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Review Article

A Review on Recognition of Various Peroxidases

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Abstract: Peroxidases, widespread in the biological world, are a kind of oxidoreductases important in biological antioxidant defense systems. For great potential value of them in physiology, biochemistry and treatment of industrial pollutants, etc., they receive extraordinary attention, and also a large number of peroxidases have been found and studied on the nature and catalytic properties of them to date. Especially, horseradish peroxidase is first found and given more researches on the protein structure, catalytic mechanism and practical applications, and even modified by gene engineering and protein engineering to improve its catalytic performance. In addition, also there are numerous peroxidases that are derived from various sources or used for catalytic oxidation of different substrates, which have many differences from horseradish peroxidase in amino acid composition, active site structure, catalytic characteristics and corresponding applications. To better study and selectively employ peroxidases as biocatalysts, it is critical to comprehensively recognize various peroxidases, so as to present a reference for the in-depth and accurate studies on them in the future. In this paper, various peroxidases usually focused by enzymologists are reviewed for better mastering the completed research work on them, understanding the main interests in them, and knowing the advantages and disadvantages of them. Moreover, the research aspects that hereafter may be focused on are prospected.

Keywords: Peroxidase, Catalysis, Review, Application

1. Introduction

Peroxidase (POD) is an enzyme that can catalyze various biosynthesis and biodegradation reactions, which commonly requires peroxides, usually being hydrogen peroxide, as oxidant to take part in these reactions [1]. PODs are widely existed in the biological world, included in cells of many plants, animals and microbes [2]. POD has a history of more than 200 years since its discovery firstly [3], and has been studied on the catalytic characteristics and applications for the potential values in physiology, biochemistry and treatment of industrial pollutants as well as other aspects [4]. Because PODs have numerous types and diverse functions, it is necessary to understand them comprehensively.

Now, there have been some reviews on PODs to show one or more catalytic characteristics of them possibly employed in some applications. But they commonly are introductions on individual PODs, e.g., horse-radish peroxidase [5], lactoperoxidase [6], thyroid peroxidase [7], glutathione peroxidase-1 [8], catalase-peroxidase [9], microperoxidase [10], etc. Or they are some monograph reviews on PODs in special aspects such as self metabolism [11], catalytic thiol chemistry [12], clinical measurement [13], and effect on food flavor [14], etc. In addition, the abundant experimental research literatures on PODs almost are beyond count, for the attractive catalysis and application potential of them [15]. In this context, a problem emerges gradually, that is to say that it is difficult to distinguish all kinds of PODs, especially those commonly researched or employed as biocatalysts. They usually have some similarities, but they are also very different and easy to be confused, which is not conducive to research on them. So it is very critical to recognize and distinguish various PODs for the convenience of pertinent researches on them. Hitherto, the review on recognition of various PODs has not seen by us. Therefore, we investigated a large number of literatures, carried out a comprehensive analysis on them, and given this overview, in which a few PODs usually focused by enzymologists are reviewed for the

purpose of fine differentiating, in-depth research and effective application on various PODs, and also the future research trend of peroxidases are also proposed.

2. Horseradish Peroxidase

2.1. Discovery and Catalytic Mechanism

Horseradish peroxidase (HRP) is a POD discovered first and studied much. In the year 1810, Planche, L. A. reported an interesting phenomenon that horseradish caused guaiaceae tincture to change color, and essentially it is the in-horseradish peroxidase that catalyzed hydrogen peroxide to oxidize guaiaceae tincture and form dark color poly phenols [3]. In the following two centuries, HRP was widely studied and it is found that HRP is a glycoprotein combining with heme oxide, namely it is mainly composed of a single peptide chain and dark brown heme, i.e., iron porphyrin [15]. HRP has four isozymes named HRPA, HRPB, HRPC and HRPD, respectively [16]. Among them, HRPC is the richest in protein content and studied thoroughly in structure and catalytic mechanism [17, 18]. The catalytic mechanism is considered as three cycled reactions, that is to say that the ground HRPC binds to hydrogen peroxidase and forms oxo Fe (IV) porphyrin π positive ion radical, called the compound I, which is the main active intermediate in catalysis [19], and then the intermediate is reduced into the compound II and the ground HRPC in order via two successive single electron reduction reactions [18] (Figure 1). Here the compound I and II all are high active oxidants, having redox potential near +1 V [20]. The further researches show there is an extremely stable compound 0 generated before the compound I [19]. And the X-ray diffraction technology has captured another intermediate compound III in the enzymatic reaction cycle and the dioxygen bond of compound III has the high activity of superoxide [4]. It is just these active intermediates that give HRPC excellent catalytic activity [21].

