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Crystallization and preliminary X-ray crystallographic analysis of type II dehydroquinase from *Helicobacter pylori*

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The enzyme 3-dehydroquinase catalyzes the interconversion of 3-dehydroquinate and 3-dehydroshikimate. The enzymes are classified into two groups, type I and type II, which have different biochemical and biophysical properties and act with different mechanisms. The type II dehydroquinase of *Helicobacter pylori*, a dodecameric enzyme, was overexpressed in *Escherichia coli*. The recombinant protein has been crystallized at 296 K using polyethylene glycol (PEG) 4000 as a precipitant. Native X-ray diffraction data have been collected to 2.5 Å resolution using synchrotron radiation. The crystals are cubic and belong to the space group $P4_232$, with unit-cell parameters $a = b = c = 98.91$ Å. The asymmetric unit contains one subunit of recombinant type II dehydroquinase, with a corresponding V_M of 2.18 Å^3 Da^{-1} and a solvent content of 43.6%.

1. Introduction

The enzyme dehydroquinase (3-dehydroquinate dehydratase; E.C. 4.2.1.10) catalyzes the interconversion of 3-dehydroquinate and 3-dehydroshikimate. This reaction is a common step in two important pathways: the shikimate pathway for aromatic biosynthesis and the catabolic pathway for quinate metabolism (Haslam, 1974; Bentley, 1990; Dewick, 1993). The seven-step shikimate pathway in plants and microorganisms leads to the synthesis of chorismate, a precursor for numerous aromatic compounds such as aromatic amino acids, folic acid, ubiquinone and vitamin E (Hawkins *et al.*, 1993; Bottomley, Hawkins *et al.*, 1996). Although the shikimate pathway is ubiquitous in bacteria, fungi and plants, it is absent from mammals (Kishore & Shah, 1988; O'Callaghan *et al.*, 1988). Therefore, enzymes of shikimate pathway including dehydroquinase are potential targets for antimicrobial agents or herbicides.

Dehydroquinases fall into two groups that have different biochemical and biophysical properties and no sequence similarity (Kleanthous *et al.*, 1992). Type I enzymes are generally found in biosynthetic pathways, use a Schiff-base intermediate formed at the conserved lysine residue and catalyze the elimination of water with *syn* stereochemistry (Shneier *et al.*, 1991; Chaudhuri *et al.*, 1991). They have subunit molecular masses of ~25 kDa and form dimers in the case of monofuntional enzymes such as *E. coli* type I dehydroquinase (Chaudhuri *et al.*, 1991). Type II enzymes have been found in both biosynthetic and catabolic pathways. They have smaller subunit molecular masses than type I (16–18 kDa), oligomerize into dodecamers of ~200 kDa and are heat-stable. They catalyze the elimination reaction with *trans* stereochemistry without involvement of covalent intermediates and do not require metals or cofactors for activity (Harris *et al.*, 1993; Bottomley, Hawkins *et al.*, 1996). The structure of the type II dehydroquinase from *Mycobacterium tuberculosis* has been reported (Gourley *et al.*, 1999), but more structural information is needed for inhibitor design and structural comparisons.

H. pylori, a Gram-negative pathogen, has been recognized to be connected with the onset of gastritis, peptic ulcer and gastric cancer. The gene encoding the type II dehydroquinase of *H. pylori* (aroQ) was sequenced and its product showed a strong identity to other members of the type II family of dehydroquinases (Bottomley, Clayton *et al.*, 1996). Here, we report the crystallization conditions of type II dehydroquinase from *H. pylori* as well as preliminary X-ray crystallographic data.

2. Experimental

2.1. Protein expression and purification

The aroQ gene was amplified by the polymerase chain reaction using the *H. pylori* genomic DNA as template. The amplified DNA was inserted into the *Nde*I/*Bam*HI-digested expression vector pET-21a (Novagen). The protein was overexpressed in soluble form in *E. coli* strain B834(DE3) (Novagen) upon induction by 0.5 m*M* isopropyl-β-D-thiogalactopyranoside (IPTG) at 303 K. Cells were

grown in Luria–Bertani medium for 4 h after IPTG induction and were harvested by centrifugation at 4200*g* (6000 rev min^{-1}; Sorvall GSA rotor) for 10 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer (50 m*M* Tris–HCl pH 7.5, 100 m*M* NaCl, 1 m*M* β-mercaptoethanol, 1 m*M* EDTA) and was then disrupted by ultrasonication. The crude cell extract was centrifuged at 36 000*g* (18 000 rev min^{-1}; Hanil Supra 21K rotor) for 40 min at 277 K. The supernatant fraction was heated and maintained between 348 and 353 K for 10 min and then placed in an ice bath for 10 min. The cell extract was centrifuged at 36 000*g* (18 000 rev min^{-1}; Hanil Supra 21K rotor) for 50 min at 277 K. The supernatant was subjected to ion-exchange chromatography on a Q-Sepharose column (Amersham-Pharmacia) which was previously equilibrated with buffer *A* (50 m*M* Tris–HCl pH 7.5, 1 m*M* β-mercaptoethanol, 1 m*M* EDTA) and the protein was eluted with a linear gradient of 0–0.5 *M* NaCl. Final purification of the enzyme was performed by gel filtration on a HiLoad XK16 Superdex 200 prep-grade column (Amersham-Pharmacia) which was previously equilibrated with buffer *A* containing 200 m*M* sodium chloride. This procedure yielded approximately 100 mg of homogeneous type II dehydroquinase from a 1.5 l culture. The protein solution was concentrated using a YM10 membrane (Amicon) to about 20 mg ml^{-1}. The protein concentration was estimated by measuring the absorbance at 280 nm employing the calculated molar extinction coefficient of 5120 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/).

