bearing Le^a, Le^b and Le^x antigens, mono- and difucosylated blood group H substances, blood group A and B substances, *N*-glycans,and glycoproteins were used as probes for lectin binding studies. Using hemagglutination inhibition, affinity electrophoresis, microprecipitation technique, equilibrium dialysis, and affinity chromatography, quantitative parameters of lectin-carbohydrate interaction were estimated and compared with those of other fucose-binding lectins *Ulex europaeus I* and *Lotus tetragonolobus.*

S8.27

Carbohydrate Specificity of Lectin from Horse Chestnut (*Aesculus hippocastanum L.*)

V. A. Antonjuk, *Z. N. Nysenko, *E. A. Erokhina, **V. E. Piskarev, **A. M. Sakharov*

*Scientific-Production Co-operative "Lectinotest", Medical Institute, Pekarskaya street, 69, 290000 Lvov, Ukraine; *N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of Russia, 117913 Moscow, Russia; **Institute of Food Compounds, Academy of Sciences of Russia, Vavilov street, 28, 117813 Moscow, Russia.*

A. hippocastanum L. lectin was isolated by affinity chromatography on hen egg white based sorbent. Lectin is a tetrameric 132 kD protein. Hemagglutination inhibition studies with monosaccharides showed the lectin affinity to Neu5NAc. More detailed binding studies with a series of the different Neu5NAcα2→3 or Neu5NAcα2→6 containing oligosaccharides from milk, urine, or glycoproteins indicated that the *A. hippocastanum* lectin has higher affinity for α2→6 -linked Neu5NAc. Comparison of the *A. hippocastanum* lectin carbohydrate binding properties showed some similarity with other Neu5NAc-specific lectins from plant *(Sambucus nigra, Sambucus racemoca)* or crab *(Cancer antennarius).* At the same time the lectins differed in agglutination of erythrocytes from various species. Thus, the lectin may be used for discrimination of the type of Neu5NAc linkage in sialoglycoconjugates in addition to other sialic acid specific lectins.

S8.28

Exploring Molecular Basis of Protein-Sugar Interactions

A. Surolia and K. D. Puri

Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560 012, India.

Contrary to the generally held view that the binding of sugars to proteins is stabilized exclusively through hydrogen bonding, our thermodynamic studies on the ligand binding to winged bean basic (WBA I) and acidic (WBA II) lectins reveal that orientation effects and non-polar forces, respectively, play crucial roles in these recognitions. WBA I binds to blood group A_1 antigen preferentially over B but not H or Le^a or Le^b structures and recognizes A blood group antigen only through one of its surfaces such that both of its fucosyl residues are oriented towards solvent. WBA II on the other hand strongly prefers terminally monofucosylated sugars with fucose linked α1-2 to the non-reducing end galactose. 2′-fucosyllactose (2-FL) being the most complementary ligand of WBA II. Type I (LNF I) and type III H-antigenic structures are poorly recognized. L-fucose in α1-3 linkage to the penultimate glucose as in 3-fucosyllactose and difucosyllactose and α1-4 and α1-3 linked fucose to *N*-acetylglucosamine as in LNF II and LNF III respectively, sterically prevent the access of these sugars to the binding site. The unique ability of WBA II to distinguish between terminally monofucosylated H-antigen from internally monofucosylated or difucosylated H-antigen should make it a valuable tool for the structural studies of glycoconjugates. WBA II exhibits positive entropy change for the binding of 2-FL indicating for the first time the predominance of non-polar forces in protein-sugar recognitions. Loci involved in polar and non-polar interactions between 2′-FL and WBA II would be discussed.

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(2) Matsuda, T. *et al.* (1989) *Mol. Immunol.*, **26**, 189.
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(4) Schwarz, F. P. *et al.* (1991) *J. Biol. Chem.*, **266**, 24344.
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8.29

Exploring Molecular Features of Protein-Sugar Interactions

K. D. Puri and A. Surolia

Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560 012, India.

Thermodynamic and kinetic investigations of the carbohydrate binding specificity of winged bean basic agglutinin (WBA I) highlighted the following features: WBA I recognizes blood group A antigen strongly over B antigen but not H, Le^a, Le^b or I antigens. Fucosyl residues of the blood group A-penta (GalNAcα(L-Fucα2)3Galβ(L-Fucα3)4Glc) thermodynamically favour the interaction of the binding epitope (GalNAcα3Galβ4Glc) indicating the important part played by sugars not involved directly in binding, favourably orienting the interacting regions of sugars. The binding sites of WBA I are specific for the sugar as well as for the alkyl substituent. The anomeric configuration of the galactoside residues bound to WBA I leads to a major change in the binding of 4-Methylumbelliferyl (MeUmb) substituent. Relatively slow binding of MeUmb derivatives of GalNAc was due to higher value of the activation energies for the association reactions indicating that sufficiently large amount of energy has to be expended for the binding process to take place, presumably due to a requirement of considerable reorientation of water molecules around acetamido group and/or the corresponding *loci* from the protein. Binding of blood group A reactive sugars as well as MeUmb galactosides is enthalpically driven with little or no change in heat capacity. This together with enthalpy-entropy compensation observed for these processes underscore the importance of water reorganization as being one of the principal determinants of protein-sugar interactions.

(1) Khan, M. I. *et al.* (1986) *J. Biol. Chem.*, **261**, 11586.
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S8.30

Formation of Homogeneous Cross-linked Lattices Between the 14 KDa Galactose-Specific Animal Lectin and Glycoproteins