Covalently Attached Multilayer Assemblies of Diazo-resins and Glucose Oxidase

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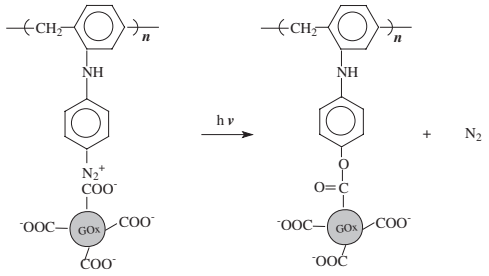
Stable multilayers of glucose oxidase were uniformly assembled on an Au electrode surface or a quarze slide by UV irradiation of ionic self-assembled multilayer films of diazo-resins (DAR) and glucose oxidase (GOx). By adopting this simple method, we achieved much greater control over the structure of the multilayer film, and we are able to analyze the catalytic responses of this GOx-modified electrode to glucose.

Layered construction of proteins into organized systems has attracted considerable attention in recent years due to its potential application in the areas of bioelectronic and biooptical devices, biosensors, and so on.[1] There have been a number of approaches for constructing multilayer protein films on the surface of solid matrix, including a layer-by-layer deposition of proteins on the surface of an electrode through a coupling reagent[2–4] and consecutive adsorption of positively and negatively charged polyelectrolytes and proteins on a solid surface through an electrostatic force of attraction.[5–8] The above methods have proven to be effective and successful ways to fabricate multilayer thin films containing proteins. However, there are some drawbacks in those techniques. The former procedure is complex and somewhat tedious, and the multilayer protein films fabricated by the latter are not stable enough. Therefore, their developments and applications are limited, and the quest for the molecularly organized and stable protein thin films is still a challenge. Here we introduce a new method to fabricate covalently attached multilayer films containing glucose oxidase by UV photoreaction of diazonium and carboxylate groups at the interface of DAR and GOx. This method combines the simplicity of the ionic self-assembly technique and the good stability of the covalently attached multilayer films. Thus it promises to be a very efficient method to prepare stable multilayer films containing GOx.

UV–vis spectra were obtained using a Shimadzu 3100 UV–vis-NIR spectrophotometer. IR spectra were obtained using a Bruker IFS66V FTIR instrument. Cyclic voltammetry (CV) was performed with a CHI 660A Electrochemical Workstation (CH instruments, USA) in a conventional three-electrode cell. The working electrodes (WE) used were modified gold electrode (Model CHI101, 2 mm-diameter). A twisted platinum wire was used as the counter electrode (CE) and a saturated calomel electrode (SCE) as the reference electrode (RE). DAR was synthesized according to previously reported procedure.[9] GOx from Aspergillus niger (100 units/mg) was obtained from Sigma. A freshly cleaned substrate (quartz or CaF_2 slide) was first immersed in 0.9 vol% aqueous cationic poly(diallyldimethylammonium chloride) (PDDA) solution for 20 min. After rinsing with deionized water, the substrate was alternately dipped into 0.1-M phosphate buffer solution (pH 6.8) of GOx (3 mg/mL) and then DAR (1.5 mg/mL) for 20 min, with intermediate water washing and drying. Multilayer films can be formed by repeating the above steps in a cyclic fashion. The above deposition process was conducted in the dark. Finally, irradiating the multilayer films with UV light for a given time is required to convert the ionic interaction of neighboring layers into covalent bonds. Fabrication of the multilayer films on a gold electrode is similar to that of above system by substituting PDDA with 3-mercapto-1-propanesulfonate (MPS).

The deposition process of the multilayer enzyme film was followed by UV–vis spectroscopy (Figure 1). The absorbance at 380 nm is attributed to the π–π^* transition of the diazonium group.[10] The absorbance at 380 nm increased in proportion to the number of deposited layers, suggesting the formation of enzyme multilayer films on the quartz slide. Moreover, the amount of DAR immobilized upon each deposition was constant, demonstrating a uniform deposition process.

Owing to the well-known photoreaction of diazonium and carboxylate groups,[11] the above assembled films containing eight bilayers of DAR/GOx were irradiated with a 30-W medium mercury lamp with appropriate filter ($360\,\text{nm} < \lambda < 380\,\text{nm}$) at a distance of 16 cm. As shown in Figure 2, the absorbance at 380 nm decreased gradually with irradiation time due to the decomposition of the diazonium group. From the inset it can be seen that the photodecomposition of the film follows the kinetics of a first-order reaction. The decrease of absorbance at 380 nm indicates the formation of a covalent linkage. The conversion of the ionic bonds to covalent bonds can be depicted as follow:



The photoreaction of diazonium and carboxylate groups in multilayer films was further confirmed by IR spectroscopy (not shown). Three absorbances at 2168 cm^{-1}, 1578 cm^{-1}, and 1113 cm^{-1} assigned to the stretching vibrations of $-N_2^+$, $-COO^-$, and N–O the complexes of diazonium and carboxylate ($-N{\equiv}N^+ \rightarrow OCO^-$), respectively, disappeared completely after irradiation. These results were consistent with that of UV–vis spectroscopy.

