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Hydrogen Peroxide Biosensors Based on Horseradish Peroxidase and Hemoglobin

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Abstract

This review focuses recent contributions in the development of the electrochemical biosensors for hydrogen peroxide (H_2O_2), based on the Horseradish Peroxidase (HRP) and Hemoglobin (Hb) modified electrodes. Various modified electrodes have been used for the determination of H_2O_2 by using electrochemical methods. HRP and Hb are the mostly used materials for the modification of electrodes. Here, we summarize the recent trends of the modification of electrodes, as well as development of the electrochemical H_2O_2 biosensors. In addition, the detection mechanism of H_2O_2 for different modified electrodes has been described.

Keywords: Hydrogen peroxide; Biosensors; Electrochemical methods; Horseradish peroxidase; Hemoglobin

Introduction

Hydrogen peroxide (H_2O_2) plays a pivotal role in various industrial applications [1]. It is an essential mediator in pharmaceutical, clinical, and environmental research. In addition, it is a byproduct of highly selective oxidases and an important contaminant in several industrial products and wastes [2]. Therefore, a reliable and economical method for the determination of H_2O_2 is of great significance.

Several methods, such as titrimetry [3], spectrometry [4], chemiluminescence [5], fluorimetry [6], chromatography [7], and electrochemical techniques [8,9], have been reported for this purpose. Among these techniques, the electrochemical techniques are preferable because of their simplicity, low cost, high sensitivity, and selectivity [10-16]. In particular, an amperometric biosensor is an attractive tool for the detection of H_2O_2. Other electrochemical methods such as cyclic voltammetry, linear sweep voltammetry and differential pulse voltammetry are also used for the development of H_2O_2 biosensor.

In the electrochemical methods, different types of modified electrodes have been used. Horseradish Peroxidase (HRP) enzyme is one of the mostly used materials for the modification of electrode. Different mediator such as ferrocene, hydroquinone, catechol, methylene blue, methylene green, nile blue, potassium hexacyanoferrate, thionine, toluidine blue etc. have been used with the enzyme modified electrodes. However, mediator-free HRP based biosensor was also reported. In this case, the direct electrochemistry of HRP enzyme plays vital role. Hemoglobin (Hb) protein is also used for the modification of electrodes. In most case, mediator is not necessary for protein modified electrodes. Enzyme free or protein free modified electrodes were also reported for the electrochemical method based H_2O_2 biosensor. Some other materials such as nanomaterials, conducting polymers, metal oxides, quantum dots, dendrimer, bilayer lipid membrane, kieselguhr membrane, ionic liquid, liquid crystal, etc. have been used with enzyme and protein modified electrodes. The sensitivity and selectivity of the H_2O_2 biosensor depends on how the electrodes are modified by different materials.

In this review, we have focused on the strategy of the surface modification with HRP and Hb, for the determination of H_2O_2 based on electrochemical methods.

Detection Mechanism of H_2O_2 Biosensor

The detection mechanism of H_2O_2 biosensor depends on how the electrode is modified and whether the mediator is used or not. Direct electron transfer between enzymes and electrodes is not easy and therefore, need to use mediator. The role of the mediator is to shuttle electrons efficiently between electrode and enzyme. In the presence of Mediator (M), the reaction mechanism of the H_2O_2 biosensor based on the HRP enzyme modified electrodes can be summarized as follows [17]:

$$H_2O_2 + HRP_{red} \rightarrow H_2O + HRP_{ox}$$

$$HRP_{ox} + M_{red} \rightarrow HRP_{red} + M_{ox}$$

$$M_{ox} + 2H^+ + 2e \rightarrow M_{red}$$

Net reaction: $H_2O_2 + 2e + 2H^+ \rightarrow 2H_2O$

First, H_2O_2 in the solution is reduced by the immobilized HRP. Then the reduced HRP is regenerated with the aid of the mediator, while the mediator itself is oxidized in the enzymatic reaction. Finally, the oxidized mediator is electrochemically reduced on the electrode, leading to an increase in the reduction current, as shown in figure 1.

Enzyme (HRP) or protein (Hb), in some modified electrodes, shows

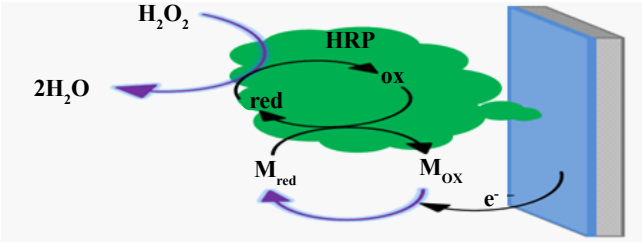

Figure 1: Schematic diagram of the reaction sequence within the enzyme electrode.

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direct electrochemistry. In this case, the mediator is not necessary. In the presence of H_2O_2, the reduction current of voltammogram for the direct electron transfer of enzyme or protein generally increases due to the electrocatalytic behavior of immobilized enzyme or protein to the reduction of H_2O_2. Furthermore, the reduction peak current increases with increasing H_2O_2 concentration. The electro catalytic mechanism could be expressed as follows [18,19].

Enzyme/protein (reduced form) $+H_2O_2 \rightarrow$ Enzyme/protein (oxidized form) $+H_2O$

Enzyme/protein (oxidized form)$+2e+2H^+ \rightarrow$ Enzyme/protein (reduced form) $+H_2O$

Net reaction: $H_2O_2+2e+2H^+ \rightarrow 2H_2O$

Enzyme (or protein) is efficiently converted to its oxidized form, which is then reduced at the electrode surface by the direct electron transfer. Therefore, the reductive current increased in the presence of H_2O_2.

