Functionalization of a Poly(amidoamine) Dendrimer with Ferrocenyls and Its Application to the Construction of a Reagentless Enzyme Electrode

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Poly(amidoamine) dendrimers having various degrees of modification with the redox-active ferrocenyls were prepared by controlling the molar ratio of ferrocenecarboxaldehyde to amine groups of dendrimers. By alternate layer-by-layer depositions of partial ferrocenyl-tethered dendrimers (Fc-D) with periodate-oxidized glucose oxidase (GOx) on an Au surface, an electrochemically and enzymatically active multilayered assembly of enzyme was constructed. The resulting GOx/Fc-D multilayer-associated electrodes were electrochemically analyzed, and the surface concentration of ferrocenyl groups, active enzyme coverage, and sensitivity were estimated. A 32% dendrimer modification level of surface amines to ferrocenyls coverage, and sensitivity were correlated to the number of deposited bilayers. From this result, it seems that the electrode sensitivity is directly controllable, and the multilayer-forming strategy with partial ferrocenyl-tethered dendrimers is useful for the construction of reagentless biosensors.

Research of molecularly organized nanostructures is of great interest in the areas of analytical chemistry, molecular device technology, and biotechnology. Especially in the fields of biosensors and bioelectronic devices, many efforts have been devoted to the construction of nanostructures containing biomolecules such as enzymes,2,3 antigens–antibodies,4,5 ion channels,6,7 peptides,8 and nucleotides.9,10 Accordingly, development of new methods and implementation of novel materials for the construction of desirable biocomposite nanostructures have been the subject of intensive research.

Highly branched dendritic macromolecules (dendrimers)11–16 have been recently recognized as a promising candidate for a building unit of the organized nanostructures. Dendrimers with high generation numbers offer some favorable advantages over the entangled linear or partly branched polymers, e.g., structural homogeneity, controllable composition, comparable size to the participating biomolecules, internal porosity, and the multiple homogeneous chain ends valuable for the conjugating reactions. With these unique characteristics, there have been a number of approaches to the construction of dendrimer-containing nanostructures, e.g., deposition of dendritic multilayers via Pt2+ complexation,17 electrostatic interaction,18 and reaction with grafted copolymers.19 Furthermore, surface groups of dendrimers can be chemically functionalized through the synthetic manipulation, and the resulting dendrimers expand their application in the related areas.20–25

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The purity and modification levels were determined with UV/visible and $^1$H NMR spectroscopies. The GPC purified Fc-D fractions were collected, and the purity of the product was verified with thin-layer chromatography. The degree of ferrocene modification was determined spectrophotometrically at 438 nm using a ferrocene standard curve in methanol (See Table 1, vide infra). The $^1$H NMR spectrum was also achieved to verify the completeness of conjugation and reduction reactions. The GOx/Fc-D multilayer construction. Before the layer-forming processes, electrodes were polished with 1- and 0.25-$\mu$m diamond slurries, subjected to ultrasonication in doubly distilled water for 2 min to remove residues from polishing materials, and treated with an etching solution of HNO$_3$/HCl/H$_2$O (1:3:4, v/v/v) for 1 min. After cleaning with ultrasonication, the electrode was polished with a 0.05-$\mu$m alumina slurry to a mirror finish and thoroughly cleaned. Voltammetric cycling in 0.5 M H$_2$SO$_4$ solution was performed to check the surface contamination. The multilayer-forming process (Figure 1B) started with the introduction of amine functionalities on the Au surface by the chemisorption of cystamine dihydrochloride (20 mM, 1 h). The resulting aminated Au surface was then cleaned to remove physically adsorbed monomers and then modified with the enzyme monolayer (E1D0) by dipping in 40 $\mu$m periodate-oxidized GOx solution in 0.1M phosphate buffer (pH 6.8) for 1 h at room temperature. The GOx/Fc-D bilayer (E1D1) was formed by immersing the resulting enzyme monolayer in an aqueous Fc-D solution containing 120 $\mu$m dendrimer, dissolved in 0.1 M phosphate buffer (pH 6.8) for 1 h, followed by rinsing with buffer solution. The Schiff bases formed were reduced by dipping in 5 mM solution of cyanoborohydride at 4 °C in the dark during 30 min. The remaining carbaldehyde groups on the periphery of GOx were blocked with 10 mM ethanolamine (pH 9.5, titrated with concentrated HCl) for 30 min to avoid self-polymerization. Alternating repetition of the above procedure led to the GOx/Fc-D multilayer with the desired number of bilayers (EnDn).

