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Peroxidase, an Example of Enzymes with Numerous Applications

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The enzyme peroxidase is a heme or iron-porphyrin protein that belongs to a large family of enzymes called the oxidoreductases. Their function mainly is to oxidize molecules at the expense of hydrogen peroxide. They are widely distributed in living organisms, and usually show dramatic colour-product formation as a result of their catalytic effect. They generally catalyse many oxygen transfer reactions involving hydrogen peroxide or anyone of the many other peroxides as electron acceptors and substrates. This ability of reducing peroxides at the expense of electron donating substrates is what marks peroxidases as ubiquitous and very important enzymes with many biotechnological applications. Not surprisingly therefore peroxidases play many important roles in different areas of biotechnology. Among others, these include such diverse areas as bioenergy, bioremediation, dye decolorization, humic acid degradation, paper and pulp and textile industries among many others. An important reason for this ability is the different areas from which peroxidases could be sourced as the function of many peroxidases show variations according to its source. This is a character that differentiates peroxidases from many other biological catalysts. Among the many different types of peroxidases are the heme peroxidases which mainly come from plants and fungi and include among others lignin peroxidases, manganese peroxidases and versatile peroxidases. Some important types of peroxidases from humans and animals are glutathione peroxidase, thyroid peroxidase, lactoperoxidase, salivary peroxidase and thyroid peroxidase.

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1. INTRODUCTION

Peroxidase is a heme or iron-porphyrin protein that belongs to a large family of enzymes called the oxidoreductases. Their function mainly is to oxidize molecules at the expense of hydrogen peroxide. They are widely distributed in living organisms, and usually show dramatic colour-product formation as a result of their catalytic effect [1,2]. The earliest report on peroxidase activity was perhaps in 1855 by Schonbein, who observed that certain organic compounds could be oxidized by dilute solutions of hydrogen peroxide in the presence of substances occurring in plants and animals [3]. The name peroxidase was first given by Linossier, upon isolating it in 1898 from pus [1]. Studies carried out in the period up to 1918 showed that peroxidase activity was found widely in plants, where they occur in the different parts such as seeds and in cell walls of woody plants [4]. They are also found among animals and microorganisms. The occurrence of peroxidases in multiple forms was only established when the zymogram technique was developed by Hunter and Market in 1957, although the phenomenon has been suspected for many years earlier. It is now established that peroxidases occur in several isoforms; as a matter of fact, peroxidase are thought to have the highest number of isoforms in plants, as seen in horseradish peroxidase (HRP) where up to 42 isoforms are known [1,5].

Peroxidases show relative specificity towards a wide range of hydrogen donors such as phenolic substances, cytochrome c, nitrite, leuco-dyes, ascorbic acid, indole amines and certain inorganic ions especially the iodide ion. However, besides these peroxidasic oxidations of electron donor molecules, various reactions have been found to be catalyzed by peroxidases. These include aerobic oxidations of dihydrooxyfumarate, indole-3-acetic acid (IAA), triose reductone, NADH and naphthohydroquinone; hydroxylation of aromatic molecules, formation of ethylene, halogenations and antimicrobial activity [1]. The common feature of all of these reactions is the involvement of hydrogen peroxide (H_2O_2). However, from the point of view of function, peroxidase is similar to oxidase, because it involves electron transfer. The foregoing reactions are not exhaustive; peroxidases have the capacity to catalyze a large number of biochemical reactions. In plants, animals and microorganisms, they apparently fulfil many different functions, yet many of these functions are not completely established. One fact however, is that they have been shown to be ubiquitous and possess intensely high biochemical versatility [6].

2. HISTORY

Peroxidase has a rich history that spans many centuries and civilizations. As far back as about the year 1500, a product that contributed to the development of peroxidase were discovered or "gifted" to Europe: guaiacum which were essentially chips from the Caribbean trees *G. santum* and *G. officinale* that cured syphilis when added to water in steam bath [7]. Although that cure only lasted for about two centuries, at its end a constituent of guaiacum, guaiacol, became a trusted bacteriostatic (tuberculostatic) agent as well as to cure cough. However, the application that guaiacum or guaiacol finds its most reliable used is in the test for occult blood in faeces, which in principle is a peroxidatic reaction [8].

