X-RAY CRYSTALLGRAPHIC STUDY OF AGGLUTININ, ARGINASE AND CARDIOTOXIN I-IV

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The ribosome inactivating proteins (RIPs) play a significant role in hydrolyzing C-N glycosidic bond of rRNA thereby inhibiting protein synthesis. Two classes of RIPs have been found: class I contains a single chain A and class II contains two chains (A and B). The toxophoric A chain inhibits protein synthesis and the B chain with two D-galactose moiety binding sites facilitates translocation of class II RIPs into cell. Due to the absence of B chain, the translocation of class I RIPs into cell is very difficult. The seeds of the plant, *Abrus precatorius* which is ubiquitous in all tropical and subtropical regions, contains two kinds of class II RIP, the cytotoxic abrins (ABRa,ABRb,ABRc,ABRd) and the low-*toxic Abrus precatorius* agglutinin(AAG). Despite a high sequence homology of 77.8%, the biological and pharmacological activities of ABRa and AAG are contrasting. The interest has been stimulated in part by the use of A chain in the preparation of immunotoxins for cancer chemotherapy.

Arginase catalyses the hydrolysis of L-arginine to urea and L-orthinine which is used in the biosynthesis of proline, glutaminic acid, spermine, spermidine and putresine. This enzyme is indispensable in the liver of ureolytic animals. It is an inhibitor to lymphocyte proliferation. The deficiency of arginase may be hereditary.

Cardiotoxin I-IV from Taiwanese cobra venom are small, membrane active, water soluble, basic and highly homologous polypeptides (6-7kDa). These may cause depolarization of excitable membranes, hemolysis, modulation of membrane enzyme activity and cytotoxicity.

The molecular functional roles of RIP activity and toxicity, catalytic activity of arginase in the urea cycle of human liver and a search for small molecules as possible antidotes against snake bite are called for to elucidate their structures as tentative first steps. Recently, we have crystallized these proteins.

The diffraction data of AAG were collected at the beamline BL41XU at Sring-8 with MarCCD detector at 1Å wavelength and 0.1mm beamsize. The data collection was done by the oscillation mode with $\Delta\varphi$ being 0.8^0 with an exposure time 3 secs per frame. The crystals belong to the space group $P4_22_12$ with cell dimension $a=b=141.9$, $c=105.6$ Å. The data set was processed using the package MOSFLM. The processed data showed a large number of weak reflections at high resolution. From our in-house preliminary x-ray experiment, we expect these crystals could diffract upto a resolution ~2 Å using synchrotron radiation. But the usable data set is only upto 7 Å resolution. The same phenomenon happened to arginase and cardiotoxins that they either did not diffract at all or diffracted in low resolution. We could index the diffraction pattern of arginase in tetragonal space group with cell dimension likely to be $a=b=140.2$, $c=109.6$ but even failed to index in the case of cardiotoxins. This is due to the deterioration of crystal quality of agglutinin, arginase and cadiotoxins crystals during transportation to Japan. Attempts are made to rectify these problems during next data collection such as carrying the boxes in a controlled temperature, using cryopectants etc.

Ultra-High Resolution X-ray Structure Analysis of [NiFe] hydrogenase

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Introduction

Hydrogenases catalyze the reversible oxidation of molecular hydrogen.

Four types of hydrogenases, Fe, Ni-Fe, Ni-Fe-Se and non-metal hydrogenases have been known. Except for non-metal hydrogenase, X-ray crystal structures of all metal containing hydrogenases have been reported [1,2,3]. [NiFe]hydrogenase has an unusual active site which contains one Ni atom and one Fe atom. Fe atom has four non-protein ligands. Three of them are diatomic ligands (SO, CO and CN) and the remaining one is a monatomic ligand (S or SH). Two thiolate side-chains of cysteinyl residues are coordinated to the Ni atom, two other thiolate side-chains of cysteinyl residues are coordinated to both the Ni and Fe atoms as bridges.

Experiments, Results and Discussions

The ultra-high resolution X-ray data collection experiments for hydrogenase from D. v. Miyazaki F was performed on one crystal at 100 K using a Mar-CCD detector system at the beam line of BL41XU at SPring-8. Two data sets at different resolution ranges (∞-1.35 Å, 0.4° oscillation/image, 35 s exposure time; ∞-1.8 Å, 1.0° oscillation /image, 1 s exposure time) were collected in order to maximize the coverage of the dynamic range of the intensities. The intensity data in the resolution range of 20.0 to 1.35 Å were processed with Rmerge of 6.4 %. A total of 161267 unique reflections were obtained by using the program packages of MOSFILM and CCP4.

Since the intensity data at 100 K were in good quality, the structure of hydrogenase has been successfully refined with crystallographic R = 0.128 at 1.35 Å resolution by using the program, SHELX-97. The current electron density map clearly shows the existence of the precipitant agent, MPD (2-methyl-2, 4-pentandiol) molecules on the surface of the enzyme. Further refinement using the higher resolution data (1.1 Å) is now in progress.

References

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