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Dendrimer Encapsulated Pt Nanoparticle-Immobilized Glassy Carbon Electrode with High Electrocatalytic Redox Activity to Hydrogen Peroxide and Its Application for Biosensing

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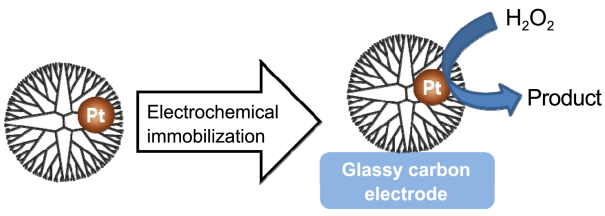
Dendrimers, which are highly-branched monodisperse macromolecules with well-defined size, shape, and surface functionality, have been widely used in the modification of electrode surfaces due to their good biocompatibility and adequate functional groups.[1] The intriguing properties of dendrimers allow the dendrimer-coated surfaces to be utilized in a variety of fundamental and applied researches. For example, the use of dendrimers has been proposed for the design of microarray chips because dendrimer-immobilized surfaces provide a unique three-dimensional architecture with flexible branched arms connected to numerous functional groups. This allows for the effective immobilization of biomolecules with a high density as well as minimization of biomolecule denature.[2] In addition, dendrimer-immobilized surfaces have been proven to be useful in improving sensitivity as well as selectivity for biosensing applications.[3] Recently, we also reported facile surface modification of carbon-based materials with amine-terminated dendrimers and its applications to construct catalytic bifunctional surfaces for electrochemical reactions of chemicals such as hydrazine and oxygen.[4]

Here, we report the electrochemical modification of glassy carbon electrodes (GCEs) *via* electrooxidative coupling of the dendrimers encapsulating electrocatalytic Pt nanoparticles and the promising potential of the resulting dendrimer encapsulated Pt nanoparticle-decorated GCEs as biosensing platforms. Since Pt nanoparticles were encapsulated inside the cores of dendrimers, the electrooxidative coupling of the dendrimers onto GCEs resulted in modification of GCEs with the catalytic Pt nanoparticles. As illustrated in Scheme 1, we synthesized dendrimer encapsulated Pt nanoparticles (Pt DENs, diameter 2.1 ± 0.3 nm) using amine-terminated sixth-generation polyamidoamine (G6-NH_2 PAMAM) dendrimers and immobilized the Pt DENs on GCEs *via* electrooxidative coupling of the terminal amino groups of dendrimers. The resulting Pt DEN-immobilized GCEs showed much higher electrocatalytic activity toward redox reactions of H_2O_2 than that shown by bare GCEs. The enhanced electrocatalytic activity of Pt DEN-decorated GCEs was further applied to glucose sensing.



Scheme 1

Figure 1 shows a TEM image and a corresponding histogram of the particle size distribution of the as-synthesized Pt DENs denoted as G6-NH_2(Pt_{250}), where the numerical subscript of Pt represents the original $PtCl_4^{2-}$:G6-NH_2 ratio used for the synthesis and thus the average number of Pt atoms in each dendrimer. The Pt nanoparticles were found to be nearly monodispersed in size and rarely aggregated due to stabilization of the nanoparticles *via* their encapsulation inside the dendrimers (Figure 1).[4b,5] The measured average diameter (2.1 ± 0.3 nm) of the Pt DENs was slightly larger than the theoretical value (1.9 nm) for nanoparticles containing 250 Pt atoms (Figure 1, inset),[6] which is presumably

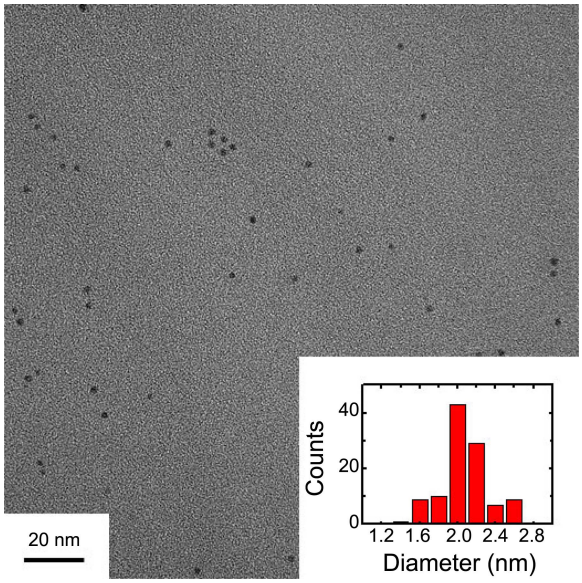


Figure 1. Typical transmission electron microscopy (TEM) image of G6-NH_2(Pt_{250}) DENs. Inset shows a corresponding particle size distribution of the G6-NH_2(Pt_{250}) DENs. The average G6-NH_2(Pt_{250}) DEN diameter is 2.1 ± 0.3 nm.

