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Sensitive reagentless electrochemical immunosensor based on an ormosil sol–gel membrane for human chorionic gonadotrophin

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Abstract

A new organically modified silicate (ormosil) material was synthesized as a matrix to encapsulate enzyme labeled antibody for preparation of immunosensors. The ormosil matrix was prepared by hydrolyzing tetraethyoxysilane and (3-aminopropyl)triethoxysilane in weak alkali solution. It possessed three-dimensional ordered nanoporous structure with high electrical conductivity and good mechanical stability. Its hydrophilicity provided a microenvironment for retaining the biological activity of the immobilized protein. Particularly, using horseradish peroxidase-labeled human chorionic gonadotrophin antibody (HRP-*anti*-hCG) as a model, the immobilized HRP showed direct electron transfer at about −35 mV with a rate constant of $15.8 \pm 3.8\,s^{-1}$. By a simple one-step immunoreaction between human serum chorionic gonadotrophin (hCG) in sample solution and the immobilized HRP-*anti*-hCG, the differential pulse voltammetric peak current of HRP decreased linearly with an increasing hCG concentration from 0.5 to 50 mIU/ml with a relatively low limit of detection of 0.3 mIU/ml at 3σ. Excellent analytical performance, fabrication reproducibility and operational stability of the proposed biosensor indicated its promising application in clinical diagnostics.

Keywords: Immunosensors; Ormosil; Sol–gel; Enzyme labeled antibody; Human chorionic gonadotrophin

1. Introduction

Electron transfer reaction between biomolecules is of fundamental importance in life science and design of bioelectronics devices (Blonder et al., 1996; Tyan et al., 2005), biosensors (Fan et al., 2002), and optobioelectronic systems (Borger et al., 2001). The direct electron transfer (DET) of heme proteins such as cytochrome c (Lojou and Bianco, 2000), myoglobin (Ray et al., 2005), hemoglobin (Han et al., 2002), horseradish peroxidase (HRP) (Liu and Ju, 2002) and other proteins (Chen et al., 2001) has been extensively used for preparation of biosensors. This strategy avoids the trouble of adding a mediator to the sample solution and the pollution of some mediators in detection systems (Xiao et al., 2000; Tian et al., 2002). Considerable attempts are to explore new methods or synthesize new matrices to immobilize enzymes or proteins while retaining their native electrical activity and function. A series of inorganic porous materials such as clay (Lei et al., 1999), montmorillonite (Lei and Deng, 1996), porous alumina (Ikeda et al., 1998) and sol–gel matrix (Dai et al., 2003), etc. have been shown to be promising as immobilization matrices for DET of immobilized redox proteins. Among these sol–gel technology provides a unique means to prepare a three-dimensional network suited for the encapsulation of a variety of biomolecules (Li et al., 1996; Walcarius, 2001).

Sol–gel-derived materials are particularly attractive for biosensor fabrication, because they can be prepared under ambient conditions and exhibit tunable porosity, high thermal stability, chemical inertness and negligible swelling in aqueous solution. Organically modified silicate (ormosil) sol–gel materials prepared by the sol–gel process have become an attractive field of study due to the individual covalently bound precursor, which can be used to alter pore size (Widera et al., 2005), hydrophobicity (Pagliaro et al., 2006) and flexibility of materials or introduce a specific function group (Rottman et al., 2001) into the matrix as needed to improve material performance. Therefore, ormosil holds much promise in the development of chemical sensors (Collinson et al., 2000;

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Gerritsen et al., 2000; Neiman et al., 2002; Lee et al., 2005). Due to the intrinsic nanoporous property of sol–gel-derived material, the solid support prepared by dropping coating can be utilized as a matrix for antibody immobilization. This architecture not only provides a very hydrophilic interface for retaining the bioactivity and improving the stability of the immobilized enzyme labels and immunocomponents, but also promotes the electrical communication between redox sites of enzyme labels and sensing surface for direct electrochemical immunoassay of proteins.