HRP based H_2O_2 Biosensors

Mediated HRP based biosensor

HRP is one of the most extensively studied and commonly used enzymes for the construction of H_2O_2 biosensors. It contains heme as a prosthetic group, which is the protein active site, along with the heme-iron Fe (III). It can catalyze the oxidation of a wide variety of substrates by H_2O_2. Moreover, the reduced form of HRP can be chemically reoxidized by H_2O_2. Generally, direct electron transfer between HRP and an electrode is difficult because the active sites of HRP are deeply buried in a thick protein shell, and because the large distance between the active sites and the electrode surface will slow down the electron transfer. Electron transfer via a mediator, however, is more effective for establishing an electrical connection between the redox centers and the electrode. Lin et al. [20] fabricated a H_2O_2 biosensor by immobilizing HRP on the methylene blue modified graphite electrode. Here, methylene molecule act as a mediator, which could shuttle electrons between immobilized HRP and graphite substrate. Both ferrocene [21] and carboxylic acid functionalized ferrocene [22], have been used as a mediator with the HRP for the construction of H_2O_2 biosensor. Wang et al. [23] used potassium hexacyanoferrate with the peroxidase for the fabrication of biosensor, and found a detection limit of 0.5 μM for H_2O_2. A lower detection limit was obtained when they used tetra thiafulvalene [24], and methylene green [25], as a mediator. Wang et al. [26] utilized catechol mediator with the HRP enzyme for the construction of biosensor. They have used sol-gel/organic hybrid composite material for the immobilization of HRP. For the covalent immobilization of HRP, Gao et al. [27] used thionine modified surface, where thionine itself act as a mediator. A successful entrapment of HRP in a gelatin matrix was proposed by Yao et al. [28], for the development of H_2O_2 biosensor. The same group also used nano composite of methylene blue and silicon oxide as mediator to construct a biosensor with HRP, co-immobilized in the gelatin matrix and cross-linked with formaldehyde [29].

Nano materials along with mediator have been widely used for the development of HRP based H_2O_2 biosensor. Among the different nano materials, gold nano particles are the mostly used materials for the immobilization of HRP. Lei et al. [30] used nano gold film modified surface for the efficient and stable immobilization of HRP. The modified electrode exhibited electro catalytical response to the reduction of H_2O_2. A linear range from 6.1 μM to 1.8 mM, with a detection limit of 6.1 μM was obtained. Lei et al. [31] immobilized HRP labeled nano-gold on a silica sol-gel/alginate hybrid film and found a better performance, compared with the nano gold film modified surface. A more better result was obtained when the immobilizing matrix was replaced with the poly (2,6-pyridinedicarboxylic acid) [32,33], as reported a nano-Au monolayer for the immobilization of HRP. They used thiol functional group-derived Carbon Ceramic Electrode (CCE) and obtained monolayer through covalent linkage between nano-Au and thiol group on the surface of CCE. The fabricated biosensor by immobilizing HRP showed a linear range of 12.2 μM to 1.1 Mm, with a detection limit of 6.1 μM. A lower detection limit was obtained by using PAMAM dendrimer/cyst amine to support the nano-Au monolayer [34]. A Layer-by-layer (LBL) assembly of nano-Au and Toluidine Blue (TB) was also reported for the fabrication of a mediated H_2O_2 biosensor [35]. Zhu et al. [36] used gold nanoparticles, nafion, Polythionine (PTH), and gelatin as matrixes for the immobilization of HRP and construction of H_2O_2 biosensor, as well. The biosensor showed the catalytic reduction of H_2O_2 and the obtained detection limit was 20 μM. Ahammad et al. [16] lowered the detection limit to 1.5 μM by using gold nanoparticle-adsorbed conducting PTH modified GCE [37]. The fabrication process of the biosensor is shown in figure 2. PTH nanowires have also been synthesized by electro deposition in porous Anodic Aluminum Oxide (AAO) template, and used to encapsulate HRP and nano-Au by in situ electrochemical copolymerization [38]. The resulting PTHNWs–HRP–nano-Au film modified electrode showed to be excellent amperometric sensors for H_2O_2. For the fabrication of a H_2O_2 biosensor, Jia [39] immobilized HRP-Au nanoparticles on a viologen-modified Glassy Carbon Electrode (GCE) by amino cation radical oxidation in basic solution. Viologen acts as a mediator and a covalent linker between GCE and the Au nanoparticles. A nanocomposite of gold nanoparticles–bacterial cellulose nano fibers was also described for the immobilization of HRP [40]. The constructed biosensor showed electro catalytic activities to the reduction of H_2O_2, in the presence of the mediator hydroquinone (Figure 2).

Carbon nanotubes are also used for the development of H_2O_2 biosensor. Tripathi et al. [41] developed an amperometric H_2O_2 biosensor by entrapping HRP in an ormosil composite, doped with ferrocene monocarboxylic acid–bovine serum albumin conjugate and Multiwall Carbon Nanotubes (MWCNTs). The proposed H_2O_2 biosensor exhibited a linear range of 0.02–4.0 Mm, with a detection limit of 5.0 μM. The detection limit is lowered to 0.5 μM, when the fabrication of the biosensor was based on the co-immobilization of HRP, Methylene Green (MG), and MWCNTs, within ormosils [42]. It is still better compared with the Layer-by-layer (LBL) assembly of the nanocomposite of Methylene Blue-Multiwalled Carbon Nanotubes (MB-MWNTs) and HRP [43]. However, a higher sensitive H_2O_2 biosensor can be constructed by using the nanocomposite of brilliant cresyl blue-MWCNTs [44], nile blue-MWCNTs [45] and toluidine blue-MWCNTs [46], methylene blue-MWCNTs [47]. Moreover, a very high sensitive H_2O_2 biosensor can be constructed by wrapping the nanocomposite of MB-Sodium Dodecylsulfate (SDS) with the MWCNTs [48].