However, the real studies of peroxidases dates to the nineteenth century with the beginning of the investigations of numerous plant and animal tissues using various organic compounds whose colour properties change as their oxidation states change [9]. The first of such studies was that reported in 1855 by Schonbein, of the abilities of various tissues of plants and animals to oxidize guaiac, which in principle, implies the presence of peroxidase activity. Thereafter, followed the oxidation of guaiac by pus first by Klebs in 1868 and then Struve four years later in 1872 [9,10]. Various other works followed these initial ones, including those of Felix Hoppe-Seyler in the 1883 as well as Paul Ehrlich in 1885; Rohmann and Spitzer in 1895, Spitzer alone in 1897, Linossier in 1898 and thereafter, that of Raudnitz rounding off that remarkable peroxidatic century in 1899. Table 1 gives a sneak peek of the specific contributions of these persons, while the works of Agner[10] and Clark [9] provide detailed explanations and specific contributions of these persons and many more with regard to those early developments in the history of peroxidases.

Another important development within the period that merits a brief mention here, is the debate over the identities and names of peroxidase and the related enzymes oxidases and oxygenase.

Their observation then, that peroxides seemed to be present (ubiquitous) in most biological materials under aerobic conditions obviously prompted Bach and Chodat to suggest a general mechanism where (oxygenase + peroxidase) = oxidase [8]. The equation below depicts that observation better.

$$\text{Oxygen} \xrightarrow{\text{Oxygenase}} \text{Peroxide} \xrightarrow{\text{Peroxidase}} \text{Oxidase}$$

Ultimately however, these same authors, together with Linnoisier were able to separate the activities of peroxidases from those of oxidases and establish their proper identities. It is for these reasons, but especially the fact that his observations came earlier that those of Bach and Chodat by about 5 years (1898 vs 1903) that Linossier is usually credited with being the person who first gave the name peroxidase to posterity.

3. REACTION MECHANISMS OF PEROXIDASES

Research over time has shown that peroxidases oxidize reducing substrates (usually represented as AH), either in the presence of hydrogen peroxide (this is called the classical peroxidase cycle, see above), or molecular oxygen, called the oxidase cycle [11,12]. In peroxidase catalysed reactions several intermediate products termed compounds I, II or III as the case may be are formed before the final product.

Peroxidase compound I and II are formed in the presence of low peroxide concentration only. The green compound I which appear first is further transformed to compound II through reduction by an electron donor [1,11]This characteristic one or two-electron oxidation of donor molecules by peroxidases is shown below:

$$\text{Peroxidase} + H_2O_2 \longrightarrow \text{Compound I} + H_2O$$
$$\text{Compound I} + AH_{2\,(\text{reduced})} \longrightarrow \text{Compound II} + AH^-_{(\text{oxidized})}$$
$$\text{Compound II} + AH_{2\,(\text{reduced})} \longrightarrow \text{Peroxidase} + AH^-_{(\text{oxidized})}$$
$$2AH^- \longrightarrow A + AH_2 \text{ (or AH – AH)}$$

Thus, compounds I and II are considered to be obligatory enzyme intermediates in an overall peroxidase reaction that eventually regenerates the original peroxidase (ferriperoxidase). The intermediate that is called compound III occurs when there is a large or excess concentration of peroxides (H_2O_2) and iron is in the ferrous state. It is believed that it is formed by the combination of superoxide, generated by the oxidation of the excess H_2O_2, with the ferric enzyme. Nevertheless, compound III is not a catalytically active intermediate [13].

