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A review on mechanism of laccase action

Pankaj Kumar Chaurasia*, Rama Shanker Singh Yadav, Sudha Yadava

Department of Chemistry, D.D.U. Gorakhpur University, Gorakhpur- 273 009, (U.P.), (INDIA)

E-mail : pankaj.chaurasia31@gmail.com

ABSTRACT

The present review demonstrates, purely, the mechanism of laccase action on the different types of substrates as phenolic or non-phenolic substrates. Role of mediators in the oxidation of non-phenolic substrates by laccases are also described in this review. Since a large number of reviews and literatures on laccases are available to describe its different properties and biotechnological applications so its biotechnological applications are described only in brief.

KEYWORDS

Laccases;
Copper centres;
Mediators;
Wood rotting fungi.

INTRODUCTION

Laccase [benzenediol: oxygen oxidoreductase; E.C. 1.10.3.2] is a polyphenol oxidase, which belongs to the superfamily of multicopper oxidases[1,2] and catalyzes[3-5] the four electron reduction of molecular oxygen to water. Laccase is one of the few enzymes that have been studied since the end of 19th century. It was first demonstrated in the exudates of *Rhus vernicifera*, the Japanese lacquer tree[6] and subsequently was demonstrated as a fungal enzyme as well[7]. At present, there is only one bacterium, *Azospirillum lipoferum*, in which a laccase type phenol oxidase has been demonstrated[8].

Laccases are the lygnolytic enzymes and aboundantly occur in the fungal systems[4] mainly in ascomycetes, deuteromycetes and basidiomycetes. They occur in fungal causative agents of the soft rot, in most white rot causing fungi, soil saprophytes, and edible fungi. These laccase producing fungi are generally called wood degrading fungi. White rot fungi are the highest producers of the laccases but also litter decomposing and ectomicorrhizal fungi secrete laccases.

However, it should not be generalized that only the fungal system has ability to produce the laccases. Laccase production has never been demonstrated in lower fungi, that is,

Zygomycetes and Chytridiomycetes[9]. Several reports can be referred, in the literature on production of laccase in ascomycetes such as Gaeumannomyces graminis[10], Magnaporthe grisea[11], and Ophiostoma novo-ulmi[12], Mauginella[13], Melanocarpus albomyces[14], Monocillium indicum[15], Neurospora crassa[16], and Podospora anserina[17]. In addition to plant pathogenic species, laccase production was also reported for some soil ascomycete species from the genera Aspergillus, Curvularia and Penicillium[18–20], and in some freshwater ascomycetes[21].

MECHANISM OF LACCASE ACTION AND ROLE OF MEDIATORS

Laccase only attacks the phenolic compounds leading to Cα oxidation, Cα-Cβ cleavage and aryl-alkyl cleavage. Laccases, typically, contain three types of copper, one of which gives it its characteristic blue colour. Similar enzymes lacking the Cu atom responsible for

the blue colour are called 'yellow' or 'white' laccases, but several authors do not regard them as true laccases. Also to perform their catalytic functions, laccases depend on Cu atoms that are distributed at the three different copper centres[22] Figure 1 viz. Type-1 or blue copper centre, Type-2 or normal copper centre and Type-3 or coupled binuclear copper centres, differing in their characteristics electronic paramagnetic resonance (EPR) signals[23,24]. The organic substrate is oxidized by one electron at the active site of the laccase generating a reaction radical which further reacts non- enzymatically. The electron is received at type1 Cu and is shuttled to the trinuclear cluster where oxygen is reduced to water.

It has been proposed that the mononuclear copper type1 function as the primary electron accepter extracting electron from the reducing substrate and delivering it to the trinuclear site where oxygen is reduced to water and the oxidized form of the enzyme is regenerated Figure 2.