Chitosan (CHIT) is extensively used for the development of

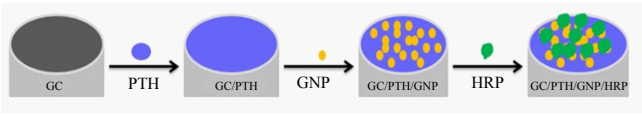

Figure 2: Schematic diagram of the fabrication of the GCE/PTH/GNP/HRP biosensor electrode [37].

mediated HRP based H_2O_2 biosensor. Wang et al. [49] described the fabrication of a H_2O_2 biosensor *via* an easy and effective enzyme immobilization method with the "sandwich" configuration: ferrocene-CHIT: HRP: CHIT-glyoxal. The biosensor surface was cross-linked with glyoxal, in order to prevent the loss of immobilized HRP. Ferrocene was selected and immobilized on the GCE surface as a mediator. Wang and Zhang [50] prepared a sol-gel organic-inorganic hybrid material from natural CHIT and tetrakis(2-hydroxyethyl) orthosilicates. An amperometric H_2O_2 biosensor was developed by immobilizing HRP onto the hybrid gel matrix. The linear range for the determination of H_2O_2 was found to be from 1.0 μM to 0.3 Mm, with a detection limit of 0.4 μM. Another organic–inorganic hybrid was prepared by Chen et al. [51] by dispersing colloidal carbon microspheres in CHIT solution. Then, they constructed a H_2O_2 biosensor by entrapping HRP in hybrid material. Also, organic-inorganic hybrid material composed of zirconia-CHIT sol–gel and Au nanoparticles have been used for entrapping HRP and fabricating H_2O_2 biosensor, as shown in figure 3 [52]. A little lower detection limit was obtained than the previous one. Fe_3O_4/CHIT modified GCE can be used for the immobilization of HRP and fabrication of H_2O_2 biosensor, as well [53]. However, the sensitivity of the biosensor is not as good as previous one. The sensitivity of the biosensor increased when magnetic carbon-coated iron nanoparticles were used to immobilize HRP on the surface of a PTH modified GCE, in combination with CHIT and cross-linking of glutaraldehyde. A very high sensitive biosensor was reported by Liu et al. [54], based on HRP and γ-Al_2O_3/CHIT composite film at a GCE. The sensitivity of the biosensor again decreased when HRP and CHIT-wrapped $NiFe_2O_4$ nanoparticles [55], or MgO nanoparticles-CHIT composite matrix [56], were used. Kafi et al. [57] developed a H_2O_2 biosensor based on the co-immobilization of HRP and CHIT, onto Au-modified TiO_2 nanotube arrays. Electrochemical measurements show that the Au-modified TiO_2 nanotube arrays provide excellent matrices for the immobilization of HRP, and that the optimized electrochemical biosensor exhibits long linearity, a low detection limit, high stability, and very good reproducibility for the detection of H_2O_2 (Figure 3).

Various polymer films have been reported for the development of mediated HRP based H_2O_2 biosensor. Qu et al. [58] proposed a H_2O_2 biosensor by encapsulating HRP in situ, in poly (neutral red) nanowires (PNRNWs) by electrochemical copolymerization. The PNRNWs showed excellent efficiency of electron transfer between the HRP and the GCE for the reduction of H_2O_2, and the PNRNWs–HRP modified GC electrode showed to be excellent amperometric sensors for H_2O_2 at -0.1V, with a linear response range of 1 μM to 8 mM. For high enzyme loading density and long-term retention of bioactivity, Zeng et al. [59] synthesized a looped HRP-poly amidoamine nanohybrid

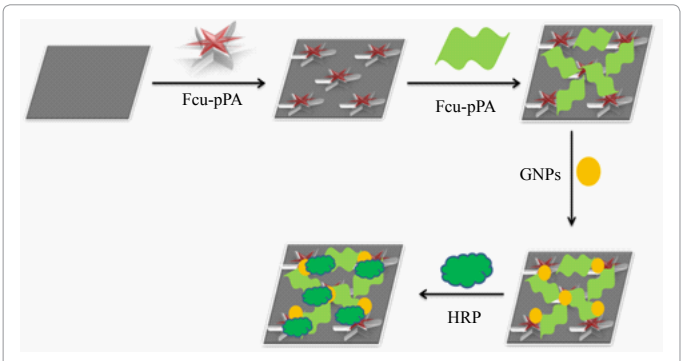

Figure 3: Illustration of the fabrication process of the biosensor (redrawn from reference [52], [80]).

for the construction of a H_2O_2 biosensor. Polythiolated-β-cyclodextrin polymer also synthesized for immobilizing adamantane-modified HRP via supramolecular associations [60]. Then the enzyme-containing electrode was used as an amperometric H_2O_2 biosensor. Another amperometric H_2O_2 biosensor was fabricated by immobilizing HRP on a GCE by poly (glycidyl methacrylate-co-vinylferrocene) (poly(GMA-co-VFc)) film [61].

A polymeric electron transfer mediator containing copolymers of Gycidyl Methacrylate (GMA) and Vinylferrocene (VFc), with different molar ratios, was prepared by free-radical copolymerization. The mediated H_2O_2 biosensor showed a fast response of less than 4 s, with a linear range 2.0-30.0 mM. Some other meterials, such as β-cyclodextrin-branched carboxymethylcellulose residues [62], manganese oxide [63], zinc oxide [64], magnetic dextran microsphere [65], bilayer lipid membrane [66], and ionic liquid [67], are reported for the immobilization of HRP and development of mediated H_2O_2 biosensor. Table 1 summarizes characteristics of the mediated HRP based H_2O_2 biosensors.

Mediator-free HRP based biosensor

The direct electrochemical behavior of the enzyme or protein at the electrode surface provides a foundation for the fabrication of the mediator-free biosensors. It simplifies the preparation processes of sensing devices and avoids the toxicity of the mediator, in comparison with the mediator based biosensors. It features the advantages of high sensitivity and selectivity, and therefore, attracts considerable attention.

Depending on the immobilizing matrix, HRP exhibited direct electrochemical behavior towards the reduction of H_2O_2. For example, a quasi-reversible electron transfer was observed in the absence of mediator, when HRP is incorporated in a salt bridge-supported Bilayer Lipid Membrane (sb-BLM), modified with Lauric Acid (LA). Zhang et al. [68] utilize this quasi-reversible electron transfer to construct a H_2O_2 biosensor.