4. CLASSIFICATION OF PEROXIDASES

The major criteria used in the classification of peroxidases is the presence or absence of a heme [13]. According to the RedOxiBase (previously called PeroxiBase, essentially because it now aims to centralize most of the oxido-reductase superfamilies) database, which since 2004 had been dedicated to providing information on the peroxidase superfamilies, peroxidases can be either heme or non-heme containing proteins [14]. According to the database, relevant phylogenetic trees show that peroxidases comprise of 13 major groups, which in turn are subdivided into more than 60 classes. It is estimated that the heme peroxidases make up approximately 80% of all known peroxidases, while the heme-free ones make up the remaining 20% [13]. Previously, the heme group was subdivided into the two major super families of peroxidase-cyclooxygenase superfamily (PCOXS) and the peroxidase-catalase superfamily, PCATS[13-16]. However, the RedOxiBase database show that the heme group has now been subdivided into many more superfamilies (Table 2) comprising (i) catalase superfamily (heme, monofunctional) (ii) Di haem cytochrome C peroxidase superfamily (iii) Dyp-type peroxidase superfamily (iv) non-animal peroxidase superfamily: class I (v) non-animal peroxidase superfamily: class II (vi) non-animal peroxidase superfamily: class III (vii) peroxidase-cyclooxygenase superfamily (animal peroxidases). Sometimes the non-animal peroxidase superfamily can be combined into a unit bringing it to about 5 superfamilies altogether.

The details of the heme-containing superfamilies and the different classes they belong to are shown in Fig. 1. The figure also shows the non-heme containing superfamilies and their different classes. The first heme containing superfamily, the catalase superfamily (also called hydroperoxidase II, Kat or HPII) contain catalases which are heme-containing enzymes that are able to dismutate H_2O_2. Such dismutations typically result in the formation of harmless water and molecular oxygen [17].

Table 1. Peroxidases, overview of important historical developments in the second half of the 19th century

1855 Schonbein	Guaiac oxidation by plant and animal tissues
1883 Hoppe-Seyler	"Activation" of oxygen by tissues
1885 Ehrlich	Indophenol blue reactions of living tissues ("oxygen need") "der ungesattigte Zustand des Protoplasmas"
1895 Rohmann and Spitzer	Indophenol blue oxidation by tissue ferments
1897 Spitzer	Iron bound to the oxygen activator
1898 Linossier	Hydrogen peroxide required for leukocyte oxidation reactions
1899 Raudnitz	Two distinct activities of tissue ferments: catalases and peroxidases

Adapted from Clark, 2000 [9].

Table 2. The peroxidase classes and superfamilies

Peroxidase type	Superfamily
Haem peroxidases	
	Catalase superfamily (heme, monofunctional)
	Di-haem cytochrome C peroxidase superfamily
	Dyp-type peroxidase superfamily (Dye-decolorizing peroxidases)
	Non Animal peroxidase superfamily: Class I
	Non Animal peroxidase superfamily: Class II
	Non Animal peroxidase superfamily: Class III
	Peroxidase-Cyclooxygenase superfamily (Animal peroxidases
Non-Haem peroxidases	
	Alkylhydroperoxidase D-like family
	Glutathione peroxidase family (Thiol peroxidase superfamily)
	Haloperoxidase superfamily
	Manganese catalase family (pseudocatalase)
	NADH peroxidase/oxidase family
	Peroxiredoxin family (Thiol peroxidase superfamily)

Haem and heme are supposedly mere spelling differences and so are often used interchangeably. Combining the three non-animal superfamily into a single unit result in just 5 instead of 7 superfamilies in total. Adapted from RedOxiBase [14].

The enzyme function as a key enzyme in the protection of all types of organisms against reactive oxygen species, hence its occurrence in all types of microorganisms including anaerobic types, plants and animals [18,19]. The second heme containing superfamily, the di-haem cytochrome C peroxidase superfamily typically contains two heme containing proteins in addition to other cytochrome c peroxidases. They usually reduce hydrogen peroxide to water using c-type heme as an oxidizable substrate. The protein structure comprises two domains each of which contain one c-type heme group and a calcium-binding site at the domain interface [20].

