

Sensitive electrochemical biosensing of H_2O_2 based on cobalt nanoparticles synthesised in iron storage protein molecules, ferritin

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Abstract: In this report, a highly sensitive electrochemical biosensor based on cobaltferritin immobilised on a self-assembled monolayer modified gold electrode for determination of hydrogen peroxide (H_2O_2) in phosphate buffer solution (pH 7.5) was investigated. The modified electrode showed excellent electrochemical activity for oxidation of H_2O_2 . The response to H_2O_2 on the modified electrode was examined using linear sweep and differential pulse voltammetries. In phosphate buffer (pH 7.5, 0.1 M), the fabricated biosensor exhibited a linear dependence ($R=0.989$) on the concentration of H_2O_2 from 2.49×10^{-9} to 1.91×10^{-8} M, a high sensitivity of $-0.4099 \mu\text{A/nM}$ and detection limit of 2.48×10^{-9} based on a signal-to-noise ratio of 3. Charge transfer coefficient (α) and the exchange current (i_0) of oxidation for H_2O_2 were found to be 0.57 and 7.55 A, respectively. It has been shown that, this modified electrode is able to determine H_2O_2 with a high sensitivity, low detection limit and high selectivity.

1 Introduction

Reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), hydroxyl radical, superoxide anion and peroxyxynitrite contribute to some diseases, such as Parkinson disease. Among the ROS, H_2O_2 is the most durable species which is the product of the reactions catalysed by a large number of oxidases. The physiological levels of H_2O_2 are involved in degradation and formation of reactive free radicals which can cause damages to parts of cells such as proteins, DNA and cell membranes [1]. It is widely used in the food industry to sterilise equipment related to mixing, transporting, bottling and packing [2]. In many countries, H_2O_2 has been accepted as a food additive of controlling the growth of micro-organisms, bleaching, removing glucose from dried eggs and controlling microbial growth in stored milk before cheese making [3]. H_2O_2 has been applied in the pulp and paper industry in recent decades [4]. Furthermore, H_2O_2 and its derivatives are powerful oxidising agents, which can therefore be employed in the synthesis of many organic compounds [5, 6]. Therefore determination of H_2O_2 concentration is practically important. Many selective and sensitive methods have been developed to determine H_2O_2 , such as titrimetry and spectrophotometry [7–9]. However, the existing methods usually cannot offer high sensitivity, reliability and operational simplicity at the same time, and often undergo interferences. Moreover, they may suffer from time–cost

and use of expensive reagents. Electroanalysis is suitable to determine H_2O_2 since it can attain low detection limit and rapid response time owing to direct reduction or oxidation of H_2O_2 [10]. Among the electrochemical techniques, a biosensor based on direct electron transfer (DET), that is, the third generation biosensor, between an electrode and immobilised biomolecules has gained great interest; the absence of mediators is the main advantage, providing them with higher selectivity, both because they should operate in a potential range closer to the redox potential of the biomolecule and are therefore less prone to interfering reactions. It should be noted that the absence of another reagent in the reaction sequence simplifies the reaction system. Direct electrochemistry of redox proteins or enzymes has been the research interest for many years, since they can be considered as good models for mechanistic studies of their electron transfer activity in biological systems [5, 11].

Ferritins are a class of iron storage and mineralisation proteins found throughout the animal, plant, and microbial kingdoms. Iron is stored within the protein shell of ferritin as a hydrous ferric oxide nanoparticle with a structure similar to that of the mineral ‘ferrihydrite’. The eight hydrophilic channels that traverse the protein shell are thought to be the primary pathways which facilitate iron entrance to the interior of eukaryotic ferritins. Twenty-four subunits construct the protein shell and, in mammalian ferritins, are of two types, H and L, which have

complementary functions in iron uptake [12]. As the naturally occurring iron core of ferritin can be removed in vitro by chemical and electrochemical reduction, ferritin has been used as a biological template for nanomaterial synthesis [13–15]. In addition, findings that ferritin can be reconstituted with various electroactive materials (CdS, Co, Mn, Ni, Pd and Pt) make ferritin suitable for biosensor and biofuel cell applications [16]. The method for immobilisation of redox proteins onto the electrode surface is the challenging issue to fabricate biosensors based on the DET of redox proteins. Ferritin is not a redox protein; however, it has been suggested that an electron transfer step is involved in the loading and unloading of iron into the apoferritin shell [11, 17].

