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An electrochemical glutathione biosensor: Ubiquinone as a transducer

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ABSTRACT

In this paper, coenzyme Q_{10} (Ubiquinone, CoQ_{10}) was used for the first time as a transducer to construct electrochemical biosensor for effectively detecting γ-L-glutamyl-L-cysteinyl-glycine (glutathione, GSH). CoQ_{10} modified electrode was fabricated by attaching its gel mixed with multi-walled carbon nanotubes (MWNTs)/ionic liquid (IL). In the optimum conditions, based on the increasing of reduction peak current of CoQ_{10} caused by GSH through voltammetric technology, it was found that the peak current of CoQ_{10} was linear with the concentration of GSH in the range from 4.0×10^{-9} to 2.0×10^{-7} mol L^{-1} at the pH 7.00, and the limit of detection was 3.2×10^{-10} mol L^{-1} ($S/N=3$). The results revealed that this method could be used to determine GSH in actual blood samples with the superiority of excellent selectivity, high stability and sensitivity. The strategy explored here might provide a new pathway to design novel multi-function transducers for detecting GSH, which has unique characteristic and potential application in the fields of sensor and medical diagnosis.

1. Introduction

Coenzyme Q_{10} (2,3-dimethoxy-5-methyl-6-decaprenyl-1, 4-benzoquinone, CoQ_{10}) is a fat soluble, vitamin-like quinine commonly known as ubiquinone or ubidecarenone [1,2], which is found to play an important role in biological systems and respiratory chains of mitochondria [3,4], existing in humans, most mammals and other edible vegetables oil [5–7]. CoQ_{10} is also a kind of electronic receptor [8], which can take part in electron and proton transfers [9–11], photosynthetic reaction [12–14], undergoing oxidation and reduction through a free-radical intermediate [15], etc. Because of the critical role of CoQ_{10} in biochemistry [16], Ma and co-workers reported that the functionalization of CoQ_n was embedded in a biomimetic membrane to mimic the initial stages of respiration [6], Murai revealed that the interactions of nicotinamide adenine dinucleotide (NADH)/CoQ_n oxidoreductase in mitochondrial were explored by photoaffinity labeling [17]. However, due to the strong hydrophobility of CoQ_{10}, its electrochemical investigations can only be performed in non-aqueous solutions. Therefore, only few methods had been reported for the immobilization of unfunctionalized CoQ_{10} on the electrode surface directly so far. Hence it still remains a great challenge to immobilize the unfunctionalized CoQ_{10} on the electrode surface and explore its electrochemical behaviors.

γ-L-Glutamyl-L-cysteinyl-glycine (glutathione, GSH) is a tripeptide, which contains an unusual peptide link between the amine group of cysteine and the carboxyl group of the glutamyl side chain [18–21]. GSH can be used as an indicator of some human diseases [22–27], including Alzheimer's, Parkinson's diseases, diabetes, macular degeneration and HIV disease. In addition, GSH shows other crucial functions [28,29], for example, Kalgutkar et al. revealed that GSH was a kind of nucleophilic reagent compound, which was prone to nucleophilic displacement reaction under some conditions [30–33], a few reports showed that nanomaterial modified electrodes had superior electrocatalytic properties for detecting GSH [34,35]. Till now, many kinds of methods have already been reported to research GSH, such as high performance liquid chromatography [36], spectrofluorimetry [37], spectrophotometry [38] and potentiometry [29,39]. However, most of these methods were intricate and time costing, so it remains a challenge to develop a simple, rapid and sensitive method for detecting GSH in biological samples.

It is interesting to further investigate the properties of CoQ_{10} with facile and sensitive electrochemistry method. However, there are very few examples of studies on CoQ_{10} for transducer element and biological applications [5,40–42], in this paper, CoQ_{10} was used as an attractive sensing element and a transducer. Initially, CoQ_{10} was dissolved in a mixed solution of benzene derivative and multi-walled carbon nanotubes (MWNTs)/ionic liquid (IL) to form a homogeneous gel, which was then successfully immobilized on the electrode surface. GSH determination in actual blood was evaluated at the modified electrode by simple and rapid voltammetric method. It was shown that the biosensor developed here exhibited advantages of facile set-up, high sensitivity, fast

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response and good stability, resulting from the combination of strong hydrophobility, high viscosity of ionic liquid, extremely high surface area and high conductivity of carbon nanotubes. The CoQ_{10}-based biosensor is expected to be applied for medical diagnosis, where GSH is involved.

2. Experimental

2.1. Chemicals and reagents

Coenzyme Q_{10} (CoQ_{10}) was purchased from Aladdin (Shanghai, China), nitrobenzene (NB) (Shanghai chemical Reagent Co. Ltd.) was the highest purity and was used as received. Multi-walled carbon nanotubes (MWNTs, 95% purity, diameter 20–40 nm, length 1–2 μm) were purchased from Shenzhen Nanotech port Co. Ltd. (Shenzhen, China) and purified by refluxing and as-received MWNTs in sulfuric acid and nitric acid (the volume ratio is 3:1) mixture solution for 8 h before use. Room-temperature ionic liquid (1-butyl-3-methylimidazolium hexafluorophosphate, $[BMIm]PF_6$, IL, 99% purity) was purchased from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences (Lanzhou, China), glutathione (GSH, 98% purity) was purchased from Shanghai source leaves biological technology Co. Ltd. (Shanghai, China). 0.1 mol L^{-1} phosphate (KH_2PO_4/K_2HPO_4) buffer solution (PBS, pH = 7.0, containing 0.1 mol L^{-1} KCl) was used as supporting electrolyte. Blood samples were obtained from a local hospital and prior to use, samples were centrifuged for 5 min at 5000 rpm in order to separate serum from plasma, and then, the obtained blood serum and plasma samples were diluted 10-fold and spiked with known amount of standard GSH solution. The anticoagulant was not added in blood samples. All other reagents were used as analytical grade. Aqueous solutions were prepared with doubly distilled water, and solutions were deoxygenated by bubbling with nitrogen for 10 min. All experiments were performed at room temperature (22 ± 2 °C).

