol dehydrogenases, such as the 890-residue alcohol dehydrogenase/acetaldehyde dehydrogenase/pyruvate–formate–lyase deactivase from *E. coli*, the 870-residue alcohol dehydrogenase 2 from *Entamoeba histolytica*, and the 860-residue alcohol dehydrogenase/acetaldehyde dehydrogenase/pyruvate–formate–lyase deactivase from *Clostridium acetobutylicum* (NCBI protein sequence databases, Altschul, Gish, Miller, Myers & Lipman, 1990; Reid & Fewson, 1994).

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