A common methodology to immobilise biomolecules on gold metal surfaces can be through thiolated molecules forming self-assembled monolayers (SAMs) [11, 17–19]. These monolayers in some cases enable the oriented anchorage of biomolecules, thus achieving a high improvement in biosensor behaviour [18]. The biosensors with self-assembly technique can remarkably diminish non-Faradaic background currents resulting in improved sensitivity [20]. Therefore self-assembly technology was used to immobilise cobaltferritin on the gold electrode to fabricate cobaltferritin biosensor. The DET of cobaltferritin was investigated at gold electrode and found an electrochemical response at the modified electrode using dithiobis-*N*-succinimidyl propionate (DTSP). DTSP forms a stable SAM on a gold surface owing to the strong S–Au covalent bond, whereas the other side is reacted with protein. The surface-electrochemical behaviour of cobaltferritin on gold electrode, modified by DTSP has already been reported [17].

In this study, the electrochemical activity of the modified electrode for oxidation of H_2O_2 has been investigated. To evaluate the possible analytical application of the modified electrode, it has been used for the detection of H_2O_2 at nanomolar concentration range.

2 Experimental methods

2.1 Reagents and apparatus

Horse spleen apoferritin was purchased from Sigma and was used without further purification. All other chemicals were purchased from Merck.

Electrochemical experiments were performed using an electroanalyser (SAMA 500). A conventional three-electrode cell was used with a saturated Ag/AgCl (3 M NaCl) electrode as the reference electrode, a platinum wire as the counter-electrode and a gold electrode as the working electrode. All experiments were carried out at room temperature.

2.2 Preparation of cobaltferritin immobilised on a SAM-modified gold electrode

According to our previous work, apoferritin was used as a constraining reaction environment for the synthesis of cobalt nanoparticles. Under a range of synthetic conditions the protein cage remains unaltered [13]. Self-assembly technique provides a facile method to immobilise cobaltferritin and it can be utilised to provide a matrix for the fabrication of biosensors. Therefore the cleaning of the gold electrode is a critical important step in SAM formation which should be accomplished systematically. Electrode

pretreatment consisted of manually polishing the electrode surface with alumina slurry of 0.5 mm, until the surface looks a mirror. Then, the electrode was sonicated in pure ethanol for 5 min. After the mechanical cleaning, the electrodes underwent a chemical treatment by immersion in a piranha solution (1:3 mixture of 30% H_2O_2 –cons- H_2SO_4) for 5 min, and then the electrode was thoroughly washed with water. The next step was the gold electrode modification process using DTSP, which undergoes dissociative chemisorption onto the gold electrode. The DTSP solution was dissolved in DMSO, 5 mM. The modified electrode was prepared by spreading 5 μl of 5 mM DTSP solution onto the gold electrode with a microsyringe. Cobaltferritin immobilisation on DTSP-modified gold electrode was performed by dipping in 1 ml of cobaltferritin for 18 h at 4 °C with an applied potential of 1.0 V against Ag/AgCl, and then was rinsed with 100 mM HEPES buffer containing 60 mM of NaCl (pH 8.7). Protein was then covalently attached to the modified gold electrode through the reaction of the terminal succinimidyl groups with amino groups of cobaltferritin molecules [17].

3 Results and discussion

3.1 Electrochemical oxidation of H_2O_2 at cobaltferritin immobilised on DTSP-modified gold electrode

Electrocatalytic oxidation of H_2O_2 using immobilised cobaltferritin on DTSP-modified gold electrode was investigated by recording linear sweep voltammograms. Fig. 1 shows linear sweep voltammograms of modified and unmodified electrodes in the presence of H_2O_2 (1 mM) at 0.1 M phosphate buffer (pH 7.5). As shown for bare gold electrode, no anodic response of H_2O_2 can be seen in the potential range from -1.1 to -0.4 V (Fig. 1b). However, at the cobaltferritin immobilised on DTSP-modified gold electrode, the oxidation current was greatly increased, indicating that the anodic oxidation of H_2O_2 could be catalysed at modified electrode [1, 21–23].

Therefore immobilisation of cobaltferritin on DTSP-modified gold electrode is a suitable protocol for fabrication of third generation biosensors [21]. The main objective of this investigation is determination of H_2O_2 .

In principle, any electrode reaction is a heterogeneous redox reaction. If its rate depends exclusively on the rate of mass transfer, then we have a mass-transfer-controlled

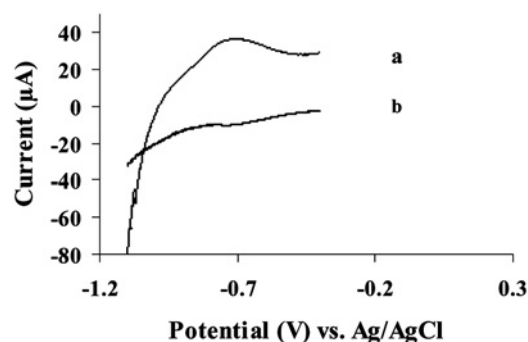


Fig. 1 Linear sweep voltammograms of

a Cobaltferritin immobilised on DTSP-modified gold and *b* Au electrodes in 1 mM hydrogen peroxide in 0.1 M phosphate buffer at 100 mVs

