Acta Cryst. (2002). A**58** (Supplement), C283

STRUCTURAL STUDIES OF THE SHANK SAM DOMAIN

M. Baron J. U. Bowie
University of California, Los Angeles Chemistry and Biochemistry 611 Charles E. Young Drive East LOS ANGELES CA 90095 USA

Shank is a new family of master scaffolding proteins in the postsynaptic density of eukaryotic neurons that interacts with cytosolic and membrane proteins. Shanks amplify intracellular signals involved in neurite outgrowth, cell migration and cytoskeletal organization. Shanks contain a C-terminal sterile α motif (SAM) domain. SAM domains are discrete protein modules found in diverse eukaryotes and have been shown to form polymers. The wild-type Shank 3 SAM domain is insoluble due to polymerization. To disrupt polymerization and solubilize the domain, point mutations were made at all conserved but potentially exposed sites. Four different mutants were found to be soluble and have been crystallized. Electron microscopy has shown that one N-terminal mutant forms fibers, suggesting that Shank SAM domains can polymerize at the neuron membrane. Polymerization of Shank would allow associated proteins to be clustered and thus communicate and to amplify intracellular signals.
Thanks to USPHS National Research Service Award GM07185.

Keywords: POLYMER, SIGNAL TRANSDUCTION, CRYSTALLOGRAPHY

Acta Cryst. (2002). A**58** (Supplement), C283

STRUCTURAL BASIS OF GATING BY THE OUTER MEMBRANE TRANSPORTER FecA

A.D. Ferguson[1] R. Chakraborty[2] B.S. Smith[1] L. Esser[3] D. van der Helm[4] J. Deisenhofer[1]
[1]Department of Biochemistry and the Howard Hughes Medical Institute, University of Texas Southwestern Medical Center Biochemistry 5323 Harry Hines Boulevard DALLAS TEXAS 75390-9050 USA [2]Department of Health Sciences, College of Public and Allied Health, East Tennessee State University, PO Box 70673, Johnson City, Tennessee 37614, USA [3]Laboratory of Cell Biology, NCI, National Institutes of Health, 37 Convent Drive, Bethesda, Maryland 20892, USA [4]Department of Biochemistry and Microbiology, University of Victoria, PO Box 3055, Victoria, British Columbia V8W 3P6 Canada

Siderophore-mediated acquisition systems facilitate iron uptake across the bacterial cell envelope. The crystallographic structure of the integral outer membrane transporter FecA from *Escherichia coli* with and without dinuclear ferric citrate has been determined at 2.5 and 2.0 Å resolution by multiple anomalous dispersion phasing. FecA is composed of three domains: a 22-stranded β-barrel, the plug, and the amino-terminal extension. Ferric citrate binding triggers a conformational change within the extracellular loops that close the external pocket of FecA. These data establish the structural basis of gating for this family of energy-dependent receptors, and suggest a four-stage siderophore transport mechanism.

Keywords: MEMBRANE PROTEIN TRANSPORTER IRON

Acta Cryst. (2002). A**58** (Supplement), C283

THE STRUCTURE ANALYSIS OF HUMAN MRP14, A CALCIUM-DEPENDENT REGULATOR PROTEIN IN INFLAMMATORY PROCESS

H. Itou[1] M. Yao[1] I. Fujita[1] N. Watanabe[1] M. Suzuki[2] J. Nishihira[2] I. Tanaka[1]
[1]Hokkaido University Division of Biological Sciences, Graduate School of Science Kita-Ku, Kita 10 Nishi 8 SAPPORO HOKKAIDO 060-0810 JAPAN [2]Central Reserch Institute, School of Medicine, Hokkaido University

Human MRPs (hMRP14 and hMRP8) are calcium-binding proteins from the S100 family of proteins. These proteins are co-expressed in myeloid cells, and play an indispensable role in calcium-dependent functions during inflammation. This role includes the activation of Mac-1, the β2 integrin which is involved in neutrophillic adhesion to endothelial cells. The crystal structure of holo hMRP8 had already been analyzed by our group, and we also succeeded in structure analysis of holo hMRP14 at 2.1 Å resolution. hMRP14 is distinguished from other S100 member proteins by its long C-terminal region. The structure analysis shows that this C-terminus is extensively flexible. In this crystal structure of hMRP14, CHAPS molecules bind to the hinge region that connects two EF-hand motifs, which suggests that this region is a target-binding site of this protein. Based on a structural comparison of hMRP14 with hMRP8 and human S100A12 (hS100A12) that is another homologue protein, the character of MRP8/14 hetero-complex and the functional significance of the flexibility of the C-terminal region of hMRP14 are discussed. MRPs alter their functions depending on their different formation of dimers; homo- or hetero-dimer. It has been proposed that this functional change of MRPs plays important roles in inflammatory process. Both homo-dimer structures are now available, and the structure analysis of hetero-dimer is indispensable for further understanding of MRPs. Recently we succeeded in obtaining the crystal of this MRP8/14 hetero-complex, which diffracted to 3.3 Å resolution. Sample preparation and crystallization experiments for improving the crystal are now in progress.

Keywords: CALCIUM-BINDING PROTEIN,HUMAN MRP14,S100 PROTEIN

Acta Cryst. (2002). A**58** (Supplement), C283

CRYSTAL STRUCTURES OF NK1-HEPARIN COMPLEXES REVEAL THE BASIS FOR NK1 ACTIVITY AND ENABLE ENGINEERING OF POTENT AGONISTS OF THE MET RECEPTOR

D. Lietha[1] D. Y. Chirgadze[2] M. Mulloy[3] T. L. Blundell[2] E. Gherardi[1]
[1]MRC Centre Growth Factors Group Hills Road CAMBRIDGE CB2 2QH UK [2]Department of Biochemistry, University of Cambridge, UK [3]NIBSC, South Mimms, UK

Hepatocyte growth factor/scatter factor (HGF/SF) and its splice variant NK1 are plasminogen related growth factors. NK1 consists of the N-terminal (N) and the first kringle (K) domain and requires heparan sulphate or heparin for activity. Both growth factors and their receptor MET play major roles in development and when mis-expressed can lead to tumour growth and metastasis. Agonists are sought for use in tissue regeneration and wound healing, whereas antagonists would provide important drugs for cancer therapy. We have solved two X-ray crystal structures of NK1-heparin complexes at 2.3 and 3.0 Å resolution. The structures reveal an NK1-dimer and define a main heparin-binding site in the N domain and further contacts between heparin and the K domain. Biochemical analysis of mutant NK1 shows that residues in the N domain binding site are required for oligomerisation, whereas residues in the K domain are not. Biological assays reveal that the N domain mutants lack biological activity, but mutation of K domain residues that contact heparin yields NK1 variants with increased activity. We uncover a complex role for heparan sulphate where binding to the N domain is essential for oligomerisation and activity, whereas binding to the K domain leads to reduced activity and we exploit the K domain interaction in order to engineer potent receptor agonists. We further suggest that dual (positive and negative) control may be a general mechanism for heparan sulphate-dependent regulation of growth factor activity and lend support to the hypothesis that the NK1 dimer originates by domain swapping.

Keywords: HEPATOCYTE GROWTH FACTOR NK1 PROTEIN ENGINEERING