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Review

Copper active site in polysaccharide monooxygenases

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ABSTRACT

The increasing demand for the next generation of renewable fuels has driven extensive research in carbohydrate-active enzymes involved in converting plant biomass to fermentable sugars. Standing out of new enzymes discovered recently are polysaccharide monooxygenases (PMOs) that activate O_2 with a Type 2 mono copper active site for the regioselective hydroxylation of the glycosidc linkages. This hydroxylation leads to the cleavage of glycosidic linkage on the substrate surfaces, creating new chain ends on the substrate surface that can be further processed by canonical glycoside hydrolases. PMOs can thus significantly boost the activity of industrial cellulases and have great potentials in the biofuel industry. Extensive research in the last 8 years have shed lights into the nature of the copper active site and how it activates O_2. While other aspects of PMOs have been thoroughly discussed in recent outstanding reviews, the coordination chemistry aspects of the copper active site have not been reviewed in details. This article will provide in-depth analysis of the copper active site and surrounding residues, including structure, electronic and spectroscopic properties, copper-binding affinity, redox properties, modulation of geometry and activity by second-sphere residues, substrate binding affinity, O_2 intermediates, and initial mechanistic insights, as well as some relevant model complexes. This review will also identify some key areas that need further work and predict the trend in PMOs research in the coming years.

Contents

Abbreviations: AA10, auxiliary activity family 10, cellulose- and/or chitin-active bacterial PMO; AA11, auxiliary activity family 11, chitin-active fungal PMO; AA13, auxiliary activity family 13, starch-active fungal PMO; AA9, Auxiliary activity family 9, cellulose-active fungal PMO; AAQO, aryl-alcohol quinone oxidase; BDE, bond dissociation energy; CAZy, carbohydrate active enzyme; CBH, cellobiose hydrolase; CBM33, carbohydrate binding module family 33; CBP21, Chitin binding module family 21; CDH, cellobiose dehydrogenase; Cel3, cellotriose; Cel6, cellohexaose; CW EPR, continuous wave EPR; dSpy, distorted square pyramid; DβM, dopamine β-monooxygenase; EPR, electron magnetic resonance spectroscopy; EXAFS, Extended X-ray Absorption Fine Structure; FAD, flavin adenine dinucleotide; GDH, glucose dehydrogenase; GH, glycoside hydrolase; GH61, glycoside hydrolase family 6; GOX, glucose oxidase; H-bonding, hydrogen bonding; His1, N-terminal histidine residue of PMO that coordinates the copper center; HisN, another histidine residue coordinating the copper center; HRP, horseradish peroxidase; ITC, isothermal titration calorimetry; *Kd*, dissociation constant; LPMO, lytic polysaccharide monooxygenase; MM, molecular mechanics; NMR, nuclear magnetic resonance spectroscopy; PASC, phosphoric acid swollen cellulose; PDB ID, protein data bank identification; PHM, peptidylglycine α-hydroxylating monooxygenase; PMO, polysaccharide monooxygenase; QM, quantum mechanics; Sbpy, square bipyramid; SHE, standard hydrogen electrode; Spy, square pyramid; Ss, seesaw; Tbpy, trigonal bipyramid; XANES, X-ray Absorption Near Edge Structure; XAS, X-ray absorption spectroscopy; XG14, a mixture of xyloglucan oligomers dominated by XXXGXXXG species where G and X are glucose and glucose with a xylose substitution, respectively; XG225, xyloglucan from tamarind seed with average M.W. of 225 kDa; XRD, X-ray diffraction crystallography.