Different nano materials are widely used for the development of mediator-free HRP enzyme based biosensor. Liu and Ju [69] proposed a renewable reagentless H_2O_2 biosensor based on the direct electron transfer of HRP, which was immobilized on a gold nano particle-modified carbon paste electrode. Electrochemical methods were used to investigate the direct electrochemistry of HRP. The biosensor displayed an excellent electrocatalytic response to the reduction of H_2O_2, without the aid of an electron mediator. Gold nanoparticle-modified ITO electrode was also used for the immobilization of HRP and investigation of the direct electrochemistry of HRP [70,71]. However, the performance of the mediator-free biosensor increased when gold nanoparticle and HRP embedded simultaneously on sol–gel network [72]. Yin et al. [73] reported the preparation of HRP–Gold Nanoparticles (GNPs)–Silk Fibroin (SF) modified GCE by one step procedure, and investigated the direct electrochemistry of HRP at the modified electrode. The fabricated mediator-free biosensor showed an excellent and quick electrocatalytic response to the reduction of H_2O_2. A very sensitive mediator-free biosensor was prepared based on the layer-by-layer assembly of Mercapto Propionic Acid (MPA), Cystine-based polymethylene-bridged cyclic bisureas (CBU)/GNPs and HRP on gold electrode [74]. A lower detection limit of 50 nM for H_2O_2 was obtained. Gold electrode was further modified with one-dimensional gold nanowires (Au NWs) and TiO_2 nanoparticles (nano-TiO_2) [75]. However, the sensitivity of the biosensor using the modified electrode was not as high as the previous one. A better performance of the biosensor was obtained when a layered Calcium carbonate–Gold

Modified Electrode	Mediator	Linear range	Detection Limit (μM)	Reference
GE/MB/HRP	methylene blue	10–560 μM	3.0	[20]
SGCE/PPy/HRP	ferrocene carboxylic acid	0.9 μM–0.2 mM	50.0	[22]
GCE/SG/HG/HRP	potassium hexacyanoferrate	0.1 μM–3.4 mM	0.5	[23]
SGOIH/HRP	Tetrathiafulvalene	up to 1.3 mM	0.3	[24]
SPCE/ TH/HRP	thionine	5–65 μM	0.5	[27]
GCE/GNP/HRP	Hydroquinone	6.1 μM–1.8 mM	6.1	[30]
GCE/SSG/AH/GNP/HRP	Hydroquinone	12.2 μM–1.5 mM	0.6	[31]
PPDA/GNP/HRP	Hydroquinone	0.3 μM–2.0 mM	0.1	[32]
CSG/GNP/HRP	Hydroquinone	12.2 μM–1.1 mM	6.1	[33]
Cys/PAMAM/GNP/HRP	Hydroquinone	10 μM–2.5 mM	2.0	[34]
TB/GNP/HRP	toluidine blue	0.2 μM–8.6 mM	70 nM	[35]
GL/PTH/GNP/HRP	Polythionine	50 μM–30.2 mM	20.0	[36]
GCE/PTH/GNP/HRP	Hydroquinone	5.0 μM–0.2 mM	1.5	[37]
PTHNW/GNP/HRP	Polythionine	0.5 μM–13.0 mM	0.3	[38]
GCE/BAPV/GNP/HRP	BAPV	0.2 μM–2.0 mM	0.1	[39]
BCN/GNP/HRP	Hydroquinone	0.3 μM–1.0 mM	0.1	[40]
FMC/BSA/MWNTs/OC/HRP	ferrocene carboxylic acid	20 μM–1.0 mM	5.0	[41]
MG/MWCNTs/HRP	methylene green	0.5 μM–20.0 μM	0.5	[42]
MB/MWCNTs/HRP	methylene blue	4.0 μM–3.8 mM	1.0	[43]
BCB/MWCNTs/HRP	brilliantcresyl blue	0.3 μM–10.0 mM	0.1	[44]
NB/MWCNTs/HRP	nile blue	0.2 μM–38.0 mM	0.1	[45]
TB/MWCNTs/HRP	toluidine blue	up to 40 mM	1.7	[46]
MB/SDS/MWCNTs/HRP	methylene blue	0.2 μM–1.4 mM	5 nM	[48]
GCE/FC-CHIT/HRP/CHIT-GLY	Ferrocene	35.0 μM–1.1 mM	8.0	[49]
GCE/CMS/CHIT/HRP	Hydroquinone	0.1–1.6 mM	0.93	[51]
ZrO_2/CHIT/GNP/HRP	2,6-pyridinediamine -copper	0.8 μM–3.7 mM	0.3	[52]
GCE/ Fe_3O_4/CHIT/HRP	methylene blue	0.2–12.0 mM	100	[53]
GCE/ γ-Al_2O_3/CHIT/HRP	ferrocene carboxylic acid	0.5 μM–0.7 mM	70 nM	[54]
GCE/ $NiFe_2O_4$/CHIT/HRP	ferrocene carboxylic acid	10.0 μM–2.0 mM	2.0	[55]
Nano-MgO/CHIT/HRP	Hydroquinone	0.1 μM–1.3 mM	50 nM	[56]
TiO2 nanotube/CHIT/HRP	methylene blue	5.0 μM–40.0 mM	2.0	[57]
GCE/ PNRNWs/HRP	neutral red	1.0 μM–8.0 mM	1.0	[58]
GCE/PAMAM/HRP	Hydroquinone	3.1 μM–2.0 mM	0.8	[59]
GDE/PTHCD/HRP	Hydroquinone	28.0 μM–5.5 mM	7.0	[60]
PGMAVF/HRP	Ferrocene	2.0–30.0 mM	2.6	[61]
ZnO/CHIT/HRP	Hydroquinone	0.5 μM–70 mM	0.3	[64]
GCE/MDMS/HRP	Hydroquinone	0.2 μM–0.7 mM	78 nM	[65]
PLM/PDA/HRP	Hydroquinone	0.3 μM–3.1 mM	0.1	[66]
GCE/PTBA/RTIL/HRP	Hydroquinone	5.0 μM–17.5 mM	0.5	[67]