**Instrumentation.** Electrochemical measurements were performed with a BAS CV-50W electrochemical analyzer (W. Lafayette, IN). A standard three-electrode configuration with a platinum gauze counter electrode and an Ag/AgCl (3M NaCl, BAS) reference electrode was used. All experiments were performed at room temperature (25 ± 2 °C) under argon atmosphere, unless otherwise specified. The glucose stock solutions were prepared in phosphate buffer (0.1 M, pH 7.0) and were allowed to mutarotate overnight before use. The electrolyte solutions were dedioxygenated with argon bubbling for 20 min before each voltammetric run.

The $^1$H NMR spectra were recorded on a Bruker AM X FT500 NMR spectrometer, and the UV/visible absorption spectra were registered on a Shimadzu UV2100 spectrophotometer at a controlled temperature of 25 °C.

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RESULTS AND DISCUSSION

Preparation of the Ferrocene–Dendrimer Conjugates. In this work, dendrimers were introduced for dual purposes: the building unit for the multilayered biocomposite film and the immobilizing matrix of the electron-transferring mediators for reagentless biosensing. In this regard, surface amine groups from the fourth generation PAMAM dendrimers (having 64 surface amines) were partially functionalized with ferrocenyls, which are used as electron-transferring mediators in the electrocatalytic reaction of GOx (Figure 1A). To obtain the ferrocenyl-tethered dendrimers (Fc-D) with various functionalization levels, the molar ratio of reacting ferrocenecarboxaldehyde to surface amines from dendrimers was changed in the reaction mixture. The functionalization level of ferrocenyl for each sample was determined by UV/visible absorption spectrophotometric titration with a ferrocene standard curve in methanol. Functionalization levels of dendrimers ranged from 4 to 80% depending on the molar ratio between ferrocenyls and amine groups from dendrimers (see Table 1). The maximum modification level was ~80% under the reaction conditions, which corresponds to the modification of ~51

Figure 1. (A) Schematic representation of semisynthetic preparation of partial ferrocenyl-tethered G4 PAMAM dendrimer (Fc-D). (B) Organization of the multilayered GOx/Fc-D network on the Au electrode surface. See Experimental Section for details.
amino groups of 64 for the G4 PAM AM dendrimers to ferrocenyls, and this limitation seems to be due to the steric hindrance by the conjugated ferrocenyl groups.

**Multilayer Construction with Fc-D and IO₄⁻-Oxidized GOX.** With the prepared partial ferrocenyl-tethered dendrimers and periodate-oxidized GOX, the multilayered enzyme assemblies containing electron-transferring ferrocenyl moieties were constructed on Au electrodes for the reagentless glucose biosensing. The multilayer-forming strategy is similar to our previous report, as schematically shown in Figure 1B. The key to this process is the formation of Schiff bases between amine groups of the partially modified dendrimers (Fc-D) and carbaldehyde groups of periodate-oxidized GOX. Through alternate depositions of periodate-oxidized GOX and Fc-D, multilayer networks with the desired number of bilayers (EnDn) were prepared. The multilayered network containing dendrimers as a building unit represents a number of advantages for the biosensing purpose, which stem from the unique characteristics of dendrimers: (1) the structural homogeneity (molecular weight, size, repeating units) and high density of identical functional chain end groups of dendrimers enables the molecularly organized and structurally stabilized constructs, (2) the compatible size of dendrimers with the biomolecules and inherent solubility facilitate the functionalization and nanostructure construction reactions with proteins, nucleic acids, etc., and (3) the internal porosity of the dendrimers, especially with high generation number, preserves the permeability of the resulting nanostructure, still maintaining the rigidity of construct, which is very important for the multilayered electrode. Accordingly, we believe that the biocomposite nanostructures adopting dendrimers as building units address special interest in the areas of biosensing and biomimetic film technology for catalysis and/or separations.

**Electrochemical Characterization of GOX/Fc-D Electrodes with Various Ferrocenyl Functionalization Levels.** On the basis of the above considerations, we attempted the preparations of mono- and multilayered GOX/Fc-D films on Au electrodes by using the synthesized Fc-Ds having various functionalization levels. The resulting electrodes were electrochemically characterized in terms of the surface concentration of electron-transferring ferrocenyls ($\Gamma_{SO}$), the active enzyme coverage ($\Gamma_{GOX}$), and the electrode sensitivity.

