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Electrodeposition of enzymes-integrated mesoporous composite films by interfacial templating: A paradigm for electrochemical biosensors

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ABSTRACT

The development of nanostructured electrodes for electrochemical biosensors is of significant interest for modern detection, portable devices, and enhanced performance. However, development of such sensors still remains challenging due to the time-consuming, detriment-to-nature, and costly modifications of both electrodes and enzymes. In this work, we report a simple one-step approach to fabricating high-performance, direct electron transfer (DET) based nanoporous enzyme-embedded electrodes by electrodeposition coupled with recent progress in potential-controlled interfacial surfactant assemblies. In contrast to those previously electrodeposited mesoporous materials that are not bioactive, we imparted the biofunctionality to electrodeposited mesoporous thin films by means of the amphiphilic phospholipid templates strongly interacting with enzymes. Thus, phospholipid-templated mesoporous ZnO films covalently inlaid with the pristine enzymes were prepared by simple one-step electrodeposition. We further demonstrate two examples of such hybrid film electrodes embedded with alcohol dehydrogenase (ADH) and glucose oxidase (GOx), which are effectively employed as electrochemical biosensors for amperometric sensing of ethanol and glucose without using any electron relays. The favorable mass transport and large contact surface area provided by nanopores play an important role in improving the performance of these two biosensors, such as excellent sensitivities, low detection limits, and fast response. The matrix mesoporous films acting as effective electronic bridges are responsible for DET between enzyme molecules and metal electrode.

1. Introduction

Construction of nanoarchitectured electrodes is of fundamental significance for the development of electrochemical biosensors in order to meet the requirements of promoting electron transfer, increased sensitivity, fast response, long-term stability, and the miniaturization of sensors [1–7]. A variety of nanomaterials, such as carbon nanotubes (CNTs) [8–12], gold nanoparticles (AuNPs) [13–17], and nanopores [18], have thus been utilized as the elements of high-performance enzymatic electrochemical biosensors in view of their intrinsic electrocatalytic activity as well as usage as electrical connectors to improve electrical transduction and contact between enzymes and electrodes. Particularly, direct electron transfer (DET) between enzymes and electrodes can be optimized by modifying the electrode surface with carbon nanotubes [9] and gold nanoparticles [13–17]. However, to achieve effective DET, two issues are generally encountered with previous methods. One involves complicated chemical fabrication and multiple steps, including the modification of both redox enzymes and underlying electrodes, processing of preformed nanomaterials, and subsequent integration of the modified enzymes and nanomaterials on the pretreated electrode. The other issue is the uniform spatial distribution of enzyme molecules on the electrode surface to ensure the stability of performance. In order to retain the original function of biomolecules and to avoid the laborious and costly modifications of analytes and electrodes, it is desirable to streamline the fabrication procedures of biosensors by facile and reliable enzyme immobilization on the electrode surface to promote DET between active sites of enzymes and electrode [5].

Alternatively, nanoporous electrodes represent a promising electrode design for amperometric biosensors with enhanced signal response and selectivity taking account of nanoporous materials possessing high specific surface area and suited pore size/channels for transport of current and analytes [19]. Recently, there has been growing interest in the electrodeposition of ordered nanostructured composites composed of structure-directing organic and inorganic ingredients, such as ZnO [20–24], $Ni(OH)_2$ [25], SnO_2 [26], and $Co(OH)_2$ [27], from dilute surfactant solution by virtue of the self-assembly of metal ions and the

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concentrated surfactant templates close to the electrode surface. This electrodeposition strategy gains access to one-step synthesis of various organic–inorganic composite films, through which functional organic components are readily incorporated. Furthermore, the organic-inorganic binding affinity in these mesoporous composite films allows for a structural and electronic coupling with enzymes and analytes, especially when the thickness and pore sizes of the mesoporous composite films are comparable to those of enzymes and analytes, respectively. Additionally, electrodeposition is a simple low-temperature technique that is compatible with biomolecules. The unique advantages of electrodeposition, combined with the diversity of composite deposits, could enable one to establish an effective platform for manufacturing many biofunctional devices.

However, in the previous examples, the structure-directing organics serving as the interfacial templates are not bioactive, so that no progress has been made to impart the nature of biofunctionality and biocompatibility to the composite films thus far. Hence, this gives us impetus to choose amphiphilic biomolecules, typically, 1,2-dipalmitoyl-sn-glycero-3-phosphate monosodium salt (DPGP), as the structure-directing organic mimicking the natural biomembranes to template the ordered mesostructures. More importantly, enzymes, such as alcohol dehydrogenase (ADH) and glucose oxidase (GOx), are known to exhibit a strong affinity to phospholipids with an amphiphilic structure, leading to the incorporation of enzymes into the phospholipid bilayers due to both their hydrophobic interactions with acyl chains of phospholipids and the electrostatic interactions with the charged phospholipid headgroup [28–31]. The formation of such enzyme–phospholipid complexes facilitates the immobilization of enzyme by means of co-immobilization of phospholipid. More specifically, the enzyme natural conformation is preserved within the amphiphilic structure of phospholipids, so will be the favorable orientation of the polypeptide moiety, thereby consolidating the accessibility of analytic substrates [30,31].

