Structure Analysis of Blastcidin S Deaminase by MAD

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1. Introduction

With synchrotron radiation, the multi-wavelength anomalous diffraction (MAD) method [1,2], which gives phases from a single anomalous scatterer, has been developed. Anomalous scattering contributes a small portion of diffraction intensity so that the accuracy of intensity data is definitely important. The PX branch of RIKEN beamline I has been designed based on a "trichromatic concept" to optimize for the MAD data collection [3]. To realize such an experimental environment, the trichromatic concept has been introduced by developing high quality diamond crystals. The main feature of trichromatic concept is to minimize systematic errors during anomalous scattering measurements for the MAD. As a first experiment, the MAD data collections of a zinc protein have done.

A zinc protein, BS deaminase (BSD, E.C. 3.5.4.23) is an enzyme that catalyzes the conversion of BS into deaminohydroxy-BS and is produced by a fungi, *Aspegillus terreus* Strain S-712 (ATCC 28865) [4]. BSD was first found in a degradation enzyme for BS as an agricultural medicine. BSD has been effectively used as a reporting marker of transformation in genetic engineering involving eukaryotes. Furthermore BS belongs to the cytidine deaminase (CDA) super family and has a common sequence cluster for zinc metal coordination. BSD contains one zinc atom in a subunit that has an amino acid length of 130 and a molecular weight of 13,000. Biochemical studies indicated that BSD activity is expressed in the tetrameric form of the enzyme.

2. MAD Experiment

An orthorhombic crystal of BSD belonging to space group $P2_12_12_1$ has unit cell dimensions of a = 54.7 Å, b = 69.8 Å, and c = 145.8 Å [5]. One symmetric unit contains 1 tetramer of BSD and as an anomalous atom, one zinc atom is in a monomer. An aliquot size crystal of BSD is soaked in an anti-freeze buffer containing 25% of 2-methyl-2,4-pentanediol (MPD) for 15 minutes and the crystal is then frozen in liquid nitrogen. The data collection was undertaken with a cold N2-gas stream at 100 K. MAD data collection of BSD was performed by using the trichromator at RIKEN beamline I (BL45XU). MAD data sets at three-wavelength are taken from single crystal without changing any setting by trichomator. The edge (1.2822 Å) and peak (1.2818 Å) wavelength of the zinc absorption edge were determined by measuring the XANES profile of BSD crystal. XANES profile is shown in Fig. 1. All diffraction images were recorded by using an imaging plate detector; RIGAKU RAXIS-IV.

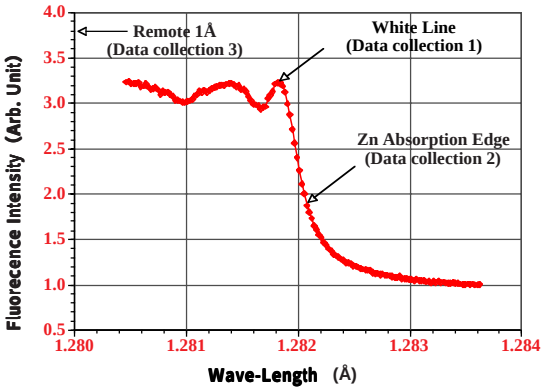

Fig. 1. XANES profile of BSD crystal.

Native data for refinement was also collected at RIKEN beamline I. The diffraction image sets of the BSD crystal in the different wavelengths are processed by DENZO. The statistics for the diffraction data set for MAD phasing are presented in Table 1.