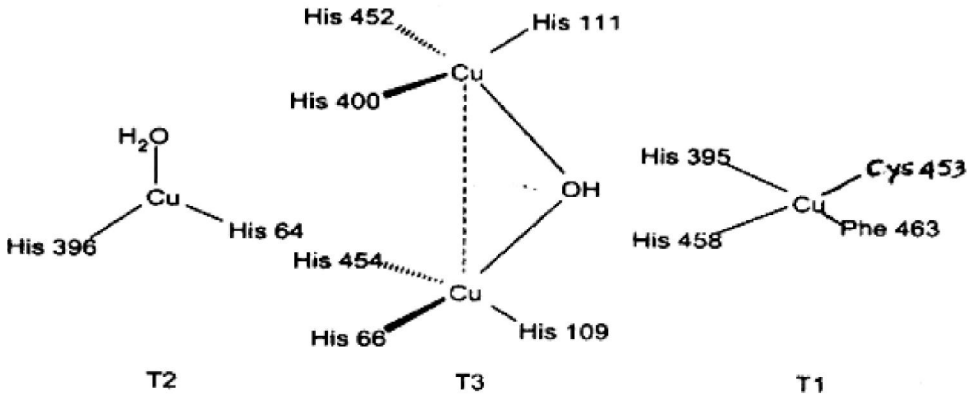

Figure 1 : Three different types of copper centres in laccase to perform their catalytic function.

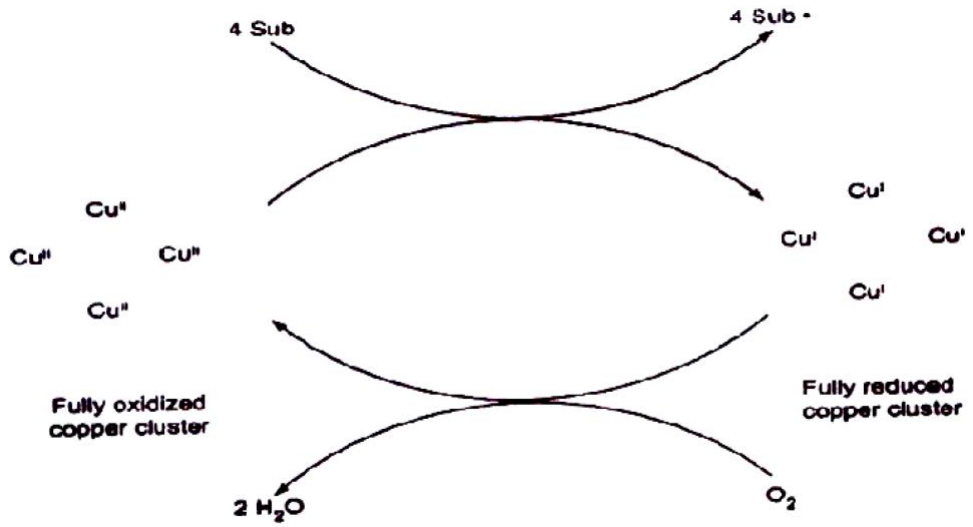

Figure 2 : Schematic representation of fully reduced and fully oxidized copper cluster during the reaction of laccase with substrate.

The substrate range of laccase can be extended to non-phenolic subunits by the inclusion of mediators also known as enhancers because its use enhances the catalytic performance of laccases to a very large extent. After the discovery of high redox potential mediators, laccases become much more significant in the area of several biotechnological applications. The well known mediators are ABTS (2,2' [Azino-bis-(3-ethylbonzthiazoline-6-sulphonic acid) diammonium salt]) and HOBT (1-hydroxybenzotriazole). An ideal mediator is that which can perform many cycles without degradations and without any side reactions. The oxidized mediator formed during the course of laccase catalyzed reaction can oxidize non-phenolic substrate, non-enzymatically. The difference between the redox potentials of the substrate to be oxidized and T1 copper ions is the driving force of the reaction. Mediators are a group of low molecular weight compounds with high redox

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potential (above 900 mV) which have ability to enhance the catalytic activity of laccases and that acts as a sort of 'electron shuttle': once it is oxidized by the enzyme generating a strongly oxidizing intermediate that is known as co-mediator or oxidized mediator, it diffuses away from the enzymatic pocket and in turn oxidizes any substrate that, due to its size could not directly enter into the active site. Due to its large size and steric hindrance, enzyme and polymer do not have to interact in a direct manner, then, the use of mediators allow the oxidation of polymers by side-stepping the inherent steric hindrance problems[25]. Alternatively, the oxidized mediator could rely on an oxidation mechanism not available to the enzyme, thereby extending the range of substrates accessible to it[26]. It is therefore of primary importance to understand the nature of the reaction mechanism operating in the oxidation of a substrate by the oxidized mediator species derived from the corresponding mediator investigated. In the laccase-dependent oxidation of non-phenolic substrates, previous evidence suggests an electron-transfer mechanism with mediator ABTS, towards substrates having a low oxidation potential. Alternatively, a radical hydrogen atom transfer route may operate with N-OH type mediators, if weak C-H bonds are present in the substrate[27].

