Indium tin oxide with zwitterionic interfacial design for biosensing applications in complex matrices

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Biosensing interfaces consisting of linker molecules (COOH or NH2) and charged, anti-biofouling moieties (−SO3− and N3−, Me3) for biosensing applications were prepared for the first time by the in situ deposition of mixtures of aryldiazonium cations on indium tin oxide (ITO) electrodes. A linker molecule is required for the attachment of biorecognition molecules (e.g., antibodies, enzymes, DNA chains, and aptamers) to the transducer surface. The attached molecules improve the biosensing sensitivity and also provide a short response time for analyte detection. Thus, the incorporation of a linker and an anti-biofouling molecule is an important interfacial design for both affinity and enzyme-based sensors. The relative adsorption behavior and electrochemical measurement were studied for (1) an individual compound and (2) a mixture of anti-biofouling zwitterionic molecules together with linker molecules (combination 1: 4-sulfophenyl (SP), 4-trimethylammoniumphenyl (TMAP), and 1,4-phenylenediamine (PPD); combination 2: 4-sulfophenyl (SP), 4-trimethylammoniumphenyl (TMAP), and 4-amino benzonic acid (PABA) of aryldiazonium cations grafted on an ITO electrode. The mixture ratios of SP:TMAP:PPD and SP:TMAP:PABA that provided the greatest resistance to non-specific protein adsorption of bovine serum albumin labeled with fluorescein isothiocyanate (BSA-FTC) and coenzyme labeled with rhodamine 6G (R6G-Cy3) were determined by confocal laser scanning microscopy (CLSM). For the surface anti-biofouling study, we used 2-[(2-methoxyethoxy)-ethoxy]actic acid (OEG) as a standard control because of its prominent anti-biofouling properties. Surface compositions of combinations 1 and 2 were characterized using X-ray photoelectron spectroscopy (XPS). Field emission scanning electron microscopy (FE-SEM) was used to characterize the morphology of the grafted films to confirm the distribution between linker and anti-biofouling molecules grafted onto the ITO surfaces. Combination 1 (SP:TMAP:PPD) with a ratio of 0.5:1.5:0.37 exhibited the best anti-fouling capability with respect to resisting the non-specific adsorption of proteins.

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1. Introduction

Biofouling is the accretion of proteins, cells, and other biological materials onto a bare electrode surface. For fast clinical screening, immunosensors are designed to detect disease analytes such as nucleic acids, proteins or other disease biomarkers at very low concentrations from complicated sample [1-5]. Such an accumulation of biofouling substances leads to reduced analyte diffusion and perfusion to the sensor interface, which eventually causes a decrease in sensor response and sensitivity. Polymers such as polyurethane, polyvinyl chloride, poly(dimethylsiloxane) and poly(methylacrylate) have been used as anti-fouling agents for biosensing surfaces, where they provide selective transport of a targeted analyte to the biosensing interface while preventing biofouling components from reaching the electrode [6-10]. Although these polymers are exceptionally suitable in clinical monitoring, their functional stability is greatly affected by the biocompatibility of the biosensor materials in contact with the biological sample [11,12]. Poly(ethylene glycol) (PEG) and self-assembled monolayers with oligo(ethylene glycol) chains (OEG-SAMs) have been used to reduce the non-specific adsorption of non-desired proteins [13]. The activity of anti-biofouling coatings in complex samples, e.g., human sera, depends on several factors, including the concentrations and chain lengths of PEG and OEG and the temperature. At 37°C, the coated surface can adsorb additional non-specific proteins. Moreover, surfaces grafted with PEG and OEG are prone to auto-oxidation in most biologically relevant solutions for in vivo studies [6,14,15]. Zwitterionic polymers such as phospho-rycholine, sulfobetaine, and carboxy betaine, which contain both
only formed with the present of PPD. The EDX results confirmed the deposition of GNP onto modified ITO electrode surfaces at an atomic ratio of 0.1.7 (Fig. 5(c)). The linkages of amine in the mixture of SP: MAP: PDD to GNP was more likely to occur by dehydrogenation from an amine group because of its stronger covalent bond. Such observations confirmed the homogenous grafting of the linker molecule (PPD) onto the ITO surface, unlike the SP: MAP: PABA mixture, which was dominated with PABA molecule (XPS results are shown in Table 2). The image of an SP: MAP: PABA-modified ITO electrode showed a thin, smooth, and featureless morphology indicating that no GNP could be deposited onto this type of surface (Fig. 5(d)). An obvious difference was noticed in images between the bare and SP: MAP: PABA modified ITO surfaces which captured at 1500x magnification (Fig. 5(d)).

4. Conclusions

A biosensing interface consisting of anti-fouling and linker molecules based on in-situ generated aryldiazonium cations was described. The differences in electrochemical behaviors among all types of modified surfaces (either with the individual compound or a mixed layer of aryldiazonium cations) were investigated and evaluated. An anti-fouling study utilizing coaxial laser scanning microscopy showed that ITO surfaces coated with mixed layers of SP: MAP: PDP 0.5:1:100 (mix 3) or SP: MAP: PABA 0.5:1:100 (mix 3) exhibited better anti-fouling capabilities against both BSA-FITC and RBTC-Cyt c compared to ITO surfaces coated with PPD: PABA: OEG. However, PPD: PABA: OEG coated ITO surfaces showed lower amounts of BSA-FITC adsorbed compared to SP: MAP: PABA coated ITO surfaces. The mixture of SP: MAP: PDP showed better resistance to nonspecific adsorptions of BSA-FITC and RBTC-Cyt c compared to SP: MAP: PABA. Analyses of XPS survey spectra further confirmed the deposition of SP: MAP: PDP and SP: MAP: PABA onto ITO surfaces. FE-SEM and EDX analyses showed dense and homogeneous distributions of GNP, which indicated a good distribution of linker and anti-fouling molecules on the ITO sensing surfaces. This study highlights the prospective use of aryldiazonium-cation-derivative/witnerion compounds and linker molecules for the easy fabrication of ITO biosensing interfaces. These interfaces can repel undesirable anionic and cationic proteins and detect target analytes in highly complicated biological matrices, such as blood, serum, and urine.

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