Dyp-type peroxidase superfamily, which is the third heme containing peroxidase superfamily, are typically detected among the fungi, mycetozoa, bacteria and archaea. A distinctive characteristic of a branch of the superfamily is the possession of twin-arginine dependent signal sequence (TAT domain) which typically accompanies exported proteins with bound redox cofactors. It is also important to note that Dyp-type peroxidases from fungal microorganisms show no homology whatsoever to other fungal peroxidases, such as fungal derived lignin peroxidase and manganese peroxidases; they also lack the typical heme-binding region conserved among the plant peroxidase superfamily [21,22].

The fourth, fifth and sixth heme containing peroxidase superfamilies are the non-animal peroxidases: Classes I, II and III. They used to

be classed as part of the PCAT (peroxidase-catalase) superfamily and even before then as the plant, fungal and bacterial heme peroxidase group. They constitute one of the most studied super families of the non-animal heme peroxidases [16]. The class I peroxidases are typically not glycosylated and also do not have signal peptides, calcium ions or disulfide bridges. Examples include ascorbate peroxidase APx, Catalase peroxidase CP, cytochrome C peroxidase CcP[23]. The class I peroxidase play important roles in the prevention of oxidative stress in bacteria due to their unique ability to catalytically dismutate H_2O_2 and produce molecular O_2 [16].

The Class II of the non-animal peroxidase superfamily are exclusive fungal peroxidases and include important fungal ligninases such as lignin peroxidase (LiPs), manganese peroxidases (MnPs) and versatile peroxidases (VPs) among others. These are extracellular heme enzymes that are involved in the degradation of lignin which is the second most abundant organic compound on Earth after cellulose, and typically has a slow degradation rate [14,24]. The third class, class III, of the non-animal peroxidase superfamily (Class III peroxidases or secreted plant peroxidases) are only found in plants, where they form large multigenic families. Their primary sequences differ in some points from those of classes I and II. However, their 3-dimensional structures are very similar to those of Class II. In addition, they also possess calcium ions, disulfide bonds and an N-terminal signal for secretion [14]. An important attribute of the class III peroxidases is their ability to undertake a second cyclic reaction distinct from the peroxidative one. During this hydrolytic cycle, the peroxidases pass through an Fe (II) state and use mainly the superoxide anion (O_2^-) to generate hydroxyl radicals (OH). It is this ability to use these two cycles that furnish the class III peroxidases with the ability to participate in many different plant processes starting from germination to senescence, such as, auxin metabolism, cell wall elongation and stiffening, protection against pathogens, wound healing, lignification among many others [14,16]. Important examples of peroxidases from this class include horseradish peroxidase (HRP), soybean peroxidase (SBP), peanut peroxidase, turnip peroxidase and also sorghum peroxidase which have been a major part of our research in recent years [25-28].

The last superfamily, the Peroxidase-Cyclooxygenase superfamily are the animal peroxidases which previously constituted one of the two major super groupings of the peroxidase superfamily. This superfamily comprises animal peroxidases such as myeloperoxidase (MPO), lactoperoxidase (LPO), thyroid peroxidase (TPO), Eosinophil peroxidase (EPO) etc. In addition, representatives of this peroxidase class are also found in plants where they constitute the class known as alpha-dioxygenases (DiOx); they are a family of fatty acid metabolizing enzymes found only in plants. Another enzyme found in this super family is the dual oxidase (DuOx) which also function in fatty acid metabolism [14]. Details of the non-heme peroxidase classification are not provided here but can be found in the RedOxiBase database [14].

5. APPLICATIONS OF PEROXIDASES

The current global interest in peroxidases derives from the multitude of applications to which it can be subjected to in different aspects of life and industry. The basis for the applications of peroxidases lies in their ability to catalyse the oxidation of a wide variety of substrates, using H_2O_2 or other peroxides. In addition, the reduction of peroxides at the expense of electron-donating substrates makes peroxidases useful in a number of industrial and analytical applications.Presently, Peroxidases are widely used in clinical biochemistry and enzyme immunoassays [29,30].

