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Crystallization and preliminary crystallographic analysis of D-serine dehydratase from chicken kidney

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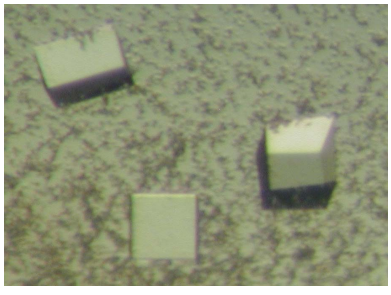

D-Serine dehydratase purified from chicken kidney was crystallized by the hanging-drop vapour-diffusion method using PEG 4000 and 2-propanol as precipitants. The crystal belonged to space group *P*422, with unit-cell parameters a = 105.0, c = 81.89 Å, and diffracted to 2.09 Å resolution. An attempt to solve the structure using the MAD method is in progress.

1. Introduction

D-Serine is known to activate *N*-methyl-D-aspartate (NMDA) receptors as a co-agonist of L-glutamate (Labrie & Roder, 2010). Since the NMDA receptor is likely to be involved in higher brain functions such as learning and memory, the metabolism of D-serine has been studied in order to understand the molecular mechanism of higher brain functions. In mammals, D-serine is mainly degraded by D-amino-acid oxidase (DAO; Verrall *et al.*, 2010; Pollegioni & Sacchi, 2010) and is partly dehydrated by a bifunctional serine racemase/dehydratase (Pollegioni & Sacchi, 2010). Although D-serine is widely believed to be degraded by DAO in vertebrate brains, our recent study showed that there was no detectable DAO activity in chicken brain. Rather, D-serine in chicken brain was degraded by D-serine dehydratase (DSD; Tanaka *et al.*, 2007), which catalyzes the β-elimination of water from D-serine to form pyruvate and ammonia. We also found that chicken DSD (chDSD) was highly expressed in the kidney. In order to understand the molecular mechanism of D-serine degradation in chicken brain, biochemical and biological analysis of chDSD was initiated.

Examination of the chDSD gene sequence revealed that chDSD belongs to an enzyme family distinct from that of well known bacterial DSDs (Tanaka *et al.*, 2008). chDSD shows weak amino-acid sequence similarity to alanine racemases from bacteria and exhibits 29% amino-acid sequence identity to DSD derived from *Saccharomyces cerevisiae* (scDSD; Tanaka *et al.*, 2008; Ito *et al.*, 2008). Biochemical analyses showed that chDSD is a pyridoxal 5′-phosphate (PLP)-dependent enzyme like bacterial alanine racemases and scDSD. chDSD is expected to be a member of the fold-type III enzymes such as alanine racemase (Grishin *et al.*, 1995).

It is known that the PLP–D-serine Schiff base is primed for five distinct reactions: decarboxylation, racemization, transamination, $C^{\alpha}-C^{\beta}$ cleavage (retro-aldol cleavage) and β-elimination (dehydration) (Toney, 2005). After forming the Schiff base, a carbanion is formed by heterolytic cleavage of any one of the bonds to C^{α} (except for the C—N bond). Racemization, transamination and β-elimination of serine follow cleavage of the C^{α}—H bond. Dunathan's hypothesis proposed that the cleavage site is determined by the stereoelectronic configuration of the Schiff base in the enzyme active site (Dunathan, 1966). Biochemical analysis showed that chDSD only catalyzes the dehydration of D-Ser; although the primary structural similarity between chDSD and bacterial alanine racemases suggests that their active-site structures are also similar, chDSD does not show a detectable level of racemase activity (Tanaka *et al.*, 2008). The molecular mechanism of the high reaction specificity of chDSD could therefore provide novel insights into the molecular evolution of the

PLP-dependent enzymes, particularly the evolution of their active-site structures. However, this mechanism remains elusive owing to the lack of three-dimensional structural information for chDSD. To address this problem, we initiated a crystal structure analysis. Here, we report the crystallization and preliminary X-ray crystallographic data of chDSD.