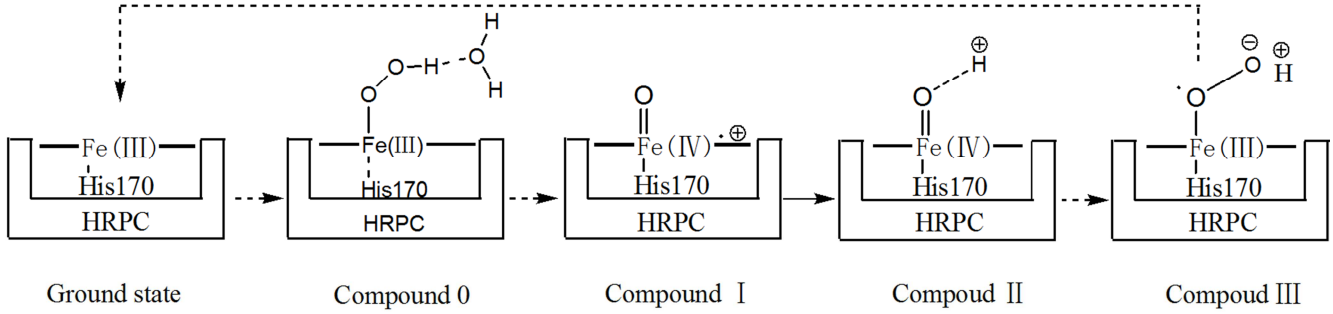

Figure 1. *Ground state and four active intermediates of HRPC in catalysis.*

2.2. Modification and Application Researches

In order to improve the stability of HRP, chemical modification, site directed mutation and direct evolution have ever been employed [20, 22]. On the other hand, HRP was explored in areas such as organic synthesis, biotransformation, enzyme coupling assay, chemiluminescence analysis, immunodiagnosis, targeted tumor therapy and wastewater treatment, etc., and also applied in small batch of organic synthesis, e.g., N-and O-dealkylation, oxidative coupling, selective hydroxylation and oxygen transfer process [18, 20, 23, 24]. Especially, HRP has great application potential in environmental remediation, and it can be used for degradation of phenols [25] and steroids [26]. In addition, HRP can also be used in the field of preparation of special materials, such as the creation of skin like polysaccharide fold film by its catalytic oxidation [27]. In view of the production rate and price limitation of HRP, in order to realize its industrial application, in addition to reducing the preparation cost, it is necessary to control the use method and dosage of oxidant hydrogen peroxide to avoid enzyme deactivation [28], and to prepare engineering and immobilized HRPs so as to improve the stability and catalytic efficiency of them [21, 29].

3. Other Peroxidases

3.1. Chloroperoxidase

Chloroperoxidase (CPO) is firstly separated from marine fungus *Caldariomyces fumago* in the 1960s [30], and, at present, thought to be the most abundant in catalysis in POD family, which has extensive substrate adaptability and can catalyze both halogenation and oxidation reactions [31]. Moreover, CPO has the catalytic properties of POD, catalase and cytochrome P-450 at the same time [32]. In addition, CPO can also catalyze some special reactions, such as carbazole polyhalide [33], aminobenzenesulfonic acid degradation [34], indole oxidation to oxidized indole [35], and furfuryl alcohol epoxidation combined with glucose oxidase [36]. Its catalytic activity can be improved by site-specific mutagenesis [37], and can also be adjusted by additional reagents, e.g., chitosan improving the chlorination activity [31], mercaptoacetic acid increasing the oxidation activity [38]. Based on the catalytic properties of CPO, some applications have been explored in the treatment of dye wastewater, the synthesis of chiral compounds, the purification of petroleum, and the preparation of bioelectrode [32].