In general, DET of the heme enzyme labeled on antibody is fairly difficult due to the relatively large size of antibody, which blocks the electron transfer of labeled enzyme. In previous works we observed the DET of HRP-labeled on the carcinoma antigen-125 (Dai et al., 2003) and human serum chorionic gonadotrophin (hCG) (Chen et al., 2005) antibodies, which was encapsulated in a titania sol–gel film by the immunoreaction with corresponding antigen immobilized in the film. The DET signal was thus used for immunoassay of these antigens. However, the electrochemical signal from the DET is weak due to the relatively slow electron transfer rate.

In this work a new sol–gel-derived material, ormosil sol–gel, was synthesized for trapping the enzyme labeled antibody, studying the DET of the trapped enzyme and preparing one new kind of immunosensors. The ormosil sol–gel was prepared by hydrolyzing tetraethyoxysilane (TEOS) and (3-aminopropyl)triethoxysilane (APTES), which have also been used as two prescurosrs for syntheses of several solid silicas under acidic conditions (Zhou et al., 1998; Wang et al., 2005). Using HRP-labeled hCG antibody as a model the heme enzyme label immobilized in this matrix showed a more excellent DET behavior than those observed in our previous works (Dai et al., 2003; Chen et al., 2005). Upon the formation of immunocomplex the DET signal decreased due to the increasing spatial blocking and dielectric constant of the microenvironment around HRP molecules, thus a sensitive immunosensor for hCG was constructed.

hCG is a 37 kDa glycoprotein hormone. This is the first glycoprotein produced by trophoblasts of the placenta during pregnancy and is secreted by trophoblastic neoplasms and a variety of nontrophoblastic tumors. Detection of hCG in urine or serum is thus employed in all modern immunological pregnancy tests and for monitoring of trophoblastic diseases (Albert and Danny, 1999). In an earlier study, a sandwich-type electrochemical immunoassay (Aizawa et al., 1979) and a separation-free immunosensor using a gold-coated microporous nylon membrane to immobilize antibody (Duan and Meyerhoff, 1994) for hCG have been reported, and various immunoassay kits and strategies for hCG measurements have also been proposed, including an indirect perfluorosulfonated ionomer-coated electrochemical immunosensor in connection with HRP (Chetcuti et al., 1999), an electrochemical flow immunoassay system using capillary columns and ferrocene-conjugated immunoglobulin G (Lim and Matsunaga., 2001). In this report, the constructed immunosensor and suggested detection strategy appeared to be simple, sensitive, practical, and low-cost in clinic diagnostic.

2. Materials and methods

2.1. Chemicals

hCG enzyme-linked immunosorbent assay (ELISA) kits were purchased from BioCheck, Inc. (Foster city, USA). The ELISA kits consisted of a series of hCG standard solutions with different concentrations from 0 to 150 mIU/ml, a stock solution of HRP-labeled hCG monoclonal antibody from mouse and a buffer for in vitro diagnostic incubation. APTES was the product of Sigma Chemical Company (St. Louis, MO, USA). TEOS with analytical grade was obtained from Shanghai Chemical Company. All other reagents were of analytical grade. All solutions were made up with twice distilled water.

2.2. Apparatus

Electrochemical measurements were performed on a CHI 660B electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China). All experiments were carried out using a conventional three-electrode system with a modified graphite electrode (GE) as working, a platinum wire as auxiliary and a saturated calomel electrode as reference electrodes. Scanning electron microscope (SEM, LEO 1530 VP, Germany) was used for morphological analysis of different films at 5.00 kV. FTIR spectra were obtained on a Nexus 870 (Nicolet) FTIR instruments at room temperature.

2.3. Preparation of hCG immunosensor

The graphite electrode (GE, 4 mm in diameter), which was homemade with spectral grade graphite, was polished to a mirror finish using 0.3 and 0.05 μm alumina slurry (Beuhler) followed by rinsing thoroughly with twice distilled water. After sonicated successively in 1:1 nitric acid, acetone and twice distilled water, the electrode was rinsed with twice distilled water and allowed to dry at room temperature. The active surface-area of the electrode obtained from the cyclic voltammograms of 1.0 mM K_3[Fe(CN)6] in 0.1 KNO_3 solution at the scan rates from 20 to 200 mV/s to be 0.19 cm^2.