First, the surface concentration of ferrocenyl groups for the ESD5 multilayered electrode constructed with Fc-Ds having various degrees of functionalization was traced by cyclic voltammetry. The cyclic voltammograms were typical for the surface-immobilized ferrocenyl groups (see Figure 3A, vide infra), and the surface concentrations of the immobilized ferrocenyl moieties were calculated by the integration of charges passed during the oxidation or reduction process from respective voltammograms. By the successive GOX/Fc-D bilayer depositions, gradual increments in charge passed were registered from respective voltammograms, and the surface concentrations of immobilized ferrocenyl groups for each electrode constructed with Fc-Ds were estimated. The surface concentrations of immobilized ferrocenyl groups ($\Gamma_{SO}$) for each ESD5 electrode were tabulated in Table 1. As can be noted from the registered values, the surface concentration of ferrocenyls increased as the functionalization level of Fc-D enhanced up to 71% suggesting that the amount of immobilized electroactive ferrocenyls at the electrode surface increased correspondingly. For the Fc(80%)-D-associated electrode, however, the registered surface concentration of ferrocenyl functionality dropped abruptly. This result might be due to the reduced conjugating ability of highly functionalized dendrimers bearing few surface amines for the multilayer construction. The shortage of reactive surface amine groups and/or the steric hindrance by the conjugated ferrocenyl groups seems to result in poor conjugation between enzymes and dendrimers. Additionally, it is very interesting that the electrode constructed with the Fc-Ds having low modification levels (4–23%) showed underestimated surface concentrations of ferrocenyls after multilayer construction compared to those constructed with Fc-Ds having moderate or high modification levels. When the electrode was constructed with Fc-

### Table 1. Electrochemical and Bioelectrocatalytic Characteristics of the GOX/Fc-Dendrimer Multilayer-Associated Electrodes with Various Ferrocenyl Functionalization Levels

<table>
<thead>
<tr>
<th>entry</th>
<th>% mod</th>
<th>$\Gamma_{SO}^{a}$, $(10^{10}$ mol cm$^{-2}$)</th>
<th>$\Gamma_{GOX}^{b}$, $(10^{12}$ mol cm$^{-2}$)</th>
<th>sensitivity$^{c}$ $(\mu$A mmol$^{-1}$ cm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 (1)</td>
<td>1.2</td>
<td>4.5 ± 0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>8 (2)</td>
<td>3.8</td>
<td>4.8 ± 0.1</td>
<td>3.3</td>
</tr>
<tr>
<td>3</td>
<td>23 (5)</td>
<td>13</td>
<td>4.6 ± 0.2</td>
<td>6.1</td>
</tr>
<tr>
<td>4</td>
<td>32 (7.5)</td>
<td>23</td>
<td>4.6 ± 0.1</td>
<td>7.4</td>
</tr>
<tr>
<td>5</td>
<td>40 (10)</td>
<td>35</td>
<td>2.7 ± 0.1</td>
<td>6.8</td>
</tr>
<tr>
<td>6</td>
<td>71 (25)</td>
<td>44</td>
<td>1.8 ± 0.1</td>
<td>3.7</td>
</tr>
<tr>
<td>7</td>
<td>80 (30)</td>
<td>23</td>
<td>1.5 ± 0.1</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*a* Calculated from the respective absorption spectra. In parentheses: the amount of reacted ferrocenecarboxaldehyde (milligrams in 4 mL of reaction mixture) for each sample. See Experimental Section. *b* The values were registered by integrating the charge passed during the anodic scan. The Au electrode area was 0.033 cm$^2$. *c* The values were estimated from kinetic analysis based on the known rate constants (refs 2 and 5). *d* Calculated from the anodic signals at $+370$ mV for each ESD5 electrode.
(4%)-(4%) - $\Gamma_f$, for ES5 was estimated to be $\sim 1.2 \times 10^{-10}$ mol cm$^{-2}$, which corresponds to only 3% of the $\Gamma_f$ of Fc(40%)D-associated electrode. This can be attributed to the fact that, with slightly functionalized Fc-Ds, only a portion of immobilized ferrocenyls are electrochemically communicable with each other in the multilayered construct due to a rather wide gap between ferrocenyls.