OXIDATION OF ABTS BY LACCASE

Most commonly used mediators are the ABTS and the triazole 1-hydroxybenzotriazole (HOBT)[28-30]. ABTS is the best organic redox mediator. Its use for oxidation of non-phenolic lignin structures gave impetus to search for new laccase mediators. It was generally believed that ABTS oxidized to ABTS cation radical by removal of its one electron during the course of its enzymatic reaction, responsible for the oxidation of non-phenolic substrates but now it has been cleared after the spectrochemical and electrochemical studies that ABTS undergoes two step oxidation reaction during the course of it enzymatic oxidation reaction by laccase i.e. firstly convert into the ABTS cation radical and then undergoes its slow oxidation into ABTS dication as following way: various laccases readily oxidize ABTS, by free radicals, to the cation radical $ABTS^{+\cdot}$ Figure 3(a) and the concentration of the intensely colored, green-blue cation radical can be correlated to the enzyme activity ($\varepsilon 418 = 36000\ M^{-1}cm^{-1}$). It is well known that cation radicals represent an intermediate oxidation step in the redox cycle of azines and, upon extended oxidation and abstraction of the second electron, the corresponding dications can be obtained Figure 3(b). These cation radical and dication play role in the oxidation of the substrates, non-enzymatically. The redox potentials of $ABTS^{+\cdot}$ and $ABTS^{2+}$ were evaluated as 0.680 V and 1.09 V respectively[31].

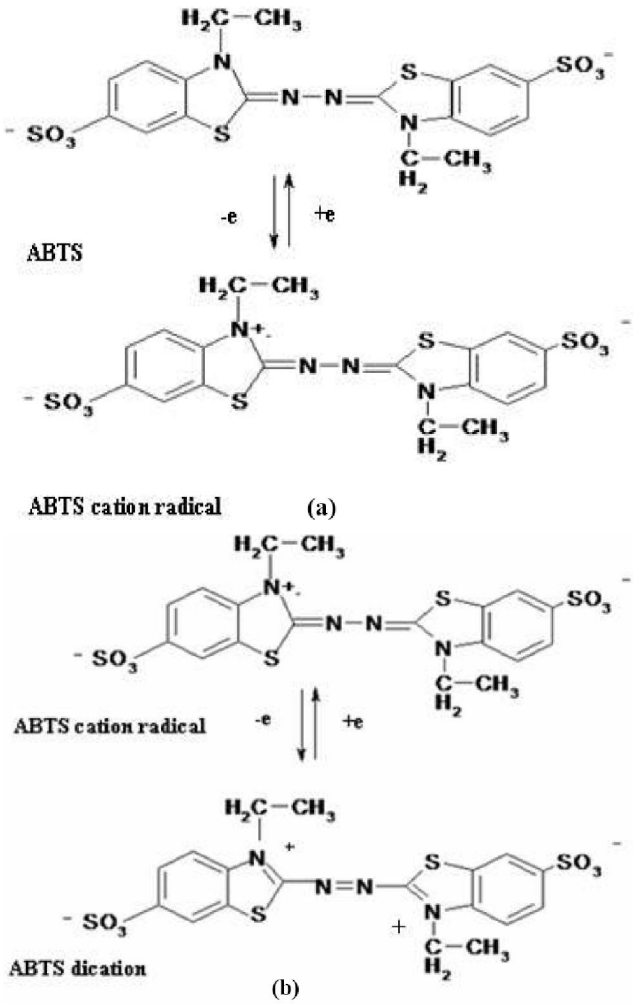

Figure 3 : Oxidation of ABTS by laccase to ABTS cation radical (a) and ABTS dication (b) to perform the oxidation reaction of substrates.

OXIDATION OF HOBT BY LACCASE

HOBT is another important organic redox mediator. HOBT belongs to the N-heterocyclic coumpounds bearing N–OH groups mediators[32]. Consuming oxygen HOBT is converted by the enzyme into the active intermediate, which is oxidized to a reactive radical (R–NO.) and HOBT redox potential has been estimated as 1.1-1.2 V[33].

