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Two staphylococcal sialic acid binding proteins from the superantigen superfamily

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Staphylococcus aureus is a significant human pathogen. Among its large repertoire of secreted toxins is a group of staphylococcal superantigen-like proteins (SSLs). These are homologous with superantigens (SAgs) but do not have the same activity. We have shown that two of these proteins, SSL5 and SSL11, bind to human granulocytes and to the cell surface receptors for human IgA (FcaRI) and P-selectin (PSGL-1) in a sialic acid-dependent manner. We co-crystallized both SSL5 and SSL11 with the tetrasaccharide sialyl-Lewis X (sLeX), a key determinant of PSGL-1 binding to P-selectin. The structures of the SSL5-sLeX and SSL11-sLeX complexes were refined at resolutions of 1.65 Å and 1.6 Å, respectively. Both SSLs share the same SAg-like fold and both show a similar tendency to form dimeric structures in the crystal. In both complex structures, sLeX binds to a specific site on the surface of the C-terminal domain, in a conformation identical to that bound by P-selectin. Conservation of the key carbohydrate-binding residues indicates that this ability to bind human glycans is shared by a substantial sub-group of the SSLs. This indicates that the ability to target human glycans is an important property of this group of toxins. Structural comparisons also show that the sialic acid binding site in SSL5 contains a substructure that is shared by other sialic acid binding proteins from bacteria and viruses and represents a common binding motif.

Keywords: superantigen-like toxins, microbial pathogenesis, staphylococcus aureus

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Crystal structure of the extracellular protease of the nosocomial pathogen stenotrophomonas maltoph

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Stenotrophomonas maltophilia is a multiresistant pathogen increasingly emerging in the hospital environment. In immunosuppressed patients these bacteria may cause severe infections associated with tissue lesions like pulmonary haemorrhage. This indicated proteolysis as a possible pathogenic mechanism in these infections. Indeed, a new protease with broad specificity has been found to be secreted by S. maltophilia [Windhorst et al., J Biol Chem 277, 11042]. The gene, termed StmPr1, was analyzed; it codes for a 63 kDa precursor, which is processed to the mature protein of 47 kDa. The enzyme is an alkaline serine protease which, by sequence homology and enzymic properties, can be further classified as a new member of the family of subtilases. However biochemical studies with the purified protein indicated that the StmPr1 protease differs substantially from so far known subtilisins in molecular size and in substrate specificity. In order to gain insights into the structure-function-relationship the StmPr1 gene was overexpressed in E. coli and processed to the active enzyme. Crystals of the native and inhibited enzyme could be grown after the protease was allowed to truncate from the C-terminus to a 36 kDa active core protein. Diffraction data sets of the native and inhibited enzyme were collected to 1.5 Angstrom resolution applying synchrotron-radiation, and the 3D-structure was solved. Compared to known baterial proteases it revealed a new overall fold and architecture of the active site. The high resolution structure can serve to develop specific agents for a targeted therapy of St. maltophilia infections.

Keywords: crystal structure, bacterial, protease

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Crystal structure of a sigma28-regulated non-flagella virulence protein *from campylobacter jejuni*

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Campylobacter jejuni, a Gram-negative motile bacterium, is a leading cause of human gastrointestinal infections. The mechanism of C. jejuni mediated enteritis appears to be multi-factorial. The flagella of C. jejuni play complex roles in virulence of this human pathogen. Of note, in the absence of a specialized type III secretion system, the C. jejuni flagella filament secretes several non-flagellar proteins to the extracellular milieu, some of which are involved in virulence. Cj0977 is a recently identified virulence factor in C. jejuni, and is expressed by a sigma28 promoter which controls late genes in the flagellar regulon. A Cj0977 mutant was fully motile but significantly reduced in invasion of intestinal epithelial cells in vitro. In an effort to gain insight into the structural basis of C. jejuni virulence, we set out to solve the crystal structure of Cj0977. The crystal structure reveals that Cj0977 adopts the 'hot-dog' fold, which is characterized by 6 beta-stranded antiparallel beta-sheet wrapping around an alpha-helix. The characteristic hot-dog fold proteins are found in certain coenzyme A compound binding proteins with numerous oligomeric states. Structural comparison with other known hot-dog fold proteins suggest a possible link between virulence and membrane lipid biosynthesis. This work was supported by Robert A. Welch Foundation grant # E-1616 to H.J.Y.

