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Crystallization and preliminary X-ray structure analysis of isocitrate dehydrogenase from two hyperthermophiles, *Aeropyrum pernix* and *Thermotoga maritima*

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Isocitrate dehydrogenase (IDH) catalyses the dehydrogenation and decarboxylation of isocitrate to α-ketoglutarate and CO_2 with NAD or NADP as cofactor. IDH from *Aeropyrum pernix* is the most thermostable IDH identified. Crystals of *A. pernix* IDH diffracted to 2.6 Å with synchrotron radiation and belong to space group $P4_32_12$. IDH from *Thermotoga maritima* is the only IDH that has been characterized as homotetrameric and might be an evolutionary link between two different IDH subfamilies. *T. maritima* IDH crystals diffracted to 2.8 Å with Cu $K\alpha$ radiation and belong to space group $P2_12_12_1$. The structures will be helpful in the study of the factors responsible for thermostability and the evolutionary relationships of IDHs.

1. Introduction

Isocitrate dehydrogenase (IDH) is an enzyme in the TCA cycle which catalyses the consecutive dehydrogenation and decarboxylation of isocitrate to α-ketoglutarate and CO_2 with NAD^+ or $NADP^+$ as cofactor (Bolduc *et al.*, 1995; Hurley *et al.*, 1991; Stoddard *et al.*, 1993). Together with isopropylmalate dehydrogenase, it forms a family of metal-dependent β-decarboxylating dehydrogenases which are structurally and mechanistically related to each other. They also share a nucleotide-binding site that is different from the Rossmann fold found in many other dehydrogenases (Hurley *et al.*, 1989; Imada *et al.*, 1991; Rossmann *et al.*, 1974). Only a few conserved amino acids are responsible for the discrimination between closely related substrates and cofactors used by this family (Chen *et al.*, 1997; Dean *et al.*, 1996; Doyle *et al.*, 2001; Hurley *et al.*, 1996; Imada *et al.*, 1998; Miyazaki & Oshima, 1994). Some IDHs, however, show dual coenzyme specificity (Steen *et al.*, 1997 and references therein).

IDH evolved early and is widely distributed in archaea, bacteria and eukarya, as is reflected by its diverse primary structures, different oligomeric states and different cofactor specificity. At present, three IDH subfamilies can be phylogenetically distinguished from the cloned sequences (Steen *et al.*, 2001). Subfamily I contains all archaeal and most bacterial IDHs, with a sequence identity within the group of 45–75%. They have been characterized as homodimeric and mostly as NADP-dependent. Subfamily II is formed by eukaryotic homodimeric NADP-IDHs and several bacterial NADP-IDHs, whereas subfamily III is represented by eukaryotic hetero-oligomeric NAD-IDHs and the NADP-IDH from *Thermus thermophilus*. Isopropylmalate dehydrogenase constitutes a fourth subfamily to which tartrate dehydrogenase and homoisocitrate dehydrogenase have also been proposed to belong (Chen & Jeong, 2000; Howell *et al.*, 2000; Tipton & Beecher, 1994). There are also monomeric IDHs without significant overall sequence identity to the other IDH subfamilies (Steen *et al.*, 1998 and references therein).

In order to study the factors responsible for extreme thermostability and to enhance our knowledge about the origin and evolution of IDH, we aim towards the determination of the crystal structures of IDH from two hyperthermophiles: the archaeon *Aeropyrum pernix* and the bacterium *Thermotoga maritima*. IDH from *A. pernix* (*Ap*IDH) is the common homodimeric NADP-dependent form of IDH that has been previously identified in organisms from all three domains of life and has a molecular weight of 47.9 kDa (Steen *et al.*, 2001). However, it is the most thermostable IDH characterized so far, with a melting temperature of 383 K and optimal activity at or above 368 K (Steen *et al.*, 2001). In contrast, IDH from *Escherichia coli* (*Ec*IDH) is completely inactivated after 10 min incubation at 313 K (Miyazaki, 1996). Despite this, the identity between their sequences is 48%, which suggests homologous three-dimensional structures and makes them suitable for comparison of their structural features. To our knowledge, no crystal structure of a hyperthermostable IDH or an IDH from the archaeal domain has so far been determined.

The NADP-IDH from *T. maritima* (*Tm*IDH) is so far the only hyperthermophilic representative within the second subfamily. It has a melting temperature of 371.4 K and

Table 1
Data-collection statistics.

Values in parentheses refer to data in the highest resolution shell.

