Crystallography

5A, 17A, NW12A, NE3A/2009G592

Crystal Structure of a Zinc-Dependent D-Serine Dehydratase from Chicken Kidney

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Introduction

D-Serine is a physiological co-agonist of the N-methyl-D-aspartate (NMDA) receptor. It regulates excitatory neurotransmission, which is important for higher brain functions in vertebrates. In mammalian brains, D-amino acid oxidase (DAO) degrades D-serine [1]. However, we have found recently that in chicken brains the DAO is not expressed and instead a D-serine dehydratase (DSD) degrades D-serine. The primary structure of the chicken DSD (chDSD) shows significant similarities to those of metal-activated D-threonine aldolases, which are fold-type III PLP-dependent enzymes, suggesting that it is a novel class of DSD. chDSD catalyzes the dehydration of D-serine to form pyruvate and ammonia. In the catalytic reaction, a PLP-D-serine Schiff base is formed at the first step. Our biochemical analysis suggested that in the following steps only the dehydration occurs despite the fact that the PLP-D-serine Schiff base is theoretically prone to five possible reactions. In order to elucidate the high reaction specificity of the chDSD, we determined the crystal structure of native chDSD, EDTA-treated chDSD, which lacks the zinc ion in the active sites, and the EDTA-treated chDSD–D-serine complex.

Methods

Native chDSD was purified from chicken kidney, and crystallized by the hanging-drop vapor diffusion method [1, 2]. EDTA-treated chDSD was crystallized by the micro-seeding method using crushed native chDSD crystals as seeds. Crystals of the EDTA-treated chDSD–D-serine complex were prepared by the soaking method.

Results

Diffraction data of chDSD crystals were collected at BL5A, BL17A, and NE3A of PF at KEK (Table 1) [3]. The diffraction data were processed using the XDS or HKL2000 program. The crystal structure of native chDSD was determined by the MAD method using zinc ion as the anomalous scatter. The crystal structure showed that chDSD is a dimeric protein and has two active sites at the dimer interface. Each active site contains a PLP molecule, which forms a Schiff base with Lys45, and a zinc ion that coordinates His347 and Cys349. Our structural and biochemical analyses suggested that the zinc ion is critical for the strict reaction specificity of chDSD. This is the first example of a PLP-containing enzyme that has a metal ion as a catalytic co-factor.

Table 1 Crystallographic summary

Crystal form	Native chDSD	EDTA-treated chDSD–D-Ser
Crystal size [mm]	0.1x 0.1 x 0.05	0.4 x 0.03 x 0.03
X-ray source	Photon Factory	Photon Factory
Beamline	BL5A	NE3A
Oscillation angle (°)	1	1
Exposure time (s)	8	2
Wavelength (Å)	1.0000	1.0000
Temperature (K)	95	95
Space group	*P*422	*P*422
Unit-cell parameters (Å)	a= b=104.6, c=81.4	a= b=105.1, c=81.9
Resolution (Å)	17.0-1.90 (2.00-1.90)	100.0-2.65 (2.79-2.65)
Unique reflections	35,919 (5,021)	13,850 (1,924)
Completeness (%)	99.2 (99.7)	99.9 (99.9)
Redundancy	6.9 (6.7)	7.0 (7.0)
Average $I/\sigma(I)$	26.5 (3.5)	15.4 (3.5)
Rmerge (%)	0.047 (0.546)	0.127 (0.588)
Rwork/Rfree	0.189/0.215	0.206/0.266

Values in parentheses are for the outermost resolution shell.

References

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