Short communication

Electrochemical sensor based on direct electron transfer of HIV-1 Virus at Au nanoparticle modified ITO electrode

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ABSTRACT

In this study, for the first time, an electrochemical detection method was proposed to detect direct electron transfer signal from HIV-1 Virus. Au nanoparticles were fabricated on the Indium Tin Oxide coated glass (ITO) electrode by electrochemical deposition to improve the surface area to provide better electron-transfer kinetics, and higher background charging current. On the Au nanoparticle modified ITO electrode, antibody fragment was immobilized by self-assembly method with gold-thiol interaction and different concentrations of HIV-1 Virus like particles (VLPs) were applied for the direct determination. Due to the advantage of fabricated Au nanoparticle’s excellent electrical and mechanical properties, HIV-1 VLPs were successfully detected from 600 fg/ml to 375 pg/ml. Furthermore, since the proposed electrochemical virus sensor is designed for direct determination without any labeling structure, the electrochemical communication between redox target and electrode surface will reduce interference reactions and simplifies the detection system with small amount of reagent and better stability. Therefore, highly sensitive virus sensor was developed to detect HIV-1 Virus in a label free system.

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1. Introduction

Since the development of electrochemical sensor by Clark and Lyons in 1962, much attention has been paid to generate more efficient biosensors (Wang et al., 2008). Initial study was focused on electrochemical sensing of simple metabolites such as urea, lactate or glucose, but now it has been extended to complex molecules such as proteins and viruses. For electrochemical detection of biomolecular interaction, there must be an electroactive region which will undergo a redox reaction at the electrode and thus it is known as an indirect detection method forming DNA hybridization, sandwich assay with electrochemical label such as redox enzyme complex (horseradish peroxidase or alkaline phosphatase) (Albers et al., 2003; Ding et al., 2008). Generally, these electrochemical sensors have the virtue of high sensitivity and selectivity, but it requires more fabrication steps and time consuming. Moreover in enzyme based electrochemical sensors, in which enzyme is crucial to the environment, often suffer from unstable response and poor reproducibility (Toghill and Compton, 2010). To overcome these problems, several efforts have given to develop electrochemical sensor based on direct electron transfer between biomolecules and electrode without any mediators (Cui et al., 2007; Yu et al., 2008). The direct electron transfer between redox site of biomolecule and electrode surface could provide an easier way for label-free and sensitive biosensors. This method of detection will not only eliminate interference reactions (as the sensing potential is closer to the potential of the redox material), but also simplify the detection system with better stability (Zhang et al., 2008). Unfortunately, direct electrochemistry of most redox biomolecules on conventional electrodes is a great challenge because of deeply buried redox- active center inside the molecule (Riklin et al., 1995). Several works has been performed to detect virus based on DNA hybridization or sandwich assay, however, virus detection based on the direct electron transfer of virus particle has not been reported yet. Interest in nanotechnology has revealed new possibilities in developing more accurate measurement of specific electrochemical properties (Merkoci 2007; Park et al., 2002). The higher surface-to-volume ratio of nanostructure enhances their electrochemical properties and increases the sensitivity. Metallic nanoparticles, nanorods, and carbon nanotubes are merely some of the familiar materials that are emerging as candidates to develop highly sensitive future electrochemical sensors (Wanekaya et al., 2006; Stadler et al., 2007). In this study, for the first time, HIV-1 Virus was directly measured by direct electron transfer based on Cyclic Voltammmogram (CV) without use of any mediators. On the Au nanoparticle modified Indium Tin Oxide coated glass (ITO) electrode, antibody fragment was immobilized by self-assembly method with gold-thiol interaction and different
concentrations of HIV-1 Virus like particles (VLPs) were applied for the determination.