Herein, considering that ZnO-based matrix is well biocompatible, nontoxic, cost-effective, fast electron transport, and high electron mobility [32,33], we thus explore a newly–designed strategy to directly immobilize pristine redox enzymes, including ADH and GOx, onto the underlying electrodes by growing nanostructured thin composite films on working electrodes through cathodic deposition from a H_2O/*N*-methyl-2-pyrrolidone (NMPD) solution of $Zn(NO_3)_2{\cdot}6H_2O$ and a complex template of enzyme molecules bound to DPGP bilayers. Then, DET-based electrodes are realized via hybrid ZnO film bridge between the active sites of enzymes and the underlying electrode. We demonstrate that the enzyme-immobilized mesoporous ZnO film electrodes have been successfully used for amperometric biosensing of ethanol and glucose. In contrast to the complex nanoengineering routes to electrical connectors of biosensors, our protocol opens up a facile and promising way to design biosensors with optimal performance and reproducibility and allows an extension of this innovative strategy to the integrated assembly of a vast category of enzymes and mesoporous metal oxide matrices for extensive biosensing applications. In addition, we are able to simply enlarge the deposition electrode area to validate a scalable fabrication technique for biosensing.

2. Experimental

2.1. Materials and Instrument

All reagents were commercially available and used as received. Zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$) (98%), 1,2-dipalmitoyl-sn-glycero-3-phosphate monosodium salt (DPGP, ≥99%), dihydronicotinamide adenine dinucleotide (β-NADH, ≥97%, stored at −20 °C), β-NAD^+ from yeast, alcohol dehydrogenase (ADH) from saccharomyces cerevisiae protein (≥300 units/mg protein), and glucose oxidase (GOx) from *Aspergillus niger* (Type X-S, lyophilized powder, 100–250 units/mg, stored at −20 °C) were purchased from Sigma-Aldrich. Glucose, *N*-methyl-2-pyrrolidone (NMPD), KH_2PO_4, and K_2HPO_4 was purchased from Alfa-Aesar. Bovine plasma was commercially available from Beijing Biodee Biotechnology Corporation. Water used in this work was supplied from a Milli-Q water purifying system (>18 MΩ cm).

All electrodepositions of the composite films and electrochemical impedance spectroscopy (EIS) were performed on a PARSTAT 2273 potentiostat/galvanostat (Advanced Measurement Technology Inc.) by using three-electrode cells. All amperometric measurements were conducted on a CHI 660D electrochemical analyzer (CH Instruments, Chen-Hua Co., Shanghai, China) in 0.10 M PBS (ADH pH = 7.2; GOx pH = 7.4, N_2-purged) using a conventional three-electrode glass cell at 30 °C. The bovine plasma was passed through a 0.45 μm-diameter pore size filter before use. The filtered plasma was N_2-purged and then spiked with glucose to adjust the concentration ranging from 0.5 to 100 mM. The corresponding calibration plots were made from three separately prepared hybrid film electrodes. All other amperometric measurements were carried out in 0.10 M phosphate buffer solution (PBS, pH = 7.4) in pure water. The reference electrode was a saturated calomel electrode (SCE, E = 0.241 V vs SHE at 25 °C). For the electrodeposition experiments, gold plates served as the working electrode and a large surface area platinum plate was used as the counter electrode. The gold or platinum plate electrodes were prepared by thermally evaporating 10 nm of chromium and then 50 nm of Au on clean glass slides, or by depositing 10 nm of titanium followed by 50 nm of platinum on clean glass slides by sputter coating, respectively. For ESI and electrochemical measurements, the as-prepared film electrodes and a platinum wire were used as the working electrode and the counter electrode, respectively. Impedance measurements were carried out with a 2.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe and 0.10 M KNO_3 as the supporting electrolyte. The results were recorded potentiostatically by applying an ac voltage of 5 mV amplitude within a frequency range of $0.01–10^5$ Hz. The working electrode area was maintained at 1.0 cm^2 during the electrochemical measurements.

2.2. Electrodeposition

The cathodic deposition of ZnO/biomolecules composite films were carried out both potentiostatically and thermostatically at −0.5 V (vs SCE) for 60 min from a 0.02 M zinc nitrate solution containing the anionic surfactant DPGP, or a binary system composed of DPGP and ADH (or GOx). The cell temperature was set at 35 °C for all depositions. To fully dissolve DPGP that is insoluble in water, 25 wt % of NMPD was added into the plating solution. The weight percent of DPGP and the weight ratio of DPGP to ADH or GOx in the deposition bath were 0.02 wt% (corresponding to a saturation solution under this condition) and 4/1, respectively. The fully water miscible NMPD solvent is biocompatible and also helpful for maintaining bioactivity of enzyme, as shown in the recent studies of enzymatic catalysis [34,35]. To avoid denaturing the protein, the concentration of $Zn(NO_3)_2$ and NMPD should be as low as possible while maintaining both necessary conductivity of the electrolyte and the concentration of DPGP in solution. Meanwhile, to achieve sufficient content of inorganic moiety in the deposit for constructing high-quality nanostructured films, the concentration of $Zn(NO_3)_2$ was optimized at 0.02 M. For each deposition, the total mass of the plating solution was 20.00 g. For electrochemical measurements of the amperometric biosensors, the as-prepared film electrodes were thoroughly rinsed with a H_2O/NMPD (v/v 1:1) mixed solvent and then stored in 0.1 M PBS (pH = 7.0) at 4 °C. The composition of the