As mentioned above, the active enzyme coverage in the multilayered electrode might vary with the modification level of dendrimers, affecting the bioelectrocatalytic reaction. To estimate the active enzyme coverage in the multilayered electrode, the bioelectrocatalytic responses from the GOx/Fc-D electrodes were kinetically analyzed in the presence of freely diffusing electron-transfering mediators. Under the condition of ferrocenemethanol (0.1 mM) in electrolyte, total enzyme coverage within the multilayer, including enzyme molecules, which are active but which are not electrically connected to immobilized ferrocenyl groups, can be estimated from kinetic analyses on the basis of previous work of Savant's group[5], and ours[26]. As shown in Table 1, the active coverage of GOx was conserved at $\sim (4.6 \pm 0.2) \times 10^{-12}$ cm$^{-2}$ for the ES5 electrodes with Fc-D modification levels ranging from 4 to 32%. But, for the multilayered electrodes constructed with Fc-D having high-level modification (40, 71, and 80%), the active enzyme coverage decreased as the functionalization level increased. In the case of Fc(80%)D-associated electrode, the active coverage of GOx was $\sim 1.5 \times 10^{-12}$ cm$^{-2}$, which corresponds only to $\sim 30\%$ of those constructed with the Fc-Ds having low modification levels. This result seems to be caused by the fact that the shortage of reactive surface amine groups from Fc-Ds and/or the steric hindrance by the conjugated ferrocenyl groups reduces the conjugation capability of dendrimers with enzyme molecules, leading to low enzyme coverage.

The bioelectrocatalytic signal amplification during the multilayer-depositing process (E1D1-E5D5), in the absence of free diffusing mediators, was traced for respective GOx/Fc-D electrodes as a function of deposited bilayer numbers (Figure 2), and the calculated electrode sensitivity is shown in Table 1. The anodic currents sampled at 370 mV (vs Ag/AgCl) for 20 mM glucose concentration increased correlative to the number of deposited bilayers, except for the Fc(4%)D- and Fc(8%)D-associated electrodes which showed a saturation of signal from $n = 4$. The maximum electrode sensitivity was observed with the Fc(32%)D-associated electrode.

These observations led us to categorize the functionalization level of dendrimers with ferrocenyls into three subgroups. The first is the low-tethering group including Fc(4%)D and Fc(8%)D, which represents the optimal conjugation with enzyme, but is not sufficient for efficient bioelectrocatalysis. The second is the moderate-tethering group including Fc(23%)D and Fc(32%)D, exhibiting both the optimal conjugation and electrocatalytic activity for satisfactory bioelectrocatalysis. The third is the high-tethering group having modification levels of 40% and above, which results in inefficient bioelectrocatalysis due to the lowered conjugation ability with enzyme molecules, despite the high surface concentration of ferrocenyl groups. On the basis of these results, the optimal modification level of dendrimers with ferrocenyls was determined to be $\sim 32\%$ which used as the building block for the GOx/Fc-D multilayered electrode in this work.

**Figure 3.** (A) Cyclic voltammograms of the GOx/Fc(32%)D multilayer associated electrodes under the potential scan rate of 50 mV/s: (a) E1D1, (b) E3D3, and (c) E5D5-associated Au electrodes. All curves were registered in 0.1 M phosphate buffer (pH 7.0) under Ar. (B) Coulometric analysis of the surface density of ferrocenyls with added bilayer numbers, n. The values were registered by integrating the charge passed during the anodic scan. Electrode area was 0.033 cm$^2$.

Electrochemical Characteristics of GOx/Fc-D Electrodes Constructed with Fc(32%)D. Multilayered GOx/Fc-D electrodes were constructed with the Fc(32%)D and electrochemically characterized in more detail. Figure 3A shows the cyclic voltammograms from one (E1D1), three (E3D3), and five (E5D5) GOx/Fc-D bilayer-associated gold electrodes in 0.1 M phosphate buffer. The E1D1 electrode exhibited a ferrocene/ferricinium redox wave with a small peak separation ($\Delta E_p$) of $\sim 23$ mV at 50 mV/s, which is typical for the electrochemically active motifs immobilized on the electrode surface. The full width at half-maximum ($\Delta E_{1/2}$) of $\sim 100$ mV was registered, which is close to the ideal value for the immobilized species of 90.6 mV. Also, the multilayered GOx/...
Fc-D-associated electrodes showed surface waves, but the redox peaks were slightly more separated as the number of added bilayers increased (29 mV for E3D3- and 45 mV for E5D5-associated electrodes), which seems to be due to the rather slower charge transfer displayed by the ferrocenyls as the successive enzyme/dendrimer layers deposited. The most important finding, however, is that the surface concentration of ferrocenyls increased as the multilayer formation proceeded, indicating that the growth of GOx/Fc-D film on an Au surface and the electrical connectivity of ferrocenyl groups in the network are maintained. Control experiments revealed that the electrodes fabricated with native GOx and Fc-D, instead of periodate-oxidized GOx, did not show reproducible and increasing redox waves, indicating the failure in multilayer formation.

