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Crystallization and preliminary crystallographic analysis of human serine dehydratase

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L-Serine dehydratase (SDH) catalyzes the pyridoxal phosphate (PLP) dependent deamination of L-serine to yield pyruvate. Recombinant human serine dehydratase was crystallized by the hanging-drop vapour-diffusion method. Crystals were grown at 291 K using $(NH_4)_2SO_4$ as precipitant. Diffraction data were obtained to a resolution of 2.5 Å from a single frozen crystal using Cu $K\alpha$ radiation. The crystal belongs to space group $I422$, with unit-cell parameters $a = 157.4$, $b = 157.4$, $c = 59.2$ Å, $\alpha = \beta = \gamma = 90^\circ$. The asymmetric unit contains one molecule and has a solvent content of about 46%.

1. Introduction

L-Serine dehydratase (SDH; deaminase; EC 4.2.1.13) catalyzes the pyridoxal phosphate (PLP) dependent deamination of L-serine to yield pyruvate. The rat liver enzyme also catalyzes the conversion of L-threonine to α-ketobutyrate and ammonia by the same mechanism and is therefore thought to be identical to L-threonine dehydratase (EC 4.2.1.16; Nakagawa, 1971). The primary structures of biosynthetic (Lawther *et al.*, 1987) and biodegradative (Datta *et al.*, 1987) threonine dehydratases from *Escherichia coli*, D-serine dehydratase from *E. coli* (Schiltz & Schmitt, 1981; Marceau, McFall *et al.*, 1988) and biosynthetic threonine dehydratase from yeast (Kielland-Brandt *et al.*, 1984) are now known and it has been shown that significant sequence homology exists between these enzymes (Datta *et al.*, 1987). In human liver SDH, PLP binds to Lys41 to form a Schiff base and its encompassing amino-acid sequence, Ser-Xaa-Lys-Ile-Arg-Gly, is well conserved among SDHs from human (Ogawa, Gomi *et al.*, 1989), rat (Ogawa, Konishi *et al.*, 1989), tomato (Samach *et al.*, 1991), *Escherichia coli* (Datta *et al.*, 1987; Eisenstein *et al.*, 1995) and yeast (John, 1995). The active sites are highly homologous with those of the microbial threonine dehydratases. Moreover, these enzymes have a glycine-rich sequence in a region 100–130 amino-acid residues downstream of the PLP-binding lysyl residue (Datta *et al.*, 1987). The importance of this motif in the interaction with the coenzyme was demonstrated by the finding that substitution of the glycine residues by aspartic acid residues impairs PLP binding to *E. coli* D-serine deaminase (Marceau, Lewis *et al.*, 1988). These two conserved sequences suggest that SDHs and TDHs have evolved from a common ancestral protein (Datta *et al.*, 1987).

In mammals, SDH is found predominantly in the liver. Extensive studies have been carried out on SDH from rat and the enzyme has been found to play an important role in gluconeogenesis; its activity is induced by the consumption of high-protein diets, starvation and other treatments (Snell, 1984; Ebara *et al.*, 2001). However, little is known about the physicochemical and enzymatic properties of human SDH, as human liver exhibited low SDH activity (Yoshida *et al.*, 1969). It has been suggested that vertebrates catabolize serine by the consecutive actions of serine hydroxymethyltransferase and glycine-cleavage enzymes rather than by SDH under physiological conditions (Kikuchi, 1973; Xue *et al.*, 1999). It has also been reported that a case of non-ketotic hyperglycinaemia was apparently a consequence of a hereditary deficiency of threonine dehydratase (Krieger & Booth, 1984). In addition, serine dehydratase activities were absent in human colon carcinoma and rat sarcoma (Snell *et al.*, 1988). As a result, SDH is thought to be related to non-ketotic hyperglycinaemia and tumours. This prompted us to examine the properties of human SDH.

There is presently no known structure of SDH. In this study, the crystallization and preliminary crystallographic analysis of human SDH are reported. The structure of this protein will be helpful in the illustration of the properties and function of SDHs.

2. Materials and methods

2.1. Protein expression and purification

The coding sequence for human serine dehydratase (35 kDa) was amplified from RT-PCR products from human liver using the polymerase chain reaction (PCR) method. Two PCR primers, 5′-CCG GAA TTC ATG ATG TCT GGA GAA CCC C-3′ and 5′-TCC GCT

CGA GCT TGG GCA ACC TAT TTG T-3′, were designed. The PCR product was restricted with *Eco*RI and *Xho*I, purified and ligated into *Eco*RI- and *Xho*I-restricted sites of the pET28a(+) vector (Novagen Inc.) with a T7 tag. A further transformation into *E. coli* DH5α competent cells was performed and positive clones with an insert of the right size were identified by double digestion with *Eco*RI and *Xho*I. The recombinant plasmid was transformed into *E. coli* strain BL21 (DE3) and the transformants were selected on LB agar plates containing 25 µg ml^{-1} kanamycin. The cells were then cultured at 310 K in LB medium containing 50 µg ml^{-1} kanamycin. When the culture density reached $A_{600} \simeq 0.8$, the culture was induced with 0.5 m*M* IPTG and was grown for an additional 4 h before the cells were harvested.