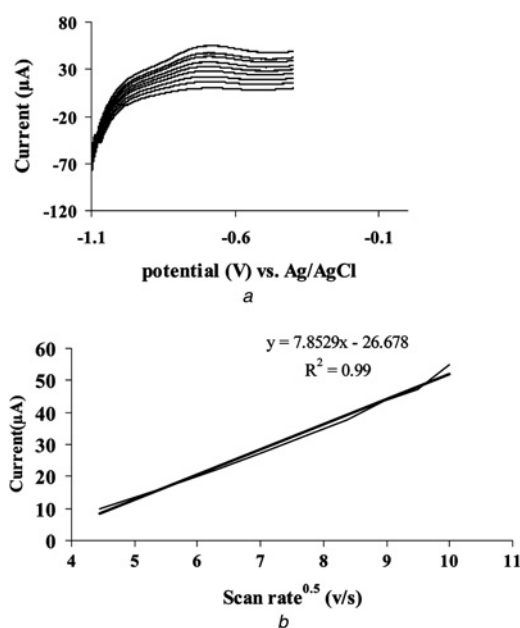


Fig. 2

a Linear sweep voltammograms of cobaltferritin immobilised on DTSP-modified gold electrode in 0.1 M phosphate solution containing 1 mM H_2O_2 at various scan rates: 20, 30, 40, 50, 60, 70, 80, 90, 100 mV/s
b Plot of I_{p_a} versus $v^{1/2}$

electrode reaction. If the only mechanism of mass transfer is diffusion (i.e. the spontaneous transfer of the electroactive species from regions of higher concentrations to regions of lower concentrations), then we have a diffusion-controlled electrode reaction. Fig. 2a shows the linear sweep voltammograms of 1 mM H_2O_2 solution at different scan rates. The oxidation peak increased with increasing scan rates and a linear relationship is found between the peak current and square root of scan rate, with a correlation coefficient of 0.99 (Fig. 2b). This behaviour indicates a diffusion-controlled process at the electrode surface [22, 24, 25].

In order to obtain some information about the rate-determining step, the Tafel plot is a useful tool to evaluate kinetic parameters. Therefore a Tafel-like plot was drawn using the data derived from the rising part of the current–voltage curve recorded at a scan rate of 20 mV/s for 1 mM H_2O_2 (Fig. 3). The slope of the Tafel plot is equal to $n(1 - \alpha)F/2.3RT$ or to 7.336 decade/V, the charge transfer coefficient (α), estimated from the slope was found to be 0.57, indicating that an electron process was involved in the rate determining step. The exchange current, i_0 , can also be estimated from the intercept of the Tafel plot it was found to be 7.55 A [5, 26, 27].

3.2 Linear range and limit of detection

Cobaltferritin immobilised on DTSP-modified gold electrode is very suitable to fabricate the third generation of mediatorless biosensors for determination of H_2O_2 . Since differential pulse voltammetric (DPV) is much more current sensitive than linear sweep voltammetry, this method was employed in order to estimate the low detection limit. Fig. 4 shows voltammograms of decrease in peak current with H_2O_2 concentration, and the inset shows the calibration curve of the biosensor for the successive

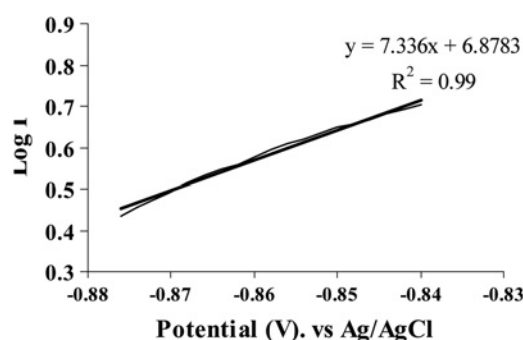


Fig. 3 Tafel plot derived from the rising part of voltammograms recorded at a scan rate 20 mV/s

addition of different concentrations of H_2O_2 . Co(II) ions are produced in the electrochemical reactions. These electroactive species in the presence of H_2O_2 , outside of ferritin cavity, can be converted to $\text{CoO}(\text{OH})$ precipitate which is a non-electroactive species [28]. Therefore the current is reduced as the concentration of H_2O_2 is increased.

