A facile method of achieving low surface coverage of Au nanoparticles on an indium tin oxide electrode and its application to protein detection†

Md. Abdul Aziz, Srikanta Patra and Haesik Yang*

Received (in Cambridge, UK) 12th May 2008, Accepted 10th July 2008
First published as an Advance Article on the web 4th August 2008
DOI: 10.1039/b808026g

Low surface coverage of Au nanoparticles on an indium tin oxide electrode for sensitive electrochemical detection was achieved using electrostatic adsorption of $AuCl_4^-$ followed by reduction.

Au electrodes have been widely used as working electrodes in electrochemical sensors.[1] However, such electrodes exhibit high and potential-dependent background current in aqueous solution because of their high capacitive current and complex surface redox reactions.[2] This undesirable behavior makes it difficult to achieve high signal-to-background ratios (*i.e.*, low detection limits).[3] Compared to bulk Au, Au nanoparticles (NPs) show better electrocatalytic activities for many electroactive species.[4] Indium tin oxide (ITO) electrodes exhibit poor electrocatalytic activities, but favorably low and flat background current.[5] Thus, the modification of ITO electrodes with a low surface coverage of Au NPs could allow high electrocatalytic activities along with low background current. Moreover, use of minimal Au may reduce the cost of electrochemical sensors.

The modification of electrode surfaces using metal NPs can be achieved *via* the electrodeposition of metal ions or the immobilization of presynthesized metal NPs.[6] However, the fast rate of electrodeposition makes it difficult to control the size and surface coverage of NPs, hence low surface coverage of metal NPs is not readily achieved with either technique.[6e,7] The formation of metal NPs using electrostatic adsorption of metal ions followed by reduction is another strategy. For example, Pt NPs on amine-functionalized carbon nanotubes and Au NPs on amine-functionalized Si substrates were synthesized.[8] Similarly, the formation of Pd, Pt, and binary Pt–Ru NPs on Nafion and functionalized carbon has been reported.[9] The charge density of the electrode determines the surface density of adsorbed metal ions, which in turn limits the size and surface density of NPs formed from the adsorbed ions. A large number of metal ions is required to form a single NP. Therefore, low surface densities of metal NPs could be achievable with this preparation method.

3-Aminopropyltriethoxysilane (APTES) has been commonly used to prepare amine-functionalized self-assembled monolayers on ITO electrodes.[10] The amine group is protonated at neutral pH, and, as a result, a negatively charged metal complex is readily bound to the protonated amine group.[8,11] Chemical reduction of such bound ions allows the facile formation of metal NPs. In biosensors, the immobilization of biomolecules on biosensing surfaces is a critical step. The Au NPs formed on APTES-modified ITO electrodes could allow easy immobilization of biomolecules *via* simple nonspecific binding in place of covalent modification.[12] Moreover, it is known that proteins adsorbed on Au NPs retain activities for a long time.[12]

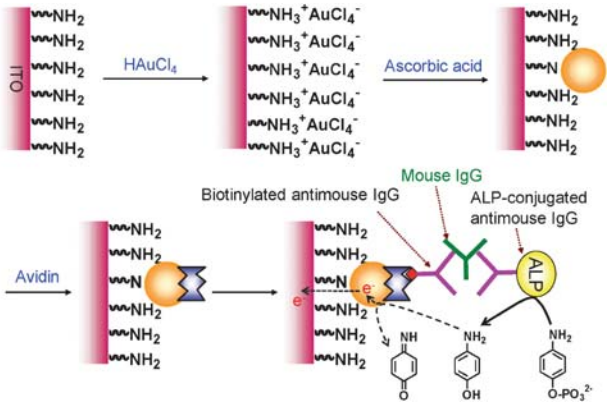

Fig. 1 Schematic representation of the formation of Au NPs on an APTES-modified ITO electrode, and the preparation of an electrochemical immunosensor for detecting mouse IgG. It is not clear whether the formed Au NPs are in direct contact with the ITO surface or not.

Herein, we describe a facile method whereby low surface coverage of Au nanoparticles on APTES-modified ITO electrodes can be achieved. In addition, we report on the application of this technique in the construction of an electrochemical immunosensor. Au NPs were formed by electrostatic adsorption of $AuCl_4^-$ followed by reduction with ascorbic acid. This simple preparation method allowed highly sensitive detection of mouse IgG.

