PS04.11.13 CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION STUDY OF TRICHOANTHES 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. It consists of two peptide chains, each with approximately 30KD molecule weight. TKL has diverse biochemistry, physiology and toxicology activities and binds strongly to galactose and lactose. It shows immunological cross-reactions with both lectin in contained in seeds of Ricinus communis and trichosanthis, another interesting protein from Trichosanthes Kirilowii maxim with anti-AIDS effects. There is high structural similarity between the A-chain of ricin and trichosanthis. It is important to determine TKL structure and to compare the structural aspects of TKL, ricin and trichosanthis 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 0.5M reservoir solution, containing 0.5M Li2SO4 and 15% PEG-6000. The crystals belong to an orthogonal space group with unit cell parameters of a=44.7 Å, b=99.5 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. Lobanov1, Jung-Kay Chiul2, Chi-Hung Siu1, 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, MSS 1A8 Canada.

Discoïdin I is a β-galactoside binding lectin involved in Dicyostelium discoideum cell adhesion. Starvation of slime mould amoebae results in the expression of the lectin and the formation of fruiting bodies. Discoïdin I is physiologically active as a tetramer. The discoïdin 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

PS04.11.15 STRUCTURE OF A GALACTOSE-SPECIFIC C-TYPE ANIMAL LECTIN. Anand R. Kolatkar and William J. 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-acetylglactosamine (GalNAc). Three amino acid substitutions and insertion of a glycine-rich loop in wild-type mannos-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 Ca2+ at site 2 and form hydrogen bonds with amino acid residues that also coordinate the Ca2+. Galactose specificity is conferred by a glycine-rich loop which holds Thr188 in a position optimal for packing against the apolar face of the galactose ring, which prevents mannose binding by steric exclusion. The structure of the N-acetylglactosamine (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 (Iobs, 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 Thr188 -> 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 His202 is too distant from the acetamido group of GalNAc to make direct contact. It is possible that His202 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 Roeyl, (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 pilatus, hydrolyzes the central β(1-4) glycosidic bond between the core N-acetylglucosamine residues of asparagine-linked oligosaccharides. It requires the following minimum substrate: -Man α(1-3)Man α(1-6)Man β(1-4)GlcNAc β(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(α/β)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 A (D130A, Glu (D130E), 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 (P21) than the wild-type enzyme (P42,2), 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 P42,2), 0.38 Å; E132A (P21), 0.54 Å; and, D130N + E132Q (P21), 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.