There is a linear relation between response current and peroxide concentration in the range of 1.91×10^{-8} – 2.49×10^{-9} . The detection limit (signal to noise of 3) and sensitivity were 2.48×10^{-9} M and $-0.4099 \mu\text{A/nM}$, respectively. Table 1 indicates the detection limit, linear calibration range and sensitivity for H_2O_2 determination of this biosensor are comparable and even better than those have been reported for other modified electrodes [1, 6, 29–31].

3.3 Selectivity and analyses of the H_2O_2 biosensor

Seven interfering substances were used to evaluate the selectivity of the electrode. The interferents selected for this study include those that are likely to appear in biological and food samples. The influence of possible interfering species on the current response of the sensor was examined and is given in Table 2. The current response obtained with 1:2 concentration ratio of H_2O_2 and the interfering species was compared with the results obtained with that of pure H_2O_2 alone. The degree of interference from the substances can be judged from the value of the current ratio. In our

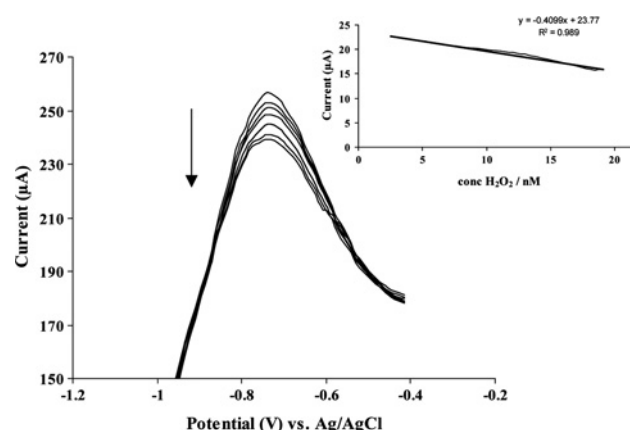


Fig. 4 Differential pulse voltammograms cobaltferritin immobilised on DTSP-modified gold electrode in 0.1 M phosphate buffer (pH 7.5) containing different concentrations of H_2O_2

Inset: calibration curve of the biosensor as a function of H_2O_2 concentrations

Table 1 Performances of the cobaltferritin immobilised on DTSP-modified gold electrode H₂O₂ biosensor compared to previously reported biosensors

H ₂ O ₂ biosensor	Sensitivity	Detection limit	Linear range	References
cobaltferritin/DTSP/Au	−0.4099 $\mu\text{A/nM}$	2.48 nM	2.49 nM–0.0191 μM	present work
Frt/CoOx NPs/GCE	0.2826 $\mu\text{A mM}^{-1}$	18 μM	0.2–15 mM	Moghaddam <i>et al.</i> [1]
HRP/Au/CPE	–	0.21 μM	0.48–50 μM	Liu and Ju [6]
TH/CCE	–	1.38 μM	3.44 μM –3.07 mM	Thenmozhi <i>et al.</i> [29]
HRP/Au/cys/nafion/Pt	–	0.105 μM	0.35 μM –5.87 mM	Liu <i>et al.</i> [30]
Zr(AMP)2/Mb/GCE	–	0.06 μM	up to 148.47 μM	Qiao <i>et al.</i> [31]

Table 2 Effect of interferents in the determination of H₂O₂ with cobaltferritin immobilised on DTSP-modified gold electrode

Interferents	Current ratio
uric acid	0.98
glucose	1.04
citric acid	1.02
acetic acid	1.03
ascorbic acid	1.07
SO ₄ ^{2−}	1.03
CO ₃ ^{2−}	0.98

Current ratio was obtained by comparing the current response of 2.6×10^{-4} M H₂O₂ + 5.2×10^{-4} M interferents with 2.6×10^{-4} M H₂O₂.

experiments, the seven tested substances did not interfere with the electrode. Thus, the sensor is capable of giving a remarkable selectivity for the determination of H₂O₂.

4 Conclusions

In this study, the gold electrode was modified using succinimidyl alkanedisulfide compound, which underwent dissociative chemisorption onto the gold electrode. Cobaltferritin was then covalently attached to the modified gold electrode through the reaction of the terminal succinimidyl groups with amino groups of ferritin molecules.

It proves that immobilised cobaltferritin still preserves its biological activity. Compared to the bare gold electrode, the cobaltferritin immobilised on DTSP-modified gold electrode displays high electrocatalytic activity towards the oxidation of H₂O₂, a linear dependence ($R=0.989$) on the H₂O₂ concentration from 2.49×10^{-9} to 1.91×10^{-8} M, a high sensitivity of $-0.4099 \mu\text{A/nM}$. The electro-oxidation of nanomolar H₂O₂ solutions was performed using modified gold electrode. Significantly lower detection limit, greater analytical sensitivity and stability response of this modified electrode compare favourably with all other modified electrodes employed as H₂O₂ sensors.

5 References

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