Some suggested novel applications of peroxidases include treatment of waste water containing phenolic compounds, synthesis of various aromatic chemicals and removal of peroxide from materials such as foodstuffs and industrial wastes [31]. Being the most known source, horseradish root tubers are commonly employed as a commercial source for peroxidase production [32-34]. However other cultivated species may provide peroxidases exhibiting similar or better properties, especially recombinant species [35]. We are presently working on developing sorghum peroxidase as a possible alternative to HRP [25-28]. Some applications of peroxidase include the following.

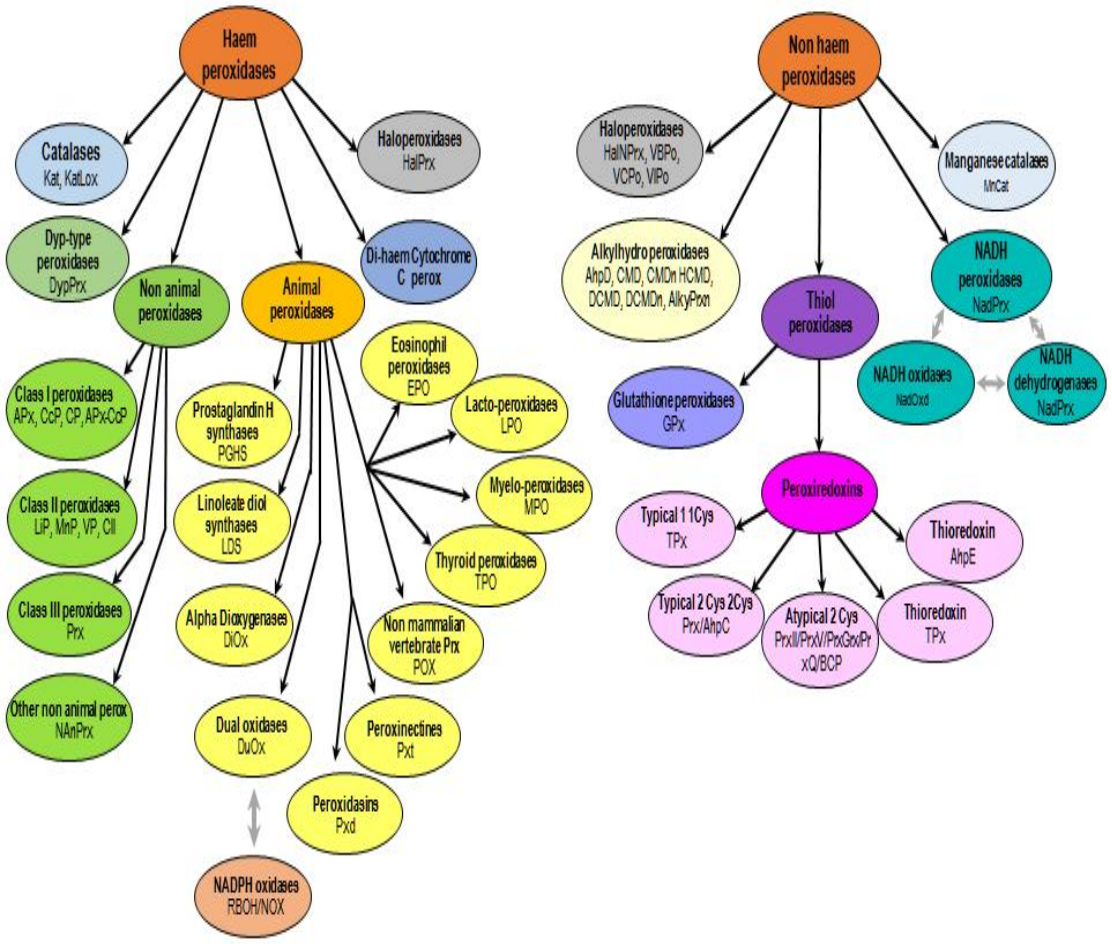

Fig. 1. Schematic representation of the classification of peroxidases. The black arrows link classes, families or superfamilies of peroxidase proteins originating from the same ancestral sequence, while the grey arrows link two different peroxidase protein classes that share common domains.

