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The framework of polysaccharide monooxygenase structure and chemistry

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Polysaccharide monooxygenases, or PMOs (also known as lytic PMOs or LPMOs), are a group of enzymes discovered in recent years to catalyze the oxidative degradation of carbohydrate polymers. The PMO catalytic domain has a β-sandwich fold that bears a strong resemblance to both immunoglobulin (Ig) and fibronectin type III (FnIII) domains. PMOs are secreted by fungi and bacteria, and there is recent evidence for their roles in pathogenesis, in addition to biomass processing. This review addresses the biological origins and functions of emerging PMO families, as well as describes the aspects of PMO structure that support the chemistry of copper-catalyzed, oxidative polysaccharide degradation.

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Introduction

Polysaccharide monooxygenases, or PMOs, are copper-dependent enzymes that utilize molecular oxygen to cleave glycosidic bonds. In recent years, they have been discovered as secreted enzymes in fungi and bacteria, where they are used in the conversion of polymeric carbohydrates to monomeric sugars utilizable as a carbon source. Within the past year, PMOs have been implicated in the infection cycles of microbial pathogens and viruses, suggesting an array of biological functions. Biochemical and genetic studies have informed on the activities and functions of these enzymes; spectroscopic and computational investigations have provided insight into the properties of the copper center and proposed reaction mechanisms. Nevertheless, it has been the role of structural studies, particularly early PMO structural studies, to uncover the novelty of this enzyme family (Table 1).

Early PMO structures [1–3], even those with a non-functional metal bound, exposed an unexpected framework for catalysis — a flat active site tendered by various loops spread from a compact and rigid *beta* core. These structures and subsequent ones with the functional copper in place [4] implied a new activity, namely that of oxidative polysaccharide depolymerization. Continued structural study of fungal and bacterial PMOs [5–9,10••,11•] provided important foundations for probing function and mechanism in these 'early' families. With the latest structural appearances of PMOs with novel substrates or origins [12•,13•,14], and perhaps biological functions [15], the landscape of PMO action is diversifying, and there is much on the horizon.

A superfamily of PMOs

PMOs were first classified into glycoside hydrolase family 61 (GH61) and carbohydrate-binding module family 33 (CBM33), for fungal (cellulose-active) and bacterial (chitin-active) enzymes, respectively. With the finding that these domains catalyzed a copper-dependent oxidative reaction [4,17], the name polysaccharide monooxygenase, or PMO, was adopted, and the GH61 and CBM33 enzymes were reclassified in the Carbohydrate-Active enZyme (CAZy) database as Auxiliary Activity families 9 and 10 (AA9 and AA10), respectively [18]. Some investigators then began using the names lytic PMO or LPMO. It was discovered that many AA10 enzymes could oxidize cellulose, either exclusively or in addition to chitin [10••,19]. Fungal PMOs active on chitin [12•] and starch [20•] were reported and classified respectively as AA11 and AA13. Activity toward hemicelluloses was found in an AA9 PMO from *Neurospora crassa* [21]; this is not strictly a new activity as oxygen insertion still occurs at a β-1,4-glycosidic bond, as in cellulose. This was also the only PMO known to cleave soluble cellooligosaccharides until the recent characterization of an ortholog [22].

In the past year, studies have emerged linking AA10 PMOs to bacterial pathogenesis. Two enzymes, GbpA and lmo2467, had previously been described as virulence factors for *Vibrio cholerae* and *Listeria monocytogenes*, respectively [23,24] and have in the past year been characterized as chitin-active PMOs [25••,26••]. Putative PMOs have also been implicated in virulence associated with *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Enterococcus faecelis* [27]. A predicted PMO was recently implicated

in viral pathogenesis: Chiu *et al.* reported the structure of fusolin [16••], a viral spindle protein found in insect poxviruses that had been classified as AA10 in CAZy along with gp37 from baculoviruses. Both gp37 and fusolin have been previously found to bind to purified chitin [28,29]. PMO activity has yet to be demonstrated; however, fusolin mutants with disrupted metal-binding sites do not enhance virulence [28].

Phylogenetic analyses have yet to elucidate many of the evolutionary relationships among PMO families (Figure 1); such studies are challenging due to low amino-acid homology [30••]. Book *et al.* have proposed that AA9 and AA10 families share an ancient ancestral protein and that current selection pressures are driving the expansion of cellulolytic capabilities in AA10 proteins [31•]. Intense competition between fungi and bacteria for the same ecological niches could have fostered the spread of PMO genes; although horizontal gene transfer in eukaryotes is not well understood, transfers between bacteria and fungi have been known to occur [32]. The discovery of PMOs in viruses suggests additional vectors for PMO gene transmission.

