X-RAY CRYSTALLGRAPHIC STUDY OF AGGLUTININ AND CARDIOTOXIN III

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Introduction

The seeds of the plant, *Abrus precatorius* contains two kinds of ribosomc inactivating proteins (RIP), the cytotoxic abrins [ABRa,ABRb,ABRc,ABRd] and the low-toxic *Abrus precatorius* agglutinin (AAG). AAG is a class II RIP, and 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. Despite a high sequence homology of 77.8%, the biological and pharmacological activities of extremely lethal ABRa (LD_{50}=20 _g/Kg) and less lethal AAG (LD_{50}=5mg/Kg) are contrasting. The interest has been stimulated in part by the use of A chain in the preparation of immunotoxins for cancer chemotherapy. The 3-D structure of AAG will provide structural basis for these differences and augment the knowledge of their specificity towards binding oligosaccharides/polysaccharides and toxicity.

The lethal action of the snake venoms is mainly due to the presence of two types of highly homologous toxins, namely, the cardiotoxin and the neurotoxins, having small molecular weight [6.5-8.0kDa] crosslinked by four disulphide bonds at identical positions in their amino acid sequences. Cardiotoxins display a wide variety of biological activities, such as, depolarization and contraction of muscular cells upon binding to the cell membrane and the disruption of the membrane organization, lysis of various types of cells like erythrocytes, epithelial cells, fetal lung cells, cytotoxic to certain type of tumor cells and inhibitor to the activity of protein kinase C and Na^+-K^+ATPase. Cardiotoxins have been shown to act as potent inhibitors to coagulation and platelet aggregation. Even though an impressive amount of research work has been devoted to these toxins, their mode of action is still controversial as the existence of specific receptors is unclear. In order to understand these biochemical functions and an anticipation of potential medical applications, the determinations of the 3D structures of cardiotoxins have attracted much interest.

Experiments and preliminary results

The diffraction data of AAG were collected in cold nitrogen gas stream using 20% PEG400 as cryoprotectant and recorded on ADSC-CCD detector system at the beam line BL40B2. The X-ray wavelength was 1.00 , the oscillation range was 0.7^0 and the crystal to detector distance was 200 mm. Analysis of the diffraction pattern and of systematic absences allowed to assign AAG crystals to the primitive tetragonal space group $P4_12_12/P4_32_12$, with cell parameters a=b=136.29, c=212.03 . The crystals diffracted up to 2.85 .

X-ray diffraction data on cardiotoxin III from the venom of the Taiwan cobra, *Naja naja atra* were also collected in the beam time, supplementing the mother liquor solution with 33% glycerol as a cryoprotectant. The X-ray wavelength was 1.00 , the oscillation range was 0.7^0 and the crystal to detector distance was 250 mm. The crystals diffracted up to 2.9 with cell dimensions of a=76.29, b=92.83, c=139.47 with space group $P222_1$. Structure determinations by the molecular replacement method are in progress.

Visualisation of the Lipid Bilayers in the Crystals of Membrane Proteins

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We have been working on the structure determination of Ca^{2+}-ATPase from sarcoplasmic reticulum and have already succeeded in determining the structure with 2 Ca^{2+} bound in the transmembrane high affinity sites and that in the absence of Ca^{2+} but in the presence of thapsigargin, a potent inhibitor.

One interesting aspect of our crystals of Ca^{2+}-ATPase is that they have exogenous lipids, which are expected to form lipid bilayers in the crystals. The presence of very strong lamellar reflections at the positions deviated from the reciprocal lattice points confirmed this idea. Sections of the crystals embedded in plastic showed that at the very edge of the crystals lipid bilayers form loop structures connecting different layers.

Therefore these crystals offer a unique opportunity to study the lipid-protein interactions in a great detail. Also, by solving the crystal structure of the Ca^{2+}-ATPase in the absence of Ca^{2+}, we showed that the transmembrane helices undergo dramatic rearrangements with surprisingly large components normal to the membrane (*Nature* 418: 605-611, 2002). Therefore, it is interesting to know how the lipid bilayers behave in the two crystals. Specifically, we would like to know if the bilayers move together with the transmembrane helices.

To visualise the bilayers, we need data for very low-resolution reflections that are neglected in the ordinary crystallographic analyses. The phase information for such low resolution reflections cannot be obtained reliably by multiple isomorphous replacements. Alternatively, contrast variation method can be used for this purpose. As a medium for contrast variation, we initially tried aurothioglucose, which has been used in electron microscopy for the same purpose. The presence of gold in the compound allows us to utilise anomalous scattering information as proposed by Fourme (*Acta Cryst.* D56: 1288-1303, 2000). As a first attempt, crystals of Ca^{2+}-ATPase with bound Mg/F, a phosphate analogue, were used. However, we found that the range of aurothioglucose concentration was rather limited, only up to 15% (plus 8% sucrose), although the changes in unit cell dimensions were insignificant. Sucrose could be increased to as high as 30%, but the a-axis dimension increased (176.6 to 179.8 Å at 30% sucrose). The ranges of variation of glycerol and polyethyleneglycol concentrations allowed were much more limited. So, it appeared most practical to use the combination of aurothioglucose and sucrose for contrast variation.

With the improvement of the optics system, it was possible to record the reflections between 1/150 $Å^{-1}$ and 1/4 $Å^{-1}$ using the ADSC CCD detector. Because the crystals diffracted better than this, we wanted to collect full sets of data using R-Axis IV++. However, due to apparent lack of long term stability of the system as well as the smallness of the crystals, we had to conclude that it was not practical to collect full sets of data, in particular of MAD data, using this beam line.