Crystallography

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Crystallographic analysis of D-aspartate oxidase (DDO)

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Introduction

In the mammalian neuroendocrine system, D-aspartate (D-Asp) regulates the synthesis and secretion of hormones. The metabolism of D-Asp by D-aspartate oxidase (DDO) and its regulation have, therefore, attracted attention of many researchers. DDO, which has an FAD molecule as a redox-active cofactor, catalyzes dehydrogenation of D-Asp to form iminoaspartate. The resultant iminoaspartate is non-enzymatically hydrolyzed to 2-oxo acid and ammonia immediately after the release from DDO, whereas the reduced FAD reacts with O_2 to return to oxidized state.

A kinetic study of DDO showed that DDO has two distinct reaction cycles. First one is a typical catalytic cycle of FAD-containing enzymes, in which oxidized-form DDO binds a substrate at the beginning of the catalytic reaction. The other reaction cycle begins with the substrate binding to reduced-form DDO. The kinetic study suggested that the two reaction cycles proceed with different catalytic competence under physiological D-asp and O_2 concentrations [1]. Therefore, the reaction of DDO would be regulated in a rather complicated manner *in vivo*. In order to elucidate the relationship between the catalytic cycles and physiological functions of DDO, we planed to analyze the catalytic mechanism of DDO on the basis of the crystal structures of reaction intermediates of DDO. Here, we report the crystallographic analysis of the meso-tartrate and L-tartrate binding forms of DDO.

Methods

Recombinant porcine DDO was purified as described in Yamamoto *et al*. [1]. DDO was co-crystallized with its inhibitors, meso-tartrate and L-tartrate. Crystals grew to their full size in 1 week with approximate dimensions of 0.3 x 0.1 x 0.02 mm^3. After the crystal was soaked in the cryoprotectant solution for 10-20 sec, it was mounted on a cryo-loop and flash-cooled in an N_2 stream.

Results

Diffraction data for the DDO crystals were collected using an ADSC Quantum 270 detector on beamline BL-17A of the Photon Factory. The diffraction data were processed and scaled using the *XDS* program suite (Table 1). Crystal structures of the DDO-meso-tartrate and DDO-L-tartrate complexes were determined by the molecular-replacement method with program *MOLREP* in the *CCP4* program suite. Crystallographic refinements of these structures are in progress.

Table 1 Data-collection statistics

Crystal form	meso-tartrate complex	L-tartrate complex
X-ray source	Photon Factory	Photon Factory
Beamline	BL-17A	BL-17A
Oscillation angle (°)	0.5	0.5
Exposure time (s)	15	15
Wavelength (Å)	1.0000	1.0000
Temperature (K)	95	95
Space group	$P2_1$	$P2_1$
Unit-cell parameters (Å, °)	a=79.4, b=144.0, c=80.5, β=100.9	a=79.2, b=144.9, c=80.5, β=100.5
Resolution (Å)	17.0-1.77 (1.86-1.77)	17.0-2.09 (2.21-2.09)
Observations	846,198 (117,716)	521,008 (81,482)
Unique reflections	170,360 (23,502)	104,361 (16,005)
Completeness (%)	99.8 (99.9)	99.3 (99.1)
Redundancy	5.0 (5.0)	5.0 (5.1)
Average $I/\sigma(I)$	22.7 (4.4)	19.9 (5.5)
Rmerge (%)	0.043 (0.493)	0.054 (0.437)

Values in parentheses are for the outermost resolution shell.

References

[1] Yamamoto, A. *et al*., *J. Biochem*., **141**, 363-376 (2007).

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