The bacterial cell pellet was resuspended in lysis buffer (20 m*M* Tris–HCl pH 7.6, 500 m*M* NaCl, 10 m*M* imidazole, 10% glycerol) and homogenized by sonication. The lysate was centrifugated at 20 000*g* for 30 min to remove the cell debris. The supernatant was applied to an Ni^{2+}-chelating column (1 ml Ni^{2+}–NTA agarose) and the contaminant protein was washed off with lysis buffer. The target protein was eluted with eluting buffer (20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 200 m*M* imidazole, 10% glycerol). The protein was concentrated with an Ultrafree 10 000 NMWL filter unit (Millipore) and was further purified on a Superdex-75 column (Pharmacia) in buffer *A* (20 m*M* Tris–HCl pH 7.6, 150 m*M* NaCl). The purified protein was analyzed by SDS–PAGE.

2.2. Crystallization

The purified protein was concentrated to 20–30 mg ml^{-1} in 20 m*M* Tris–HCl pH 7.6, 150 m*M* NaCl. Crystallization was performed by the hanging-drop vapour-diffusion method at 291 K in 16-well plates. 2 µl protein solution was mixed with 2 µl reservoir solution containing 0.8 *M* ammonium sulfate in 0.1 *M* Tris–HCl buffer pH 7.0–8.0 and the mixture was equilibrated against 200 µl reservoir solution at 291 K. The lemon-shaped crystal appeared after 2–3 d.

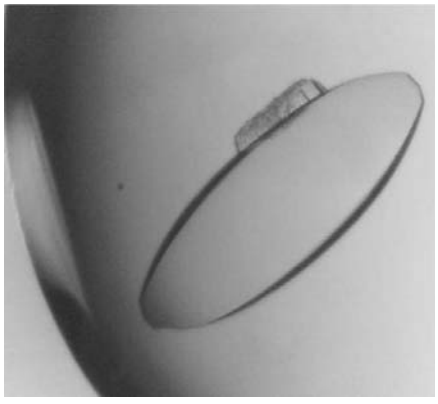

Figure 1
A crystal of SDH. The crystal is about 0.3 × 0.5 × 1.5 mm in size.

2.3. X-ray crystallographic studies

Preliminary diffraction data sets were collected at room temperature in-house using a Rigaku RU-2000 rotating-anode Cu $K\alpha$ X-ray generator operating at 48 kV and 98 mA (λ = 1.5418 Å) with a MAR 345 image-plate detector. The beam was focused using Osmic mirrors. For a more detailed analysis, flash-cooled crystals were used. Crystals were immersed in cryoprotectant for 5–10 s, picked up in a loop and then flash-cooled in a stream of nitrogen gas cooled to 100 K. The cryoprotectant was prepared by adding 30% glycerol to the mother-liquor reservoir. All intensity data were indexed, integrated and scaled using the *HKL* programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results

Crystallization trials were conducted at 291 K in 16-well plates using the hanging-drop vapour-diffusion method. Hampton Research kits (Riverside, CA, USA) were used to supply sets of screening reagents for initial screening. Although wide ranges of conditions were screened, the crystallization of human SDH was initially unsuccessful. Eventually, we found the protein to crystallize in a solution containing 0.8 *M* ammonium sulfate and 0.1 *M* Tris–HCl pH 7.0 or pH 8.0, but the crystals were twinned and were unsuitable for X-ray diffraction. Further optimization was performed and better crystals were obtained using 0.8 *M* ammonium sulfate as precipitant in Tris–HCl pH ~7.2. Drops containing 2 µl protein solution and 2 µl reservoir solution were equilibrated against 200 µl reservoir solution (Fig. 1). The crystals produced using the optimum reservoir solution condition were compact and stable, as demonstrated by their X-ray diffraction to 2.4 Å resolution after storage for about 20 d at 291 K (Fig. 2). A set of data were subsequently collected from this crystal, which belonged to space group *I*422 with unit-cell parameters a = 157.4, b = 157.4, c = 59.2 Å, $\alpha = \beta = \gamma = 90^\circ$. Scaling and merging of the crystallographic data resulted in an overall $R_{\rm merge}$ of 9.9% and an $R_{\rm merge}$ in the highest resolution shell (2.60–2.50 Å) of 36.6%. The value of the Matthews coefficient (Matthews, 1968) is 2.29 Å^3 Da^{-1} for one molecule in the asymmetric unit and the estimated solvent content is 45.9%. Complete data-collection statistics are given in Table 1.