GE: Graphite Electrode; MB: Methylene Blue; HRP: Horseradish Peroxidase; SGCE: Sol–gel Derived Composite Carbon; PPy: Polypyrrole; GCE: Glassy Carbon Electrode; SG: Sol-gel; HG: Hydro Gel; SGOIH: Sol-gel Organic-Inorganic Hybrid; SPCE: Screen-Printed Carbon Electrode; TH: Thionine; GNP: Gold Nanoparticle; SSG: Silica Sol-Gel; AH: Alginate Hybrid; PPDA: Poly(2,6-pyridinedicarboxlic acid); CSG: Carbon Sol-Gel; Cys: Cystamine; TB: Toluidine Blue; PTH: Polythionine; GL: Gelatin; PTHNW: Polythionine Nanowire; BAPV: N,N′-bis(3-aminopropyl-4,4′-bipyridinium) Tetrabromide; BCN: Bacterial Cellulose Nanofibers; FMC: Ferrocene Mono Carboxylic acid; BSA: Bovine Serum Albumin ; MWCNTs: Multi-Walled Carbon Nanotubes; OC: Ormosil Composite; MG: Methylene Green; BCB: Brilliant Cresyl Blue; NB: Nile Blue; TB: Toluidine Blue; SDS: Sodium Dodecylsulfate; FC: Ferrocene; CHIT: Chitosan; GLY: Glyoxal; CMS: Carbon Micro Spheres; PNRNWs: Poly (neutral red) Nanowires; PAMAM: Poly(amidoamine); PTHCD: Polythiolated-β-Cyclodextrin; GDE: Gold Electrode; PGMAVF: Poly(glycidylmethacrylate-co-vinylferrocene); MDMS: Magnetic Dextran Microsphere; PDA: Polydopamine; BLM: Bilayer Lipid Membrane; PTBA: Poly(thiophene-3-boronic acid); RTIL: Room Temperature Ionic Liquids

Table 1: Characteristics of the mediated HRP based H_2O_2 biosensors.

Nanoparticles ($CaCO_3$–GNPs) inorganic hybrid composite was used for the immobilization of HRP [76].

Carbon nanotubes are widely used for the development of mediator-free HRP based H_2O_2 biosensor. Qian and Yang [77] developed an amperometric H_2O_2 biosensor, based on cross-linking HRP by glutaraldehyde with multiwall carbon nanotubes/CHIT (MWNTs/CHIT) composite film coated on a GCE. The linear range of detection towards H_2O_2 was from 16.7 μM to 0.7 mM, with correction coefficient of 0.998. A nanocomposite film of Tetrathiafulvalene–Tetracyanoquinodimethane (TTF–TCNQ)/MWCNTs modified gold electrode was prepared for the immobilization of HRP [78]. The prepared enzyme electrode was used for the bioelectrocatalytic reduction of H_2O_2, without using any mediator. A remarkable detection limit of 1.5 nM was achieved when HRP was covalently attached to layered nonoriented MWNTs modified electrode [79]. The composites of MWCNTs and core–shell organosilica@CHIT crosslinked nanospheres as an immobilization matrix for the construction of an amperometric H_2O_2 biosensor was described by Chen et al. [80]. HRP was immobilized onto the composite film through electrostatic interaction between oppositely charged organosilica@

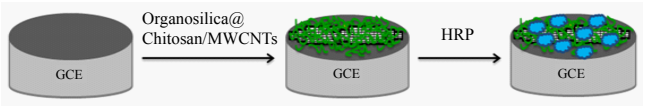

Figure 4: Illustration of the preparation process of the biosensor (redrawn from reference [80]).

CHIT nanospheres and HRP, as shown in figure 4. The direct electron transfer of HRP was achieved at HRP /organosilica@CHIT/MWNTs/GCE, which exhibited excellent electrocatalytic activity for the reduction of H_2O_2. Huang and Tsai [81] found direct electrochemistry and bioelectrocatalytic ability of HRP towards the reduction of H_2O_2 at the Multiwalled Carbon Nanotube/Alumina-coated Silica (MWCNT/ACS) nanocomposite modified GCE. The HRP/MWCNT/ACS nanobiocomposite modified GCE was prepared by casting HRP onto the surface of GCE. Direct electrochemistry of HRP was also achieved when HRP was cross-linked with MWCNTs, by using glutaraldehyde and bovine serum albumin. The sensitivity was found a little higher without using alumina-coated silica with MWCNTs. Besides CNTs, some other nanomaterials have been used for the development of HRP based mediator-free H_2O_2 biosensor, such as graphene [82-84], quantum dots [85], silver nanoparticles [86,87], silver nanowires [88], dendritic silver/silicon dioxide nanocomposites [89], graphene nano platelet [90], etc. (Figure 4).

Conducting polymer has been widely used for the construction of HRP based mediator-free H_2O_2 biosenosr. Kong et al. [91] investigated the direct electron transfer process of immobilized HRP on a conducting poly (5, 2': 5', 2''-terthiophene-3'-carboxylic acid). The HRP was immobilized by covalent bonding between amino group of the HRP and carboxylic acid group of the polymer. Amine group containing conducting polymer also can be used for the immobilization of HRP, by using cross-linking agent. For example, a H_2O_2 biosensor is constructed by cross-linking between HRP and Polyaniline (PANI), using glutaraldehyde as a cross-linking agent on F-doped Tin Oxide (FTO) electrode [92].The immobilization of HRP onto ordered mesoporous polymaniline was reported by Xua et al. [93], to construct a mediator-free H_2O_2 biosensor. The mesoporous polyaniline film was fabricated by electrodepositing from the hexagonal lyotropic liquid crystalline (LLC). The proposed biosensor combined the advantages of the good conductivity of polyaniline and the higher surface area of the ordered mesoporous film. The PANI nanofibers also reported for the immobilization of HRP [94]. Pt Nanoparticle (PtNP) was electrochemically deposited on the PANI nanofibers, which was electropolymerized on a gold electrode surface. Then, the hybrid film of GNPs, CHIT, and HRP was cast onto the modified electrode to form a stable biofunctional film. A higher sensitive H_2O_2 biosensor can be fabricated by using the hybrid film of chitosan and HRP onto the nanocomposite film of Au-Pt alloy Nanoparticles (NPs) and polyaniline nanotube [95].

