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Nanozeolite-assembled interface towards sensitive biosensing

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Abstract

A biocompatible surface has been constructed on the electrode surface via layer-by-layer assembly of beta-nanozeolites and polydiallyldimethylammonium (PDDA) for the adsorption of enzymes towards sensitive biosensing. The film assembly process and enzyme adsorption were monitored by Quartz Crystal Microbalance measurements. The nanozeolite film exhibited an amazing adsorption capacity (about 350 mg g^{-1}) for tyrosinase as a model enzyme. The tyrosinase biosensor showed a high sensitivity (400 μA mM^{-1}), short response time (reaching 95% within 5 s), broad linear response range from 10 nM to 18 μM, very low detection limit (0.5 nM) and high operational and storage stability (more than 2 months). The apparent Michaelis–Menten constant K_M^{app} was calculated to be 24 μM using phenol as the substrate. The assembly-controlled nanozeolite film could provide a biocompatible surface for the interaction study between enzymes and target molecules.
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1. Introduction

Surfaces play an important role in biology and medicine research since most biological reactions occur at surfaces and interfaces [1–3]. Controllable functionalization of the solid substrates' surfaces is critical for bioanalysis [4], including biosensing [5], enzymatic reaction [6,7], etc. Tailored surface properties such as tunable reactivity, biocompatibility, or wettability could be obtained by different approaches of surface modification, so that the design of biofunctional surface is of great interest in bioanalysis research [8–11].

Enzymes exhibit a number of features that make their use advantageous as compared with the conventional chemical catalysts. However, this is hampered by lack of stability and reusability. Compared to free enzyme in solution, the immobilized enzyme is more stable and resistant to the environmental changes by providing molecular level interactions with the substrates [12,13]. The main challenge is to develop a simple and general technique to engineer the surface for the immobilization of biomolecules. The layer-by-layer (LbL) technique is a facile method to engineer surface with targeted properties for the construction of biofunctional films [14–16]. The procedure is based on the alternative deposition of the oppositely charged polyelectrolyte [17], proteins [18], ceramics, or charged nanoparticles [19] on a charged surface by attractive electrostatic force. The functional surface made by alternate adsorption of charged nanoparticles and polyelectrolytes provides a route for extending three-dimensional "molecular" architecture in a direction perpendicular to the solid support [20]. This multilayer film maintains surface characteristics of nanoparticles and has good thermal and mechanical stability. Biomolecules embedded into such multilayer film could keep a secondary structure close to their native form, which is crucial to biological analysis [21].

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Up to now, many advanced functional materials have been used to immobilize biomolecules on the solid substrates, such as mesoporous silicates, zeolites and polymers. With the development of nanotechnology, many nanomaterials [22] have been reported in the application for fabricating the functional surfaces due to their large surface area for biomolecular immobilization. Nanozeolites possess special surface properties, including large and clean surface without any protection or surface-modifying agent, adjustable surface charge and composition, and stable colloidal property in both aqueous and organic solutions, which make them suitable to fabricate functional surface for the immobilization of biomolecules.

We report herein a biocompatible interface composed by a beta-nanozeolite three-dimensional architecture on an indium tin oxide (ITO) electrode using LbL assembly technique. The large surface area and unique surface property of the nanozeolite matrix resulted in a high enzyme adsorption capacity, and the enzyme adsorbed in this film retained its activity to a large extent. Adjusting the nanozeolite-assembled layers, thus regulating the amount of the enzyme immobilized, could facilely control the biocatalytic property of the enzyme electrode. The amperometric response of the enzyme electrode was acquired to probe the trace phenol as proposed.

2. Experimental section

2.1. Reagents

Tyrosinase (from mushroom, EC 1.14.18.1, $M_r \sim 125\,000$) was purchased from Fluka. Poly (diallyldimethylammonium chloride) (PDDA, $M_w < 200\,000$), poly(sodium 4-styrenesulfonate) (PSS, $M_w \sim 70\,000$) were obtained from Aldrich. 3-mercapto-1-propanesulfonate (MPS, 90%) were purchased from Aldrich. All chemicals were of analytical grade and were used without further purification. All solutions were prepared with bidistilled water.