2. Materials and method

2.1. Materials

The broadly HIV-1 neutralizing gp120 monoclonal antibody (gp120Mab), 2G12, was purchased from Polymun Scientific (Vienna, Austria). 2-mercaptoethylamine (2-MEA), casein and phosphate buffered saline (PBS) (pH 7.4, 10 mM) were purchased from Sigma (St Louis, MO, USA). The reduction of disulfide bond in the antibody (IgG) heavy chain was carried out in the phosphate–ethylenediaminetra acetate acid (PBS–EDTA) buffer (pH 6.0, 1L base: sodium phosphate 100 mM, EDTA 5 mM) purchased from Sigma (St Louis, MO, USA). Dulbecco’s Modified Eagle’s Medium (DMEM) glutamax, Fetal bovine serum (FBS), antibiotics (penicillin–streptomycin) were obtained from Gibco (Invitrogen, Grand Island, USA). All solutions were prepared with distilled and deionized water by Millipore [(Milli-Q) water (DDW)] and all other chemicals used in this study were of reagent grade and obtained commercially.

2.2. Production of HIV-1 like particles

HEK293 cells were co-transfected with CaCl₂ transfection with two different plasmids, pCMV-dR8.74 and pDOLHIVenv, which contain sGag and Pol, and Env components of HIV-1. Kost et al. (1991) co-transfected HEK293 cells were cultured in DMEM medium with 10% FBS and 1% streptomycin at 37 °C for 90 min and residual 2-MEA was removed. The surface morphologies of the aggregates. Fig. 1(A) illustrates the current density increased rapidly during the first 2 ms and gradually decreased to a stationary value at approximately 50 ms. This gradual decrease of current is due to the limited AuCl₄⁻ diffusion to the surface, which is related to nucleation and growth of Au deposition. The current transient profile demonstrates the initial nucleation and growth process during metal deposition (Pletcher et al., 2001). SEM images of the fabricated Au nanoparticles on an ITO surface (Fig. 1(B)) clearly demonstrate that this electrochemical deposition condition results in surfaces with highly uniform Au dot distribution. The fabricated Au dot was approximately 10–20 nm in diameter, separated by 10 nm. As a result, the presence of large number of Au nanoparticles on the electrode leads to the increment of the total surface area of electrode. Fig. 1(C) shows the voltammograms of different electrodes in 0.1 M PBS (pH 7.4), at a scan rate of 50 mV/s. As can be seen, there is no observable faradaic current on the bare ITO electrode. However, Au nanoparticle modified ITO electrode shows a remarkable oxidation peak at 0.137 V and a reduction peak at 0.100 V. This sharp oxidation peak is due to oxide formation and the occurrence of Au stripping in the presence of phosphate ion, which forms a complex ion with Au⁺³ (Richardson et al., 2003). In addition, a large background current was observed for an Au nanoparticle modified ITO electrode in comparison with bare ITO electrode which is due to the large electroactive area. Therefore, the Au nanoparticle modified ITO electrode displays an advantage for providing better electron transfer kinetics as compared with bare ITO electrode.

2.2. Electrochemical behavior of biomolecules on an Au nanoparticle modified ITO electrodes

The formation of protein layer on the electrode yielding a hydrophobic surface that perturbs the interfacial electron transfer rates between the electrode and the electrolyte buffer solution. This

Instruments, USA) controlled with “general purpose electrochemical system” software. A three electrode system comprised of Au nanoparticle modified ITO electrode as the working, a platinum wire as the counter, and Ag/AgCl as reference electrodes were used at a scan rate of 50 mV/s. In order to minimize the error, all the data are the mean ± standard deviation of three different experiments.

3. Results and discussion

3.1. The morphology and current transient of Au nanoparticle modified ITO electrodes

Since most electrochemical reactions occur in close proximity of the electrode surface, the electrodes itself play a crucial role in the performance of electrochemical biosensors. Based on the function of chosen material to develop electrode, its surface modification or its dimensions greatly influence its sensing ability. Therefore, platinum, gold, and carbon have attracted much attention for the use due to their higher conductivity, inertness, biocompatibility and large surface area. The purpose of electrode modification is to improve the sensitivity and selectivity of the biosensor. The higher surface-to-volume ratio of nanostructure enhances the electrical properties, which makes electrode susceptible to external influences, especially when their size continues to reduce to an atomic level. Since the dimension of nanostructure become similar to the size of the target biomolecules, measurement sensitivity will increase (Patolsky et al., 2006), and also this will enhance higher capture efficiency (Nair and Alam, 2007).