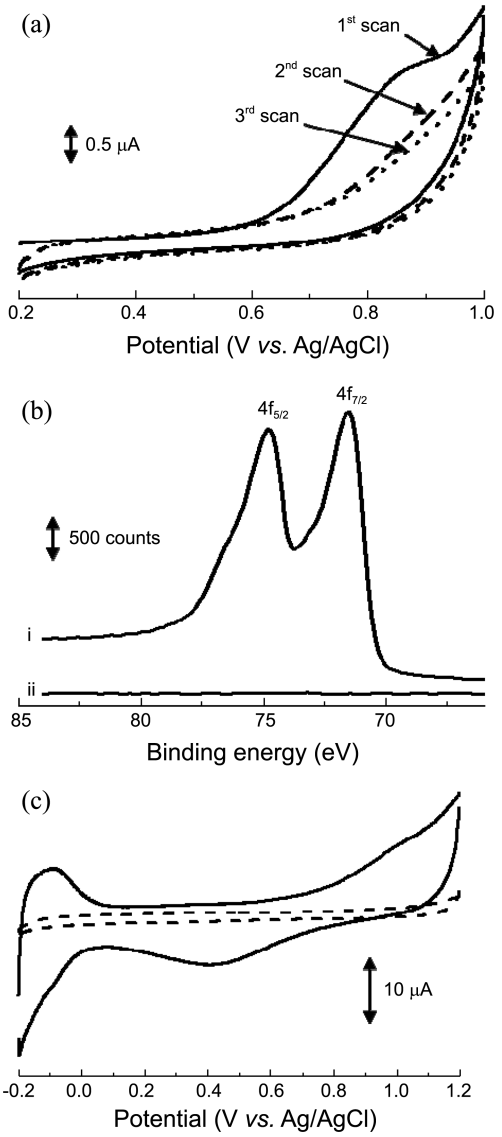


Figure 2. (a) Cyclic voltammograms obtained on a GCE in an aqueous 10 μM G6-NH_2(Pt_{250}) DEN solution containing 0.1 M $LiClO_4$. Scan rate: 0.01 V/s. (b) XPS spectra in the Pt(4f) region for (i) a Pt DEN-immobilized and (ii) a bare GCE. (c) Cyclic voltammograms of a Pt DEN-immobilized and a bare GCE in 0.5 M H_2SO_4. Scan rate: 0.1 V/s.

a consequence of a Pt loading that slightly exceeds that which can be accommodated by the dendrimer.[7]