The surface concentrations of immobilized ferrocenyl groups were traced by the integration of charges passed in the oxidation of ferrocenyl groups from respective cyclic voltammograms and plotted as shown in Figure 3B. Chronocoulometry was employed for the determination of effective electrode area, and 0.03 cm² was registered from an Anson plot. The surface concentration of ferrocenyls was linearly proportional to the number of added bilayers, and the average increment was 4.6 × 10⁻¹⁰ mol cm⁻². Interestingly, this is similar to the values for ferrocenyl alkanethiol monolayers [(3.8–5.3) × 10⁻¹⁰ mol cm⁻²], even though the electroactive groups are located at the surface of bulky (diameter, 45 Å) dendrimers in this work. Recently, Tokuhisa et al. reported a ferrocene surface concentration of 8.4 × 10⁻¹⁰ mol cm⁻² from the monolayer prepared with ferrocenyl-modified diaminobutane—dendr((propylamine)₆₄ dendrimers. In their case, the average level of ferrocenyl functionalization was 70% for the Fc-DAB(PA)₆₄ and the monolayer was prepared by simple adsorption on a bare Au electrode surface. In this work, on the other hand, the immobilization of Fc-D was made onto the platform of preformed enzyme submonolayer. Therefore, the observed surface concentration and linear increment in ferrocenyl loading strongly suggest the molecularly organized and electrically connected nanoscale structures, supporting the applicability of this multilayer-forming strategy to biosensing interface.

The finding that GOx/Fc-D electrodes represent reversible surface waves in cyclic voltammetry, typical for the immobilized redox-active species, indicates that the ferrocenyl groups tethered to dendrimers and networked with biomolecules are efficiently connected on Au electrodes for facile charge transfer. To confirm the organized formation of a GOx/Fc-D multilayer network on the Au surface and the electrical connectivity between the immobilized ferrocenyls, the electrochemical characteristics of multilayered assembly were further investigated. The cyclic voltammograms of ESDS-associated electrode versus potential sweep rates are shown in Figure 4A. The registered voltammo-

![Figure 4](image-url)

**Figure 4.** (A) Cyclic voltammograms of the GOx/Fc(32%)-D multilayer-associated electrode, ESDS, at different potential scan rates: 5, 25, 50, 100, and 200 mV/s (from inside voltammograms) in 0.1 M phosphate buffer (pH 7.0) under an Ar atmosphere. (B) Dependence of the anodic peak currents on the potential sweep rates.

grams were typical for the surface waves, as evidenced by the small peak separation and the full width at half-maximum of ~100 mV. Also, both the anodic and cathodic peak currents were directly proportional to the potential scan rates in the range of 5–100 mV s⁻¹, suggesting facile charge-transfer interactions (Figure 4B). However, at higher scan rate, the peak currents leveled off from direct linearity, implying the limitation in the electron-transferring process.

The bioelectrocatalytic signals from mono- and multilayered GOx/Fc-D electrodes were traced by cyclic voltammetry in 20 mM glucose solution (Figure 5). All the cyclic voltammograms registered from GOx/Fc-D electrodes in the presence of glucose substrate were typical for the enzyme-catalyzed and -mediated voltammograms, and the anodic currents from multilayered electrodes were significantly enhanced. However, there existed hysteresis phenomena in cyclic voltammograms, especially for the electrodes with high layer numbers, possibly due to the inhibition...
The anodic currents sampled at 370 mV vs a Ag/AgCl reference electrode were directly proportional to the number of deposited bilayers. The calculated sensitivities from the electrodes were 1.35 $\mu$A M$^{-1}$ glucose cm$^{-2}$ for E1D1 and elevated to 4.21 (E3D3) and 7.38 (E5D5) by additional bilayer depositions. From this result, it seems that the immobilized ferrocenyls are able to electrochemically crosstalk with each other throughout the entire assemblies, and consequently, the electrochemical amplification reaction takes place efficiently.