Hence to develop highly ordered nanostructure, Tween 20 was used as a surfactant to modify the interfacial properties of both the agglomeration of Au precursor and the electrodes and to control the morphology of the aggregates. Fig. 1(A) illustrates the current-time curve at a potential of −0.9 V for 30 s. The current density increased rapidly during the first 2 ms and gradually decreased to a stationary value at approximately 50 ms. This gradual decrease of current is due to the limited AuCl₄⁻ diffusion to the surface, which is related to nucleation and growth of Au deposition. The current transient profile demonstrates the initial nucleation and growth process during metal deposition (Pletcher et al., 2001). SEM images of the fabricated Au nanoparticles on an ITO surface (Fig. 1(B)) clearly demonstrate that this electrochemical deposition condition results in surfaces with highly uniform Au dot distribution. The fabricated Au dot was approximately 10–20 nm in diameter, separated by 10 nm. As a result, the presence of large number of Au nanoparticles on the electrode leads to the increment of the total surface area of electrode. Fig. 1(C) shows the voltammograms of different electrodes in 0.1 M PBS (pH 7.4), at a scan rate of 50 mV/s. As can be seen, there is no observable faradaic current on the bare ITO electrode. However, Au nanoparticle modified ITO electrode shows a remarkable oxidation peak at 0.137 V and a reduction peak at 0.100 V. This sharp oxidation peak is due to oxide formation and the occurrence of Au stripping in the presence of phosphate ion, which forms a complex ion with Au⁺³ (Richardson et al., 2003). In addition, a large background current was observed for an Au nanoparticle modified ITO electrode in comparison with bare ITO electrode which is due to the large electroactive surface area (Arrigan, 2004). Therefore, the Au nanoparticle modified ITO electrode displays an advantage for providing better electron transfer kinetics as compared with bare ITO electrode.

3.2. Electrochemical behavior of biomolecules on an Au nanoparticle modified ITO electrodes

The formation of protein layer on the electrode yielding a hydrophobic surface that perturbs the interfacial electron transfer rates between the electrode and the electrolyte buffer solution. This
is because of the binding of fragmented antibody on the electrode surface which retards the interfacial electron transfer and eventually blocks the electron transfer, which will be reflected as reduced electrical response. Fig. 2(A) shows the significant signal reduction of the electrical response due to the immobilization of fragmented antibody on the electrode surface. In addition, the less efficient penetration of interfacial electron transfer due to antibody immobilization made it more difficult for oxidation reaction to occur on the electrode surface, which results in shifting of oxidation peak position to higher potential. When HIV-1 VLPs interacts with immobilized antibody on the electrode surface, the electrical response of oxidation and reduction peak has been decreased more and shifted to higher potential than antibody immobilized layer. This result might be due to the thicker protein layer formed by HIV-1 VLPs, which was covered by glycoproteins. Moreover, since the oxidation peak potential position was different from antibody immobilized layer, it could be expected that HIV-1 VLP might possess electrochemically active site to oxidize. When immunoreactions occur between the HIV-1 VLPs and fragmented antibody
immobilized on the Au nanoparticle modified ITO electrode, the surface component of HIV-1 VLPs exposed to the electrode and electrolyte were only gp120 and lipid layer. Since, there is no electrochemical activity on lipid layer, it was expected that gp120 might possess electrochemical activity for oxidation. The structure of gp120 has been identified which is composed of 500 amino acid residues and with oligosaccharide structures as shown in Fig. 2(B) (Modrow et al., 1987). It is known that, among the many amino acids sulfur containing cystine residue is the only responsible amino acid for electrochemical activity because of the presence of sulfur as shown in the Fig. 2(C). Moreover, it was experienced that the oxidative site of cystine is limited to its disulfide bond which means that the oxidation was not related to the carboxyl or amino groups (Zong et al., 2010). Furthermore, it is reported that changes in the redox status of the disulfides of gp120 post-synthesis occur in relation to HIV entry into the target cell (Ryser et al., 1994; Berger et al., 1999), which represents the redox activity of disulfide bond of cystine residues. Since gp120 possesses nine disulfide bonds, which can be oxidized in electrochemical processes, the expected oxidation mechanism of cystine has been clarified. It could be expected that the disulfide bond of cystine residue could be oxidized to either sulfenic, sulfonic or sulfonic acid as shown in the Fig. 2(D).

