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Biosensing interfaces based on the dendrimer-underlying layer on gold

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Abstract

Structually organized mono- and multilayers were developed on gold for the catalytic and affinity biosensing using hyper-branched dendrimers. For the catalytic biosensing interface, a new approach to construct a multilayered enzyme film on the electrode surface was developed. The film was prepared by layer-by-layer depositions of dendrimers and periodate-oxidized glucose oxidase. The voltammograms obtained from the GOx/dendrimer multilayered electrodes revealed that bioelectrocatalytic response is directly correlated to the number of deposited bilayers. From the analysis of voltammetric and ellipsometric signals, the coverage of active enzyme per layer during the layering steps was estimated, demonstrating the spatially-ordered multilayer formation. As an extension of the study, dendrimers having various degrees of ferrocenyl modification were prepared and used. The resulting electrodes were electrochemically characterized, and the density of ferrocenyl groups, active enzyme coverage, and sensitivity were estimated. For the affinity-sensing surrface, a biosensor system based on avidin-biotin interaction was developed. As the building block of affinity monolayer, G4 dendrimer having partial ferrocenyl-tethered surface groups was prepared and used. And the biotinylated and electroactive dendritic monolayer was used for the affinity-sensing surface interacting with avidin. Electrochemical characterization of the resulting biosensor was conducted using free enzyme in electrolyte in terms of degree of surface coverage with avidin and subsequent surface shielding.

Introduction

Driven by interest in designing interfaces at the molecular level, research on the investigation of organized thin films has developed into an important area in analytical chemistry and molecular device technologies. As a building unit for the organic thin films, highly branched dendritic macromolecules are of great interest.[1),2)] They possess a unique surface of multiple chain-ends, and the number of surface groups can be precisely controlled as a function of synthetic generations. The high concentration of functional end groups of dendrimers enables synthetic modifications for the molecularly ordered nanostructures.

Here, we report a new approach to construct a thickness-tunable enzyme multilayer film on the

Au electrode surface for use as a biosensing interface. The multilayered enzyme film was prepared by using G4 dendrimers and periodate-oxidized GOx *via* layer-by-layer depositions on Au. Based on voltammetric and ellipsometric tests, we demonstrate that the enzyme/dendrimer multilayer is formed in a spatially-ordered manner and stable on Au, and the resulting electrode is useful for the biosensing application. In addition, we attempted to develop a reagentless biosensor using the functionalized dendrimers. G4 dendrimers were partially modified with the redox-active ferrocenyls, and the resulting Fc-Ds were used for the construction of multilayered assembly of enzymes via layer-by-layer depositions with periodate-oxidized glucose oxidase. By taking into consideration the surface concentration of ferrocenyls, active enzyme coverage, and the electrode sensitivity, degree of functionalization was optimized. Analytical performance of the resulting biosensor was evaluated in terms of sensitivity and stability.

Also, for an affinity biosensing interface with high sensitivity and selectivity, construction of molecularly organized sensing surface representing high density of ligand groups with adequate accessibility, fulfilling efficient affinity reaction and easy signal generation, is required. In this respect, much effort has been devoted to the development of affinity sensors based on SAMs, silane modified layers, and polymer grafted layers. The establishment of an effective biosensing interface especially draws a growing quest in diagnostic research fields.

In this report, an electrochemical affinity biosensor based on avidin-biotin interaction was developed. As the affinity surface, a monolayer of biotinylated Fc-D was constructed on gold electrode. A monolayer of double functionalized dendrimer plays a role as a molecular gate for free diffusing and signaling molecules in electrolyte. Non-labeled free GOx in electrolyte, as a diffusional tracer, generates an electrochemical signal, depending on the degree of coverage of the sensing surface with avidin. The kinetic analysis using biotinylated glucose oxidase, forming an enzyme adlayer on the avidin pretreated surface, was also carried out to demonstrate the active coverage and the spatial organization of proteins on the derivatized gold electrode.

Experimental

Detailed experimental steps are described in the literature.[3),4),5)]

Results and discussion

A. Catalytic biosensor First, we focused on the exploitation of a simple process leading to the formation of a multilayered GOx/dendrimer assembly on the Au electrode surface. Through alternate depositions of IO_4^--oxidized GOx and amine-terminated dendrimers, multilayers with the desired thickness can be prepared. To evaluate the usefulness of this strategy, we have prepared the multilayered assemblies and investigated their bioelectrocatalytic characteristics. From voltammetric tests, anodic responses were remarkably increased for the multilayered

electrodes due to the increased amount of enzyme deposited on Au. The anodic plateau current showed an almost linear relationship with the number of deposited bilayers. Thus, it is thought that each bilayer contains same amount of enzyme, and the multlayer is constructed in a spatially-ordered manner. Also, electrocatalytic responses from the GOx/dendrimer associated electrodes were kinetically analyzed. The coverage of active GOx per bilayer was registered, and total coverage was linearly proportional to the number of bilayers. For the direct tracing of multilayer growth, ellipsometric measurements were performed. Film thickness was found to increase in linear proportion to the layer numbers, and the increment in thickness per bilayer was *ca.* 52 Å. To address the analytic usefulness of multilayered enzyme electrodes, calibration curves were obtained. The result shows that the sensitivity of the electrode can be adjusted by modulating the deposition number of GOx/dendrimer bilayers.