A schematic representation of the preparation of Au NPs on APTES-modified ITO electrodes is shown in Fig. 1. The amine-functionalized ITO electrodes were immersed in an aqueous solution containing 1 mM $HAuCl_4$ (pH 3.13) for 30 min. At this pH, a significant proportion of the Au precursor exists as $AuCl_4^-$, which is electrostatically bound to protonated amine groups on ITO electrodes.[13] After the electrodes were rinsed with water, the bound $AuCl_4^-$ was reduced in an aqueous solution containing 0.5 mM ascorbic acid.

To confirm the formation of Au NPs on APTES-modified ITO electrodes, SEM images were obtained (Fig. 2). No significant difference between SEM images taken before (Fig. 2a) and after (Fig. 2b) the formation of Au NPs on APTES-modified ITO electrodes was observed. However, some brighter spots in Fig. 2b, compared to Fig. 2a, might be associated with Au NPs.

Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, Busan 609-735, Korea. E-mail: hyang@pusan.ac.kr; Fax: +82 51 516 7421; Tel: +82 51 510 3681

† Electronic supplementary information (ESI) available: Experimental details, SEM images, and cyclic voltammograms. See DOI: 10.1039/b808026g

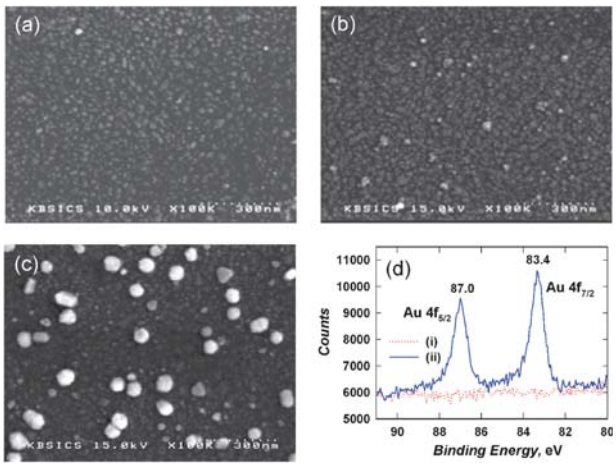

Fig. 2 SEM images of APTES-modified ITO electrodes (a) before and (b) after the formation of Au NPs. (c) SEM image of an Au NP- and APTES-modified ITO electrode after the sizes of Au NPs were increased. (d) XPS spectra of APTES-modified ITO electrodes before (i) and after (ii) the formation of Au NPs.

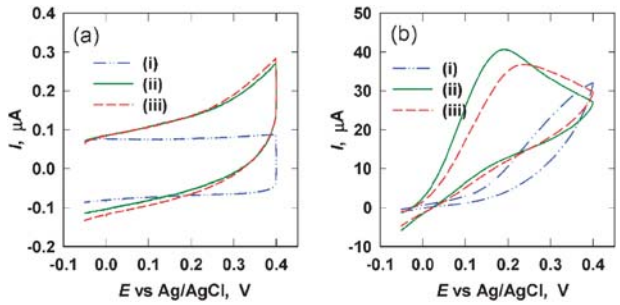

Fig. 3 Cyclic voltammograms obtained at modified ITO electrodes in (a) a Tris buffer solution (pH 9.0) and (b) a Tris buffer solution containing 1.0 mM AP (at a scan rate of 20 mV s^{-1}) [(i) APTES-modified ITO electrode, (ii) Au NP- and APTES-modified ITO electrode, and (iii) avidin-, Au NP-, and APTES-modified ITO electrode].

It seemed that Au NPs were not sufficiently large to be distinguished in SEM images because a high number of $AuCl_4^-$ molecules adsorbed onto an APTES-modified ITO surface is required to form a single Au NP. Small Au NPs on ITO electrodes can act as seeds for further growth of Au NPs. To clearly see the presence of Au NPs, SEM images were obtained after increasing the size of Au NPs by employing an aqueous solution containing 0.3 mM $HAuCl_4$ and 0.3 mM $H_2NOH{\cdot}HCl$ for immersion times of 5 min (Fig. 2c) and 20 min (Fig. S1b in ESI†). The formation of larger NPs indicated the presence of small seed NPs on the APTES-modified ITO electrodes.[14] The surface density of Au NPs was not high, suggesting that the surface coverage of seed Au NPs was low. As a control experiment, APTES-modified ITO electrodes were immersed in the same solution for 20 min. The formation of Au NPs was not observed (Fig. S1a in ESI†), indicating that Au NPs grew only when small Au NPs already existed on ITO electrodes.