3.3. Cyclic voltammetry for detection of different concentrations of HIV-1 VLPs

Earlier studies reported that the virus detection based on electrochemical method was focused and developed only by mediator based with enzyme or redox labels. But, here in this study HIV-1 VLPs were directly determined by localized antibody fragment and target analytes on an Au nanoparticle modified ITO electrode. Fig. 3 (A) shows the cyclic voltammograms for different concentrations of HIV-1 VLPs from 600 fg/mL to 375 pg/mL on the Au nanoparticle modified ITO electrode. Upon addition of HIV-1 VLPs, the oxidation and reduction current peak increased with increasing concentration of HIV-1 VLPs. The Au nanoparticle modified ITO electrode enhanced the electrochemical conductivity compared to that of the bare electrode which is related to the increase of the electrode’s active surface area. Furthermore, the 3D structure of the fabricated Au nanoparticle might be able to activate the deeply buried redox-active center inside the virus component. Moreover, these results might be related to moderate electrocatalytic activity of the Au nanoparticle modified ITO electrode, which was obtained by modifying poorly electrocatalytic electrode (ITO) with a highly electrocatalytic material (Au). This might enable the electrode to obtain high signal-to-background ratios compared to those of Au and Pt electrodes (Das and Yang, 2009). Therefore, the Au nanoparticle modified ITO electrode displayed advantages related to providing better electron-transfer kinetics than that of bare electrodes, and the catalytic properties of Au nanoparticles might increase the oxidation of HIV-1 VLPs at the Au nanoparticle modified ITO electrode. Hence, various concentration of HIV-1 VLPs ranging from 600 fg/mL to 375 pg/mL was successfully measured by Au nanoparticle modified ITO electrode based on direct electrochemical determination. The calibration plot, acquired from oxidation peak of gp120 at ca. 0.153 V shows a linear relation in a range from 600 fg/mL to 375 pg/mL with a correlation coefficient of 0.958 (Fig. 3(B)). Table S1 provides a comparison of the analytical properties between previously reported HIV-1 detection methods. Compare to mass (Lu et al., 2012) and optical methods (Biancotto et al., 2009; Tang and Hewlett, 2010) electrochemical method is inexpensive, robust, and highly sensitive. However, it still requires labeling mediator for sensitive detection (Kheiri et al., 2011; Gan et al., 2013). Apart from these drawbacks, proposed Au nanoparticle modified ITO electrode could be used to develop a highly sensitive direct (label free) electrochemical biosensor for determination of low concentration of HIV-1 Virus.

4. Conclusion

In this study, electrochemical detection method was proposed to detect HIV-1 Virus. Au nanoparticles were fabricated on the ITO electrode by electrochemical deposition to improve the surface area to provide better electron-transfer kinetics, and higher background charging current. On the Au nanoparticle modified ITO electrode, antibody fragment was immobilized by self-assembly method with gold-thiol interaction and different concentrations of HIV-1 VLPs were applied for the direct determination. Due to the excellent electrical and mechanical properties of fabricated Au nanoparticle, HIV-1 VLPs were successfully measured from 600 fg/mL to 375 pg/mL. In addition, the proposed electrochemical sensor was designed for direct determination, the electrochemical communication between redox target and electrode surface reduced interference reactions and simplified the detection system with small amount of reagent and better stability. Therefore, it was able to develop a highly sensitive virus sensor to detect HIV-1 Virus in a label free system.

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Appendix A. Supporting information

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