MS5-P43 Structure and function of lytic polysaccharide monooxygenases

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The drive for energy security and environmental sustainability has led to the development of biofuels derived from waste biomass such as lignocellulose, chitin etc., generically known as second generation biofuels. However, the degradation of these substrates is difficult due to their high complexity and recalcitrance. Classic glycoside hydrolases (GHs) can achieve this degradation, but their efficiency is limited. A major recent breakthrough has been the discovery of novel mononuclear Cu oxygenases termed "lytic polysaccharide monooxygenases" (LPMOs)[1] that oxidatively open-up the crystalline biomass rendering it accessible to GH action. LPMOs are found in a number of sequence-based families (www.cazy.org) where they generically termed "Auxiliary Activities" and are classified into families AA9, AA10, AA11 and AA13. Recent structural insight has revealed that the majority of structures present a flat surface, where catalysis takes place at the Cu centre. I shall present details of new LPMO structures, demonstrate how these enzymes produce aldonic acid products and show how photo-reduction in the synchrotron X-ray beam complicates electron density interpretation.

[1]Hemsworth *et al.*,: Recent insights into copper-containing lytic polysaccharide mono-oxygenases. Curr. Opin. Struct. Biol. 2013, 23, 660–668.

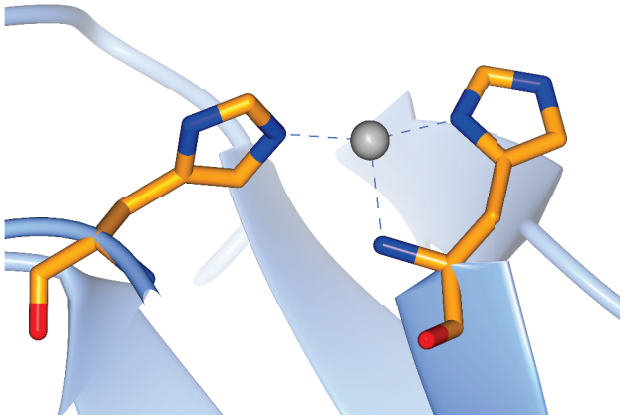

Figure 1. Histidine brace. The copper is coordinated by the imidazole and main-chain amino group of the N-terminal histidine and a further histidine imidazole group. The metal displays a Cu (I) coordination in the crystal structure (in contrast with the EPR results), due to radiation damage.

Keywords: Lytic polysaccharide monooxygenases, LPMO, copper-dependent oxygenase

MS5-P44 Structural basis of the peptidoglycan binding to LytA, the major pneumococcal autolysin

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Autolysins are bacterial cell wall hydrolytic enzymes that mediate antibiotic induced lysis and may function as important virulence factors for bacterial pathogens. The cell wall of S.pneumoniae is a complex three-dimensional network of peptidoglycan (PG) and teichoic acids which protect the cells against mechanical stress and internal osmotic pressure and serves as a scaffold for many associated proteins that modulate the cell wall activity or interacts with the environment. The major autolysin of S.pneumoniae, LytA breaks the PG by hydrolyzing the amide bond between glycan chains and peptide stems. LytA associates with the cell wall through its C-terminal choline-binding domain by non-covalent interactions with phosphocholine moieties of the pneumococcal teichoic acids.

We present the three-dimensional structure of the major autolysin LytA of Streptococcus pneumoniae. The crystal structure of two separate LytA domains combined with small angle X-ray scattering of the full length protein reveal that LytA homodimer adopts a cherry-like conformation with two catalytic domains located far from each other, in trans configuration relatively to the plane of the two C-terminal choline-binding domains.

The 1.05Å crystal structure of the catalytic domain exhibits a prominent Y-shaped binding crevice that can accommodate a peptidoglycan fragment of four/five saccharides and a stem pentapeptide. The active site contains a zinc ion bound to two histidine residues and one aspartate, located at the bottom of the branch point of the crevice. Co-crystallization of the inactive mutant of the LytA catalytic domain and a peptidoglycan fragment produced crystals of the complex diffracting to the same resolution as substrate-free LytA. This allowed us to model more than 100 atoms of the peptidoglycan fragment. The structure displays the specific conformation of four sugars in the active site of autolysin different from any other peptidoglycan fragment. The importance of protein-sugar interactions far from the catalytic site was confirmed by mutational studies. We hypothesize that substrate requirements restrict LytA to the sites on the cell wall where nascent peptidoglycan synthesis occurs.

Keywords: Peptidoglycan, autolysis