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 2µl sample solution with concentration of 8.3mg/ml TKL and 2µl reservoir solution, equilibrated against 500µl reservoir solution, containing 0.5M Li_2SO_4 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[1], Jung-Kay Chiu[1], Chi-Hung Siu[2] and James M. Rini[1]. Departments of Molecular and Medical Genetics and Biochemistry[1], Banting and Best Department of Medical Research and Department of Biochemistry[2], University of Toronto, Toronto, Ontario, M5S 1A8 Canada

Discoidin I is a β-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 $CoCl_2$. The protein-sugar mixture contains 10 mg/ml protein and 100 mM thio-di-galactoside 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^{2+} at site 2 and form hydrogen bonds with amino acid residues that also coordinate the Ca^{2+}. Galactose specificity is conferred by a glycine-rich loop which holds Trp^{189} 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^{202} —> 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^{202} is too distant from the acetamido group of GalNAc to make direct contact. It is possible that His^{202} 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[1,2], and Patrick Van Roey[1], (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-β-*N*-acetylglucosaminidase H, an endoglycosidase secreted by *Streptomyces plicatus*, hydrolyzes the central β(1-4) glycosidic bond between the core N-acetylglucosamine residues of asparagine-linked oligosaccharides. It requires the following minimum substrate: -Manα(1-3)Manα(1-6)Manβ(1-4)GlcNAcβ(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 $(\alpha/\beta)_8$-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 ($P2_1$) than the wild-type enzyme ($P4_32_12$), 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 $P4_32_12$), 0.38 Å; E132A ($P2_1$), 0.54 Å; and, D130N + E132Q ($P2_1$), 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.