Table1. Statistics of data collection and phase analysis

Wavelength/Å	1.2818 (peak)	1.2822 (edge)	1.0000 (remote)
Resolution/Å	2.2	2.2	2.2
No. of obs. ref.	68,612	68,580	70,398
No. of indep ref.	20,194	20,220	20,468
I/σ(I)	8.6	12.3	13.3
Completeness	0.692	0.693	0.723
R_{merge}/%	5.8	4.8	4.8

Calculated Bijvoet Patterson maps and dispersive Patterson maps show several clear peaks indicating the possibility of interoperation. The phase of the BSD crystal is calculated as MAD phasing using three wavelengths with using MLPHARE. Phasing power at peak and edge wavelength was 1.42 and 1.33, respectively. Initial figure of merit was 0.49 at 2.2 Å resolution, and final figure of merit was improved to 0.90 after solvent flattering and non-crystallographic symmetry average. Electron density maps drawn after density modification are presented in Fig. 2. The maps clearly show that the location of zinc atom is in the middle of a subunit and that Cys residues coordinate the zinc atom. The model building is done by program o.

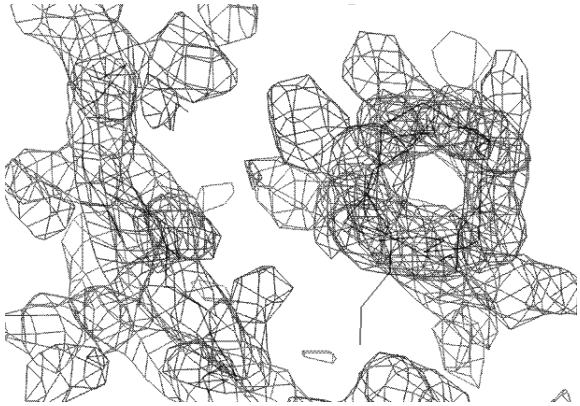

Fig. 2. Initial electron density map calculated from MAD phase of α-helix region.

First, we assign main chain atoms and clear side chains. Then, refinements are made using partial structures. Repeating the processes of model amendment and map omission confirms the construction of the final model, but last four amino acid residues are not assigned. A refinement process is started while maintaining non-crystallographic symmetry between a subunit and another subunit. When the R factor is reduced to 0.25, the employed constraint is removed. By using diffraction data up to 1.7 Å resolution, the structure is reasonably solved at an R factor of 0.2.

3. Structure of BSD

The overall structure of the enzyme has similarity at the zinc-binding site with that of the catalytic domain of CDA [6,7]. Resulted organization of BSD and CDA have similar active structure. These similarities suggest that CDA and BSD have a common reaction mechanism that implies the formation of a tetrahedral intermediate of cytidine and zinc site. The BSD molecule has a pseudo dimer organization derived from homo-tetrameric form with two by two formation and has dimensions of 35 x 35 × 30 Å. Each subunit takes the same folding patters except the C-terminal region.

The structure of N-terminal half of a monomer begins an α-helix. This α-helix is embraced by anti-parallel β-sheet including three β-strands. Additional α-helix is protruded from the other side of the β-sheet wall. Very short β-strand and continuos loop connects the latter half of monomer. This portion contains two anti parallel β-strands and two α-helices. The over all structure of a BSD monomer can be interpretative as an anti-parallel "Greek key" β-sheet that found in CDA.

4. Disscussion and Summary

The data collection of BSD under the trichromatic concept has successfully achieved to obtain straight determination of phase information from MAD of zinc atom. The trichromatic concept at RIKEN beamline I may make easy to obtain the initial phase from a single crystal with an anomalous scatter.

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References

[1] W.A. Hendrickson, Science **254** (1991) 51.
[2] W.A. Hendrickson and C.M. Ogata, Methods in Enzymol. **276** Ed. Carter, C.W. Jr. and Sweet, R.M. (1997) 494.
[3] M. Yamamoto *et al.*, J. Synchrotron Rad. **5** (1998) 222.
[4] M. Kimura *et al.*, Biochim. Biophys. Acta. **1219** (1994) 65.
[5] M. Nakasako *et al.*, Acta Cryst. **D54** (in press).
[6] L. Betts, *et al.*, J. Mol. Biol. **235** (1994) 635.
[7] D.C Carlow *et al.*, Biochemistry **37** (1998) 1199.