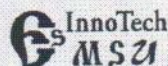


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Lytic polysaccharide monooxygenases: structure, mechanism and function

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Lytic polysaccharide monooxygenases (LPMOs) are metal-dependent enzymes that catalyze the oxidative cleavage of various polysaccharides. The oxidative nature of LPMOs has been discovered in 2010-2011, although these enzymes had previously been known as proteins of the CBM33 and GH61 families in the Carbohydrate Active Enzymes (CAZy) database. LPMOs preferentially acting on cellulose are recently reclassified into families 9 and 16 of Auxiliary Activities (AA9 and AA16) in the CAZy database, while those acting on other polysaccharides (chitin, xylans, starch, etc.) are the members of the AA10, AA11, AA13, AA14 and AA15 families. All known LPMOs share similar structure of the active site that contains a copper ion coordinated by the histidine brace; the first of two conserved histidines represents the N-terminal residue of the mature protein. LPMOs cleave a polysaccharide chain with oxidation occurring either at C1 or C4 atom of the glycoside ring with formation of aldonic acid or 4-keto-aldose, respectively; the third group of these enzymes generate products oxidized at both C1 and C4 position. In the first studies, it was believed that molecular oxygen is involved in the formation of the reactive intermediate with copper. More recently, hydrogen peroxide, rather than O₂, has been suggested to be a true co-substrate for LPMOs, while the latest studies indicate that both O₂ and H₂O₂ can be utilized by LPMOs as co-substrates. Cellulose-oxidizing LPMOs have attracted the attention of researchers as enhancers of hydrolytic performance of cellulases in the enzymatic saccharification of cellulose, a key step in the growing biotechnology for production of second-generation biofuels and chemicals from renewable lignocellulosic biomass.

The characteristic features of AA9 family LPMOs from fungi *Trichoderma reesei*, *Thielavia terrestris*, *Myceliophthora thermophila* and *Penicillium verruculosum* will be discussed in this report, covering such properties as the enzyme activity based on the recently developed highly-sensitive fluorimetric assay of the oxygen consumption rate or an alternative assay based on using H₂O₂ as a co-substrate; the influence of pH and effectors on the LPMO activity; enzyme thermostability; a synergism between LPMOs and individual cellulases or their multienzyme cocktails in saccharification of cellulosic and lignocellulosic substrates. The role of a cellulose-binding module (CBM) in LPMO functioning will also be discussed, and the CBM effects on the activity and substrate specificity of a chimeric LPMO, obtained using a protein engineering technique, will be demonstrated.