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Ionic liquid based polymeric liposomes: A stable and biocompatible soft platform for bioelectrochemistry

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ABSTRACT

Polymeric liposomes (denoted as ILs-polysomes) are a biocompatible and conductive nanomaterial, which was first utilised as the electrode material for immobilising and biosensing redox enzyme horseradish peroxide (HRP). The morphology and surface property of IL-polysomes was characterised and systematically compared with unpolymerised ionic liquid based liposomes (denoted as ILs-liposomes). Differing from IL-liposomes, IL-polysomes preserves their original morphology and bilayer membrane structure on glassy carbon (GC) electrodes due to the cross-linking of polymerised lipids, thus exhibiting excellent stability and specific biocompability. Because of the existence of imidazolium ionic liquid moieties on the outer surface, IL-polysomes displays a positive charge in aqueous solution, leading to oppositely charged HRP self-assembling onto the vesicles to form HRP/IL-polysomes/PVA/GC electrodes. Owing to the combined merits of ILs and liposomes, electron transfer between HRP-Fe^{III}/Fe^{II} redox couples of immobilised enzymes and GC electrodes can be achieved. Therefore, HRP/IL-polysomes/PVA/GC electrodes exhibited good electrocatalytic performance toward the electrocatalysis of H_2O_2. Accordingly, IL-polysomes could act as an efficient charged platform for the self-assembled redox enzymes to realise direct electrochemistry. IL-polysomes have a promising application in the fabrication of third-generation electrochemical biosensors.

1. Introduction

There has been increased interest in the fabrication of an enzyme electrode to achieve direct electrochemistry, which can be then potentially utilised for the construction of novel electrochemical biosensors, biofuel cells and enzymatic reactors [1–4]. Moreover, the research into the direct electron transfer (DET) of redox enzymes has also attracted considerable attention for illustrating the electron transfer mechanism of biological systems [5–8]. Unfortunately, it is still a great challenge to achieve the DET for most redox enzymes on conventional electrodes due to their deeply buried electroactive sites and the unfavourable orientations of enzymes at electrodes [9,10]. Recently, it has been found that the proper use of conductive and biocompatible nanomaterials in the construction of enzyme electrodes is crucial to keep enzymatic bioactivities and promote direct electrochemistry [11–13].

As a well-known biomimetic nanomaterial, liposomes, which have a supramolecular assembly composed of an amphiphilic bilayer enclosing an aqueous interior volume, have attracted intensive interest for various biological applications, especially in the field of electrochemical biosensors [14–16]. It has been proven that the unique bilayer membranes of liposomes, which are similar to biological cell membranes, can provide a biocompatible environment to stabilise the conformation of immobilised enzymes [17]. Hence, prepared liposomes based enzymatic biosensors exhibit good analytical performance. However, liposomes also suffer the major drawback of limited physical stability in working environments [18,19]. To be specific, liposomes are prone to fusion with solid surfaces or other bio-substances. The inherent instability of liposomes not only leads to damaging their unique bilayer membrane and morphology, but also destroys the specific biocompability of the vesicles, thus further affecting the performance of the biosensors [20]. For this reason, many researchers are making great efforts to improve the stability of liposomes [21–23]. The introduction of covalent interaction between self-assembled lipids has been found to be an efficient strategy to form robust liposomes [24–26]. For instance, the lipids can be functionalised with polymerisable C = C double bond. Cross-linking modified amphiphiles after the formation of vesicles via polymerisation efficiently fixes the unique shape and structure of supramolecular assembles, thus endowing the liposomes with remarkably high physical stability [27–30]. Moreover, besides the polymerisable moieties introduced to improve the liposomal stability, it is desirable to tag other

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specific chemical functionalities onto lipids to enhance biosensor performance [31].

Being a kind of organic salt composed of an organic cation and various anions, ionic liquids (ILs) have attracted a lot of attention in redox enzymes direct electrochemistry because of their remarkable properties, especially good conductivity [32]. However, the liquid character of ILs at room temperature presents some difficult drawbacks, such as poor processability and the need for encapsulation due to possible leakage [33]. Based on this viewpoint, the concept of supported ILs (SILs) was developed to improve the applicability of ILs in various biosensors [34]. After the integration of ILs with other substrates via surface modification or polymerisation, the resulting non-liquid state SILs can present the unique properties of ILs together with the intrinsic properties of substrates [35]. Using this design concept, biocompatible SILs have also been fabricated using liposomes as the supporting matrix. For instance, Garcia et al. established a novel IL based polymerised liposome (ILs-polysomes) via the polymerisation of self-assembled IL modified lipids [36,37]. As a novel vesicle, IL-polysomes not only exhibit the enhanced physical stability of liposomes, they also make transforming the existing state of ILs from fluid to solidified nanomaterial a feasible process. Moreover, the assembly combines the major advantages of the two components, which are biocompability of liposomes and conductivity of ILs. Therefore, IL-polysomes may open up new opportunities for the development of electrochemical biosensors and bioelectronics. However, to the best of our knowledge, there is no report on the introduction of such multi-functionalised liposomes in the field of biosensors.

