School of Pharmaceutical Sciences, University of Tokyo (Japan). E-mail: umeji@mol.f.u-tokyo.ac.jp

MD-1 is a 162 amino-acid glycoprotein that associates with a B-cell-specific RP105 protein on the cell surface and has a low sequence identity of 16% to MD-2 that associates with Toll-like receptor 4 and recognizes endotoxic lipopolysaccharide. MD-1 and RP105 are supposed to mediate lipopolysaccharide recognition, however, little is known about their structures and functions. To obtain structural insights into the RP105 and MD-1 system, we have determined the crystal structure of mouse MD-1.

Mouse MD-1 was expressed in yeast *Pichia pastoris*, and was purified to homogeneity. Crystallization was performed with the sitting-drop vapor-diffusion method. Two crystal forms, hexagonal and tetragonal, were obtained. The crystal structure of the hexagonal form was initially determined at 2.4 Å resolution with the single isomorphous replacement method, and then the structure was further refined to 1.65 Å resolution against the tetragonal form.

MD-1 is folded into a single domain consisting of two antiparallel β-sheets in the β-cup fold; one sheet consists of three strands, and the other of six strands. A deep hydrophobic cavity of 1,915 $Å^3$ is formed between these sheets as is MD-2. No charged residues are located on the cavity entrance. Continuous electron-densities attributable to bound phosphatidylcholine were observed in the cavity. It is likely that phosphatidylcholine is an endogenous ligand of MD-1 and plays a role in retaining the MD-1 structure since the bound phosphatidylcholine from *P. pastoris* is copurified with MD-1. Together with the binding assay with tetra-acylated lipid IVa, MD-1 is shown to be a lipid-binding coreceptor.

Keywords: receptor, immune, lipid

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Structure and mechanisms of SIGN-R1, pneumococcal and sialylated protein receptor on macrophages

Silva-Martin N.,[a] Galan S.,[a] Schauer J. D.,[b] Park G. C,[b] Hermoso J. A.[a] [a]*Departament of Crystallography and Structural Biology, Instituto de Química Física "Rocasolano", CSIC, Serrano 119. 28006 Madrid, (Spain).* [b]*Laboratory of Cellular Physiology and Immunology and Chris Browne Center for Immunology and Immune Diseases, The Rockefeller University, 1230 York Avenue, New York, NY 10021, (USA).* E-mail: xjuan@iqfr.csic.es

The intricate system of serum complement proteins provides resistance to infection. SIGN-R1 is a transmembrane receptor found in Spleen macrophages, providing resistance against blood-borne S. Pneumoniae, and that can also recognize C. albicans, HIV and M. tuberculosis. SIGN-R1 extracellular moiety comprises a neck region formed by six repeats and a carboxyl-terminal calcium-type carbohydrate recognition domain (CRD). Pneumococcal recognition by SIGN-R1 activates the classical complement pathway independent immunoglobulins, as well as promotes phagocytes processes [1]. Furthermore SIGN-R1 has been probed to be required in anti-inflammatory activity of sialylated Fc fragments [2]. In order to better understand these processes we have performed the structural determination of SIGN-R1 CRD by X-Ray crystallography. The three-dimensional structures of the SIGN-R1 CRD in complex with a pneumococcal polysaccharide analogue (dextran sulphate) and sialic acid have been obtained at 2.5Å and 2.6Å of resolution respectively. SIGN-R1 CRD structure displays the typical long-form C-type lectin-like domains (CTLDs) fold [3] with two Ca2+ sites mediating carbohydrate binding. Binding site is altered by the unusual orientation of the long loop region, moreover two additional secondary structures elements are present: a 3_{10} helix and a small β-sheet arising from the extended β-sheet 2. Unexpectedly, crystal structure reveals five sulfate binding sites not been observed previously. In addition, docking experiments with models of pneumococcal CSP (CPS14) and *Staphylococcus aureus* "poliribitol phosphate" ligands have been done. We found that sialylated-glyco-proteins could bind to the classical lectin polysaccharide recognition zone while CPS seems to have a new secondary recognition region. This two different recognition sites will allow SIGN-R1 to bind both glyco-proteins and CPS simultaneously. Structural description and carbohydrate recognition are in deep described in the poster.

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Structural basis of carbohydrate recognition by calreticulin

Guennadi Kozlov,[a] Cosmin L. Pocanschi,[b] Angelika Rosenauer,[a] David B. Williams,[b] Kalle Gehring,[a] [a]*Department of Biochemistry, Groupe de recherche axé sur la structure des protéines, McGill University, (Montréal).* [b]*Departments of Biochemistry and Immunology, University of Toronto, Toronto (Canada)*. E-mail: guennadi.kozlov@mcgill.ca

The calnexin cycle is a process by which glycosylated proteins are subjected to folding cycles in the endoplasmic reticulum (ER) lumen via binding to the membrane protein calnexin (CNX) or to its soluble homolog calreticulin (CRT). Defects in the calnexin cycle and ER protein folding in general are important for a number of diseases and conditions, ranging from cystic fibrosis to malfunctions of the ER stress response due to aging, genetic mutations, or environmental factors. Components of the calnexin cycle additionally play key roles in the assembly of major histocompatibility complex (MHC) class I molecules where CRT associates with protein disulfide isomerase ERp57, tapasin, the heavy chain/β2-microglobulin heterodimer and the TAP peptide transporter to form the peptide-loading complex. The previous crystal structure of CNX revealed two main structural components, a globular lectin domain and an extended arm-like domain, called the P-domain [1]. CNX/CRT specifically recognize monoglucosylated $Glc_1Man_9GlcNAc_2$ glycans, but the structural determinants underlying this specificity were unknown.

Here, we determined a 1.95 Å crystal structure of the CRT lectin domain in complex with the tetrasaccharide fragment from the glucosylated arm of the $Glc_1Man_9GlcNAc_2$ glycan [2]. The lectin domain shows a jelly roll fold similar to leguminous lectins and largely consists of a beta-sandwich formed by two curved beta-sheets. It also contains a single high-affinity calcium-binding site that plays an important role in stabilizing the protein but does not participate in carbohydrate recognition. The tetrasaccharide binds to a long channel on CRT formed by a concave beta-sheet. All four sugar moieties are engaged in the protein binding via an extensive network of hydrogen bonds and hydrophobic contacts. The structure explains the requirement for glucose at the non-reducing end of carbohydrate; the oxygen O2 of glucose perfectly fits to a pocket formed by CRT side chains while forming direct hydrogen bonds with carbonyl of Gly124 and the side chain of Lys111. The structure also explains a requirement for the Cys105-Cys137 disulfide bond in CRT/CNX for efficient carbohydrate binding. The Cys105-Cys137 disulfide bond is involved in intimate contacts with the third and fourth sugar moieties of the Glc_1Man_3 tetrasaccharide. Finally, the structure rationalizes previous mutagenesis