Cellulose breakdown. Insights into how fungal enzymes break down crystalline cellulose fibers may be useful for industrial processes. The false-color SEM image shows cellulose fibers in a paper towel. Each fiber is about 10 to 15 μm wide.

CHEMISTRY

How to break down crystalline cellulose

Biochemical and genomic data elucidate how a fungal enzyme attacks polysaccharides

By **Angel T. Martínez**

Biomass-degrading microorganisms use lytic polysaccharide monooxygenase (LPMO) enzymes to help digest cellulose, chitin, and starch. By cleaving otherwise inaccessible crystalline cellulose chains, these enzymes provide access to hydrolytic enzymes. LPMOs are of interest to biotechnology because efficient depolymerization of cellulose is a major bottleneck for the production of biologically based chemicals and fuels. On page 1098 of this issue, Kracher *et al.* (*1*) compare LPMO-reducing substrates in fungi from different taxonomic groups and lifestyles, based on both biochemical and genomic evidence. The results provide insights into reductive activation of LPMO that are important for developing more efficient industrial enzymes for lignocellulose biorefineries.

Early studies (*2*) on microbial cellulose degradation proposed a two-component system, in which a first, unidentified component (C_1) helps to overcome the polysaccharide crystallinity and a second component (C_x) corresponds to the glycoside hydrolases (cellulases) that were discovered at the time. Sixty years passed between these studies and the description of the missing enzyme in cellulose degradation: fungal and bacterial copper-containing LPMOs that act on crystalline polysaccharides (*3*–*5*). Addition of ascorbic acid enabled demonstration of the monooxygenase activity of these enzymes and the associated breakdown of cellulose and chitin chains (*5*). However, to understand the reaction mechanism and better use it industrially, scientists had to identify the natural LPMO reductant(s).

Since then, Phillips *et al.* have shown that another redox enzyme, cellobiose dehydrogenase (CDH), acts as a source of electrons for LPMOs (see the figure) (*6*). CDH is an oxidoreductase that oxidizes cellobiose at its flavin domain and reduces LPMOs at its heme domain after intramolecular electron transfer (*7*). CDH is likely to have a physiological role in activating fungal LPMOs, given that its electron-transfer rate is orders of magnitude faster than that of other reductants. Furthermore, Kracher *et al.* show that the two enzymes are more often found together in the genomes of white-rot, soft-rot, and plant pathogenic fungi than in the genomes of fungi with no or a limited number of cellulose-hydrolyzing enzymes.

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Other LPMO reductants in species lacking CDH are substituted phenols from lignin degradation or present as plant extractives. However, extractives, such as gallic acid, would be scarce in decayed lignocellulose. Moreover, even if phenols and quinones are among lignin-degradation products, a continuous supply is required to fully support LPMOs. Such supply would be considerably easier if these compounds were recycled by a chemical or enzymatic mechanism.

Chemical recycling has been reported in a reaction including LPMO oxidation of low–molecular mass phenols, and lignin reduction of the phenoxy radicals formed (*8*). However, Kracher *et al*. show that only phenols with very low redox potential (such as hydroquinones) can be oxidized by the enzyme; the radicals formed in these reactions are not expected to be strong lignin oxidizers. In contrast, enzymatic redox cycling of quinones is fully operative in the presence of glucose dehydrogenase (GDH). Both GDH and CDH are members of the glucose-methanol-choline (GMC) oxidoreductase superfamily, which also includes other quinone-reducing enzymes. As Kracher *et al*. show, the enzymatic mechanism for LPMO activation involving GDH is very efficient.