In this research, we firstly associated the IL-polysomes with a redox enzyme, horseradish peroxidase (HRP) to construct an integrated modified HRP/IL-polysomes/PVA/GC electrode. In order to illustrate the structural features of IL-polysomes, unpolymerised ionic liquid based liposomes (ILs-liposomes) were investigated as an analogous electrode material. Compared with IL-liposomes, IL-polysomes exhibited excellent stability maintaining their original morphology and bilayer membrane structure on GC electrodes. Moreover, HRP/IL-polysomes/PVA/GC electrodes also exhibited good HRP electron transfer properties and displayed good performance toward the electrocatalysis of H_2O_2, such as wide linear range, good reproducibility and long-term stability.

2. Experimental section

2.1. Chemicals

Horseradish peroxide (HRP) from horseradish and potassium persulfate were purchased from Sinopharm Chemical Reagent Co Ltd. 2-methylimidazole and 11-bromoundecene were purchased from J&K Scientific Ltd. All chemicals were of analytical grade and used without further purification. All solutions were prepared using Milli-Q purified water (>18.0 MQ) sterilised at high temperature.

2.2. Material characterisation

Electrochemical measurements were performed at room temperature using a BAS100B workstation. The measurements were based on a three-electrode system with the as-prepared modified electrode as the working electrode, a platinum wire as the counter electrode, and a saturated Ag/AgCl electrode as the reference electrode. Without special statement, 0.1 M pH 7.0 PBS was used as the electrolyte solution in all experiments. The buffer solution was purged with highly purified nitrogen for at least 30 min and a nitrogen atmosphere environment was kept during all electrochemical measurements.

UV–vis experiments were performed with UV-2100S spectrophotometer (Shimadzu). The FTIR spectra of samples in KBr pellets were recorded on a PerkinElmer instrument. The morphologies of various liposomes were observed utilising a Hitachi model H-800 transmission electron microscope (TEM) operated at an accelerating voltage of 100 kV and a scanning electron microscope (SEM, JEOL JSM-7400F) operating at 5 kV. For the SEM characterisation, the samples were prepared by casting on aluminium foil. The dynamic light scattering (DLS) and Zeta potential data of various liposomes were collected by using a Zetasizer Nano-ZS particle analyser (Malvern Corp, England).

2.3. Preparation of ionic liquid modified lipid N,N′-bis(10-undecenyl)-2-methylimidazolium bromide

The ionic liquid modified lipid *N*,*N*′-bis(10-undecenyl)-2-methylimidazolium bromide was prepared according to the reported method with a slight modification [36]. Typically, 11-bromoundecene (24.4 mmoL, 5.68 g), 2-methylimidazole (12.2 mmoL, 1.0 g) and triethylamine (14.6 mmoL, 1.48 g) were added to 100 mL of dried toluene. The mixture was then heated to 90 °C and constantly stirred for 48 h. After cooling to room temperature, the toluene was evaporated from the dispersion and the resulting oil product was purified by a recrystallisation procedure in acetone to form a white powder, which was the ionic liquid modified lipid.

2.4. Preparation of IL-liposomes and IL-polysomes

The prepared ionic liquid modified lipid (0.16 mmoL, 0.062 g) was dissolved in water (40 mL) and stirred continuously for 1 hour to form IL-liposomes via self-assembly.

Potassium persulfate (24 mg) was added to the solution of IL-liposomes. The solution was then purged with highly purified nitrogen for at least 30 min. Afterwards, the mixture was heated to 80 °C and stirred constantly for 24 h for polymerisation. According to the reported literature, the percentage of polymerisation for monomer is about 72% [36]. After the thermal polymerisation, the resulting dispersion containing IL-polysomes was subsequently cooled to room temperature, centrifuged and lyophilised. The IL-polysomes white powder obtained was further re-dispersed in water. The final concentration of the total lipids of IL-polysomes aqueous solution was maintained at 0.16 mM, which was the same as that of IL-liposomes aqueous solution.

2.5. Fabrication of various modified electrodes

Prior to use, glassy carbon (GC) electrodes with a diameter of 3 mm were polished on a polishing cloth with 1.0, 0.3, 0.05 μm alumina powder respectively and rinsed with deionised water followed by sonicating in acetone, ethanol and deionised water successively. Then the electrodes were dried with a purified nitrogen stream.

IL-polysomes/PVA/GC electrodes were prepared by a simple casting method. Firstly, 7 μL IL-polysomes aqueous dispersion was cast onto the GC electrode. Thereupon a beaker was used to cover the electrode so that water could evaporate slowly in air and a uniform film electrode formed. Then the prepared IL-polysomes/PVA/GC electrode was immersed into 0.10 M pH 9.7 phosphate buffer solution (PBS) containing 5 mg mL^{-1} HRP for 12 h to construct the enzyme electrode. The modified electrode was rinsed with deionised water to remove the excess HRP. Polyvinyl alcohol (PVA) sol (3%, 10 μL) was then added for encapsulation to construct HRP/IL-polysomes/PVA/GC electrode. The dried HRP/IL-polysomes/PVA/GC electrode was stored at 4 °C in a refrigerator when not in use.

For comparison, IL-liposomes/PVA/GC and HRP/IL-liposomes/PVA/GC electrodes were prepared with the same procedures as described above.

3. Results and discussion

3.1. Fabrication of HRP/IL-polysomes/PVA/GC electrodes

The fabrication of HRP/IL-polysomes/PVA/GC electrodes is shown in Schematic 1. As illustrated, the hydrophilic and hydrophobic parts,