Keywords: bacterial pathogenesis, hot-dog fold, virulence

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Allostery in p38-docking-peptide interaction-an NMR study

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MAP kinases respond to a wide variety of extracellular stimuli. These are first phosphorylated by MAP/ERK kinase kinase (MAP2K) and then the MAP kinases in turn phosphorylate specific nuclear transcription factors. These events are mediated by docking peptides, which are remote from the phosphorylation site in substrates or active site of the activators. Earlier work from our laboratory on the crystal structures of p38 with docking peptides from substrate MEF2A and activator MKK3B (Chang et al., 2002); and ERK2 with a similar peptide derived from a phosphatase (Zhou et al., 2006) showed conformational changes local to the binding site. In addition, large unexpected allosteric changes in the active site were seen in both p38 and ERK2. Recently Vogtherr et. al (Vogtherr et al., 2005) assigned 64% of the backbone and Cb shifts for p38. To see the relevance of the allostery observed in crystal structure, we conducted N15 NMR studies on p38 with docking peptides derived from MEF2A and MKK3b. Residues distal from the binding site showed moderate chemical shifts indicating docking and allostery are present in solution and is a mechanism by which specificity is achieved in these kinases.
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Zhou, T., Sun, L., Humphreys, J., and Goldsmith, E. J. (2006). Docking interactions induce exposure of activation loop in the MAP kinase ERK2. Structure 14, 1011-1019.

Keywords: allostery, MAP kinases, NMR

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Time-resolved X-ray crystallography captures transition-state-like intermediate in PYP photocycle

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Since the geometrical isomerization within the chromophore pocket of a protein is spatially restricted via media constraints such as hydrogen bonding network and confined space, the usual one-bond-flip mechanism observed in the gas and solution phases no longer holds and instead the volume-conversing mechanism has been hypothesized. Previous studies on photoactive yellow protein (PYP) predicted that a twisted form of chromophore by volume-conversing model in picoseconds regime, but its exact 3D structure has been elusive. Here we report the earliest intermediate structure (IT) of trans-cis isomerization in PYP and detailed atomic motions by picosecond X-ray crystallography. The IT intermediate is distorted such that the planarity of chromophore is broken while all three original hydrogen bonds are still intact, and resembles a theoretically predicted transition-state. Hydrogen bonds networking make this distorted structure stable as an intermediate rather than a transition state detectable with time-resolved crystallography. The carbonyl oxygen of IT is along the pathway connecting the ground state to the next intermediates, ICT and pR1, via the bicycle-pedal mechanism and hula-twist mechanism, respectively.

Keywords: time-resolved crystallography, time-resolved Laue diffraction, reaction mechanisms

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Slow ligand migration dynamics in carbonmonoxy myoglobin at cryogenic temperature

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Myoglobin (Mb) is a small globular heam protein in muscle, which reversibly binds ligands at the heam iron site deeply inside the protein matrix. This ligand dissociation can be triggered by photo irradiation [1]. The ligand binding reaction in Mb has been studied by a variety of techniques. Photolysis of carbonmonoxy myoglobin (MbCO) has been extensively studied by X-ray diffraction experiments (e.g. Laue diffraction experiments at room temperature [2,3] or monochromatic X-ray diffraction experiments at cryogenic temperature [4]). In spite of lots of known details regarding the gas ligand molecules trapped in internal cavities of Mb, there exists no direct evidence to show the migration pathways connecting these cavities. In order to explore the ligand migration pathways in myoglobin induced by ligand dissosiation, we have carried out cryogenic X-ray crystallographic investigations of carbonmonoxy myoglobin (native sperm whale MbCO) crystals illuminated by a laser. Slow ligand migration in Mb was observed at the cryogenic temperatures.
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Keywords: myoglobin, time-resolved X-ray diffraction, ligand binding

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Crystal structure of FlgD from Xanthomonas: Insights into the hook capping for flagellar assembly

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Structural genomics is crucial for understanding the intricate interactions among proteins in a whole organism. We have studied the structural genomics of Xanthomonas campestris (Xcc), a gram-negative bacterium that is phytopathogenic to cruciferous plants and causes worldwide agricultural loss. Xcc is the only bacterium known to lack a cAMP signaling system, and uses a cAMP-receptor protein like protein (CLP) system instead. Currently we are working on its flagellar structural genomics. The first crystal structure of a hook-capping protein FlgD of a microbial flagellum from the plant pathogen Xanthomonas campestris has been determined to a resolution of 2.5 Å; crystallography. The monomer comprises 221 amino acids with a MW of 22.7kD, but the disordered N-terminus