(Source: RedoxiBase database [14]).

5.1 Removal of Phenolic Contaminants and Related Compounds

Aromatic compounds, including phenols and aromatic amines, constitute one of the major classes of pollutants. They are found in the waste waters of a wide variety of industries, including coal conversion, petroleum refining, resins and plastics, wood preservation, metal coating, dyes and other chemicals, textiles, mining and dressing, and pulp and paper [36]. Most aromatic compounds are toxic and must be removed from waste waters before they are discharged into the environment. Enzymatic treatment has been proposed by many researchers as a potential alternative to conventional methods. Firstly, enzymes are highly selective and can effectively treat even dilute wastes [37]. Secondly, they are less likely to be inhibited by substances which may be toxic to living organisms and their cost could eventually be less than that of other methods, if commercially available enzymes are produced in bulk quantities. Moreover, enzymes operate over a broad aromatic concentration range and require low retention times with respect to other treatment methods [38]. Peroxidases that have been used for treatment of aqueous aromatic contaminants include horseradish peroxidase (HRP), lignin peroxidase (LiP) and a number of other peroxidases from different sources. Most applications have focussed on the treatment of

phenolic contaminants in the presence of H_2O_2 [39-41].

5.2 Decolorization of Synthetic Dyes

Dyes are used extensively for paper printing, color photography, textile dyeing and as additive in petroleum products. They have a synthetic origin and complex aromatic molecular structure. It is estimated that there are over 10,000 commercially available dyes with a production of over 7×10^5 tons per year [42]. However, about 10–15% of the synthetic dyes produced are discharged into industrial effluents [43], causing environmental problems. Many of the synthetic dyes are not amenable to conventional biological wastewater treatment because of their structures, and the treatment of effluents containing dyes usually involves physico-chemical methods. Although, currently available methods such as chemical oxidation, reverse osmosis, and adsorption, are highly efficient they suffer some disadvantages. The limitations include high cost, limited applicability, high energy input, and usually these treatments may result in the production of toxic by-products. Therefore, interest is now growing about the use of microbial degradation of dyes since this process is less expensive and represents a less intrusive alternative [44]. Some authors have shown that oxidative destruction of colored compounds is significantly stimulated by oxidative enzymes [45,46], and may be of practical interest for decolorization of synthetic dyes. Enzymes such as lignin peroxidase (LiP) and manganese peroxidase (MnP), both associated with lignin degradation, are involved in the decolorization of synthetic azo dyes such as Orange II, and others [47]. Horseradish peroxidase (HRP) is known to degrade certain recalcitrant organic compounds such as phenol and substituted phenols via a free radical polymerization mechanism. Other peroxidases have also been used.

5.3 Organic and Polymer Synthesis

The free radicals produced by peroxidase catalysis may participate in different post-enzymatic reactions. Oxidative polymerization of aromatic compounds mediated by oxidoreductases has been studied to create new functional polymers and to synthesize phenolic resins with good chemoselective conversion. This can be achieved without using toxic formaldehyde, which must be used in chemical polymerization [48].

HRP has been used to polymerize phenolic and aromatic amine compounds, while new types of aromatic polymers have been synthesized in water and in water-miscible organic solvents [49]. Cardanol is a phenol derivative having a C15 unsaturated alkyl chain with 1 to 3 double bonds at its meta position [50]. It is obtained by thermal distillation of cashew nut shell liquid, and has been used as a raw material to produce resins and friction linings. In a study reported by Kim et al. [51], soybean peroxidase catalyzed the oxidative polymerization of cardanol, using methanol, ethanol, 2-propanol, t-butyl alcohol or 1-4-dioxane. Higher yield (62%) was obtained using 2-propanol as solvent. However, the use of HRP resulted in inefficient cardanol polymerization. Additionally, these authors examined the antibiofouling capability of polycardanol by growing *Pseudomona fluorescences* on a cured polycardanol surface, and found a considerable decrease in biofilm formation. The production of conducting polymers has remarkable interest because of their wide range of applications, including anticorrosive protection, optical display, light-emitting diodes, etc. Polyaniline is one of the most extensively investigated conducting polymers because of its high environmental stability and promising electronic properties. Nowadays, polyaniline is synthesized by oxidizing monomer aniline under strongly acidic conditions and low temperature using ammonium persulfate as initiator of radical polymerization [52]. Chemical methods of polyaniline synthesis have some disadvantages. First the reaction is a radical polymerization, and hence, is not kinetically controlled and second, the reaction is not environmentally friendly because it is carried out at very low pH. For these reasons, enzymatic polymerization of aniline is an attractive alternative to the synthesis of polyaniline. Horseradish peroxidase has been used in the synthesis of the polyelectrolyte complex polyaniline [53].