As an extension of this research, dendrimers having various degrees of modification with ferrocenyls were prepared and used. Surface amines from dendrimers were functionalized with ferrocenyls that are used as electron mediators in the catalysis with GOx. Functionalization levels of dendrimers ranged from 4 to 80%. With the prepared Fc-D and periodate-oxidized GOx, the multilayered assemblies containing ferrocenyl moieties were constructed on Au. Through alternate depositions of GOx and Fc-D, multilayer networks with the desired number of bilayers were prepared. The resulting electrodes were characterized in terms of surface concentration of ferrocenyls, active enzyme coverage, and electrode sensitivity. Based on these results, the optimal modification level of dendrimers with ferrocenyls was determined to be about 32 %. Multilayerd GOx/Fc-D electrodes were constructed with the Fc(32%)-D and electrochemically characterized. The surface concentration of ferrocenyls increased as the multilayer formation proceeded, indicating the growth of GOx/Fc-D film and the electrical connectivity of ferrocenyls are maintained. The surface concentration of ferrocenyls was linearly proportional to the number of added bilayers. The bioelectrocatalytic signals from mono- and multilayered electrodes were also directly proportional to the number of bilayers. The calculated sensitivities from the electrodes were 1.35 $\mu A\ mM^{-1}\ cm^{-2}$ for E1D1, and elevated to 4.21 (E3D3) and 7.38 (E5D5). From this result, it seems that the immobilized ferrocenyls are able to electrochemically crosstalk with each other throughout the entire assemblies.

B. Affinity biosensor According to the signaling principle of the affinity biosensor adopted in this study, surface amine groups from dendrimers are used for immobilization on the electrode surface and two functionalization reactions to introduce ferrocenyls and biotinyl groups, and we thought that the Fc-D with one third functionalization might be satisfactory for the construction of affinity sensing monolayer. Thus we prepared Fc-D having 30 % modification by controlling the molar ratio between ferrocene and dendritic amines. From the photometric analysis, the percentage modification of dendritic surface amines to ferrocenyls was found to be ca. 32%.

For the construction of affinity sensing monolayer on gold electrodes, a bottom-up synthetic procedure was attempted. An amine reactive SAM was prepared, the activated succinimidyl ester groups were reacted with the amine groups of Fc-D, and biotinyl-ε-amidocaproic acid NHS ester was reacted for biotinylation of the remaining amine groups of dendritic monolayer. The covalent immobilization of ferrocenyl-tethered dendrimers on the SAM was confirmed by cyclic voltammetry. To verify the biosensor's signaling principle, dependency of the sensor signal on the avidin concentration was investigated. For this, electrodes were incubated with avidin samples of various concentrations followed by rinsing steps, and the voltammograms were obtained. The voltammograms showed essentially an inverse proportionality to the avidin concentration. From the avidin concentration of 1 ng/ml, a gradual decrease in oxidative catalytic current was observed. At the avidin concentration of 10 μg/ml, an almost identical voltammogram to the background one was registered, suggesting that the affinity sensing surface was fully covered with avidin. From the observation that the affinity biosensor exhibited a negligible signal change when the ferrocenyl-tethered dendrimer was not biotinylated, it is evident that the derived catalytic current with dependency on avidin concentration is attributed to the specific avidin-biotin recognition rather than nonspecific protein adsorption.

To confirm the proposed signaling principle and to characterize the interaction at the affinity sensing surface in more detail, bioelectrocatalytic signals from the affinity biosensor were kinetically analyzed. For this, biotin functionalized electrodes were fully covered with avidin and b-GOx, subsequently. From the voltammograms, the coverage of active b-GOx was estimated to be 2.5×10^{-12} mol/cm^2, which suggests that a spatially organized enzyme monolayer was formed on the electrode surface. To evaluate the analytical performance of the affinity biosensor for avidin, calibration experiments were performed. The calibration curve was linear ranging from 1.5 pM to 10 nM of avidin, and the detection limit was about 4.5 pM.

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