MS7-O3 Structural studies of medically-interesting protease inhibitors and lectins that belong to the β-trefoil family

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Several related proteins that belong to the β-trefoil family have been investigated by X-ray crystallography as well as by biochemical and biophysical techniques. Two of them are potent inhibitors of trypsin-related enzymes. EcTI, isolated from the seeds of *Enterolobium contortisiliquum*, inhibits the invasion of gastric cancer cells through alterations in integrin-dependent cell-signaling pathway. BbKI, found in *Bauhinia bauhinioides* seeds, is a kallikrein inhibitor with a reactive site sequence similar to that of kinins, the vasoactive peptides inserted in kininogen moieties. A much weaker protease inhibitor isolated from the bark of *Crataeva tapia* tree (CrataBL) also functions as a lectin. The amino acids sequence of CGL, a lectin isolated from the sea mussel *Crenomytilus grayanus*, is significantly different from the other three proteins.

We determined high-resolution crystal structures of free EcTI and in complex with bovine trypsin, in the process re-determining the amino acid sequence. Modeling of the putative complexes of EcTI with several serine proteases and a comparison with equivalent models for other Kunitz inhibitors elucidated the structural basis for the fine differences in their specificity. The structure of free BbKI indicated that the presence of disulfide bonds is not necessary for stabilization of the fold of the members of this family. A model of a complex of BbKI with plasma kallikrein indicates the need for mutual rearrangement of the interacting molecules.

We have also determined the high-resolution crystal structure of glycosylated CrataBL. We have shown that, as a lectin, CrataBL binds only sulfated oligosaccharides, most likely heparin and its derivatives.

CGL displays antibacterial, antifungal, and antiviral activities, and displays high affinity for mucin-type receptors, abundant on some cancer cells. We determined its crystal structure and modeled the glycan-binding pockets, based on the location of the glycerol molecules bound in the three sites exhibiting quasi-threefold symmetry.

Keywords: protease inhibitors, lectins, crystal structures

MS7-O4 Structure-function characterization reveals catalytic diversity in the galactose oxidase family

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Auxiliary Activity 5 family (AA5; http://www.cazy.org) comprises the well-studied glyoxal oxidase (AA5_1) and galactose oxidase (AA5_2) subfamilies. Despite all the known biochemical characterization, only a few structures are available for these copper radical oxidases. These enzymes employ molecular oxygen as a terminal electron acceptor to generate hydrogen peroxide (believed to be coupled to lignolytic peroxidases), independently of an organic cofactor (1,2) for their catalytic activity on galactose. This has increased the biological interest in the context of recalcitrant plant degradation by fungal saprotrophs and phytopathogens (2,3). With a combination of spectroscopic, crystallographic and biochemical studies, here we report the discovery of a new fungal AA5_2 from *Colletotrichum graminicola* (*Cgr*AlcOx). This enzyme, in contrast with its homologue, the fungal *Fusarium graminearum* galactose oxidase (*Fgr*GalOx), shows poor oxidative capability on galactose, but efficiently catalyses the oxidation of aliphatic alcohols (4,5).

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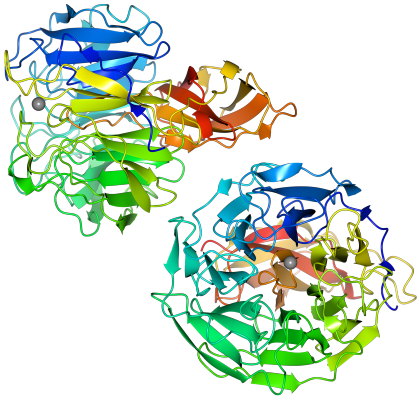

Figure 1. Lateral and rear view of the *Fgr*AlcOx structure in complex with copper (pdb 5C92). The N terminus (blue to yellow) consists of seven Kelch motifs arranged in a β-propeller, enclosing the copper-binding site. The C terminus (orange to red) displays a nine-stranded β-barrel.

Keywords: Copper, EPR, eukaryotic expression, deglycosylation

MS7-O5 Structure of the factor H-sialic acid complex links sialic acid recognition to atypical hemolytic uremic syndrome and reveals a novel conformation of the GM1 glycan

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Complement factor H (FH) ensures down-regulation of the complement alternative pathway upon interaction with specific glycans on host cells, protecting the latter from attack by the host's complement system. There is long-standing evidence that the ubiquitous mammalian glycan cap sialic acid acts as such a self marker to FH, but the low affinity of the interaction and the structural diversity of sialylated glycans have hampered structural and functional investigations. Using ligand-based NMR spectroscopy we defined the FH sialoligosaccharide specificity and found that FH binds to glycans that spot Neu5Acα(2-3)Gal caps. A previously reported crystal form of the two FH C-terminal domains 19 and 20 (1), which contain the sialic acid binding site, were refractory to glycan soaking. Co-crystallization attempts with the GM3 glycan yielded another unliganded crystal form of FH 19-20. Inspection of the protein packing in these two crystals forms prompted us to co- crystallize FH 19-20 with another FH ligand, the thioester domain (TED) of C3b, yielding FH19-20/C3b-TED crystals (2) that were successfully soaked with the GM3 and GM1 glycans. The atomic (2.2 Å resolution) structures of the ternary complexes suggest that sialic acid and the C3b TED together recruit FH to self-cells to which C3b attaches covalently by virtue of its reactive thioester bond - initiating local degradation of C3b on host cells that are covered with sialic acid (3). The structures revealed that numerous FH residues linked to atypical hemolytic uremic syndrome (aHUS), a rare hereditary disease that can lead to kidney failure, cluster in the sialic acid binding site, and their functional impairment with respect to sialic acid binding has since been confirmed (4). Our structures lend an etiological model to aHUS and also bear implications for complement evasion strategies by bacterial pathogens that cover themselves in host-derived sialic acid or recruit FH directly via its sialic acid binding site. Additionally, the complex structure with the GM1 glycan revealed a previously unreported GM1 conformation that escaped NMR-restrained modeling in the past but that is evident in the electron density and was confirmed by molecular dynamics simulations in the 10 μs time scale (5). (1) Jokiranta T.S. et al, EMBO J. 2006. (2) Morgan H.P. et al, Nat Struct. Mol.Biol, 2011. (3) Blaum B.S. et al, Nat. Chem. Biol. 2015. (4) Hyvärinen S. et al, Blood 2016. (5) Blaum B.S. et al., Glycobiology 2016.

Keywords: crystallography, glycobiology, ganglioside, NMR spectroscopy, innate immunity, molecular dynamics