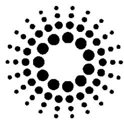

ESRF

Experiment title:

Structure Determination of Maclura Pomifera Agglutinin: a member of a new lectin family.

Experiment number:

LS 422

Beamline: BL19

Date of Experiment: from: April 30, 1997 to: May 2, 1997

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Shifts: 24

Local contact(s): Dr. Andrew Thompson

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Report:

Carbohydrate-protein interactions plays a very important roll in many surface phenomena, particularly cell-cell recognition and communication. *Maclura pomifera agglutinin* (MPA) from a plant of Moraeae family is specific for the tumor antigen Galβ1, 3GalNAc, a core structure of the O-linked type of glycopeptide.

MPA has been used in immunology as erythroagglutinin, in development biology as probes, as markers in cell differentiation and in cancer biology as labels of carcinomas cells and in glucoprotein purification. Its sequence, specificity for carbohydrate and its lack of metal ions and disulfide bonds all indicate MPA to be a member of a new lectin family. It has also been shown to be detrimental to the growth of larvae and to block HIV infection.

MPA is tetramers of subunits. Each has two chains, a and b. Chain a has 133 residues while the other is extremely small for a non-covalently attached chain, with 20 or 21 residues. The post-translational cleavages of the precursor give a one residue variation in length for MPA b-chain. It has been confirmed that the two chains come from a much longer precursor.

In the precursor, the C-terminus of the b-chain is separated by only four residues from the N-terminus of the larger a-chain. Two genetic variants were found in both the protein and DNA sequencing for MPA. Hence there is post-translational heterogeneity superimposed on the genetic variation. Surprisingly MPA has been successfully crystallized with Tumor antigen as ligand and without ligand.

MPA and the T-antigen complex was crystallized in 1989 (1). More than 40 soaking and cocrystallization experiments have been conducted in order to obtain heavy atom derivatives for isomophous replacement. Unfortunately all failed to give derivatives. In 1993 the protein was crystallized with the mercury compound p-hydroxy-mercuriphenyl sulfonic acid. Fluorescence experiment at the mercury absorption edge proved that the mercury atom was incorporated into the protein. However, multiple experiments at Brookhaven and the beamline XL2 in ERSF showed that the mercury atom is too close to the origin and the phasing power was very poor. In May 1996 a new candidate was available with double heavy atoms label, the same mercury compound and the trimethyllead as the second additive for the cocrystallization trial. The crystal obtained was of space group C222 and it diffracted to 2.4 Å using a San Diego multiwire type area detector. A new data collection trial was conducted at BL19 with the excellent help of Dr. Andrew Thompson. Two crystals were used for the data collection. For each crystal data was collected at six wavelengths, three at the mercury edge and three at the lead edge. The Rsymm for all the data sets was around 270 and the completeness was 98%. Anomalous and dispersive Pattersons all clearly showed that there are two lead sites. The FOM is 0.72 and 0.84 before and after solvent flattening. The electron density map calculated with the two lead sites was very good. The whole skeleton of the molecule, a chain plus b chain was readily traced with the program package O and the fitting of the whole molecule, backbone and side chains was completed within three days. A ribbon diagram to show the raw model was thus obtained. Currently the model is being refined. A detailed report on the refined structure will be supplied to ESRF as soon as the structure determination is completed.