Laccases: Complex Architectures for One-Electron Oxidations

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Abstract—Laccase (p-diphenol:dioxygen oxidoreductase), one of the earliest discovered enzymes, contains four copper ions in two active sites and catalyzes the one-electron oxidation of substrates such as phenols and their derivatives, or aromatic amines. This oxidation is coupled to the four-electron reduction of dioxygen to water. The catalytic mechanism has been studied for decades but is still not completely elucidated, especially in terms of the reduction of dioxygen to water. The key structural features of this enzyme are under investigation in several groups using techniques such as X-ray diffraction, electron paramagnetic resonance (EPR) spectroscopy, and site-directed mutagenesis. The high interest in laccases is explained by the large number of biotechnological applications. In this review, the most recent research on the overall structural features as well as on the structures and properties of the active sites are summarized, along with currently proposed mechanisms of reaction.

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Laccase (p-diphenol:dioxygen oxidoreductase, EC 1.10.3.2), one of the earliest discovered enzymes, is an oxidoreductase that contains four copper ions in two active sites and catalyzes the one-electron oxidation of substrates such as phenols and their derivatives, or aromatic amines. This oxidation is coupled to the four-electron reduction of dioxygen to water.

Laccases occur mostly in the kingdom of fungi [1-8], but they have also been found in numerous plants [1, 9-11], prokaryotes [12-18], and insects [19]. Their physiological roles are diverse and include lignin degradation in white-rot fungi [20], lignin biosynthesis [21] in plant cell walls, as well as other polymerization processes that are a good inspiration in biotechnology [22], plant defense [1], fungal pathogenesis [23-28] by metabolizing phytoalexins and other mechanisms, morphogenesis and copper homeostasis [16, 29, 30], and sclerotization of the cuticle in insects [31].

Laccases display remarkably broad substrate selectivity, being able to oxidize numerous substrates such as polyphenols, polyamines, α-, p-diphenols, aminophenols, aryl diamines, and ferrocyanide as well as some inorganic ions [32, 33]. This feature makes them valuable tools in biotechnological domains such as bioremediation of industrial dyes and pesticides, paper industry, ethanol production, wine industry, cosmetics, textile industry, biocatalysis, and bioanalytics. Excellent reviews on the occurrence, biochemical properties, and applications of laccases are available [34-39].

Typical laccases have four copper ions distributed in two sites that are denoted according to their spectral and structural features. Substrate oxidation takes place at the mononuclear type 1 center (T1), while oxygen reduction occurs at the tri-nuclear site composed of a type 2 (T2) and a type 3 (T3) pair of copper ions [40, 41].

OVERALL STRUCTURE

Architectural features of laccases. The three-dimensional structure of laccases has many similar architectural features with other multicopper oxidases such as ascorbate oxidase (AO), copper-dependent nitrite reductase (NR), and ceruloplasmin (CER) [16, 42]. The first solved laccase structure was from Coprinus cinereus [42], which lacks the T2-Cu center. Now there are numerous solved laccase structures from eighteen different organisms including Bacillus subtilis, Melanocarpus albomyces, Escherichia coli, Coriolopsis gallica, Trametes versicolor, Coprinus cinereus, Rigidoporus lignosus, Cerrena maxima, Trametes trogii, Coriolus zonatus, and Lentinus tigrinus.

The overall structure of the laccases is characterized by the existence of three distinct domains: A (N-terminal), B, and C (also designated as I, II, III, or 1, 2, 3) as exemplified by B. subtilis laccase in Fig. 1a. Each of the three domains displays a similar Greek key β-barrel...
architecture known as cupredoxin-like fold, with two β-sheets, each composed of four strands arranged in a sandwich topology [44]. Usually, the structure is stabilized by one or two disulfide bridges between domains A and B. The tri-nuclear center is placed between domains 1 and 3, while the type 1 center is found in domain C (C-terminal). Domain 2 does not participate directly in the formation of the active site; it most likely contributes to the stability of the protein assembly as a functional unit. In contrast to fungal laccases, bacterial laccases, such as *B. subtilis* laccase (CotA), exhibit a large loop segment (residues 341-368) that connects domains 2 and 3 (Fig. 1a) [49]. This external connection between domains 2 and 3 is also present in the CueO laccase from *E. coli*, while in fungal laccases this link is made through an internal connection. Another different feature for the CueO and CotA laccases is a distinct coiled segment formed by twenty N-terminal residues connecting the A and B domains [16]. This section, which provides hydrogen bonds allowing stabilization of the interface between domains 1 and 2 together with the external connection loop, appeared to have a noticeable similarity between the overall folds of the bacterial enzymes CotA and CueO. The CotA laccase also has a distinctive feature formed by a protruding segment composed of a short α-helix and a loop, which form a lid-like structure near the substrate-binding site (Fig. 1a). This segment was considered to be involved in substrate binding or the assembly of CotA into the spore coat [49].

Analyzing the primary structure of hundreds of laccases of all types (fungal, bacterial, plant, and insect origins) by multiple sequence alignment has led to identification of four sequence regions that have no gaps. By marking the four regions (R1-R4) in the 3D structures of any structure-solved laccase, a more or less C2-symmetric protein conformational motif can be observed that characterizes the active site apparatus of the enzymes [50]. The relative orientation of R1 and R2 is similar to that of R3 and R4, despite the fact that the sequence and structure homology is poor in the rest of the protein. However, many of the residues belonging to the four regions but not involved in the copper ligation could be critical in forming a specific and almost C2-symmetric protein conformational motif [51].

**The C-terminus in asco-laccases.** Most fungal laccases studied so far come from the basidiomycete group (especially white-rot fungi, which have excellent lignin degradation activity). Several ascomycete fungal laccases have been also purified and characterized from organisms such as *Botrytis cinerea* [52], *Trichoderma atroviride*, *Trichoderma haryianum* [53], *Paraconiothyrium variabile* [54], *Neurospora crassa* [55], Cryphonectria parasitica [56], and Sclerotinia sclerotiorum [57]. However, for only two ascomycete fungal laccases have the three-dimensional structures been solved: for *M. albomyces* [58] and very recently for *Thielavia arenaria* [59]. A processing of the C-terminus that blocks the tri-nuclear center solvent...