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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