Fig. 2d shows the X-ray photoelectron spectra of APTES-modified ITO electrodes before and after the formation of Au NPs. No peaks related to Au were observed before the formation of Au NPs [(i) in Fig. 2d]. However, after treatment of APTES-modified ITO electrodes with $HAuCl_4$ followed by reduction of the complexed Au ions with ascorbic acid, two clearly distinct peaks at 83.4 eV and 87.0 eV corresponding to Au $4f_{7/2}$ and Au $4f_{5/2}$ core-level binding energies, respectively, were observed [(ii) in Fig. 2d], confirming the presence of Au in the zero oxidation state.[15]

The presence of Au NPs on the APTES-modified ITO electrode was also evident from cyclic voltammograms (Fig. S2 in ESI†). The cyclic voltammogram obtained at an Au NP- and APTES-modified ITO electrode in 0.1 M NaOH showed the characteristic redox signal of Au (Fig. S2a in ESI†).[16] The redox currents increased when the sizes of Au NPs increased [(i) and (ii) of Fig. S2b in ESI†], again confirming the presence of seed Au NPs on APTES-modified ITO electrodes.

Cyclic voltammetry in Tris buffer (pH 9.0) was performed to measure changes in background currents and electrocatalytic activities at modified ITO electrodes (Fig. 3). The cyclic voltammogram obtained at an APTES-modified ITO electrode exhibited low and flat background currents at a range of potentials from −0.05 to 0.4 V [(i) in Fig. 3a], because of low capacitive and redox currents. Similarly, the cyclic voltammogram obtained at Au NP- and APTES-modified ITO electrodes showed a low background current, although the background current increased with potential [(ii) in Fig. 3a]. This change in background current arose because of the presence of Au NPs. However, the background current was much smaller than that of a bare Au disc electrode (Fig. S3 in ESI†). The absence of a significant increase in background current, compared to that seen with an APTES-modified ITO electrode, indicated that the surface coverage of Au NPs was very low. Importantly, the cyclic voltammogram of (ii) in Fig. 3a was highly reproducible, indicating that the formation of Au NPs on ITO electrodes was also quite reproducible. This is because the surface density of amine groups on ITO electrodes is uniform and, accordingly, the amount of bound $AuCl_4^-$, determined by the amine groups, is also uniform. The further modification of Au NP- and APTES-modified ITO electrodes with avidin did not result in any significant change in background current [(iii) in Fig. 3a].

Catalytic conversion of *p*-aminophenyl phosphate (APP) to *p*-aminophenol (AP) by alkaline phosphatase (ALP) is commonly employed to obtain amplified electrochemical signals in biosensors.[5] The anodic peak of AP should appear at a value significantly below 0.4 V to minimize the interfering anodic current of APP, because the electrooxidation of APP is considerable above 0.4 V.[5] Fig. 3b shows the cyclic voltammograms for AP electrooxidation obtained at modified ITO electrodes in a Tris buffer (pH 9.0) solution containing 1 mM AP. No anodic peak of AP at an APTES-modified ITO electrode was observed up to 0.4 V [(i) in Fig. 3b]. However, the anodic peaks at an Au NP- and APTES-modified ITO electrode appeared below 0.2 V [(ii) in Fig. 3b]. The presence of Au NPs on the APTES-modified ITO electrodes enabled the easy electrooxidation of AP, although the surface coverage of Au NPs was very low. A slight positive shift in the anodic peak of AP was observed after avidin was immobilized onto Au NPs. However, the electrocatalytic activity was still sufficiently good to electrooxidize AP at a low potential.

To apply the Au NP- and APTES-modified ITO electrodes to electrochemical biosensors, we developed a sandwich-type immunosensor for detecting mouse IgG. The detection scheme for this immunosensor is shown in Fig. 1. Biotinylated

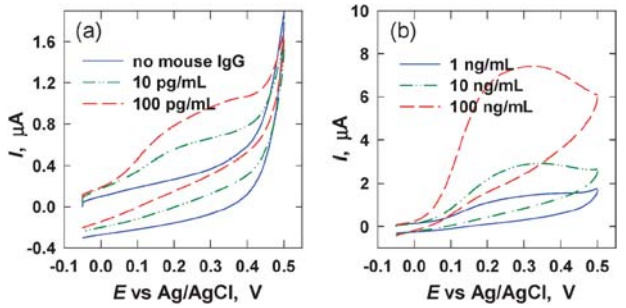

Fig. 4 Dependence of cyclic voltammograms on the concentration of mouse IgG. Cyclic voltammograms were obtained at a scan rate of 20 mV s^{-1} after incubation for 10 min in a Tris buffer containing 1.0 mM APP at (a) 0, 10, and 100 pg mL^{-1} mouse IgG; and (b) 1, 10, and 100 ng mL^{-1} mouse IgG.