Figure 6 depicts the idealized organization of the multilayered GOx/Fc-D network and the bioelectrocatalytic reaction, which is anticipated to proceed in the multilayer (EnDn)-associated Au electrodes. Each assembling component links alternately as a submonolayer and plays a role as a platform on which layer-forming counterparts are bound. This enables the formation of electron-transferring pathways from the FAD reaction center of GOx in the network to the Au electrode surface via a diffusion-like electron-hopping mechanism from the FAD prosthetic group to the adequately positioned ferrocenyl mediator, between neighboring ferrocenyls immobilized onto the surface of dendritic chain ends, inter-ferrocenyl tethered dendrimers, and from the ferrocenyls at the opening of the primary (underlying) enzyme layer to the Au electrode. In other words, it seems that the entire network is electrochemically connected with immobilized ferrocenyls so that the enzymatic reaction can be efficiently transformed to the electrical currents. Additionally, the anodic signal, which was directly proportional to the number of deposited bilayers, demonstrates that the immobilized enzyme retained its activity during the multilayer-forming and signaling processes. In this regard, it is thought that the crucial requirements for the optimal preparation of biosensing nanostructures such as structural integrity, stability, biological activity, etc., have been successfully addressed with the multilayer-forming strategy.

Analytical Performance of Multilayered GOx/Fc-D Glucose Biosensor. To evaluate the analytical performance of the multilayered GOx/Fc-D biosensors, calibration experiments were performed for the E5D5 and E1D1 electrodes (Figure 7). The amperometric signals were registered at the working potential of 370 mV vs a Ag/AgCl reference electrode under air at room temperature. With the E1D1 electrode, anodic signals were developed in correlation to the glucose level at low concentration range but leveled off at $10^{-6}$ M range when the S/N ratio was 3. This implies that the sensing capabilities such as linear detection range, signal amplification, and sensitivity can be improved and controlled by the multilayer-forming strategy.

The bioelectrocatalytic signals from the E5D5 electrodes reached 90% of the steady-state values within 6 s, implying a negligible diffusional restriction for the analytes, and the steady-state currents were maintained with no fluctuation. Stability of the E5D5 electrode was tested, and the catalytic signals were found to be maintained over 80% of the initial response even after 20 days under daily calibrations. The same electrode was subjected to daily calibrations with 10 mM glucose and was stored in 0.1 M phosphate buffer (pH 7.0) at room temperature under ambient air when not in use. This stability seems to be due to the structural

Figure 5. Cyclic voltammograms for the bioelectrocatalytic glucose oxidation by the GOx/Fc(32%)-D electrodes as a function of the number of bilayers: (a) E5D5, (b) E4D4, (c) E3D3, (d) E2D2, and (e) E1D1 in the presence of 20 mM glucose as an analyte; (f) E1D1 in the absence of glucose. All curves were registered in 0.1 M phosphate buffer (pH 7.0) under Ar. Potential scan rate was 5 mV/s.

Figure 6. Schematic representation of the idealized multilayered GOx/Fc-D network on an Au electrode surface and the anticipated biochemical and charge-transfer reactions.

The integrity of the multilayered network, which results from the multiple covalent linkages formed between enzyme molecules and dendrimers and also from the rigidity of the dendrimers as building blocks of the multilayered network. The gradual decrease in the signal during the stability test can be attributed to the erosion (dissolution) of outermost layer-forming components, as evidenced by the decrease in peak current or area from the background cyclic voltammograms (in the absence of glucose) which were registered with the used and stored electrodes.

In summary, we have demonstrated that ferrocenyl-tethered dendrimers can be effectively employed for the construction of reagentless biosensor by simple and direct layer-by-layer depositions of dendrimers and periodate-oxidized glucose oxidase on Au electrodes. The response of the enzyme electrode was significantly amplified with multilayer growth, which indicates that the electrode sensitivity is tunable, promising for the efficient fabrication of microbiosensors. In this context, we believe that the multilayered enzyme/dendrimer network prepared using partial ferrocenyl-tethered dendrimers will find applications in the field of biosensors, bioelectronic devices, and separative and catalytic biomembranes.

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