We then electrochemically immobilized the synthesized Pt DENs on the GCE surfaces, as we have reported previously.[4a,4b] In the previous reports, we demonstrated the electrochemical immobilization of amine-terminated dendrimers onto GCEs, which can be applied to the immobilization of DENs to assemble catalytic nanoparticles of uniform size on GCEs. Briefly, we immobilized the Pt DENs on GCE surfaces by scanning the potential of the electrodes three times between 0.2 and 1.0 V (*vs*. Ag/AgCl) in an aqueous 10 μM G6-NH_2(Pt_{250}) solution containing 0.1 M $LiClO_4$. Figure 2(a) shows cyclic voltammograms obtained during the immobilization process. In the first scan (solid line in Figure 2(a)), an irreversible oxidation curve appears with a peak potential of about 0.8 V, which is attributable to the electrochemical oxidation of the amine groups of dendrimers to their reactive radicals and consequent formation of carbon-nitrogen bonds on the GCE surfaces.[4a,8] In the following potential scans, the irreversible oxidation currents gradually decreased, which was previously attributed to the film formation of the dendrimers encapsulating Pt nanoparticles on the carbon electrode after the first scan.[4a,9] The immobilization of Pt DENs was then further confirmed by XPS and electrochemical measurements. Figure 2(b) shows the XPS spectra of a Pt DEN-immobilized (spectrum i) and a bare (spectrum ii) GCE in the Pt (4f) region. The characteristic Pt($4f_{5/2}$) and Pt($4f_{7/2}$) peaks were found only on the Pt DEN-immobilized GCE, which verifies the presence of Pt nanoparticles after the Pt DEN immobilization process. Cyclic voltammetry was also performed to confirm the presence of Pt DENs on the Pt DEN-immobilized electrodes. Figure 2(c) shows cyclic voltammograms of a Pt DEN-immobilized (solid line) and a bare (dashed line) GCE obtained in 0.5 M H_2SO_4 at a potential scan rate of 0.1 V/s. The Pt DEN-immobilized GCE showed characteristic redox currents similar to those of a bulk-phase polycrystalline Pt electrode, while the bare GCE showed only non-characteristic charging currents. These results indicate that Pt DENs were successfully immobilized on the GCE surfaces during the electrochemical immobilization process and the immobilized Pt DENs were electrochemically active and in good electrical contact with the underlying GCE, implying that the Pt DEN-immobilized GCE has the potential to be used as an electroanalytical platform for biosensing.

Thus far, the as-synthesized Pt DENs and the Pt DEN-immobilized GCEs have been characterized in this study. We then attempted to demonstrate the interesting use of the Pt DEN-immobilized GCEs as biosensing platforms. First, we tested the possibility of using the Pt DEN-immobilized GCE as an electrochemical biosensing platform for H_2O_2. The detection of H_2O_2 is of great interest in many bioanalytical research fields because H_2O_2 is released as an important byproduct during many enzymatic reactions.[10] As illustrated in Scheme 1, we expected that the immobilized dendrimers are permeable and the encapsulated Pt nanoparticles catalyze the redox reactions of H_2O_2 due to the dendrimer porosity and electrocatalytic activity of Pt nanoparticles to H_2O_2.[5,11] Figure 3(a) shows cyclic voltammograms for the redox reactions of H_2O_2 at a Pt DEN-immobilized and a dendrimer-immobilized (but without encapsulated Pt nanoparticles) GCE. The Pt DEN-immobilized GCE presented significant redox currents of H_2O_2 starting at around 0.25 V (solid line in Figure 3(a)), while only a small background current was observed at the dendrimer-immobilized GCE (dashed line in Figure 3(a)), which is similar to a bare GCE (data not shown). These results can be attributed to the high electrocatalytic redox activity of the Pt DEN immobilized on GCE, which enables effective low-potential amperometric sensing of H_2O_2 on the Pt DEN-immobilized GCE. Note that some of the reduction