Some other conducting polymers, copolymers and materials are reported for the construction of HRP based mediator-free H_2O_2 biosenosr such as polypyrrole [96,97], poly-(vinylferrocenium perchlorate) [98], poly 2,6-pyridinediamine [99], poly(3,4-ethylenedioxythiophene) [100], poly (N-isopropylacyamide-co-3-methacryloxy-propyltrimethoxysilane) [101], ionic liquids [102-104], 4-Carboxyphenyl [105], zirconia enhanced grafted collagen tri-helix scaffold [106], DNA films [107], Nafion–Sonogel–Carbon composite [108], silica–hydroxyapatite hybrid film [109], porous structure of screen-printed electrode [110], kieselguhr membrane [111], lipid membrane [112,113] etc. Table 2 summarizes characteristics of the mediator-free HRP based H_2O_2 biosensor.

Hb based H_2O_2 biosensor

It is well known that HRP is the most commonly used enzyme for the fabrication of H_2O_2 electrochemical biosensors. Owing to its expensiveness and unstableness, finding alternatives to reduce the cost and to improve the performance of H_2O_2 biosensors is scientifically interesting and important practically. Hb, a protein, can be utilized as the HRP substitute in the detection of H_2O_2, in virtue of the low cost and the stable property of in solution. Although Hb does not play a role as an electron transfer carrier in biological systems, it has been shown to possess enzyme-like catalytic activity. Its bioelectrocatalytic activity to reduce H_2O_2 is also well documented.

Modified Electrode	Linear range	Detection Limit (μM)	Reference
CPE/GNP/HRP	0.5–50 μM	0.2	[69]
ITO/APTMS /GNP /HRP	20.0 μM–8.0 mM	8.0	[70]
ITO/GNP/HRP	8.0 μM–3.0 mM	2.0	[71]
GDE/SSG/GNP/HRP	1.6 μM–3.2 mM	0.5	[72]
GCE/SF/GNP/HRP	10.0 μM–1.8 mM	5	[73]
GDE/MPA/CBU/GNP/HRP	0.4–0.9 μM	50 nM	[74]
GDE/nano-TiO2/AuNWs/HRP	2.3 μM–2.4 mM	0.7	[75]
GDE/ATP/CaCO3–GNPs/HRP	0.5 μM–5.2 mM	0.1	[76]
GCE/MWCNTs/CHIT/HRP	16.7 μM–0.7 mM	10.3	[77]
GDE/MWCNTs/TTF–TCNQ/HRP	5.0 μM–1.1 mM	0.5	[78]
PDDA/MWNTs/HRP	Up to 120 nM	1.5 nM	[79]
MWNTs/organosilica@chitosan/HRP	0.7 μM–2.8 mM	0.3	[80]
GCE/ACS/MWCNTs/HRP	1.0 μM–0.5 mM	0.6	[81]
GCE/CHIT/GP/HRP	5.0 μM–5.3 mM	1.7	[82]
ITO/CHIT/GP-Fe3O4/HRP	5.0 μM–3.8 mM	0.6	[83]
GP/GNP/CdTe–CdS/GNP/HRP	0.1–12.0 nM	0.03 nM	[84]
GCE/QDs/HRP	5.0 μM–0.1 mM	0.3	[85]
GDE/SNP/HRP	3.3 μM–9.4 mM	0.8	[86]
GDE/DNA/SNP/HRP	1.5 μM–2.0 mM	0.5	[87]
GDE/SNW/HRP	4.8 nM–0.31 μM	1.2 nM	[88]
GCE/DSSD/HRP	0.7 μM–0.1 mM	50 nM	[89]
TPA/SLGnP/HRP	0.6–16.8 μM	0.1	[90]
FTO/PANI/HRP	up to 20 mM	---	[92]
GCE/PANI/HRP	1.0 μM–2.0 mM	0.6	[93]
PANI/PNP/CHIT/GNP/HRP	7.0 μM–14.0 mM	2.8	[94]
Nano-PANI/GNP-PNP/CHIT/HRP	1.0 μM–2.2 mM	0.5	[95]
PPy/PNP/GNP/HRP	4.9 μM–4.8 mM	1.3	[97]
Pt/PPA/GNP/HRP	0.4 μM–1.5 mM	0.1	[99]
Au/PEDOT-PSS/HRP	0.2 μM–38.0 mM	0.1	[100]
GCE/PNM/HRP	0.2–1.4 μM	47.5 nM	[101]
GDE/DNA/ BMITB/HRP	10.0 μM–7.4 mM	3.5	[102]
β-CD//ILs/ BMIMBF4/HRP	4.0–84.0 μM	2.7	[103]
GDE/4-CP/HRP	20.0 μM–20.0 mM	5.0	[105]
SGE/HRP	4.0 μM–0.1 mM	1.6	[108]
GCE/SHAP/HRP	1.0 μM–0.1 mM	0.4	[109]
PSPCE/HRP	5.9–35.4 μM	0.5	[110]