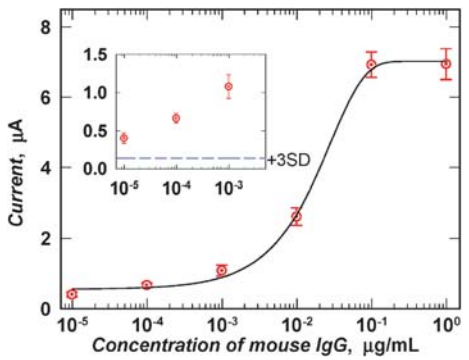

Fig. 5 Dependence of the anodic current at 0.3 V in Fig. 4 on the concentration of mouse IgG. All data were subtracted by the mean current at zero concentration of mouse IgG. The inset represents a magnification of the data points obtained at low IgG concentrations. The dashed line corresponds to three times the standard deviation (SD) of the current at zero concentration of mouse IgG.

antimouse IgG was specifically bound to avidin immobilized on Au NPs. Subsequently, target mouse IgG was captured, and then ALP-conjugated antimouse IgG was bound. ALP catalyses the conversion of APP to AP, and then the amplified AP is electrooxidized on the Au NPs to obtain an electrochemical signal.

Fig. 4 shows cyclic voltammograms obtained at different concentrations of mouse IgG. A sharp increase of anodic current above 0.4 V at low concentrations of target mouse IgG corresponds to the electrooxidation of APP. The anodic current of AP increases with the concentration of mouse IgG. In the absence of target mouse IgG, no anodic peak corresponding to the electrooxidation of AP was observed. This indicated that the nonspecific binding of ALP-conjugated IgG to the sensing surface was very low. To test the selectivity of the sensor, we also obtained a cyclic voltammogram when 100 ng mL^{-1} rabbit IgG was used in place of mouse IgG. The current at 0.3 V (Fig. S4 in ESI†) is similar to that in the absence of mouse IgG (Fig. 4a). No anodic peak related to AP electrooxidation was observed. It indicates that the sensor is highly selective to mouse IgG.

The dependence of the anodic current at 0.3 V on the concentration of mouse IgG is shown in Fig. 5. At 10 pg mL^{-1} of mouse IgG, the anodic current at 0.3 V was 0.74 ± 0.21 μA, which was sufficiently higher than the anodic current observed in the absence of mouse IgG (0.35 ± 0.13 μA) (Fig. 4a). Therefore, the detection limit for mouse IgG was 10 pg mL^{-1}, which is superior to that of general ELISA (enzyme-linked immunosorbent assay) (sub ng mL^{-1} range).

In conclusion, we have developed a sensitive electrochemical immunosensor to detect mouse IgG, using low surface coverage of Au NPs on an APTES-modified ITO electrode. Au NPs were formed on the amine-functionalized ITO surface by electrostatic adsorption of $AuCl_4^-$ followed by chemical reduction. The Au NPs formed in this way provided efficient sites for avidin immobilization. The Au NP- and APTES-modified electrodes offered both low background currents and good electrocatalytic activities, which enabled a low detection limit of mouse IgG (10 pg mL^{-1}).

This work was supported by the Nano/Bio Science & Technology Program (2005-01333) of the Ministry of Education, Science and Technology (MEST) and the Healthcare & Biotechnology Development Program (A020605 and A050426) of the Korea Health Industry Development Institute.

