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Crystallization and preliminary X-ray diffraction studies of monomeric isocitrate dehydrogenase from *Corynebacterium glutamicum*

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A monomeric isocitrate dehydrogenase has been crystallized for the first time. This enzyme catalyzes the conversion of isocitrate to oxalosuccinate and subsequently to α-ketoglutarate and CO_2; the coenzyme $NADP^+$ is reduced to NADPH during the reaction. Polyethylene glycol 2000 monomethyl ether was used to crystallize the enzyme in space group $C2$ with unit-cell parameters a = 137.1, b = 54.6, c = 126.4 Å, β = 108.2°. The very small crystal (0.05 × 0.20 × 0.05 mm) diffracted to 3.5 Å d spacing using synchrotron radiation.

1. Introduction

NADP-dependent isocitrate dehydrogenase (E.C. 1.1.1.42; IDH) catalyzes the dehydrogenation of 2*R*,3*S*-isocitrate to oxalosuccinate, followed by the decarboxylation of the intermediate to α-ketoglutarate and CO_2 (Bolduc *et al.*, 1995).

$$\text{Isocitrate} + \text{NADP}^+ \longleftrightarrow \text{Oxalosuccinate} + \text{CO}_2$$

IDH is ubiquitously present in both prokaryotes and eukaryotes, and has the major function of producing NADPH and α-ketoglutarate for biosynthetic reactions (Chen & Gadal, 1990). IDHs are usually dimeric proteins with two identical subunits of molecular mass of 40–50 kDa per subunit (Chen & Gadal, 1990). The crystal structure of *Escherichia coli* IDH shows that the substrate-binding pockets and catalytic sites of the dimeric enzymes are formed from side chains of residues donated asymmetrically by both subunits (Hurley *et al.*, 1989). In contrast, monomeric IDHs with a molecular mass of 80 kDa are found in some bacteria (Chen & Gadal, 1990); the active site of these enzymes must be constructed from the side chains of residues from a single polypeptide chain. Immunological studies suggest that monomeric and dimeric IDHs are not structurally homologous (Leyland & Kelley, 1991; Fukunaga *et al.*, 1992). The sequences of the genes encoding monomeric IDH from *Vibrio sp*. ABE-1 and *Corynebacterium glutamicum* have been reported (Ishii *et al.*, 1993; Eikmanns *et al.*, 1995). No amino-acid sequence homology could be identified between the monomeric and dimeric IDHs. These results suggest that monomeric and dimeric IDHs may have evolved independently.

The monomeric IDH from *C. glutamicum* has been recently purified to homogeneity suitable for crystallographic analysis (Bai *et al.*, 1999). Here, we report the crystallization and preliminary X-ray diffraction of this protein. These and future studies should reveal differences in how these enzymes are regulated and whether or not the monomeric and dimeric IDHs represent convergent evolution.

2. Materials and methods

2.1. Expression and purification of isocitrate dehydrogenase

The plasmid pEK-icdES1 containing the monomeric IDH gene was transformed into *C. glutamicum* ATCC13032. Aerobic growth was conducted at 303 K in a 12 l fermenter (New Brunswick Scientific) for 6–8 h, following the method of Eikmanns *et al.* (1995).

Purification of the enzyme was carried out at 277 K as described previously (Eikmanns *et al.*, 1995) with some modifications (Bai *et al.*, 1999). Two additional steps were included: gel filtration on a Sephadex G-150 column (5.0 × 100 cm) and affinity chromatography on an Affi-gel Red Sepharose CL-6B column (Pharmacia Biotech). The desalted eluent from a HR MonoQ 10/10 column was applied to an Affi-gel Red Sepharose CL-6B Column (1.2 × 35 cm) equilibrated with 25 m*M* 2-morpholino ethanesulfonic acid (MES) buffer pH 6.2, 2.5 m*M* $MnSO_4$ and 2.5 m*M* dithiothreitol (DTT) containing 20 m*M* NaCl. The enzyme was then eluted in the same buffer containing 4 m*M* trisodium D,L-isocitrate. Fractions with high enzyme activity were pooled and concentrated with Centricon-30 concentrators (Amicon) and then dialyzed against 5 m*M* MES buffer pH 6.2, 10% glycerol, 2 m*M* $MnSO_4$ and 2 m*M* DTT. The purified enzyme was concentrated to approximately 10 mg ml^{-1} and stored at 193 K.

2.2. Enzyme assay

Activity of IDH was measured at 294 K in 1 ml volume containing 100 m*M* triethanolamine buffer pH 7.6, 0.8 m*M* $MnSO_4$, 0.5 m*M* $NADP^+$ and 0.8 m*M* trisodium D,L-isocitrate (Bai *et al.*, 1999). The reduction of $NADP^+$ was monitored at 340 nm with a Hewlett–Packard 8453 single-beam diode-array spectrophotometer.

