

**PS04.11.13 CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION STUDY OF TRICHOSANTHES KIRILOWII LECTIN.** Yao-Ping Wang, Ji-Shen Pan, Ke-Yi Wang#, Ru-Chang Bi. Institute of Biophysics, Academia Sinica, Beijing 100101, P.R.China; #Institute of Biochem., Academia Sinica, Shanghai 200031, P.R.China

Trichosanthes Kirilowii lectin (TKL) is a new protein purified from a Chinese herb medicine, the tuber of *Trichosanthes Kirilowii* maxim. It consists of two peptide chains, each with approximately 30kD molecule weight. TKL has diverse biochemistry, physiology and toxicology activities and binds strongly with galactose and lactose. It shows immunological cross-reactions with both ricin contained in seeds of *Ricinus communis* and trichosanthin, another interesting protein from *Trichosanthes Kirilowii* maxim with anti-AIDS effects. There is high structural similarity between the A-chain of ricin and trichosanthin. It is important to determine TKL structure and to compare the structural aspects of TKL, ricin and trichosanthin in elucidating the structure-function relationships of these proteins at molecular level.

After screening of crystallization conditions with the conventional hanging-drop method, better TKL crystals appeared under the following conditions: a drop prepared by mixing 2 $\mu$ l sample solution with concentration of 8.3mg/ml TKL and 2 $\mu$ l reservoir solution, equilibrated against 500 $\mu$ l reservoir solution, containing 0.5M Li<sub>2</sub>SO<sub>4</sub> and 15% PEG-8000. The crystals belong to an orthogonal space group with unit cell parameters of a=44.7 Å, b=69.5 Å and c=180.9 Å, and there is one molecule in the asymmetric unit. 3 Å diffraction data were collected at room temperature, using Mar Research Image Plate System in our laboratory.

**PS04.11.14 CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION ANALYSIS OF DISCOIDIN I.** Yuri D. Lobsanov<sup>1</sup>, Jung-Kay Chiu<sup>1</sup>, Chi-Hung Siu<sup>2</sup> and James M. Rini<sup>1</sup>. Departments of Molecular and Medical Genetics and Biochemistry<sup>1</sup>, Banting and Best Department of Medical Research and Department of Biochemistry<sup>2</sup>, University of Toronto, Toronto, Ontario, M5S 1A8 Canada

Discoidin I is a  $\beta$ -galactoside binding lectin involved in *Dictyostelium discoideum* cell adhesion. Starvation of slime mold amoebae results in the expression of the lectin and the formation of fruiting bodies. Discoidin I is physiologically active as a tetramer. The discoidin domain is a protein module which has recently been identified on two proteins in the coagulation cascade (Factor V and VIII), as well as cell surface molecules, including the neural antigen A5, thought to be involved in retinal axon targeting, and the tyrosine kinase signalling receptors DDR, Ptk-3 and Tyro 10.

Discoidin I was purified from slime mold culture by affinity chromatography on Sepharose-4B. Protein was eluted by 0.3 M galactose in 20 mM Tris-HCl pH 7.2, 1mM EDTA and 150 mM NaCl. Crystals have been obtained by the hanging-drop vapor-diffusion technique. The well solution contains 1.8 M ammonium sulfate, 100 mM MES pH 6.5 and 10 mM CoCl<sub>2</sub>. The protein-sugar mixture contains 10 mg/ml protein and 100 mM thio-digalactoside in 20 mM Tris-HCl pH 7.2 and 1mM EDTA. Rod-like crystals reach dimensions of 0.2 x 0.2 x 0.8 mm within 1 - 4 weeks and diffract to at least 2.4 Å on a conventional rotating anode. The crystals grow in a trigonal space group (a=66.0 Å, b=66.0 Å, c=149.5 Å), with one molecule per asymmetric unit, yielding a solvent content of 63.5%. Native data to 2.6 Å resolution have been collected and heavy atom screening is in progress.