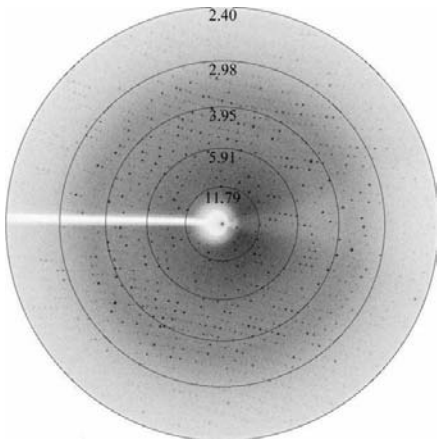

Figure 2
A typical diffraction pattern of an SDH crystal. The detector edge corresponds to 2.4 Å resolution. The exposure time was 300 s, the crystal-to-detector distance was 225 mm and the oscillation range per frame was 1°.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

Space group	*I*422
Unit-cell parameters (Å, °)	a = 157.4, b = 157.4, c = 59.2, $\alpha = \beta = \gamma$ = 90.0
Matthews coefficient (Å^3 Da^{-1})	2.29
Resolution (Å)	50–2.5
Total observations	193906
Unique reflections	94265 (12739)
Redundancy	7.4 (7.5)
Average $I/\sigma(I)$	20.03 (5.62)
$R_{\rm merge}$† (%)	9.9 (36.6)
Data completeness (%)	96.3 (98.5)

† $R_{\rm merge} = 100\sum|I_i - \langle I\rangle|/\sum I_i$, where I_i is the intensity of the observation.

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References

Datta, P., Goss, T. J., Omnaas, J. R. & Patil, R. V. (1987). *Proc. Natl Acad. Sci. USA*, **84**, 393–397.

Ebara, S., Toyoshima, S., Matsumura, T., Adachi, S., Takenaka, S., Yamaji, R., Watanabe, F., Miyatake, K., Inui, H. & Nakano, Y. (2001). *Biochim. Biophys. Acta*, **1568**, 111–117.

Eisenstein, E., Yu, H.-D., Fisher, K. E., Itacuzio, D. A., Ducotre, K. R. & Schwarz, F. P. (1995). *Biochemistry*, **34**, 9403–9413.

John, R. A. (1995). *Biochim. Biophys. Acta*, **1248**, 81–96.

Kielland-Brandt, M. C., Holmberg, S., Petersen, J. G. L. & Nilsson-Tillgren, T. (1984). *Carlsberg Res. Commun.* **49**, 567–575.

Kikuchi, G. (1973). *Mol. Cell. Biochem.* **1**, 169–187.

Krieger, I. & Booth, F. (1984). *J. Inherit.Metab. Dis.* **7**, 53–56.

Lawther, R. P., Wek, R. C., Lopes, J. M., Pereira, R., Taillon, B. E. & Hatfield, G. W. (1987). *Nucleic Acids Res.* **15**, 2137–2155.

Marceau, M., Lewis, S. D. & Shafer, J. A. (1988). *J. Biol. Chem.* **263**, 16934–16941.

Marceau, M., McFall, E., Lewis, S. D. & Shafer, J. A. (1988). *J. Biol. Chem.* **263**, 16926–16933.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Nakagawa, H. (1971). *Seikagaku*, **43**, 1–22.

Ogawa, H., Gomi, T., Konishi, K., Date, T., Nakashima, H., Nose, K., Matsuda, Y., Peraino, C., Pitot, H. C. & Fujioka, M. (1989). *J. Biol. Chem.* **264**, 15818–15823.

Ogawa, H., Konishi, K. & Fujioka, M. (1989). *Biochim. Biophys. Acta*, **996**, 139–141.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Samach, A., Hareven, D., Gutfinger, S., Ken-Dror, S. & Lifschitz, E. (1991). *Proc. Natl Acad. Sci. USA*, **88**, 2678–2682.

Schiltz, E. & Schmitt, W. (1981). *FEBS Lett.* **134**, 56–62.

Snell, K. (1984). *Adv. Enzyme Regul.* **22**, 325–400.

Snell, K., Natsumeda, Y., Eble, J. N., Glover, J. L. & Weber, G. (1988). *Br. J. Cancer*, **57**, 87–90.

Xue, H. H., Sakaguchi, T., Fujie, M., Ogawa, H. & Ichiyama, A. (1999). *J. Biol. Chem.* **274**, 16028–16033.

Yoshida, T., Kikuchi, G., Tada, K., Narisawa, K. & Arakawa, T. (1969). *Biochem. Biophys. Res. Commun.* **35**, 577–583.