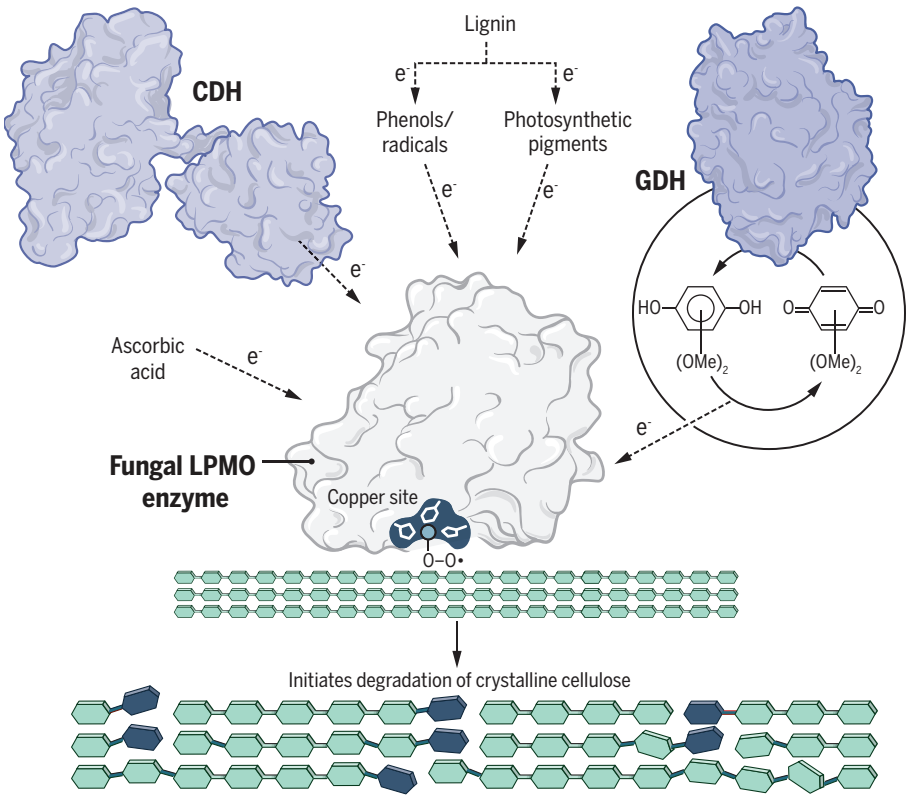
Photosynthetic pigments, once activated by light, can also transfer electrons to LPMOs and be reduced back by lignin preparations (*9*). The environmental relevance of light-driven cellulose degradation by LPMOs remains unclear, but the reaction is of applied interest because it proceeds 100 times faster than with standard reductants.

However, several questions about LPMO remain. First, although polysaccharide oxygenation takes place directly at the copper site, no agreement exists about the Cu(II) reduction site and mechanism. Several studies suggest a long-range electron transfer pathway (*10*), but others have suggested direct reducing-substrate oxidation at the same copper site (*7*). Moreover, although a Cu(II)-superoxide radical complex is formed after O_2 activation by Cu(I) (*10*), it is not clear whether this is the main catalytic species or whether a more reactive Cu(II)-oxyl radical is formed in a later step, as suggested by some molecular dynamics studies.

Second, the diversity of LPMOs remains little explored. The number of identified LPMOs in genomes has increased exponentially since 2010, with 328 genes in the family of cellulose-cleaving fungal LPMOs, 1840 genes of chitin- and cellulose-cleaving bacterial LPMOs, 66 genes of chitin-cleaving fungal LPMOs, and 14 genes of starch-cleaving fungal LPMOs. This diversity shows that LPMOs are very ancient enzymes and offers a lot of potential for biotechnology. Biochemical studies must explore expected differences in catalytic and kinetic properties between these LPMO families.

Finally, biotechnology applications of these oxidoreductases are in their infancy. The enzyme industrial sector was strongly involved in the discovery and characterization of both LPMOs that act on cellulose (*3, 4*) and starch (*11*). Since 2012, several commercial cellulase preparations have included LPMOs (*12*), resulting in improved hydrolyzability and reduced cellulose crystallinity (*13*). These cocktails now need to be formulated for every raw material, process, and application, considering both the benefit from lowering the cellulase dosage and the concomitant formation of nonfermentable oxidized sugars.

On the basis of evidence provided by Kracher *et al*., we can conclude that lignocellulose-degrading fungi developed different lifestyle-related mechanisms for electron supply to LPMO. These mechanisms operate continuously and involve oxidation of simple sugars (in the cases of CDH and GDH) or lignin (in the cases of phenols and pigments). In this way, cellulose and other polysaccharides are efficiently depolymerized to monosaccharides by the combined action of LPMOs and glycoside hydrolases. Kracher *et al*. also show that GMC oxidoreductases contribute directly (CDH) or through quinone redox cycling (GMC dehydrogenases) to activating LPMO for cellulose depolymerization. These findings reveal unexpected connections between polysaccharide and lignin biodegradation in plant materials. ■



Fuel for cellulose degradation. LPMOs are key enzymes in crystalline cellulose degradation. LPMO activity was first detected with ascorbic acid as an electron source. Since then, several natural electron sources have been identified, including enzymes (such as CDH), simple phenols, and even light-activated photosynthetic pigments. The electrons reduce a catalytic copper ion, enabling LPMO to activate O_2 by forming a reactive Cu(I)-superoxide. The latter initiates degradation by breaking down cellulose chains and lowering crystallinity. Kracher *et al*. compare the above electron sources for the enzyme and show that quinone redox cycling by GMC oxidoreductase (such as GDH) is a particularly efficient LPMO activation mechanism.

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10.1126/science.aaf8920

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Angel T. Martínez (May 26, 2016)
Science **352** (6289), 1050-1051. [doi: 10.1126/science.aaf8920]

Editor's Summary

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.