	*Ap*IDH	*Tm*IDH
Wavelength (Å)	0.8482	1.5418
Unit-cell parameters (Å, °)	$a = b = 107.8$, $c = 171.9$, $\alpha = \beta = \gamma = 90$	$a = 63.6$, $b = 88.1$, $c = 180.9$, $\alpha = \beta = \gamma = 90$
Max. resolution (Å)	2.6	2.8
Mosaicity (°)	0.5	0.5
Completeness (%)	99.9 (100)	89.0 (57.7)
$R_{\rm merge}$	0.072 (0.42)	0.090 (0.41)
No. of observed reflections	2213607	549765
No. of unique reflections	31879	22729
Average $I/\sigma(I)$	39.6 (6.67)	11.8 (1.94)

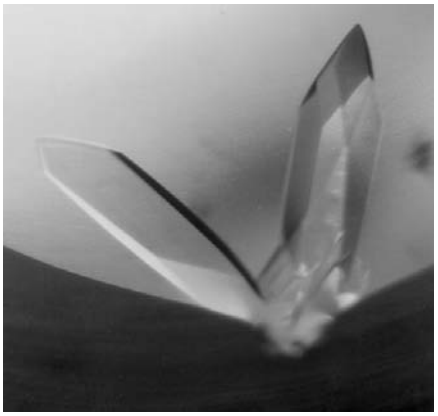

Figure 1
Tetragonal *A. pernix* IDH crystals with dimensions of approximately 0.8 × 0.2 × 0.2 mm were obtained in 12% PEG 6000, 60 m*M* $MgCl_2$, 100 m*M* sodium citrate pH 5.6.

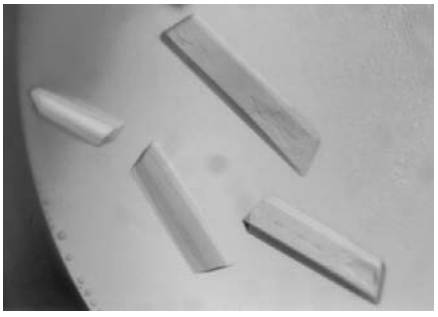

Figure 2
IDH from *T. maritima* was crystallized in 15% PEG 6000, 200 m*M* sodium acetate, 100 m*M* succinate buffer pH 6.0. The dimensions of the crystals are approximately 0.7 × 0.2 × 0.2 mm.

optimal activity at 363 K (Steen *et al.*, 2001). The sequence identity between *Tm*IDH and *Ec*IDH from subfamily I of 27.7%, compared with less than 18% between *Ec*IDH and the other IDHs in this subfamily, suggests an evolutionary relationship between these subfamilies (Steen *et al.*, 2001). However, it was recently found that under some conditions *Tm*IDH can exist as a homotetramer as well as a homodimer (Steen *et al.*, 2001). This feature is unique among characterized IDHs. The molecular weight of 45.4 kDa corresponds to bacterial and eukaryotic homodimeric NADP-IDHs, which are usually composed of subunits of 40–57 kDa. To establish a kinship between these subfamilies, we need to determine the crystal structure of *Tm*IDH and compare its three-dimensional structure with that of an IDH from subfamily I. Here, we report the crystallization and preliminary X-ray analyses of the crystals obtained.

2. Materials and methods

2.1. Crystallization

IDHs from *A. pernix* and *T. maritima* were expressed and purified as described previously (Steen *et al.*, 2001). *Ap*IDH was dialyzed against 20 m*M* Tris pH 7.4 and concentrated to a protein concentration of 15 mg ml^{-1}. A random-screening protocol with two screens developed in-house was used and the initial hits were optimized with a finer grid search. One screen is similar to the MembFac screen (Stowell *et al.*, 1993) and the other screen contains 15% of various PEGs or Jeffamins, 0.2 *M* of various salts and buffers with a pH range 4–8. The best *Ap*IDH crystals were obtained from 12% PEG 6000, 60 m*M* $MgCl_2$, 100 m*M* sodium citrate pH 5.6. *Tm*IDH in 20 m*M* Tris pH 7.4 and 10 m*M* NaCl was concentrated to 18 mg ml^{-1} and crystallized with 16% PEG 6000, 200 m*M* sodium acetate, 100 m*M* succinate buffer pH 6.0. In both cases, the sitting-drop vapour-diffusion method was used with equal volumes of protein solution and the reservoir solution in the drop.

2.2. X-ray data collection and processing

An *Ap*IDH crystal was soaked for 0.5 min in a cryoprotective buffer containing 14% PEG 6000, 60 m*M* $MgCl_2$, 100 m*M* sodium citrate pH 5.6 and 20% glycerol. The *Tm*IDH crystal was cryoprotected in 18% PEG 6000, 200 m*M* sodium acetate, 100 m*M* succinate buffer pH 6.0 and 20% ethylene glycol. The crystals were flash-frozen with boiling nitrogen at 100 K and X-ray diffraction data were collected using Cu $K\alpha$ radiation with a wavelength of 1.5418 Å from a MacScience rotating-anode X-ray generator operated at 45 kV and 110 mA. 360 images with a 0.5° rotation per image were collected from each crystal using a 30 cm MAR image plate and the program *marControl* 4.1.0. The crystal-to-detector distance and exposure time per image were 250 mm and 400 s, respectively, for the *Ap*IDH crystal and 230 mm and 500 s, respectively, for the *Tm*IDH crystal.