CPE: Carbon Paste Electrode; GNP: Gold Nano Particle; ITO: Indium Tin Oxide; GDE: Gold Electrode; SSG: Silica Sol-gel; SF: Silk Fibroin; MPA: Mercapto Propionic Acid; CBU: Cyclic Bisureas; AuNWs: Gold Nanowires; ATP: 4-aminothiophenol; APTMS, (3-aminopropyl) Trimethoxysilane; MWCNTs: Multi-Walled Carbon Nanotubes; CHIT: Chitosan; TTF–TCNQ: Tetrathiafulvalene–Tetracyanoquinodimethane; PDDA: Poly(dially dimethylammonium); ACS: Alumina-Coated Silica; GP: Graphene; QDs: Quantum Dots; SNP: Silver Nanoparticles; SNW: Silver Nanowire; DSSD: Dendritic Silver/Silicon Dioxide; TPA: Tetrasodium 1,3,6,8-Pyrenetetrasulfonic acid); SLGnP: Single-Layer Graphene Nanoplate; PANI: Polyaniline; FTO: F-doped Tin Oxide; PNP: Pt Nanoparticle; PPy, polypyrrole, Pt: Platinum; PPA: Poly 2,6-Pyridinediamine; PEDOT: Poly(3,4-ethylenedioxythiophene); PSS: Poly(styrene sulfonic acid); PNM: Poly (N-isopropylacyamide-co-3-methacryloxy-propyltrimethoxysilane); BMITB: 1-Butyl-3-Methylimidazolium Tetrafluoroborate; β-CD: β-cyclodextrin; ILs: Ionic Liquids; BMIMBF4: 1-Butyl-3-Methylimidazolium Tetrafluoroborate; 4-CP: 4-carboxyphenyl; SGE: Sonogel–Carbon Electrode; SHAP: Silica–Hydroxyapatite; PSPCE: Porous Screen-Printed Carbon Electrode

Table 2: Characteristics of the mediator-free HRP based H_2O_2 biosensor.

Carbon nanotubes and GNPs are widely used with Hb for the construction of biosensor. Qi et al. [114] designed a mediator-free H_2O_2 biosensor by immobilizing Hb on MWCNTs modified with GCE. The direct electron transfer of the Hb was observed. A linear in the concentration range from 6.0 μM to 6.0 mM, with a detection limit of 1.2 μM was obtained. The direct electron transfer of Hb also observed onto MWCNTs enhanced grafted collagen matrix [115]. However, the fabricated biosensor by this way showed lower sensitivity. A high sensitive Hb based mediator-free biosensor can be constructed by using the nanocomposite of MWNTs, CeO_2 nanoparticles, and CHIT as an immobilizing matrix [116]. The composite matrix combined the advantages of MWNTs, CeO_2 nanoparticles, and CHIT, with good electron-transfer ability, attractive biocompatibility, and fine filmforming ability, which could increase Hb attachment quantity and H_2O_2 detection sensitivity. The nanocomposite of MWCNTs and GNPs also used for the fabrication of high sensitive Hb based mediator-free biosensor [117].

Zhang and Oyama [118] prepared a modified ITO electrode surface, with spherical and rod-shaped GNPs surfactant-assisted seeding growth approach, which provided a biocompatible matrix for the immobilization of Hb. The Hb immobilized GNPs-modified ITO (Hb/Au/ITO) electrode exhibited an effective catalytic response to the reduction of H_2O_2. The linear relationship existed between the catalytic current and the H_2O_2 concentration, in the range of 10 μM to 7 mM. The detection limit (S/N=3) was 4.5 μM. The nano molar detection limit can be achieved by using gold electrode, instead of ITO [119]. In this case 1,6-hexanedithiol (HDT) need to be used as a molecule bridge. The layer-by-layer assemble of the composite of C at SiO_2 with GNPs shown in figure 5, was also reported to achieve the lower detection limit [120] (Figure 5).

Tang et al. [121] described a facile strategy of a mediator-free H_2O_2 biosensor based on the direct electrocatalysis of Hb immobilized on GNPs/1,6-diaminohexane (DAH) modified GCE. A uniform monolayer film of DAH was initially covalently bound on a GCE surface, by virtue of the electrooxidation of one amino group of DAH, and another amino group was modified with GNPs and Hb, successively. In another way, a biocompatibile nanocomposite of colloidal gold and Hydroxyapatite nanotubes (Hap) was first prepared, then the Hb was immobilized on the nanocomposite [122]. The immobilized Hb showed fast direct electron transfer and excellent electrocatalytic behavior toward reduction of H_2O_2. Another composite film of gold colloid (nano-Au)/l-cysteine (l-cys)/nano-Au/nanoparticles Pt (nano-Pt)/CHIT was also reported for the fabrication of high sensitive Hb based mediator-free H_2O_2 biosensor [123].

One-dimensional (1D) GNPs were prepared and used for the immobilization of Hb, to construct an amperometric biosensor for H_2O_2 [124]. The linear range for the determination of H_2O_2 was 0.6 to 12.4 μM, with detection limit of 24 nM was obtained. Three-dimensionally ordered macroporous gold-nanoparticle-doped titanium dioxide (3DOM GTD) film by Wei et al. [125] to fabricate a mediator-free H_2O_2 biosensor. However, the detection limit was not as low as one-dimensional (1D) GNPs. Nevertheless, three-dimensional nanoporous Au networks modified Ti substrate can lower the detection limit to 20 nM [126]. Some other nanomaterials have been reported for the construction of Hb based mediator-free H_2O_2 biosensor, such as zirconium dioxide nanoparticles [127-129], iron nanoparticles [130-132], Fe_3O_4 nanoparticles [133], Ag nanoparticles [134], Cadmium telluride (CdTe) nanoparticles [135], titania nanotubes [136], nanographene [137], activated carbon nanoparticles [138], carbon nanofiber [139], etc.

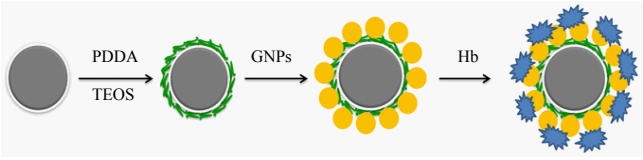

Figure 5: Procedure for preparation of Hb–GNPs–C@SiO_2(redrawn from reference [120]).