**PS04.11.15 STRUCTURE OF A GALACTOSE-SPECIFIC C-TYPE ANIMAL LECTIN.** Anand R. Kolatkar and William I. Weis, Stanford University, Dept. Structural Biology, Stanford, CA

Galactose-binding C-type lectins function in serum glycoprotein clearance, tumor cell recognition, and organization of the extracellular matrix. The crystal structure of a galactose-binding mutant of a C-type animal lectin has been solved unliganded and in complex with galactose and N-acetylgalactosamine (GalNAc). Three amino acid substitutions and insertion of a glycine-rich loop in wild-type mannose-binding protein A (MBP-A) gives a mutant (QPDWG) that exhibits specificity and affinity for galactose similar to naturally-occurring galactose-binding C-type lectins. The 3- and 4- OH groups of galactose coordinate the Ca<sup>2+</sup> at site 2 and form hydrogen bonds with amino acid residues that also coordinate the Ca<sup>2+</sup>. Galactose specificity is conferred by a glycine-rich loop which holds Trp<sup>189</sup> in a position optimal for packing against the apolar face of the galactose ring, and which prevents mannose binding by steric exclusion. The structure of the N-acetylgalactosamine/QPDWG complex shows that the 2-acetamido group of GalNAc is oriented such that it could interact with the amino acid positions identified by site-directed mutagenesis (Iobst, S.T. & Drickamer, K., *J. Biol. Chem.*, **271**, 1996, *in press*) as being important in GalNAc-specific C-type lectin binding sites. An additional mutation of Thr<sup>202</sup>  $\rightarrow$  His in QPDWG (to produce QPDWGH) exhibits an 8-fold increase in GalNAc specificity over galactose. The GalNAc/QPDWGH structure is currently being refined, and the preliminary results indicate that His<sup>202</sup> is too distant from the acetamido group of GalNAc to make direct contact. It is possible that His<sup>202</sup> affects GalNAc binding through either a bridging water or by interaction with an amino acid residue that does make direct contact with GalNAc.

**PS04.11.16 PROBING THE ACTIVE SITE OF ENDO H BY MUTAGENESIS AND X-RAY CRYSTALLOGRAPHY.** Vibha Rao<sup>1,2</sup>, and Patrick Van Roey<sup>1</sup>, (1) Wadsworth Center, New York State Dept. of Health, Albany, NY 12201, USA, and (2) Physics Dept., University at Albany, Albany, NY 12222, USA

Endo- $\beta$ -N-acetylglucosaminidase H, an endoglycosidase secreted by *Streptomyces plicatus*, hydrolyzes the central  $\beta$ (1-4) glycosidic bond between the core N-acetylglucosamine residues of asparagine-linked oligosaccharides. It requires the following minimum substrate: -Man $\alpha$ (1-3)Man $\alpha$ (1-6)Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)GlcNAc-Asn, and therefore is highly specific for high mannose and hybrid glycans. The overall fold of Endo H is that of an ( $\alpha$ / $\beta$ )<sub>8</sub>-barrel (Rao, V. *et al.*, *Structure*, **3**, 449-457, 1995). Site directed mutagenesis studies have resulted in the identification of Asp130 and Glu132 as catalytic residues. Mutations of Asp130 to Asn (D130N), Glu (D130E) and Ala (D130A) resulted in 0.1 - 1.0% activity of the wild-type enzyme. However, the Glu132 mutants, E132A and E132Q have no detectable activity. Crystal structures of three mutants, D130N, E132A and the double mutant D130N + E132Q, have been determined to 2.1 Å resolution by molecular replacement methods. Mutants of E132 crystallize in a different crystal form (P2<sub>1</sub>) than the wild-type enzyme (P4<sub>3</sub>2<sub>1</sub>2), apparently resulting from the absence of an intermolecular contact made by Glu132 in the wild-type form. Detailed comparison of the structures shows no notable change in the backbone conformations. The r.m.s. deviations of main chain atoms compared to the wild-type structure are: D130N mutant (space group P4<sub>3</sub>2<sub>1</sub>2), 0.38 Å; E132A (P2<sub>1</sub>), 0.54 Å; and, D130N + E132Q (P2<sub>1</sub>), 0.58 Å. Side-chains of hydrophobic residues in the area of the active-site vary more in conformation than those of polar residues. Hence, mutagenesis experiments and the crystal structures of the active-site mutants indicate that Glu132 is absolutely essential for the hydrolysis and that Asp130 may not participate directly in the catalysis, but may serve to create a more negative environment around Glu132.