Notes and references

1 For an overview, see: (*a*) A. P. Silverman and E. T. Kool, *Chem. Rev.*, 2006, **106**, 3775–3789; (*b*) A. N. Shipway and I. Wilner, *Acc. Chem. Res.*, 2001, **34**, 421–432; (*c*) R. Miranda-Castro, P. de-los-Santos-Álvarez, M. J. Lobo-Castañón, A. J. Miranda-Ordieres and P. Tuñón-Blanco, *Anal. Chem.*, 2007, **79**, 4050–4055.
2 (*a*) I. Zudans, J. R. Paddock, H. Kuramitz, A. T. Maghasi, C. M. Wansapura, S. D. Conklin, N. Kaval, T. Shtoyko, D. J. Monk, S. A. Bryan, T. L. Hubler, J. N. Richardson, C. J. Seliskar and W. R. Heineman, *J. Electroanal. Chem.*, 2004, **565**, 311–320; (*b*) S. E. Creager and K. G. Olsen, *Anal. Chim. Acta*, 1995, **307**, 277–289; (*c*) T. A. Ivandini, Y. Nono, A. Nakajima and Y. Einaga, *Chem. Lett.*, 2005, **34**, 1086–1087.
3 (*a*) J. S. Yoo and S. M. Park, *Anal. Chem.*, 2005, **77**, 3694–3699; (*b*) J. Das, K. Jo, J. W. Lee and H. Yang, *Anal. Chem.*, 2007, **79**, 2790–2796.
4 (*a*) J. D. Aiken III and R. G. Finke, *J. Mol. Catal.*, 1999, **145**, 1–44; (*b*) P. C. Biswas, Y. Nodasaka, M. Eyno and M. Haruta, *J. Electroanal. Chem.*, 1995, **381**, 167–177.
5 M. A. Aziz, S. Park, S. Jon and H. Yang, *Chem. Commun.*, 2007, 2610–2612 and cited references therein.
6 (*a*) M. O. Finot, G. D. Braybook and M. T. McDermott, *J. Electroanal. Chem.*, 1999, **466**, 234–241; (*b*) M. O. Finot and M. T. McDermott, *J. Electroanal. Chem.*, 2000, **488**, 125–132; (*c*) S. Guerin and G. S. Attard, *Electrochem. Commun.*, 2001, **3**, 544–548; (*d*) G. Chang, M. Oyama and K. Hirao, *J. Phys. Chem. B*, 2006, **110**, 20362–20368; (*e*) J. Zhang and M. Oyama, *Anal. Chim. Acta*, 2005, **540**, 299–306.
7 (*a*) M. S. El-Deab, T. Satomura and T. Osaka, *J. Electrochem. Soc.*, 2005, **152**, C1–C6; (*b*) O. P. Khatri, K. Murase and H. Sugimura, *Langmuir*, 2008, **24**, 3787–3793.
8 (*a*) D.-J. Guo and H.-L. Li, *Electroanalysis*, 2005, **17**, 869–872; (*b*) C. Damle, K. Biswas and M. Sastry, *Langmuir*, 2001, **17**, 7156–7159.
9 (*a*) P. Bertoncello, M. Peruffo and P. R. Unwin, *Chem. Commun.*, 2007, 1597–1599; (*b*) K. Yasuda and Y. Nishimura, *Mater. Chem. Phys.*, 2003, **82**, 921–928; (*c*) N. Fujiwara, K. Yasuda, T. Iorai, Z. Siroma and Y. Miyazaki, *Electrochim. Acta*, 2002, **47**, 4079–4084; (*d*) E. K. Tuseeva, A. A. Mikhailova, O. A. Khazova, V. A. Grinberg and K.-D. Kourtakis, *Russ. J. Electrochem.*, 2005, **41**, 1316–1324.
10 (*a*) S. Kumar and S. Zou, *Langmuir*, 2007, **23**, 7365–7371; (*b*) A. Toyota, N. Nakashima and T. Sagara, *J. Electroanal. Chem.*, 2004, **565**, 335–342; (*c*) S. Park, P. Yang, P. Corredor and M. J. Weaver, *J. Am. Chem. Soc.*, 2002, **124**, 2428–2429.
11 S. Watanabe and S. L. Regen, *J. Am. Chem. Soc.*, 1994, **116**, 8855–8856.
12 (*a*) G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, San Diego, 1st edn, 1996, pp. 593–601; (*b*) R. C. Ebersole, J. A. Miller, J. R. Moran and M. D. Ward, *J. Am. Chem. Soc.*, 1990, **112**, 3239–3241.
13 F. Moreau, G. C. Bond and A. O. Taylor, *J. Catal.*, 2005, **231**, 105–114.
14 K. R. Brown and M. J. Natan, *Langmuir*, 1998, **14**, 726–728.
15 T. F. Jaramillo, S.-H. Baeck, B. R. Cuenya and E. W. McFarland, *J. Am. Chem. Soc.*, 2003, **125**, 7148–7149.
16 S. Kumar and S. Zou, *J. Phys. Chem. B*, 2005, **109**, 15707–15713.