OXIDATION OF TMPO BY LACCASE

2,2,6,6-tetramethyl-1-piperidinyloxyl (TMPO) is also an important organic redox mediator. TMPO is more efficient than ABTS, HOBT, or the natural laccase mediator 3-hydroxyanthranilic acid. TMPO mediator is present in the solution in the form of a relatively stable N-oxyl radical, which can perform primary modification of some high potential substrates even without laccase[34]. Laccase oxidizes TMPO to produce the oxoammonium ion, which reacts with the substrate. Proton removal yields the oxidized product and the reduced form of TMPO and this reduced form is converted to the oxidized form by laccase and then to oxo-ammonium ion[34].

Other organic redox-mediators are N-hydroxyphthalamide (NHPI), and inorganic redox-mediators are iron complexes with o-phenanthroline and 4,4-dimethylbipyridine etc. These have high redox potential and can perform multiple catalytic events without chemical degradations[34,35]. Transition metal complexes have very high redox potential in comparison to the organic mediators and have also the strong tendency to oxidize non-phenolic lignin substrates directly in reaction mixture, but its use is very costly and environmentally unsafe, due to which it is restricted to the industrial use.

The different steps involve in the general oxidation reaction done by laccase with the help of mediator molecules and without the help of mediator molecules are shown in Figure 4(a-b). Figure 4(a) clearly demonstrates that the oxidation of non-phenolic substrates can be achieved in the presence of mediator molecules. Laccase firstly oxidizes the mediator molecule and then this oxidized mediator oxidizes the non-phenolic substrate. Figure 4(b) represents the direct oxidation of substrate (phenolic) without the help of any type of mediators. All the above mechanistic discussions show the burning biotechnological and several synthetic importances of laccases.

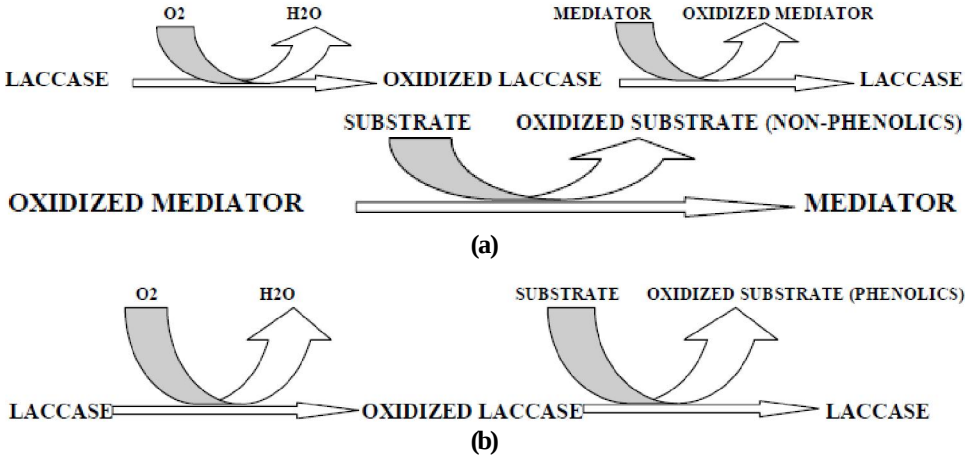

Figure 4 : Schematic representation of the role of laccase in oxidation of different types of substrates- non-phenolic substrate (a) and phenolic substrate (b).

APPLICATIONS

The ability of laccases to catalyze the oxidation of various phenolic as well as non-phenolic compounds, coupled to the reduction of molecular oxygen to water makes it valuable from the point of view of their commercial applications[4,36-38]. The biotechnological importance of laccases have increased after the discovery that oxidizable reaction substrate range could be further extended in the presence of small readily oxidizable molecules called mediators[39,40]. During the last two decades, laccases have turned out to be the most promising enzymes for industrial uses[37,38] having applications in food, pulps, paper, textile, and cosmetics industries and in synthetic organic chemistry[41-44]. Besides the above described applications of laccases, laccase are also too much valuable in medicinal synthesis such as in penicillin synthesis[45], anticancer drugs synthesis[46,47] etc.

CONCLUSIONS

The main purpose of this review is to study the mechanism of laccase action and study of applications and the synthetic importance of laccases is out of scope

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of this review. In order to explain how much a laccase can be effective in the biotechnological applications and other several applications, authors mainly focused their attention on to provide better knowledge of laccase action mechanism and the study of mechanism of laccase action, as described above, clearly demonstrates the importance of laccases in the area of biotechnological applications. Oxidizing properties of the laccase make it a very effective biocatalyst.

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