The ormosil sol was firstly prepared by mixing 150 μl of APTES, 50 μl of TEOS, 100 μl of 10 mM NaOH as catalyst, and 700 μl of H_2O in a small test tube under stirring for 4–5 min at room temperature. The ratio of APTES to TEOS used was optimized using the DET signal of the obtained immunosensors. The weak alkali medium improved the hydrolysis rate and avoided the denature of protein when used for immobilization of biomolecules. To immobilize HRP-*anti*-hCG in the silica network, 2 μl of the received HRP-*anti*-hCG mixed with 2 μl ormosil sol was dropped on the pretreated GE surface and allowed to dry for the formation of gel under ambient condition for 3 h. After the modified electrode was rinsed with doubly distilled water thrice, the immunosensor, HRP-*anti*-hCG/sol–gel/GE, was obtained. When not in use, the sensor was stored in air at 4 °C.

For morphological analysis the films were prepared in the same way on different slides dealt with nitric acid and the

mixture H_2SO_4:H_2O_2 (1:1). After coated with Au film to improve the electrical conductivity, these films were examined under a scanning electron microscope (Zong et al., 2006).

2.4. Measurement procedure

The one-step immunoreaction was accomplished on the immunosensor surface for immunoassay. Firstly, 10 μl hCG standard or sample solution was diluted with 40 μl buffer to a total volume of 50 μl in an incubation cup. The immunosensor was then incubated in the solution at 30 °C for 40 min and washed carefully twice with distilled water to obtain an immobilized immunoconjugate layer, named as hCG/HRP-*anti*-hCG/sol–gel/GE. The amount of produced immunocomplex depended on hCG concentration in standard or sample solution. After rinsing with pH 7.0 PBS, the hCG/HRP-*anti*-hCG/sol–gel/GE was detected in 0.1 M pH 7.0 PBS with differential pulse voltammetry (DPV) from +200 to −200 mV at a pulse amplitude of 50 mV and a pulse width of 50 ms. The optimal pH value of detection solution was obtained from the DPV response of the immunosensors after incubating in a solution without presence of hCG.

3. Results and discussion

3.1. Characterization of different membranes

The surface morphology of the ormosil sol–gel matrix was an important factor affecting the performance of the immunosensor. As shown Fig. 1A and B, the ormosil sol–gel membrane was uniform and incompact with a very narrow particle size distribution. The diameters of these particles were in the range 15–20 nm. Such a membrane could provide a necessary conduction pathway to assist the electron transfer between the immobilized labeled enzyme and the electrode. After HRP-*anti*-hCG was immobilized in the matrix, the structure kept being uniform and porous, and bright particles of HRP-*anti*-hCG were observed (Fig. 1C), which resulted in a good DET. Upon the formation of immunocomplex the aggregates of the protein molecules were observed, the surface became rough and uneven (Fig. 1D).