Cyclic voltammatry was used to study bioelectrocatalytic characteristics of the multilayered GOx films. Figure 3 shows the cyclic voltammograms obtained from the Au electrodes covalent-attached 0, 2, 4, 6, and 8 bilayers of GOx/DAR in the presence of 20-mM glucose. Anodic currents were remarkably increased for the multilayered electrodes due to the increased

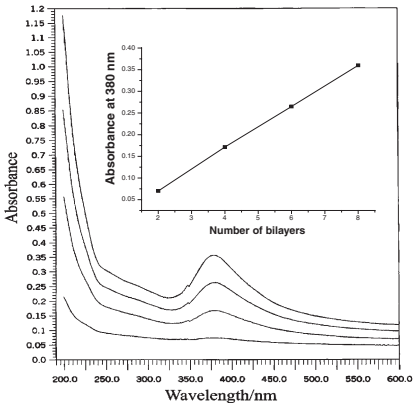


Figure 1. UV–vis absorption spectra of DAR/GOx multilayer films; from the lower to upper, the number of bilayers is 2, 4, 6, and 8. Inset shows the absorption at 380 nm vs the number of bilayers.

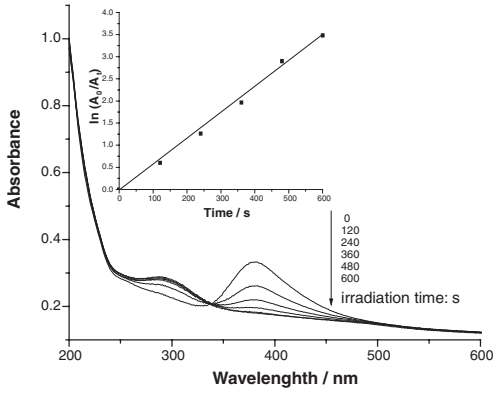


Figure 2. UV–vis absorption spectra of eight bilayers of DAR/GOx upon irradiation with UV light; Inset: Linear relationship between $\ln(A_0/A_t)$ and photoreaction time. A_0 and A_t represent the absorbances of the film before and after irradiation for different times.

amount of enzyme deposited on the Au electrode surface. As can be seen, the anodic plateau current showed an almost linear relationship with the number of assembled GOx/DAR bilayers. It means that each bilayer contains the same amount of GOx and the fabrication is a process of ordered deposition. The ability to control the surface concentration of enzyme of the electrode should be useful, especially for the electrode containing multiple enzymes with significantly different activities.

The stability of these films was investigated according to the decrease of the response of anodic bioelectrocatalytic current. The covalent-attached film electrodes showed no decrease in the current response to glucose when subjected to 50 potential cycles from 0 to 0.50 V vs SCE at a scan rate of $10\,mV{\cdot}s^{-1}$. In contrast, the decrease was obviously observed for non-irradiated film electrodes. The enzyme film electrode was measured every 3 days and keeping it in 0.1-M phosphate buffer solution (pH 6.8) at 4 °C when not in use. The current response of the covalent-attached film electrode to glucose remained almost unchanged during the first 30 days, and only 10% of the original response lost after six weeks, which is more minor than that of 50% for non-irradiated film electrode.

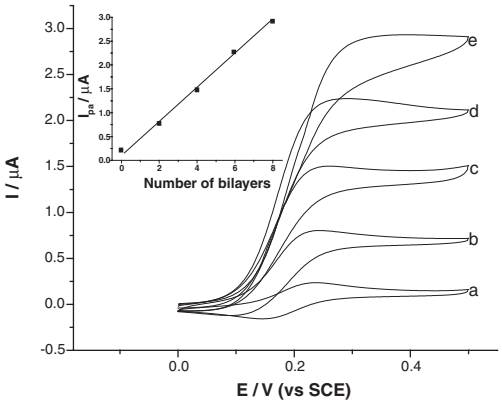


Figure 3. Cyclic voltammograms of the DAR/GOx multilayered gold electrodes in the presence of 0.1-mM ferrocenemethanol as a diffusional electron-transferring mediator: (a) 0, (b) 2, (c) 4, (d) 6, and (e) 8 bilayers in the presence of 20-mM glucose. Inset shows the relationship between catalytic currents (Ipa) and the number of bilayers. All curves were registered in 0.1-M phosphate buffer (pH 6.8) under N_2. Potential scan rate was $10\,mV{\cdot}s^{-1}$.

In conclusion, a new method to fabricate covalently attached multilayers of proteins has been developed exploiting the ionic self-assembly technique and post UV-irradiation photoreaction of the diazonium and carboxylate groups in the multilayer assemblies. This technique is a simple but efficient method to prepare stable proteins-containing multilayer films.

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