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Research review paper

Recent developments and applications of immobilized laccase

María Fernández-Fernández, M. Ángeles Sanromán, Diego Moldes *

Department of Chemical Engineering, University of Vigo, Lagoas Marcosende s/n, E-36310 Vigo, Spain

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ABSTRACT

Laccase is a promising biocatalyst with many possible applications, including bioremediation, chemical synthesis, biobleaching of paper pulp, biosensing, textile finishing and wine stabilization. The immobilization of enzymes offers several improvements for enzyme applications because the storage and operational stabilities are frequently enhanced. Moreover, the reusability of immobilized enzymes represents a great advantage compared with free enzymes. In this work, we discuss the different methodologies of enzyme immobilization that have been reported for laccases, such as adsorption, entrapment, encapsulation, covalent binding and self-immobilization. The applications of laccase immobilized by the aforementioned methodologies are presented, paying special attention to recent approaches regarding environmental applications and electrobiochemistry.

Contents

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Abbreviations: (EPR), Electron paramagnetic resonance; (LMSs), Laccase mediator systems; (ITO), Indium tin oxide; (GLU), Glutaraldehyde; (NHS), N-hydroxysuccinimide; (NPG), Nanoporous gold; (EDC), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; (PHEMAH), Poly(hydroxymethylmethacrylate-n-methacryloly-(l)-histidine-methylester); (LDH), Layered double hydroxide; (SAM), Self-assembled monolayer; (RR), Resonance Raman; (SERS), Surface-enhanced Raman scattering; (LbL), Layer by layer; (MCM), Mobil composition of matter; (CNS), Cyano-modified silica; (SBA-15), Santa Barbara amorphous; (PS), Polystyrene particles; (AAEM), β-Diketone groups; (PVAs), Poly(vinyl alcohol) cryogel particles; (CLECs), Cross-linked enzyme crystals; (CLEAs), Cross-linked enzyme aggregates; (HFBs), Hydrophobins; (PEI), Poly(ethyleneimine); (SGZ), Syringaldazine; (DMP), 2,6-Dimethoxyphenol; (PPD), *para*-phenylenediamine; (APTES), 3-Aminopropyltriethoxysilane; (MG), Methyl green; (RBBR), Remazol brilliant blue R; (PAH), Polyallylamine hydrochloride; (PSS), Polysodium 4-styrenesulfonate; (CPC-silica), Controlled-porosity carrier beads; (PEG), Polyethylene glycol; (ABTS), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); (semi-IPNs), Semi-interpenetrating networks; (MCFs), Mesostructured siliceous cellular foams; (PAHs), Polycyclic aromatic hydrocarbons; (BaP), Benzo[a]pyrene; (BPA), Bisphenol A; (DCP), Dichlorophenol; (TCP), 2,4,6-trichlorophenol; (CPs), Chlorophenols; (OMW), Olive oil mill wastewater; (TCS), Triclosan; (OMMs), Ordered mesoporous materials; (MWL), Milled wood lignin; (RKL), Residual kraft lignin; (CCE), Carbon ceramic electron; (GC), Glassy carbon electrode; (LDG), Low density graphite electrode; (DET), Direct electron transfer; (NTA), Nitriloacetic acid; (1-AP), 1-aminopyrene; (Pt), Platinum; (CC), Cyanuric chloride; (HQ), Hydroquinone; (HGA), Homogentisic acid; (CEPEI), Cetyl ethyl poly(ethyleneimine); (MB), Methylene blue; (AuNPs), Gold nanoparticles; (SPEs), Screen printed electrodes; (POMs), Polyoxometalates.

* Corresponding author at: Department of Chemical Engineering, Lagoas Marcosende s/n, 36310 Vigo, Spain. Tel.: +34 986 818723; fax: +34 986 812380.
E-mail addresses: diego@uvigo.es, diegomoldes@gmail.com (D. Moldes).

1. Laccases

Laccases (benzenediol:oxygen oxidoreductases, EC 1.10.3.2) belong to the group of blue oxidases and represent the largest subgroup of multicopper oxidases. These enzymes have been studied since the nineteenth century due to their ability to oxidize phenolic compounds, and their applications in several industrial sectors have been intensively studied as of late (Giardina et al., 2010; Loera et al., 2006; Madhavi and Lele, 2009; Morozova et al., 2007b).