5.4 Applications in the Paper Pulp Industry

White rot fungi can attack lignin and simultaneously degrade wood components to carbon dioxide and water [54]. Some of them selectively and efficiently degrade lignin rather than cellulose or hemicellulose [55]. There are drawbacks to the direct use of microorganisms for breaking down lignocellulosic materials, including degradation of cellulose fibers and longer retention times, extending to several days.

The biopulping is a process where the extracellular enzymes (hydrolytic and oxidative) produced by a white rot fungus remain adsorbed on the wood chips, degrading lignin. After the pulping process, about 10% of the lignin appears as modified lignin which is responsible for a characteristic brown color. Modified lignin can be enzymatically degraded using a biobleaching process [56]. Major lignin-degrading enzymes from basidiomycetes include manganese peroxidase (MnP), laccase and to a lower extent lignin peroxidase (LiP). MnP in the presence of Mn(II) chelated with an organic acid, Tween 80, and a H_2O_2 generating system depolymerized milled pine wood. A key element in the delignification system is Mn(III), a strong oxidizing agent, that is generated by MnP[57]. A crude MnP preparation from the marine basidiomycete *Phlebia* sp. MG60, effectively bleached a hard wood kraft pulp (UKP). When a model effluent 'white-water' was used to bleach UKP, the pulp treated with MnP from *Phlebia* sp. was brighter than that achieved using MnP from *P. Chrysosporium* [58]. Thus, MnP from *Phlebia* sp. is potentially useful in biobleaching when white-water is recycled. Wood pulp may be delignified enzymatically with good results using a LiP in the absence of peroxide, and when the enzyme is firstly chemically modified to avoid its adsorption to the pulp [58]. Enzyme pulping using MnP and laccase of *Polyporus* sp., and pectinases from *Rhizopus* sp.26R significantly reduced the amount of NaOH in a following alkaline pulping process. More studies are needed to determine if selective lignin degradation and efficient biopulping require a proper balance between lignin and cellulose degradation.

5.5 Peroxidase Biosensors

One field that offers great potential for peroxidase application is that comprising electrochemical biosensors. Recently, peroxidase-based electrodes have had widespread use in analytical systems for determination of hydrogen peroxide and organic hydroperoxides [59]. When co-immobilized with a hydrogen peroxide producing enzyme, they may be exploited for determination of glucose, alcohols, glutamate, choline [60]. As shown above, peroxidase first catalytic cycle involves reaction of the active site with hydrogen peroxide (equation 2).

$$FeIII + H_2O_2 \rightarrow [(FeIV = O) - R+\bullet] \text{ Compound I} + H_2O \quad (2)$$

Then, Compound I oxidizes a substrate to give a substrate radical and Compound II, which is reduced by a second substrate molecule, regenerating the native ferric enzyme. When an electrode substitutes the electron donor substrate in a common peroxide reaction cycle, the process is denominated as direct electron transfer. Enzymes immobilized on an electrode can be oxidized by hydrogen peroxide (equation 2) and then reduced by electrons provided by an electrode (equation 3).

$$[(FeIV = O) - R+\bullet] \text{ Compound I} + 2e^- + 2H^+ \rightarrow FeIII + H_2O \quad (3)$$