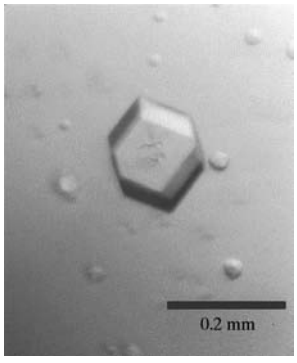

Figure 1
A crystal of type II dehydroquinase from *H. pylori*. Its approximate dimensions are 0.2 × 0.15 × 0.15 mm.

2.2. Crystallization

Crystallization was achieved using the hanging-drop vapour-diffusion method at 296 K using 24-well tissue-culture plates (Hampton Research). A hanging drop was prepared by mixing equal volumes (2 µl each) of the protein solution and the reservoir solution. The protein concentration was 20 mg ml^{-1} before mixing with the reservoir solution. Each hanging drop was placed over a 1.0 ml reservoir solution. Initial crystallization conditions were established by sparse-matrix sampling (Jancarik & Kim, 1991).

2.3. X-ray diffraction experiment

A crystal of type II dehydroquinase was transferred to a solution consisting of 40%(*w*/*v*) PEG 4000, 200 m*M* NaCl, 100 m*M* sodium citrate pH 5.8, 1 m*M* EDTA and 1 m*M* β-mercaptoethanol within a minute before being flash-frozen, increasing the concentration of PEG 4000 in three steps. A set of native X-ray diffraction data was collected at 100 K at the BL-6A experimental station of the Photon Factory, Tsukuba, Japan (Sakabe, 1991) with an ADSC Quantum 4R CCD detector. The wavelength of synchrotron X-rays was 1.000 Å. The crystal was rotated through a total of 90°, with a 1.0° oscillation range per frame. The raw data were processed and scaled using the program *MOSFLM* (Leslie, 1992).

3. Results

Recombinant type II dehydroquinase from *H. pylori* has been overexpressed in *E. coli* as a soluble form to the level of 70 mg per litre of culture. The crystals were obtained using a reservoir solution containing 100 m*M* sodium citrate buffer pH 5.8 and 28%(*w*/*v*) PEG 4000. They grew to maximum dimensions of 0.2 × 0.15 × 0.15 mm within one week (Fig. 1).

The native crystals diffracted to 3.3 Å resolution on a MacScience 2030b imaging-plate detector with double-mirror-focused Cu $K\alpha$ X-rays from a rotating-anode source. The native diffraction data were collected to 2.5 Å resolution at 100 K using synchrotron X-rays. A total of 239 220 measured reflections were merged into 6146 unique reflections with an $R_{\rm merge}$ (on intensities) of 8.6%. The merged data set is 100% complete to 2.5 Å resolution. The crystals belong to the cubic space group $P4_232$, with unit-cell parameters $a = b = c = 98.91$ (12) Å, where estimated standard deviation (e.s.d.) is given in parentheses. The presence of a single subunit of dehydroquinase in the asymmetric unit gives a crystal volume per protein mass ($V_{\rm M}$) of 2.18 Å^3 Da^{-1}, with a corresponding solvent content of 43.6% (Matthews, 1968).

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References

Bentley, R. (1990). *Biochem. Mol. Biol. Crit. Rev.* **25**, 307–384.
Bottomley, J. R., Clayton, C. L., Chalk, P. A. & Kleanthous, C. (1996). *Biochem. J.* **319**, 559–565.
Bottomley, J. R., Hawkins, A. R. & Kleanthous, C. (1996). *Biochem. J.* **319**, 269–278.
Chaudhuri, S., Duncan, K., Granham, L. D. & Coggins, J. R. (1991). *Biochem. J.* **275**, 1–6.
Dewick, P. M. (1993). *Nat. Prod. Rep.* **10**, 233–263.
Gourley, D. G., Shrive, A. K., Polikarpov, I., Krell, T., Coggins, J. R., Hawkins, A. R., Isaacs, N. W. & Sawyer, L. (1999). *Nature Struct. Biol.* **6**, 521–525.
Harris, J., Kleanthous, C., Coggins, J. R., Hawkins, A. R. & Abell, C. (1993). *J. Chem. Soc. Chem. Commun.*, pp. 1080–1081.
Haslam, E. (1974). *The Shikimate Pathway*. New York: John Wiley & Sons.
Hawkins, A. R., Lamb, H. K., Moore, J. D., Charles, I. G. & Robert, C. F. (1993). *J. Gen. Microbiol.* **139**, 2891–2899.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Kishore, G. M. & Shah, D. M. (1988). *Annu. Rev. Biochem.* **57**, 627–663.
Kleanthous, C., Deka, R., Davies, K., Kelly, S. M., Copper, A., Harding, S. E., Price, C. N., Hawkins, A. R. & Coggins, J. R. (1992). *Biochem. J.* **282**, 687–695.
Leslie, A. G. W. (1992). *Jnt CCP4/ESF–EACMB. Newslett. Protein Crystallogr.* **26**.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–493.
O'Callaghan, D., Maskell, D., Liew, F. Y., Easmon, C. S. & Dougan, G. (1988). *Infect. Immun.* **56**, 419–423.
Sakabe, N. (1991). *Nucl. Instrum. Methods A*, **303**, 448–463.
Shneier, A., Kleanthous, C., Deka, R., Coggins, J. R. & Abell, C. (1991). *J. Am. Chem. Soc.* **113**, 9416–9418.