2. Methods and results

2.1. Protein expression, purification and crystallization

ChDSD was purified from chicken kidneys as described previously (Tanaka *et al.*, 2008). Briefly, chicken kidneys were homogenized in 10 m*M* potassium phosphate pH 7.2 containing 50 µ*M* PLP. The homogenates were centrifuged at 17 700*g* for 20 min. The resultant supernatant was purified by four-step chromatography with DEAE-Sepharose (Amersham Pharmacia, Chicago, Illinois, USA), hydroxyapatite (Nacalai Tesque, Kyoto, Japan), Mono Q (Pharmacia) and TSKgel SuperSW3000 (Tosoh, Tokyo, Japan). The purified chDSD was concentrated to 14.7 mg ml^{-1} in 20 m*M* potassium phosphate pH 7.5, 1 m*M* dithiothreitol, 10 µ*M* PLP and 10 m*M* (*R*)-(−)-2,3-diaminopropionic acid hydrochloride (D-2,3-DAP) as an inhibitor.

Initial screening for crystallization was performed by the hanging-drop vapour-diffusion method using Crystal Screen (Hampton Research, Laguna Niguel, California, USA; Jancarik & Kim, 1991) at 293 K. A hanging drop was prepared by mixing 1.0 µl each of the protein and reservoir solutions and was equilibrated against 500 µl reservoir solution. Clusters of needle-shaped crystals appeared in 24 h under several conditions: Crystal Screen condition Nos. 2, 17, 22, 40 and 41. All of the reservoir conditions except for No. 2 contained polyethylene glycol (PEG) 4000 as a precipitant. After 3 d, thin plate-shaped crystals of approximately 10 µm in length were obtained using Crystal Screen condition No. 41 [10%(*v*/*v*) 2-propanol, 0.1 *M* HEPES–NaOH pH 7.5 and 20%(*w*/*v*) PEG 4000]. In order to obtain larger plate-shaped crystals, the pH value (4.6–8.5), the PEG 4000 concentration [10–30%(*w*/*v*)] and the protein concentration (4–14 mg ml^{-1}) were systematically optimized. After several trials, the optimal reservoir conditions were determined to be 12–15%(*w*/*v*) PEG 4000, 50 m*M* MES–NaOH pH 6.5 and 10%(*v*/*v*) 2-propanol using 4–8 mg ml^{-1} protein solution. Larger plate-shaped crystals (approximately 0.2 × 0.15 × 0.05 mm) were obtained in 2–3 weeks (Fig. 1). Although chDSD is a PLP-dependent enzyme and thus is typically yellow in colour, the crystals obtained were nearly colourless, suggesting that PLP had dissociated from chDSD during crystallization. In order to incorporate PLP into chDSD in the crystal, the obtained crystal was soaked in PLP solution [5 m*M* PLP, 30%(*w*/*v*) PEG 4000, 10%(*v*/*v*) 2-propanol, 100 m*M* MES–NaOH pH 6.5], resulting in yellow crystals.

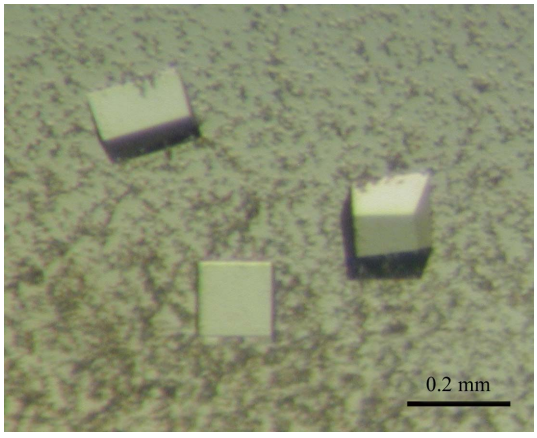

Figure 1
Crystals of chDSD purified from chicken kidney.

Table 1
Data-collection statistics.

Values in parentheses are for the outermost resolution shell.