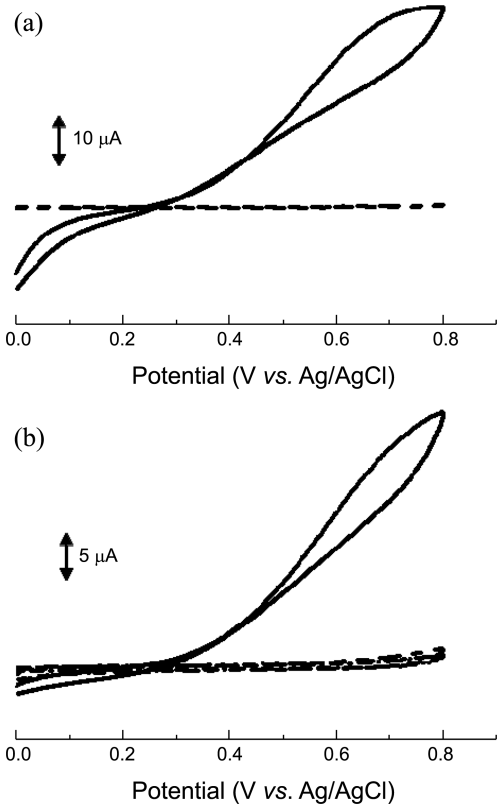


Figure 3. (a) Cyclic voltammograms of 1 mM H_2O_2 in a PBS buffer (50 mM, pH 7.4) on (solid line) a Pt DEN-immobilized and (dashed line) a dendrimer-immobilized (but without encapsulated Pt nanoparticles) GCE. Scan rate: 0.05 V/s. (b) Cyclic voltammograms of 10 mM glucose in a PBS buffer (50 mM, pH 7.4) on a Pt DEN-immobilized GCE (solid line) in the presence of, or (dotted line) in the absence of, dissolved GOx (3 mg/mL). Similarly, dashed and dashed-dotted lines represent cyclic voltammograms obtained on the Pt DEN-immobilized GCE in the presence of dissolved GOx (3 mg/mL) but without glucose, or on a dendrimer-immobilized (but without encapsulated Pt nanoparticles) GCE even in the presence of both dissolved GOx (3 mg/mL) and glucose (10 mM), respectively. Scan rate: 0.05 V/s.

current observed at the Pt DEN-immobilized GCE is also contributed by the reduction of O_2 dissolved in PBS solution (Supporting Information, Figure S1). We then extended the use of Pt DEN-immobilized GCE as a biosensing platform for detection of glucose in the presence of dissolved glucose oxidase (GOx). We expected the dissolved GOx to catalyze the oxidation of glucose in the presence of O_2 and thus to produce H_2O_2 and gluconolactone.[4b] The liberated H_2O_2 is also expected to be oxidized through the electrocatalytic reaction by the Pt DEN immobilized on GCE, as was confirmed above. We performed a set of key experiments to verify the glucose sensing ability of the Pt DEN-immobilized GCE. Specifically, a cyclic voltammogram of 10 mM glucose (O_2-saturated 50 mM PBS, pH 7.4) was obtained with the Pt DEN-immobilized GCE in the presence of dissolved GOx (3 mg/mL), which showed a significant oxidation current for liberated H_2O_2 from the enzymatic oxidation of glucose by dissolved GOx (solid line in Figure 3(b)). In the absence of dissolved GOx (dotted line in Figure 3(b)), however, we observed only a negligible oxidation current even in O_2-saturated glucose solution (50 mM PBS, pH 7.4). Similarly, no significant current was observed in cyclic voltammograms obtained with the Pt DEN-immobilized GCE in the presence of dissolved GOx but without glucose (dashed line in Figure 3(b)), or with the dendrimer-immobilized (but without encapsulated Pt nanoparticles) GCE even in the presence of both dissolved GOx and glucose (dashed-dotted line in Figure 3(b)). These results indicate that the Pt DEN-immobilized GCE can be used for biosensing of glucose due to the electrocatalytic redox activity of the immobilized Pt DENs and the compatibility with enzymatic activity of dissolved GOx.

In conclusion, we described the synthesis and electrochemical immobilization of the Pt DENs, which were clearly confirmed by TEM, CV, and XPS measurements. We also demonstrated the potential of the resulting Pt DEN-immobilized GCEs as biosensing platforms. The Pt DEN-immobilized GCEs showed high electrocatalytic activity in the redox reactions of H_2O_2, which was further applied to glucose sensing. Since a variety of electrocatalytic nanoparticles (for example, monometallic nanoparticles such as Pt, Au, Pd, Ni, Fe, and Cu and bimetallic alloy or core/shell nanoparticles[12]) can be conjugated within the dendritic nanostructures on GCEs,[4b] we envision the resulting DEN-immobilized GCEs to be employed in the development of a broad range of bioanalytical devices as facile biosensing platforms.

Experimental Section

Chemicals and Materials. Amine-terminated sixth-generation poly(amidoamine) dendrimers (G6-NH_2 PAMAM dendrimers, 5 wt % in methanol), K_2PtCl_4, $NaBH_4$, $LiClO_4$, H_2O_2, glucose oxidase, glucose and cellulose dialysis sacks (MW cutoff of 12,000) were purchased from Sigma-Aldrich (MO, USA). Glassy carbon (GC) disks and GC plates were obtained from CH Instruments (USA) and Tokai Carbon Co. (Japan), respectively. 18 MΩ·cm deionized water (Ultra370, Younglin Co, Korea) was used to prepare the aqueous solutions.

Preparation of Pt DEN-Immobilized Electrodes. Pt DENs were synthesized according to the procedure described earlier.[4b,5] In brief, 250 mol equivalent of an aqueous 10 mM k_2PtCl_4 was added to an aqueous 10 μM G6-NH_2 dendrimer solution. The mixture was then stirred for 72 h for complexation of Pt ions with the interior amines of the dendrimers. After complete reduction of the complexed Pt ions with a 20-fold excess of an aqueous $NaBH_4$ solution, the Pt DEN solution was dialyzed overnight using a cellulose dialysis sack.

