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MULTI-AUTHOR REVIEW

Laccases: old enzymes with a promising future

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In the last decades, the use of enzymes for environmental-friendly industrial processes has more and more increased, due to advancements in biotechnology, especially in the area of genetics and protein engineering, resulting not only in new products, but also in the development of new industrial processes for existing products. Laccases (benzenediol:oxygen oxidoreductases, EC 1.10.3.2) are enzymes of great relevance both as a model for structure/function relationships and as a green tool in many processes of the biotechnology industries. This is mainly due to their very broad substrate range and the fact that they only require oxygen for catalysis. In this multi-author review, the most recent studies on laccases structural features and catalytic mechanism along with analyses of potentiality of their applications are reported and examined.

A laccase was first discovered in the sap of the Japanese lacquer tree *Rhus vernicifera* in 1883 by H. Yoshida (Yoshida H., 1883, Chemistry of lacquer (urushi). J Chem Soc 43:472–486). Since then, laccases have been found in various basidiomycetous and ascomycetous fungi and, thus far, fungal laccases have accounted for the most important group of multi-copperoxidases (MCOs) with respect to number and extent of characterization. Laccases contain four copper atoms/molecule, distributed in three different copper-binding sites. Structure and organization of laccase copper-binding sites is shared by other multi-copper proteins, which have physiological roles not apparently related to phenol oxidase activity (copper tolerance, iron transport, and sporulation). Indeed, among prokaryotic MCOs, metalloxidases show a robust activity toward metals, and are involved in the metal metabolism of the microorganisms. Moreover, many laccases from prokaryotes, with a superior efficiency for oxidation of organic compounds when compared with metals, have also been identified and characterized (L. O. Martins, P. Durão, V. Brissos and P. F. Lindley; Laccases of prokaryotic origin—enzymes at the interface of protein science and protein technology).

In fungi, laccases carry out a variety of physiological roles including morphogenesis, plant pathogen/host interaction, stress defense, and lignin degradation. The majority of the fungal laccases are extracellular monomeric globular proteins of approximately 60–70 kDa with an acidic isoelectric point around pH 4.0; they are generally glycosylated, with an extent of glycosylation ranging between 10 and 25 %.

Albeit laccases have been studied for decades to rationalize their structure–function relationships, still the complex mechanism of the reaction remains a subject of debate.

Structural information on laccases is based on the crystal structures of some native laccases and of complexes between laccases and their reducing substrate. Crystal structures give valuable insights into the functional mechanisms and are the basis for the development of laccases for industrial applications (N. Hakulinen and J. Rouvinen; Three-dimensional structures of laccases). Numerous efforts to understand their reaction mechanism have been also made using site-directed mutagenesis, directed evolution and, more recently, computational approaches. (I. Pardo and S. Camarero; Laccase engineering by rational and evolutionary design). Substitutions on amino acids located in the substrate binding pocket or in the vicinity of the catalytic copper ions can affect enzyme activity. However, directed evolution studies have demonstrated

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that the overall enzyme activity is also affected by beneficial mutations located far from these sites. The advances made in the knowledge of laccase structure–function relationships can in turn facilitate the rational design of laccases.

The main contribution to the spectroscopic characterization of the copper sites was from Solomon's group (S.M. Jones, E.I. Solomon; Electron Transfer and Reaction Mechanism of Laccases). They have studied the electron transfer process in laccases, and the mechanism of O_2 reduction through spectroscopic, kinetic, and computational data. The mechanism of O_2 reaction is based on the intermediates formed during the two $2e^-$ reduction steps. The first $2e^-$ step forms the peroxide intermediate, followed by the second $2e^-$ step to form the native intermediate, which is the catalytically relevant fully oxidized form of the enzyme. The nature of the catalytic site and radical species formed has been also explored by a combined multifrequency EPR/computational approach described by Pogni et al. (R. Pogni, M.C. Baratto, A. Sinicropi, R. Basosi; Spectroscopic and computational characterization of laccases and their substrate radical intermediates).

The most recent and emerging trends of laccase uses and applications in textile and food fields, in pharmaceutical, and cosmetic industry, as well as examples concerning polymer synthesis and laccase catalyzed-grafting have been described (C. Pezzella, L. Guarino, A. Piscitelli; How to enjoy laccases). In these fields, rational and evolutionary design can guide the engineering of recombinant biocatalysts better suited for target biotransformations under defined operational conditions. The development of laccase-based biocathodes for bioelectrocatalytic oxygen reduction and the use of advanced electrode materials are described in a separated review, where the uses of laccase electrodes for in vivo application of biofuel cells are emphasized (Le Goff, M. Holzinger and S. Cosnier; Recent progress in oxygen-reducing laccase biocathodes for enzymatic biofuel cells). Laccases are old enzymes with a promising future. As a fact, the survey of the recent literature collected in this MAR emphasizes how the increased knowledge around this class of enzymes boosts their concrete exploitation in different industrial fields encouraging further research on this fascinating topic.