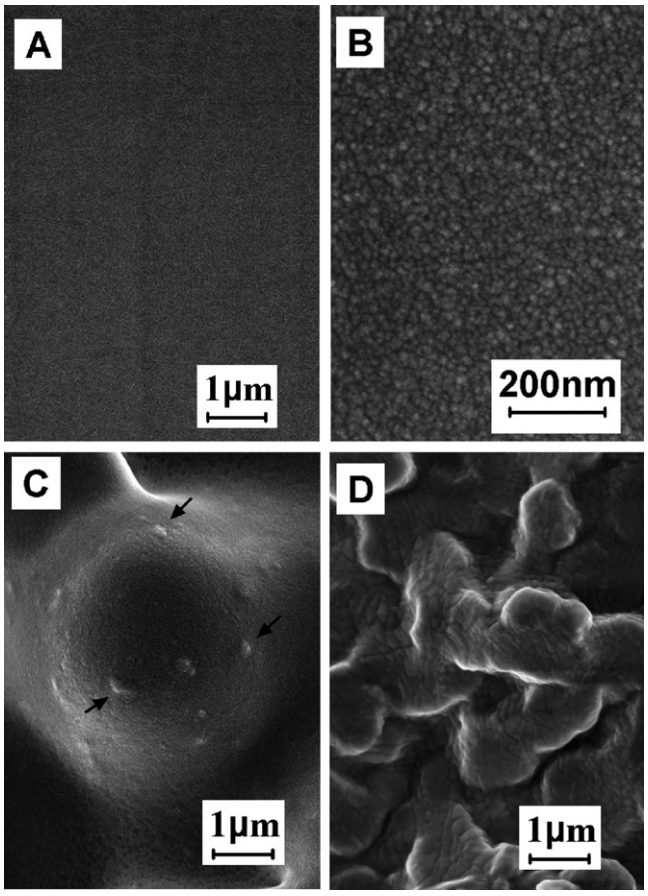

Fig. 1. Scanning electron micrographs of graphitic electrodes coated with sol–gel (A and B), HRP-*anti*-hCG/sol–gel (C) and hCG/HRP-*anti*-hCG/sol–gel (D) at ×10.0 K (A, C, D) and ×80.0 K (B).

3.2. Cyclic voltammetric behavior of modified electrodes

The cyclic voltammograms of different electrodes in 0.1 M pH 7.0 PBS were shown in Fig. 2. No response was observed at pretreated GE (curve a) and ormosil sol–gel modified GE (curve b) in the work potential range. The ormosil sol–gel modified electrode showed a less background current compared to the pretreated GE in the same medium. After mixing HRP-*anti*-hCG with the sol–gel, HRP-*anti*-hCG molecules encapsulated in the sol–gel matrix displayed the DET response of HRP label (curve c). A pair of stable and well defined redox peaks with regard to Fe(III)–Fe(II) conversion of the immobilized HRP were observed. The anodic and cathodic peak potentials were −20 and −50 mV (versus SCE) at 100 mV/s, respectively. The large quantities of hydroxyl groups in the sol–gel hybrid material could form strong hydrogen bonds with the hydroxyl and amino

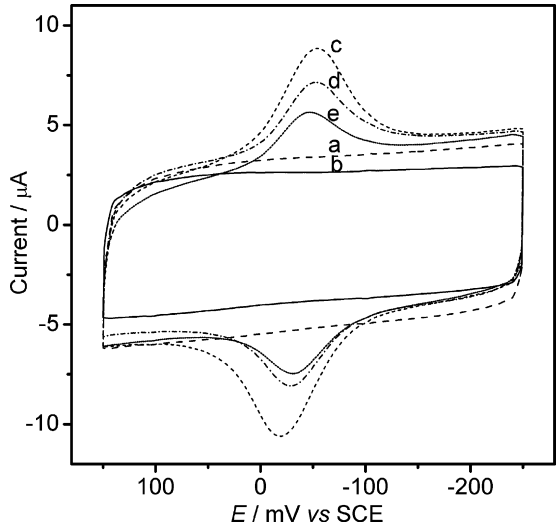

Fig. 2. Cyclic voltammograms of different electrodes in pH 7.0 PBS at 100 mV/s: (a) pretreated bare GE, (b) sol–gel/GE, (c) HRP-*anti*-hCG/sol–gel/GE, (d and e) hCG/HRP-*anti*-hCG/sol–gel/GE obtained after incubating the immunosensor in 50 μl incubation solution containing 10 μl of 5.0 (d) and 20.0 (e) mIU/ml hCG at 30 °C for 40 min.