The PMO domain

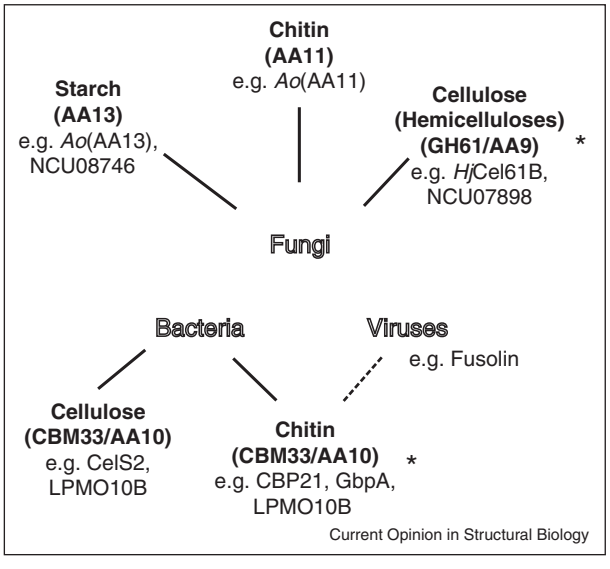
The catalytic domain of PMOs resembles both fibronectin type III (FnIII) and immunoglobulin (Ig) domains (Figure 2a). Both are β-sandwich modules of 80–100 amino acids forming seven to nine anti-parallel β-strands with Greek key motifs [33,34]. Their common jelly roll fold is widely distributed in all life forms and regularly found in extracellular proteins [35]. Protected hydrophobic cores, inter-strand backbone hydrogen bonds, and the presence of disulfide bridges (in the case of Ig domains) contribute to structural stability. *Beta*-hairpins and longer loops conjoin adjacent strands; these are perhaps most notable as the complementarity determining regions (CDRs) in antibodies and T-cell receptors.

The PMO domain is a similarly stable β-sandwich of seven to nine β-strands, but displays longer and more variable loop regions (Figure 2a). PMO domains usually comprise 200–250 amino acids, but this residue total depends on cumulative loop length. A small bacterial PMO has been reported at only ~15 kDa; homology modeling suggests abbreviated loop features limit its size [36]. Like the Ig fold, the PMO fold contains disulfide bonds, typically two or three. Scattered throughout the β-sandwich interior are conserved tyrosine and tryptophan side chains, similar to those found in the hydrophobic core of FnIII domains [34]. In PMOs these electron transfer (ET)-competent residues have ostensible roles in protein-mediated ET.

The PMO reaction involves two separate, one-electron reductions; in fungi, ET to PMOs occurs via a large, multi-domain heme protein, cellobiose dehydrogenase (CDH) [17]. Because active-site access is restricted when bound to substrate, long-range ET is probably required to funnel electrons from a remote face of the PMO to the copper center. During a catalytic cycle, the Cu(II) center must undergo reduction to Cu(I) before activating O_2; the enzyme then, in some order, performs hydrogen-atom abstraction (HAA) and oxygen insertion on the substrate C–H bond, as well as reduces the second oxygen atom to H_2O. If HAA and oxygen insertion occur before the second reduction, direct ET (implied by one docking result [37]) would require the PMO and substrate to bind and dissociate in between the two reduction events. Even if the second reduction occurs before HAA, direct ET over multiple catalytic cycles would still require the PMO to travel back and forth between substrate and redox partner.

Fungal PMOs contain putative ET pathways unique to each family; fungal PMOs that oxidize C4 in cellulose may contain a second conserved ET path [7,12•,13•,30••]. ET pathways for bacterial PMOs are not yet clear, and bacteria lack a CDH, although other prospective redox partners are under study [30••,38]. The C-terminal loop (LC) in AA9 PMOs has been suggested as a docking site for CDH [7]; however, CDH reductively activates AA13 PMOs as well [20•], which lack both this motif and a C-terminal loop. More experiments are needed to elucidate the mechanism of ET from CDH to PMO.

Figure 1



Schematic of PMO families identified to date, grouped by source organism and substrate specificity with domain annotations in parentheses. A subgroup of cellulose-active fungal PMOs cleaves β-1,4-glycosidic bonds in hemicelluloses. Some bacterial PMOs are active on both cellulose and chitin. Viral fusolins are classified by sequence and structure homology; chitin activity has not yet been confirmed. Families that contain PMOs implicated in pathogenesis (in addition to those associated with biomass processing) are marked with an asterisk.