2.3. Crystallization

Protein solution was made up to a concentration of 5 mg ml^{-1} in 2.5 m*M* MES buffer pH 6.8, 1.25 m*M* $MnCl_2$, 1.25 m*M* DTT and 5% glycerol. Reservoir solution consisted of 25% PEG 2000 monomethyl ether, 0.1 *M* Tris buffer pH 7.2 and 0.18 *M* $MgCl_2$. Crystallization was carried out by the hanging-drop method at 293 K with Linbro cell-culture plates; drops were formed from 1 µl protein solution and 1 µl reservoir solution and suspended over 1 ml reservoir solution. Within two weeks, crystals of dimensions 0.05 × 0.20 × 0.05 mm were produced (Fig. 1). One of these crystals was taken to the Photon Factory (Tsukuba, Japan) and three 2° oscillations (separated by 35° in ω) around the *b* axis were recorded on Fuji BASIII imaging plates (400 × 800 mm) with the screenless Weissenberg camera (radius 429.7 mm; Sakabe, 1983) on beamline BL18B at 290 K. These diffraction data were digitized using a Rigaku drum-scanning system and processed using *DENZO* (Otwinowski & Minor, 1997). The space group was determined to be *C*2 with unit-cell parameters a = 137.1, b = 54.6, c = 126.4 Å, β = 108.2°. The very small crystal diffracted to 3.5 Å *d* spacing. If the crystal contains one enzyme molecule in the asymmetric unit, the Matthews coefficient (V_m) is calculated to be 2.8 $Å^3\,Da^{-1}$ (Matthews, 1968) and the solvent content is estimated to be 56%. Crystallization trials are under way in order to develop suitable crystals for a complete X-ray analysis.

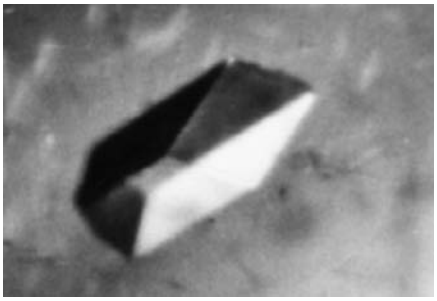

Figure 1
A crystal of monomeric isocitrate dehydrogenase from *C. glutamicum*; the crystal is 0.05 × 0.20 × 0.05 mm in size.

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References

Bai, C., Fernandez, E., Yang, H. & Chen, R. D. (1999). *Protein Expr. Purif.* **15**, 344–348.
Bolduc, J. M., Dyer, D. H., Scott, W. G., Singer, P., Sweet, R. M., Koshland, D. E. Jr & Stoddard, B. L. (1995). *Science*, **268**, 1312–1318.
Chen, R. D. & Gadal, P. (1990). *Plant Physiol. Biochem.* **28**, 411–427.
Eikmanns, B. J., Rittmann, D. & Sahm, H. (1995). *J. Bacteriol.* **177**, 774–782.
Fukunaga, N. S., Sahara, I. T., Ishii, S. A. & Suzuki, M. (1992). *J. Biochem.* **112**, 849–855.
Hurley, J. H., Thorsness, P., Ramalingham, V., Helmers, N., Koshland, D. E. Jr & Stroud, R. M. (1989). *Proc. Natl Acad. Sci. USA*, **86**, 8635–8639.
Ishii, A., Suzuki, M., Sahara, T., Takada, Y., Sasaki, S. & Fukunaga, N. (1993). *J. Bacteriol.* **175**, 6873–6880.
Leyland, M. L. & Kelley, R. (1991). *Eur. J. Biochem.* **202**, 85–93.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Sakabe, N. (1983). *J. Appl. Cryst.* **16**, 542–547.

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addenda and errata

Crystallization and preliminary X-ray diffraction studies of monomeric isocitrate dehydrogenase from *Corynrbacterium glutamicum*. Erratum

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In the paper by Audette *et al.* [*Acta Cryst.* (1999), D**55**, 1584–1585] the postal code of one of the authors was printed incorrectly. The correct version is given above. Also the reaction catalyzed by isocitrate dehydrogenase was given incorrectly in the paper; the correct reaction is given below.

$$\text{Isocitrate} + \text{NADP}^{+} \longleftrightarrow \text{Oxalosuccinate} + \text{NADPH}$$
$$\text{Oxalosuccinate} \longleftrightarrow \alpha\text{-Ketoglutarate} + \text{CO}_2 \quad .$$

References

Audette, G. F., Quail, J. W, Hayakawa, K., Bai, C., Chen, R., Delbaere, L. T. J. (1999). *Acta Cryst.* D**55**, 1584–1585.

Crystallization and preliminary X-ray analysis of the conserved domain IV of *Escherichia coli* 4.5S RNA. Erratum

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In the paper by Jovine *et al.* [*Acta Cryst.* (2000), D**56**, 1033–1037] the name of the second author was given incorrectly. The correct name should be Tobias Hainzl as given above.

References

Jovine, L., Hainzl, T., Oubridge, C. & Nagai, K. (2000). *Acta Cryst.* D**56**, 1033–1037.