Beamline	BL-5A (PF)
Oscillation angle (°)	1
Crystal-to-detector distance (mm)	284.3
Exposure time (s)	8
Wavelength (Å)	1.0000
Temperature (K)	95
Space group	*P*422
Unit-cell parameters (Å)	*a* = 105.0, *c* = 81.89,
Resolution (Å)	17.0–2.09 (2.21–2.09)
Observations	387030 (59770)
Unique reflections	27631 (4172)
Completeness (%)	99.6 (100.0)
Multiplicity	14.0 (14.3)
Average $I/\sigma(I)$	33.1 (5.1)
$R_{\rm merge}$	0.057 (0.597)
Mosaicity (°)	0.315

2.2. X-ray diffraction analysis

Diffraction data were collected at 95 K using an ADSC CCD detector on beamline BL-5A of Photon Factory (PF; Tsukuba, Japan; Fig. 2). Prior to data collection, the crystals were soaked in a cryoprotectant solution consisting of 30%(*v*/*v*) glycerol, 20%(*w*/*v*) PEG 4000, 100 m*M* MES–NaOH pH 6.5 and 10%(*v*/*v*) 2-propanol containing 5 m*M* PLP for 20 s and flash-cooled in an N_2 stream. The exposure time per frame was 8 s.

The diffraction data were processed and scaled at 2.09 Å resolution using the programs *XDS* and *XSCALE* (Kabsch, 2010). The crystals belonged to space group *P*422 (Table 1). Assuming one subunit of chDSD (40.4 kDa) per asymmetric unit of the crystal, the Matthews

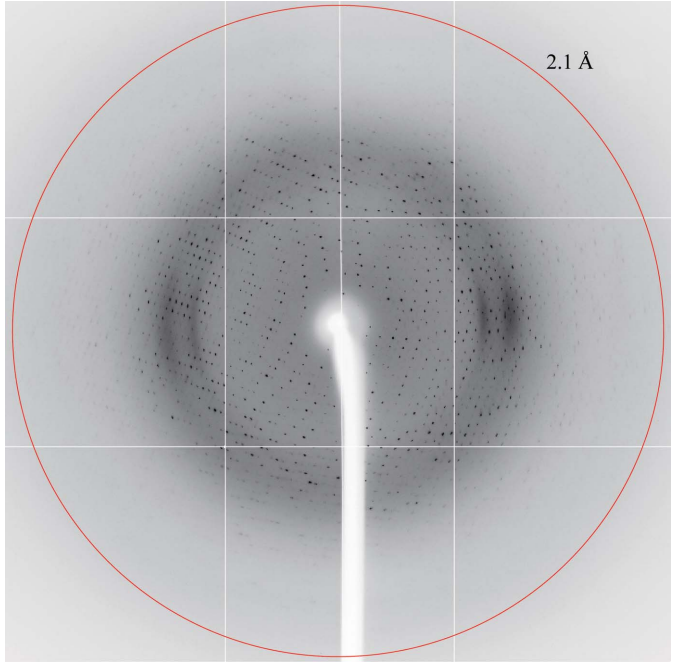

Figure 2
X-ray diffraction pattern of a chDSD crystal. The diffraction data were collected on beamline BL-5A of PF using an ADSC Quantum 315 CCD detector.

coefficient (V_M) was calculated to be 2.79 Å^3 Da^{-1}, corresponding to a solvent content of 56.0% (Matthews, 1968). This result suggests that the subunits of the dimeric chDSD molecule are related by a crystallographic twofold axis.

To prepare heavy-atom derivatives, crystals were soaked in artificial mother liquor [30%(*w*/*v*) PEG 4000, 100 m*M* MES–NaOH pH 6.5 and 10%(*v*/*v*) 2-propanol] containing 1 m*M* zinc chloride for 25 min. The crystal diffracted to approximately 2.15 Å resolution. MAD data were collected at four different wavelengths and four Zn atoms were located in the asymmetric unit of the cell using the MAD data. MAD phasing and structure determination are in progress.

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