Modified Electrode	Linear range	Detection Limit (μM)	Reference
GCE/MWCNTs/Hb	6.0- μM–6.0 mM	1.2	[114]
grafted collagen-MWNTs/Hb	0.6–30.0 μM	0.1	[115]
CHIT/MWCNTs/CeO_2/Hb	5.0 μM–0.5 mM	0.6	[116]
GNP/MWCNTs/Hb	0.2 μM–3.0 mM	80 nM	[117]
ITO/GNP/Hb	10.0 μM–7.0 mM	4.5	[118]
HDT/GNP/Hb	50.0 nM–1.0 μM	10 nM	[119]
C@SiO_2/GNP/Hb	5.0–80.0 μM	80 nM	[120]
HAP/GNP/Hb	0.5–25.0 μM	0.2	[122]
GNP/PNP/CHIT/Hb	0.1 μM–6.6 mM	45 nM	[123]
ODGNP/Hb	0.6–12.4 μM	24 nM	[124]
ITO/TiO_2/GNP/Hb	5.0 μM–1.0 mM	0.6	[125]
Ti/TDN-Au/Hb	50.0 nM–0.2 μM	20 nM	[126]
PG/ZrO_2/Hb	1.5–30.2 μM	0.1	[127]
ZrO_2/Hb	0.8 μM–0.1 mM	0.1	[128]
SA/HZMS/Hb	1.8 μM–4.9 mM	0.6	[129]
CIN/Hb	3.1 μM–4.0 mM	1.2	[130]
CFN/CHIT/Hb	50 nM–1.0 mM	10 nM	[131]
GCE/MCMS/CHIT/Hb	69.0 μM–0.3 mM	21.0	[132]
Fe_3O_4/CHIT/Hb	50.0 μM–1.8 mM	4.0	[133]
GCE/AgNPs/Hb	1.0 μM–0.1 mM	0.3	[134]
CdTe/CHIT/Hb	7.4 μM–0.7 mM	2.2	[135]
TNT/Hb	1.0 μM–0.1 mM	0.9	[136]
PP123-NGP/Hb	10.0 μM–0.1 mM	8.2	[137]
GCE/CAN/NF/Hb	0.9–17.0 mM	0.4	[138]
PG/P123/Hb	1.0 μM–0.5 mM	0.5	[142]
GCE/PAN-co-PAA/Hb	9.2 μM–2.0 mM	4.5	[143]
GCE/BMIM•PF6–MSFs/Hb	0.2–28.0 μM	80 nM	[144]
BPPF6/MWCNTs/Hb	50.0 nM – 0.7 μM	3.8 nM	[145]
GCE/LCCP/Hb	7.0 μM–0.2 mM	3.1	[146]
Fe_3O_4-GP/Hb	1.5 μM–0.6 mM	0.5	[155]

Hb: Hemoglobin; HDT: 1,6-hexanedithiol; TDN: Three-Dimensional Nanoporous; HAP: Hydroxyapatite Nanotubes; ODGNP: One-Dimensional Gold Nanoparticles; PG: Pyrolytic Graphite; BMIM: 1-Butyl-3-Methyl-Imidazolium; HZMS: Hollow Zirconium Dioxide Microspheres; SA: Sodium Alginate, CIN: Carbon-Coated Iron Nanoparticles; CFN: Cobalt Ferrite Nanoparticles; MCMS: Magnetic Chitosan Microsphere; TNT: TiO2 Nanotubes; PP123: Pluronic P123, NGP: Nanographene Platelet; CAN: Activated Carbon Nanoparticles; NF: Nafion; P123: Triblock Copolymer EO20PO70EO20; PAN-co-PAA: Poly(acrylonitrile-co-acrylic acid); BMIM•PF6: 1-butyl-3-Methylimidazolium Hexafluorophosphate; MSFs: Mesocellular Siliceous Foams. LCCP: Liquid Crystal Cubic Phase; Mb: Myoglobin; RZnOMs: Rod-constructed Zinc Oxide Microspheres; ZGS: Zwitterionic Gemini Surfactant; TATP: Triacetone Triperoxide; SWNHs: Single-walled Carbon Nanohorns; PSSF: Poly(sodium 4-styrenesulfonate); PPI: Poly(propyleneimine)

Table 3: Characteristics of the Hb based H_2O_2 biosensors.

Polymer and copolymer have been used for the development of Hb based mediator-free H_2O_2 biosensor. Jia et al. [140] prepared an unmediated H_2O_2 biosensor by co-immobilizing Hb, with platinum nanoparticles enhanced poly(chloromethyl thiirane) cross-linked CHIT (CCCS-PNs) hybrid film. CCCS could provide a biocompatible microenvironment for Hb, and PNs could accelerate the electron transfer between Hb and the electrode. A linear range for H_2O_2 from

0.4 to 44 μM, with a limit of detection of 28 nM was obtained. A film of triblock copolymer EO20PO70EO20 (P123) was used to incorporate Hb onto the surface of Pyrolytic Graphite (PG) electrode [141]. The amphiphilic, biomembrane like structure of the film provided a favorable microenvironment around Hb to retain its biological activity and native structure. Immobilized Hb in the film displayed good electrocatalytic response to H_2O_2. Another film of copolymer poly(acrylonitrile-co-acrylic acid) (PAN-co-PAA) was also used for the immobilization of Hb [142]. The Hb in PAN-co-PAA matrix acted as a biologic catalyst to catalyze reduction of H_2O_2. Some other materials have been used for the fabrication of mediator-free Hb based H_2O_2 biosensor, such as ionic liquid [143-145], liquid crystal [146], sol–gel film [147-149], kieselgubr film [150], DNA membrane [151], triton X-100 [152], niobate [153], collagen [154], magnetite–graphene [155], etc. Table 3 summarizes characteristics of the Hb based H_2O_2 biosensors.

Conclusions

The goal of this review paper was to provide an updated summary of the HRP and Hb based electrochemical H_2O_2 biosensor. Modification of the electrode is an important task for the construction of biosensor. Nanomaterials modified electrode showed better performance, in terms of sensitivity and detection limit. However, less attention has been given to the fundamental research into understanding the mechanisms by which nano materilas give such excellent electrochemical performance. Enzyme based biosensor is still dominating, although they are costly. Moreover, the activity of enzymes is affected by the temperature, pH, humidity, and toxic chemicals. Much attention should be given on the simple fabrication, and cost should be considered during the construction of the biosensor. This would be helpful for the commercial production of the H_2O_2 biosensor.

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