When an electron donor (A) is present in a peroxidase-electrode system, the direct process can occur simultaneously, with reduction of the oxidized donor A• by the electrode as shown in equation 4.

$$A\bullet + e^- + H^+ \rightarrow AH \quad (4)$$

During direct electron transfer, electrons act as the second substrate for the enzymatic reaction, resulting in a shift of the electrode potential, with the measured current being proportional to the H_2O_2 concentration [61]. This technique can also be used to quantify other metabolites, especially combined with another oxidase enzyme. Peroxidase can also interact with an electrode by mediated electron transfer, where a mediator (an electron donor, A) is transporting the electrons between the enzyme and the electrode. In this system, the enzymatically oxidized donor (A•) is thus electrochemically reduced by the electrode.

5.6 Application in Analysis and Diagnostic Kits

Horseradish peroxidase is the most commonly used enzyme for practical analytical applications. However, peroxidases from other sources appear to be a good alternative as substitutes for HRP. Lactose contained in milk and milk products is determined by various analytical methods requiring trained persons and sophisticated instruments such as a spectrophotometer, biosensors, etc. Sharma et al. [62] developed a simple and economical biostrip technology for estimation of lactose by immobilizing *β*-galactosidase, galactose oxidase and HRP onto a polymeric support, being the *β*-galactosidase the key enzyme to detect lactose. The biostrip was dipped in milk or a milk product and the color developed from an added chromogen, was used to estimate lactose in the

concentration range <20–100 g/l. Due to the peroxidase ability to yield chromogenic products at low concentrations and its relatively good stability, it is well-suited for the preparation of enzyme conjugated antibodies and application in diagnostic kits [63]. Agostini et al. [31], purified various peroxidase isoenzymes from roots and hairy-roots cultures of turnip (*Brassica napus*). They developed a diagnostic test kit for determination of uric acid. The assay was based on the following reaction:

$$\text{Uric acid} + O_2 + 2H_2O \xrightarrow{\text{Uricase}} \text{allantoin} + H_2O_2 + CO_2 \quad (5)$$

$$H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{p-(benzoquinone) monoiminephenazone} \quad (6)$$

In analytical applications the enzyme must be present in saturating amounts to make sure that the H_2O_2 produced in the test is stoichiometrically converted into a colored substance [64]. The concentration of turnip peroxidase giving a linear response with time and increasing uric acid concentration was 30 mM. Analysis of uric acid in human serum from ten different patients using either the kit containing turnip peroxidase, or a commercially available kit, gave the same results. This confirmed that the cationic peroxidase isoenzyme from turnip hairy roots could be used as a reagent for clinical diagnostic, as part of a kit where H_2O_2 is generated.

5.7 Enzyme Immunoassays

Enzyme linked immunosorbent assays (ELISA) also known as EIA, are tests designed to detect antigens or antibodies by producing an enzyme triggered change of color. To this end, an enzyme-labeled antibody specific to the antigen is needed, as well as a chromogenic substrate, which in the presence of the enzyme changes color. The amount of developed color is proportional to the amount of antigen in the test specimen. Peroxidase is probably the most well-suited enzyme for the preparation of enzyme-conjugated antibodies, which are used in enzyme-linked immunosorbent assay (ELISA) tests, due to its ability to yield chromogenic products at low concentrations [63]. Peroxidase coupled with other enzymes in polyenzymatic systems producing hydrogen peroxide is also used in the determination of many compounds, such as glucose in blood. HRP is probably the most common enzyme used as a reporter (enzyme labeled antibody) in enzyme immunoassays. ELISA tests on which peroxidase is used for labeling an antibody, have been developed for screening monoclonal antibodies against mycotoxins [64].

6. CONCLUSION

It is concluded that among the many different types of peroxidases are the heme peroxidases which mainly come from plants and fungi and include among others lignin peroxidases, manganese peroxidases and versatile peroxidases. Some important types of peroxidases from humans and animals are glutathione peroxidase, thyroid peroxidase, lactoperoxidase, salivary peroxidase and thyroid peroxidase.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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