Another *Ap*IDH crystal was flash-frozen with boiling nitrogen at 100 K in the same cryobuffer and exposed to synchrotron X-ray radiation with a wavelength of 0.8482 Å at beamline X11 at the EMBL Outstation, DESY, Hamburg. 320 images were collected using a MAR CCD detector and *MARCCD* software with a crystal-to-detector distance of 190 mm and 0.5° rotation per image. A low-resolution data set of 60 images was collected using a crystal-to-detector distance of 330 mm and 2° rotation per image.

The data were indexed and scaled with the *HKL* package (Otwinowski & Minor, 1997).

3. Results

Tetragonal *Ap*IDH crystals (Fig. 1) grew to maximum dimensions of 0.2 × 0.2 × 0.8 mm within two weeks. The unit-cell parameters, completeness and merged *R* factor are given in Table 1. Systematic absences consistent with the conditions $h = 2n + 1$ in $h00$, $k = 2n + 1$ in $0k0$ and $l = 4n$ + 1, 2 or 3 in $00l$ were observed in simulated precession photographs produced after evaluating the diffraction data in space group *P*1. They indicated the space group to be either $P4_32_12$ or $P4_12_12$. A calculated Matthews coefficient ($V_{\rm M}$) of 2.6 Å^3 Da^{-1} for 16 molecules in the unit cell indicated that the crystal contains one dimer per asymmetric unit (Matthews, 1968). The solvent content was estimated to be 52%. A solution from molecular replacement using the structure of *E. coli* IDH (PDB code 3icd) as a model has been obtained in space group $P4_32_12$ and refinement is under way.

*Tm*IDH crystals (Fig. 2) were obtained within 3 d and grew to dimensions of 0.2 × 0.2 × 0.7 mm in one week. The unit-cell parameters, completeness and merged *R* factor are given in Table 1. The space group was determined to be $P2_12_12_1$. The calculated Matthews coefficient of 2.79 Å^3 Da^{-1} for eight molecules in the unit cell indicated that the crystal contains two subunits in the asymmetric unit and corresponds to a solvent content in the unit cell of 55%.

References

Bolduc, J. M., Dyer, D. H., Scott, W. G., Singer, P., Sweet, R. M., Koshland, D. E. & Stoddard, B. L. (1995). *Science*, **268**, 1312–1318.

Chen, R., Greer, A. F. & Dean, A. M. (1997). *Eur. J. Biochem.* **250**, 578–582.
Chen, R. & Jeong, S. S. (2000). *Protein Sci.* **9**, 2344–2353.
Dean, A. M., Shiau, A. K. & Koshland, D. E. (1996). *Protein Sci.* **5**, 341–347.
Doyle, S. A., Beernink, P. T. & Koshland, D. E. Jr (2001). *Biochemistry*, **40**, 4234–4241.
Howell, D. M., Graupner, M., Xu, H. & White, R. H. (2000). *J. Bacteriol.* **182**, 5013–5016.
Hurley, J. H., Chen, R. & Dean, A. M. (1996). *Biochemistry*, **35**, 5670–5678.
Hurley, J. H., Dean, A. M., Koshland, D. E. & Stroud, R. M. (1991). *Biochemistry*, **30**, 8671–8678.
Hurley, J. H., Thorsness, P. E., Ramalingam, V., Helmers, N. H., Koshland, D. E. & Stroud, R. M. (1989). *Proc. Natl Acad. Sci. USA*, **86**, 8635–8639.
Imada, K., Inagaki, K., Matsunami, H., Kawaguchi, H., Tanaka, H., Tanaka, N. & Namba, K. (1998). *Structure*, **6**, 971–982.
Imada, K., Sato, M., Tanaka, N., Katsube, Y., Matsuura, Y. & Oshima, T. (1991). *J. Mol. Biol.* **222**, 725–738.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Miyazaki, K. (1996). *Appl. Environ. Microbiol.* **62**, 4627–4631.
Miyazaki, K. & Oshima, T. (1994). *Protein Eng.* **7**, 401–403.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Rossmann, M. G., Moras, D. & Olsen, K. W. (1974). *Nature (London)*, **250**, 194–199.
Steen, I. H., Lien, T. & Birkeland, N. K. (1997). *Arch. Microbiol.* **168**, 412–420.
Steen, I. H., Madern, D., Karlstrom, M., Lien, T., Ladenstein, R. & Birkeland, N. K. (2001). *J. Biol. Chem.* **276**, 43924–43931.
Steen, I. H., Madsen, M. S., Birkeland, N. K. & Lien, T. (1998). *FEMS Microbiol. Lett.* **160**, 75–79.
Stoddard, B. L., Dean, A. & Koshland, D. E. (1993). *Biochemistry*, **32**, 9310–9316.
Stowell, M. H. B., Chan, S. I. & Rees, D. C. (1993). UCLA Macromolecular Crystallization Workshop, June 21, 1993.
Tipton, P. A. & Beecher, B. S. (1994). *Arch. Biochem. Biophys.* **313**, 15–21.