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1. Introduction

The rapidly increasing demand for the second generation renewable fuels stimulated extensive research on carbohydrate-active enzymes in the past decade. Among a large number of new enzymes discovered in this period, polysaccharide monooxygenases (PMOs) attract the most attention from both industrial and academic sectors. On the practical level, it has been shown that combining cellulose-active PMOs with cellulases could reduce the total amount of enzymes to achieve the same hydrolysis efficiency by 2–3 folds, which could significantly reduce the cost of biomass conversion to biofuels [1,2]. Some cellulase mixtures containing PMOs are now commercially available. On the fundamental level, PMOs have brought whole new perspectives in polysaccharide metabolism in particular, and in biology in general. Since the discovery of the oxidative activity on chitin of chitin binding protein 21 (CBP21) [3] and the significant synergy with cellulase of some enzymes belonging to the glycoside hydrolase (GH) family 61 [2] in 2010, the last 8 years witnessed the remarkable growth of research on PMOs. Thus far, there are four distinct families of PMOs, which are also known as lytic polysaccharide monooxygenases (LPMOs) and have been classified into four families of Auxiliary Activity enzymes in the CAZy database (Fig. 1A) [4]. AA9 family members are fungal PMOs that act on cellulose and hemicellulose containing $\beta(1 \rightarrow 4)$ linkages of glucose [2,5–7] and glucose derivative units [8–10]. AA10 PMOs are bacterial enzymes that oxidize either chitin [3] or cellulose [11]. AA11 and AA13 are fungal PMOs oxidizing chitin [12] and starch [13,14], respectively. The terms LPMO9 and LPMO10 are also used in literatures to denote AA9 and AA10 PMOs, respectively. Previously, AA9 and AA10 family members were annotated as GH61 and CBM33, respectively.

The four PMO families are highly diverse in sequence but share many structural similarities including the conserved beta-sandwich hydrophobic core and an absolutely conserved Type-2 mono-copper active site on a solvent-exposed and relatively flat protein surface (Fig. 1B). Bioinformatic analyses using the active site features first found in AA9 and AA10 PMOs have led to the discoveries of AA11 [12] and AA13 [13] families that have almost no sequence similarities to AA9 and AA10 PMOs, emphasizing the importance of this active site throughout evolution. The solvent-exposed active site surface allows for the direct action of PMOs on the glycosidic linkages (Fig. 1C) on the surface of the insoluble substrates. Thus, it is presumed that PMOs do not need to separate individual polysaccharide chains from the substrate, a slow step required by canonical GHs [15]. It is generally accepted that the copper active site activates dioxygen and hydroxylates either C—H bond of the glycosidic linkage, forming an unstable intermediate that undergoes spontaneous elimination (Fig. 1D) [6]. Consequently, the glycosidic linkage is cleaved, generating new chain ends that can be processed by exo-acting GHs.

Three types of AA9 PMOs have been characterized using bioinformatic and biochemical analyses (Fig. 1A) [16]. Type 1 and Type 2 AA9 PMOs exclusively hydroxylate C1 and C4 positions of the glycosidic linkage, respectively; while Type 3 AA9 PMOs act on both positions (Fig. 1D). Similarly, AA10 PMOs are classified in two several subfamilies, including the well characterized chitin-active and cellulose-active subfamilies (Fig. 1D) [17]. To date, chitin-active AA10 PMOs have been shown to oxidize only C1 position (herein designated as Type1A and Type 1B AA10, Fig. 1A). Cellulose-active AA10 PMOs either act at C1 position only (Type 2 AA10) or at both C1 and C4 position (Type 3 AA10) [18]. The only AA11 PMO characterized thus far has been shown to oxidize C1 position in chitin, while mass spectrometry analyses also hinted at the formation of C4-oxidized product [12]. Several AA13 PMOs characterized to date have been shown to act only on C1 position of starch [13,14]. Table 1 lists the PMOs discussed in this review with respect to their name, classification, substrate specificity, and origin. This table will help the readers avoid the confusion of PMO naming variations in the literature. Most of these PMOs are structurally and/or spectroscopically characterized. First members of PMO families or subgroups that are well characterized biochemically are also included.

There are many excellent reviews published recently discussing many aspects of PMOs [18,38–49], including structure, mechanism, substrate specificity and regioselectivity, evolution, physiological functions, applications, etc. However, the coordination chemistry aspects of the active site in PMOs, which play a key role in the PMO reaction, have not been adequately reviewed. In this review, we will provide an in-depth analysis of the copper active site in PMOs and surrounding residues with connections to PMO reaction where possible. Inner sphere geometry, electronic properties, redox properties, and second sphere residues all contribute to governing how the copper center binds O_2 and substrate, as well as how it activates O_2 for substrate oxidation. Along this line, we will