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 Å³ is formed between these sheets as is MD-2. No charged residues are located on the cavity entrance. Continuous electron-densities attributable to bound phosphatidyldcholine were observed in the cavity. It is likely that phosphatidyldcholine is an endogenous ligand of MD-1 and plays a role in retaining the MD-1 structure since the bound phosphatidyldcholine 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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**MS50.P15**


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

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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-dependent carbohydrate recognition domain (CRD). Pneumococcal recognition by SIGN-R1 activates the classical complement pathway 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.

**Keywords:** receptor, immune, lipid

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**MS50.P16**


**Structural basis of carbohydrate recognition by calreticulin**

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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 malignancies 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 Gc,Man,GlcNAc 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 Gc,Man,GlcNAc glycans [2]. The lectin domain shows a glossy 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 Gc,Man, tetrasaccharide. Finally, the structure rationalizes previous mutagenesis

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**Keywords:** SIG-R1 S. Pneumoniae Sialic-Acid

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Fluorogen-activating proteins

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Using phage display technology, antibody V\textsubscript{H} or V\textsubscript{L} domains have been selected for their binding to non-fluorescent dye molecules. The dye molecules (fluorogens) then become fluorescent [1] while bound within a dimer of the antibody fragments. These fluorogen-activating proteins (FAPs) are being developed for use as tags for the fluorescent imaging of proteins. Antibody-based FAPs can utilize different arrangements of immunoglobulin variable light and heavy domains (V\textsubscript{L}-V\textsubscript{H}, V\textsubscript{H}-V\textsubscript{L}), with different combinations of V\textsubscript{L} and V\textsubscript{H} domains causing significant differences in fluorescence output. Crystal structures have been determined for the FAP L5-MG(L89S), in the unliganded and malachite green-bound forms, to 1.93 and 2.45 Å, respectively. The structure of the malachite green-bound L5-MG(L89S) reveals an unusual homodimeric arrangement of human lambda V\textsubscript{L} domains with the malachite green fluorogen sandwiched in between V\textsubscript{L} subunits. The dimeric arrangement of the V\textsubscript{L} domains is different from any seen thus far for V\textsubscript{L} dimers. A similar homodimer is found for in the crystal of the unliganded L5-MG(L89S), although this V\textsubscript{L} domain exists as a monomer in solution. The structural results are being used as a guide for design and improvement of future FAP constructs.


Keywords: immunoglobulin, fluorescence, antibody

Crystal structure of an aminopeptidase important in antigenic peptide processing

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Two highly homologous endoplasmic reticulum aminopeptidases, ERAP1 and 2, trim antigenic peptide precursors down to the mature epitopes for binding onto nascent MHC class I molecules. The two enzymes operate in concert and have to process a vast variety of different epitope precursor sequences. In this study we report the structure of ERAP2 at 3Å resolution and compare it to recently solved structures of ERAP1. The structure of ERAP2 resembles the structure of ERAP1 in “closed” conformation [1]. Domain IV in ERAP2 is in close proximity to domains I and II, forming a closed large internal cavity. This cavity extends away from the active site and is large enough to accommodate even the longest of antigenic peptide precursors. Amino acid side chains lining the interior of this cavity form shallow specificity pockets with distinct patterns, which can account for different specificity patterns between ERAP1 and 2. A crystallographic heterodimer formed through the interaction of the N-terminal domains may constitute a model for a proposed ERAP1/2 heterodimer. Overall, the ERAP2 structure provides insights on how two homologous aminopeptidases have evolved to be able to cooperate in order to trim a very large variety of peptidic sequences of relatively long peptides, a function fundamental to their biological role in antigen processing.


Keywords: antigen presentation, immunobiology, specificity