2.2. Construction of the tyrosinase/nanozeolite/ITO electrode

Colloidal crystals of beta-nanozeolite were synthesized in a mixture solution with the molar composition of $12(TEA)_2O{:}Al_2O_3{:}60SiO_2{:}588\ H_2O$ according to a literature method [23]. The obtained nanosized zeolites were purified by centrifugation and redispersion in a 0.1 M ammonia solution for three times. The last colloid was adjusted to a solid content of 1.0 wt% and pH 10 to make the zeolite nanoparticles stable and negatively charged. The ITO electrode was cleaned by sequentially sonication in acetone and distilled water, and then treated in a basic solution ($NH_4OH{:}H_2O_2{:}H_2O = 1{:}1{:}5$ in volume ratio). Nanozeolite modified ITO electrode was prepared by LbL assembly method: the pre-treated ITO electrode was coated with cationic PDDA solution and anionic PSS solution in the sequence of PDDA/PSS to provide a negatively charged surface. Afterwards, the electrodes were alternately incubated in PDDA solution and negatively charged nanozeolite solution for 30 min each. When this cycle procedure was repeated N times, the $(\text{nanozeolite/PDDA})_N$/ITO electrode was obtained. Tyrosinase immobilization was achieved by immersing this modified electrode in tyrosinase solution (1.0 mg mL^{-1}, 20 mM phosphate buffer solution, pH 6.0) at 277 K. Fig. 1 displays the schematic model for tyrosinase/$(\text{nanozeolite/PDDA})_N$ films on the ITO electrode.

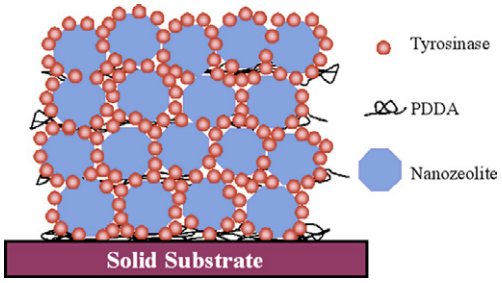

Fig. 1. Schematic diagram of tyrosinase/$(\text{nanozeolite/PDDA})_N$ film assembled on a solid substrate.

2.3. Quartz crystal microbalance measurements

The microgravimetric analysis was performed with Quartz crystal microbalance (QCM) analyzer (CHI440, CH Instruments, Shanghai, China) and quartz crystals (8 MHz) sandwiched between two Au electrodes (area 0.196 cm^2). The QCM resonator was cleaned in a mixed solution of H_2SO_4 and H_2O_2, followed by rinsing with bidistilled water and alcohol. Then the resonator was immersed into MPS solution to form a negatively charged surface. The assembly procedure of $(\text{nanozeolite/PDDA})_N$ multilayer films on QCM resonator was the same as that on ITO electrode. After each layer formed, the resonator was taken out, thoroughly rinsed with pure water and dried by N_2 blast, and then the frequency shift was measured. At last, immersed this modified resonator in tyrosinase solution (1.0 mg mL^{-1}, 20 mM phosphate buffer solution, pH 6.0) at 277 K, QCM measurement was made after rinsing and drying the resonator. The adsorbed mass of each layer was calculated with the Saurbrey equation [24]. Taking into account characteristics of the present resonators, the film mass per unit area M/A(g cm^{-2}) is given by the following equation: $M/A\ (\text{g cm}^{-2}) = -\Delta F(\text{Hz})/(1.45 \times 10^8)$.

2.4. Amperometric sensor measurements

The tyrosinase/$(\text{nanozeolite/PDDA})_N$/ITO sensor was immersed in a thermostated electrochemical glass cell containing 10 mL of 0.05 M phosphate buffer solution under different pH values (4.0–9.0) at 303 K. Under constant stirring, a current recording was started at a potential of −200 mV (versus SCE). The background current was

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