2.2. Characterization

All electrochemical experiments were performed by using CHI 832 and CHI 660 electrochemical workstation (CH Instrument Company, Shanghai, China). A three-electrode system, including a working CoQ_{10}/MWNTs/IL composite film electrode (2.0 mm diameter), a saturated calomel reference electrode (SCE) and a platinum wire counter electrode, was employed. All potentials were measured and reported according to this reference electrode. Solution pH was measured with a Sartorius basic pH meter PB-10 (RenHe Instrument Co. Ltd., Shanghai, China). SEM images were taken using a field-emission scanning electron microscope (SEM, JEOL JSM-6701F) operated at an accelerating voltage of 5 kV.

2.3. Preparation and characterization of CoQ_{10}/MWNTs/IL gel

Ionic liquids (IL) are a new type of the non-aqueous media [43], which have perfect ion conductivity [44,45]. Multi-walled carbon nanotubes (MWNTs) have highly unique electronic, mechanical, and optical properties [46,47]. The acid-treated MWNTs were dispersed into Ionic liquids (IL) to form very stable and homogeneous black gel [48] with concentration of 1 mg mL^{-1} under the assistance of ultrasonication. Then, 100 μL of 6 mmol L^{-1} nitrobenzene solution of CoQ_{10} was mixed with 200 μL MWNTs/IL gel under 10 min sonication at room temperature to give a mixture of a black gel (CoQ_{10}/MWNTs/IL gel). The resulting CoQ_{10}/MWNTs/IL gel was placed on a glass slide, which was then dried in the vacuum to remove nitrobenzene.

2.4. Preparation of CoQ_{10}/MWNTs/IL gel modified electrode

Before experiment, glass carbon electrodes (GCE) were firstly polished with 0.3 and 0.05 μm α-Al_2O_3 powder on a polishing cloth, followed by sonication with acetone, ethanol and distilled water, respectively. The prepared mixture gel was confined onto clean glass carbon electrodes by rubbing the electrode on the gel placed on a smooth glass slide [48]. Then, MWNTs/CoQ_{10}/IL nanocomposite film was immobilized on the electrode surfaces. Finally, the CoQ_{10}/MWNTs/IL gel modified electrodes were immersed into a certain concentration of GSH solution to explore electrochemical characteristics.

3. Results and discussion

3.1. Characteristics of the MWNTs/CoQ_{10}/IL nanocomposite film

The synthesis of the multifunctional nanocomposite film is started from the multi-walled carbon nanotubes (MWNTs) and coenzyme Q_{10} (CoQ_{10}) in ionic liquid (IL). The morphologies of MWNTs/IL, MWNTs/CoQ_{10}/IL and CoQ_{10}/IL film were investigated by SEM, as shown in Fig. 1A–C. The spaghetti-like tangled MWNT nanotubes can be clearly distinguished from Fig. 1A. After adding CoQ_{10} into MWNTs/IL, well-shaped MWNT nanotubes can still be seen, but it is noteworthy that apparent aggregation or crystallization of CoQ_{10} is not observed as shown in Fig. 1B, implying homogeneous dispersion of CoQ_{10} in the gel prepared. From Fig. 1C, in the absence of MWNTs, the obvious wrinkles are the cause of the IL high viscosity. The procedure of MWNTs/CoQ_{10}/IL nanocomposite film gel was repeated to obtain a dense triple-component (MWNTs, CoQ_{10}, IL) film, as shown in Scheme 1.

3.2. Electrochemical behaviour of MWNTs/CoQ_{10}/IL nanocomposite film

Fig. 2 showed the electrochemical behaviors of different samples (MWNTs/CoQ_{10}/IL, CoQ_{10}/IL, MWNTs/IL and IL) studied by cyclic voltammetric in 0.1 mol L^{-1} PBS with potential ranging from −0.4 to −0.8 V. It was found that the reduction peak of CoQ_{10} occurred at about 0.681 V on the positive scan, while on the reversed scan, no peak was observed (solid curve *a* and *b*). Compared with solid curve *b*, in the presence of MWNTs, the peak potential of CoQ_{10} did not change but the peak current (solid curve *a*) increased significantly, with difference of peak current ($i_{pa}-i_{pb}$) up to 17.69 μA. Compared with solid curve *a* and *b*, the reduction peaks of solid curve *c* and *d* were not found in the absence of CoQ_{10}. It is known that carbon nanotubes are composed of cylindrical graphite sheets having superhydrophobicity and very large van der Waals index, leading to remarkably strong hydrophobic effect in adsorption of hydrophobic organic chemicals [49]. Thus, it is possible that MWNTs may have very good adsorption to CoQ_{10} [50,51] by physical interaction. So the increased peak current may be caused by high electroconductivity and high surface area of MWNTs, leading to high amount loading of CoQ_{10}.

3.3. The effect of GSH on MWNTs/CoQ_{10}/IL nanocomposite film

The electrochemical response of GSH towards MWNTs/CoQ_{10}/IL modified electrodes was studied by CVs shown in Fig. 3. It can be seen from Fig. 3b that signal of CoQ_{10} was only observed in the absence of GSH. However, along with the addition of quantitative GSH, the reduction peak current of CoQ_{10} increased gradually (Fig. 3a), and the maximum difference of peak current ($i_{pa}-i_{pb}$) was 29.55 μA. Because CoQ_{10} is a kind of electron-defect compound, it