3.2. Cytochrome C Peroxidase

Cytochrome C peroxidase (CCP) is a POD that catalyzes

the reaction of hydrogen peroxide to oxidize cytochrome C, which plays a key role in the process of apoptosis [39]. In catalysis, when peroxide existed, electron transfers from cytochrome C to CCP and forms an intermediate similar to the compound Ⅰ in HRP catalysis, which is the Trp191 indole cation free radical [CCPFe(IV)=OTrp191$^+$], being the main active intermediate of CCP-catalytic oxidation substrate. The process of CCP catalysis is also a three-step cycle reaction, which is specially studied so as to understand the mechanism of electron transfer between proteins in biological system [40]. Further, through site mutation, combination mutation and carbonylation modification, the role of important amino acid residues in CCP in stabilizing the structure of active center, influencing the activity and specificity of enzyme catalytic reaction has ever been explored [39]. In addition to the oxidation on cytochrome C, CCP can also effectively catalyze the oxidation of phenols [41].

3.3. Glutathion Peroxidase

Glutathion peroxidase (GPX) is an important antioxidant enzyme in animals and plants, and it catalyzes the oxidation-reduction reaction of peroxide to generate water or corresponding alcohols. The oxidation-reduction reaction can remove hydrogen peroxide and other peroxides in the body, maintaining the normal physiological function of the body and delaying aging [42]. According to the existed locations in biological tissues and catalytic properties, GPXs can be divided into 8 categories GPX 1- GPX 8 [43]. According to whether selenium included in or not, GPX can also be divided into the selenium GPX and the selenium-free GPX, and the former can catalyze hydrogen peroxide and hydroperoxides, while the latter can only catalyze hydroperoxides [44]. For the selenium GPX, it is usually deemed as the organic selenium helps to improve GPX activity [45].

3.4. Lignin Peroxidase

Lignin peroxidase (LiP) is a kind of POD from fungi and can degrade lignin, and also it contains heme cofactor, sugar and calcium ion. When LiP takes H_2O_2 as auxiliary substrate, the phenol lignin model compounds is oxidized to phenoxy free radical, and the non phenol lignin model compound is oxidized to aromatic positive ion free radical. After a series of non enzyme catalyzed free radical reactions, the C-C bond on the lignin side chain breaks [46]. LiP can hydrolyze lignin in straw, forage and seed coat of feeds, and better feed quality of feed material to improve its nutritional value.

3.5. Ascorbate Peroxidase

Ascorbate peroxidase (APX) is a very good active oxygen scavenger, being a key enzyme to remove H_2O_2 [47]. APX takes the reduced ascorbic acid as the reaction substrate and catalyzes the reaction of H_2O_2 with ascorbic acid to produce monodehydroascorbic acid and H_2O, which means that APX has a high specificity and affinity for ascorbic acid [48].

3.6. Catalase-peroxidase

Catalase-peroxidase (KatG) shows double functions both peroxidase and strong catalase activities more similar to single function catalase, and the conservative amino acids on both sides of heme combined in this enzyme protein are similar to those in CCP and APX [49]. KatG, on the one hand, is more effective for the detoxification of reactive oxygen species [50], on the other hand, it can catalyze the generation of free radicals favorable in inhibiting the formation of components used to construct bacterial cell wall and thus inhibiting harmful bacteria [51].

3.7. Others

In addition, manganese peroxidase, which also catalyzes the oxidation of lignin, has a good application prospect in the degradation of organic pollutants, pulp bleaching, dye decolorization, biodegradation of lignite, treatment of agricultural wastes, and polymerization chain reaction catalysis, etc. [52]. There are also studies on myeloperoxidase, lactoperoxidase, ovopoxygenase and other peroxidases [13, 20, 53], etc.

4. Conclusions

To sum up, the researches on PODs are extensive for that they are suitable as green biocatalyst, especially having great potential in reducing phenol pollutants. However, due to the high price and poor stability of PODs, the practical application is still limited [54, 55]. In view of the advantages and disadvantages of PODs from various sources, here giving a systematic recognition on various PODs will be a basis of research work pertinently carried out in the future. As one of PODs, HRP has the earliest research, the most thorough understanding on catalytic mechanism and the most abundant explorations in application. In order to improve the yield and stability of PODs, they have been modified by genetic engineering and related biotechnology. For purpose of applications, they have been explored to catalyze different oxidized substrates. It can be concluded that the future researches on PODs will be focused on the following three aspects. (1) Taking advantage of genetic engineering and fermentation technology to improve the biological yield and catalytic stability of PODs, (2) preparing highly stable immobilized enzyme to improve the use frequency of them, and (3) taking the theoretical studies as the basis to synthesize the POD like catalytic materials.

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