1.1. Occurrence

Laccase was first discovered in the Japanese lacquer tree *Rhus vernicifera* (Giardina et al., 2010; Morozova et al., 2007b). Since then, these enzymes have been found in various plant species, insects and bacteria (Loera et al., 2006; Madhavi and Lele, 2009). However, the majority of laccases described in the literature have been isolated from higher fungi. These laccases occur in the fungal causative agents of soft rots, in most bracket fungi causing white rot, in soil saprotrophs, in plant pathogens and in many agarics, including cultivated edible fungi, e.g., champignon, *Pleurotus* and the medicinal shiitake *Lentinula edodes* (Morozova et al., 2007b). However, the most common laccase producers are nearly all wood-rotting fungi, such as *Trametes versicolor*, *Trametes hirsuta*, *Trametes ochracea*, *Trametes villosa*, *Trametes gallica*, *Cerrena maxima*, *Coriolopsis polyzona*, *Lentinus tigrinus* and *Pleurotus eryngii* (Madhavi and Lele, 2009; Morozova et al., 2007a). Additionally, laccases occur in saprophytic ascomycetes such as *Myceliophthora thermophila* and *Chaetomium thermophile*, which are involved in the humification of composts (Morozova et al., 2007b).

The best-known fungal laccases are extracellular proteins, but intracellular laccases have also been described. There are essentially three possible roles of fungal laccases: pigment formation, lignin degradation and detoxification (Loera et al., 2006).

1.2. Catalysis

Laccases have activity toward *ortho*- and *para*-diphenol groups, although their affinity is usually higher towards the latter group. These enzymes are characterized by their remarkably wide substrate specificity and a variable range of oxidizable substrates that depends on the organism producing them (Madhavi and Lele, 2009). Laccases catalyze the oxidation of a wide variety of substrates, including mono-, di-, and polyphenols, aminophenols, methoxyphenols, aromatic amines and ascorbate, with the concomitant four-electron reduction of oxygen to water (Giardina et al., 2010; Madhavi and Lele, 2009). These enzymes couple the four single-electron oxidations of the reducing substrate to the four-electron reductive cleavage of the dioxygen bond with four Cu atoms (Giardina et al., 2010). These copper atoms are classified into three groups depending on the characteristics obtained by UV/visible and electron paramagnetic resonance (EPR) spectroscopy. The type I copper (T1) is responsible for the intense blue color of the enzyme, has a strong electronic absorption approximately 600 nm and is EPR detectable. The type II copper (T2) is colorless but EPR detectable and the type III copper (T3) consists of a pair of copper atoms that give a weak absorbance near the UV spectrum and no EPR signal. The T2 and T3 copper atoms form a trinuclear cluster where the binding and multielectron reduction of dioxygen takes place (Durán et al., 2002; Madhavi and Lele, 2009).

The catalytic mechanism of the laccase enzyme starts with the donation of an electron to the substrate by the T1 copper site, followed by an internal electron transfer from the reduced T1 to the T2 and T3 copper site. The T3 copper functions as a two-electron acceptor in the aerobic oxidation process, in which the presence of the T2 copper is necessary. The reduction of oxygen to water takes place at the T2 and T3 cluster and passes through a peroxide intermediate (Durán et al., 2002; Madhavi and Lele, 2009; Morozova et al., 2007a).

Substrates with a high redox potential cannot be directly oxidized by laccases, and thus, the role of laccases in lignin biodegradation is restricted to the phenolic moieties. Laccase mediator systems (LMSs) have led to a dramatic increase in the range of laccase-oxidizable compounds (Madhavi and Lele, 2009; Morozova et al., 2007b). The so-called mediator compounds act as intermediate substrates and enable laccase to indirectly oxidize large molecules and even non-phenolic substrates (Giardina et al., 2010). An ideal redox mediator should be a good laccase substrate with stable oxidized and reduced forms and should not inhibit the enzymatic reaction. Mediator oxidation by laccase produces a high redox potential intermediate able to oxidize non-phenolic substrates. This intermediate compound is then reduced to restore its initial form and close the redox cycle (Morozova et al., 2007b). The LMSs have been successfully applied in different fields of biotechnology, such as paper pulp bleaching and delignification (Moldes et al., 2010), the degradation of polycyclic aromatic hydrocarbons (Gómez et al., 2006) and the decolorization of textile dyes (Moldes and Sanromán, 2006).

1.3. Applications

Laccases have great biotechnological potential due to their ability to oxidize a broad range of substrates that are employed in several industrial sectors. Their capacity to degrade phenolic compounds makes them appropriate for dye decolorization or the degradation of xenobiotics in the treatment of wastewaters. Electrobiochemistry has received increased attention during the last two decades. In this field, laccases have been employed for the design of biosensors, the detection of phenols in wastewaters or in food industry applications and the development of biofuel cells. Laccases have also been used in the pulp and paper industry for bleaching, delignification and for the production of novel paper products. Some potential applications are summarized in Table 1. Additionally, excellent reviews regarding laccase applications can be found in the literature (Riva, 2006; Rodríguez Couto and Toca Herrera, 2006).

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