The synthesized Pt DENs were immobilized on GCEs as we reported previously.[4a,4b] The electrochemical immobilization was carried out in a small-volume electrochemical cell using a standard three-electrode configuration with a Model 440 electrochemical analyzer (CH Instruments, USA). The glassy carbon working electrodes (GC disks or GC plates) were polished with 0.3 mm alumina powder on a polishing

cloth (Buehler, USA) followed by successive sonication in ethanol and deionized water. The electrodes were then rinsed with deionized water and blown dry with a N_2 stream. A Pt wire and a Ag/AgCl electrode were used as a counter and a reference electrode, respectively.

Instruments. Transmission electron microscopy (TEM) images were obtained using a Tecnai G^2 F30 ST TEM (FEI Co., USA). Samples were loaded on a 200 mesh carbon-coated copper grid (Ted Pella Inc., USA) and the solvent was evaporated in air. XPS spectra were collected using a PHI 5000 VersaProbe spectrometer (Physical Electronics Inc., USA). The GC plates were used to prepare the XPS samples.

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References

1. (a) Xu, L.; Zhu, Y.; Tang, L.; Yang, X.; Li, C. *J. Appl. Polym. Sci.* **2008**, *109*, 1802. (b) Lee, Y.; Kim, J.; Kim, S.; Jang, W.-D.; Park, S.; Koh, W.-G. *J. Mater. Chem.* **2009**, *19*, 5643. (c) Kim, J. M.; Ju, H.; Choi, H. S.; Lee, J.; Kim, J.; Kim, J.; Kim, H. D.; Kim, J. *Bull. Korean Chem. Soc.* **2010,** *31*, 491.
2. (a) Pathak, S.; Singh, A. K.; McElhanon, J. R.; Dentinger, P. M. *Langmuir* **2004**, *20*, 6075. (b) Ajikumar, P. K.; Ng, J. K.; Tang, Y. C.; Lee, J. Y.; Stephanopoulos, G.; Too, H.-P. *Langmuir* **2007**, *23*, 5670.
3. (a) Yoon, H. C.; Kim, H.-S. *Anal. Chem.* **2000**, *72*, 922. (b) Ledesma-García, J.; Manríquez, J.; Gutiérrez-Granados, S.; Godínez, L. A. *Electroanalysis* **2003**, *15*, 659.
4. (a) Kim, T. H.; Choi, H. S.; Go, B. R.; Kim, J. *Electrochem. Commun.* **2010**, *12*, 788. (b) Ju, H.; Koo, C. M.; Kim, J. *Chem. Commun.* **2011**, *47*, 12322. (c) Kim, J. M.; Kim, J.; Kim, J. *Chem. Commun.* **2012**, *48*, 9233.
5. Zhao, M.; Crooks, R. M. *Angew. Chem. Int. Ed.* **1999**, *38*, 364.
6. Leff, D. V.; Ohara, P. C.; Heath, J. R.; Gelbart, W. M. *J. Phys. Chem.* **1995**, *99*, 7036.
7. Ye, H.; Scott, R. W. J.; Crooks, R. M. *Langmuir* **2004**, *20*, 2915.
8. (a) Deinhammer, R. S.; Ho, M.; Anderegg, J. W.; Porter, M. D. *Langmuir* **1994**, *10*, 1306. (b) Liu, J.; Cheng, L.; Liu, B.; Dong, S. *Langmuir* **2000**, *16*, 7471.
9. Ye, H.; Crooks, R. M. *J. Am. Chem. Soc.* **2005**, *127*, 4930.
10. Esposti, M. D. *Methods* **2002**, *26*, 335.
11. Polsky, R.; Gill, R.; Kaganovsky, L.; Willner, I. *Anal. Chem.* **2006**, *78*, 2268.
12. (a) Crooks, R. M.; Zhao, M.; Sun, L.; Chechik, V.; Yeung, L. K. *Acc. Chem. Res.* **2001**, *34*, 181. (b) Scott, R. W. J.; Wilson, O. M.; Crooks, R. M. *J. Phys. Chem. B* **2005**, *109*, 692.