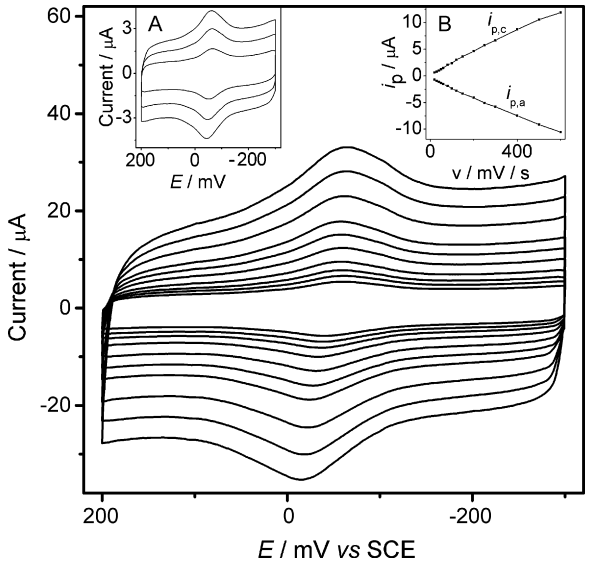

Fig. 3. Cyclic voltammograms of HRP-*anti*-hCG/sol–gel/GE in pH 7.0 PBS at 80, 100, 120, 150, 200, 250, 300, 400, 500, 600 and 700 mV/s (from lowest to highest peak current). *Inset*: (A) cyclic voltammograms of this system at 20, 40 and 60 mV/s; (B) plot of peak currents vs. scan rate.

groups in proteins (Zheng et al., 2003). These hydrogen bonds and the intermolecular interaction between antibody molecules and specific sites of silica sol–gel prevented the immobilized enzyme labeled antibody from leaking out of the thin film. On the other hand, the silica sol–gel could remain its porous structure upon the encapsulation of HRP-labeled antibody, which made the immobilized antibody to be able to bind freely with antigen in solution. After HRP-*anti*-hCG/sol–gel/GE was incubated for 40 min in 50 μl incubation solution prepared with 10 μl 5.0 mIU/ml hCG standard solution, the DET response of the immobilized HRP decreased due to the increased barrier resulted from the formation of immunocomplex (curve d). Further incubation in an incubation solution containing 20.0 mIU/ml hCG, led to similar result with greater decease in currents of both redox peaks (curve e).

Fig. 3 shows the cyclic voltammograms of HRP-*anti*-hCG/sol–gel film modified GE in 0.1 M pH 7.0 PBS at scan rates from 20 to 600 mV/s. The cyclic voltammograms at low scan rates were almost symmetrical with equal reduction and oxidation peak currents. Both the peak currents were proportional to scan rate (inset B, Fig. 3), as expected for the electrochemical behavior of redox in a thin layer. The small peak-to-peak separation indicated a fast electron transfer rate and a uniform distribution of labeled HRP in ormosil sol–gel, while the large peak-to-peak separation reported was ascribable to the various orientations of immobilized HRP molecules (Xiao et al., 2000). From the integration of the reduction peaks of the HRP-*anti*-hCG/sol–gel/GE at different scan rates, an average surface coverage of HRP-*anti*-hCG was calculated to be $(1.29 \pm 0.02) \times 10^{-9}$ mol/cm^2. The charge transfer coefficient, α, was calculated from the slope of plot of reduction peak potential versus the logarithm of scan rate to be 0.43. So the electron transfer rates k_s could be estimated with the formula $k_s = mnFv/RT$ since the peak-to-peak separation was less than 200 mV (Laviron, 1979), where m is a parameter linked to the peak-to-peak separation and could be obtained from this literature, F the Faraday's constant, R the gas constant, T the temperature, and n is the number of electron transfer. Here, $T = 291$ K and $n = 1$. The peak-to-peak separations of 20, 28, 26, 45 and 60 mV at 50, 200, 400, 600 and 1000 mV/s, respectively, produced an average k_s value of $15.8 \pm 3.8\ s^{-1}$. The peak-to-peak separation of 30 mV of HRP-*anti*-hCG/sol–gel/GE at 100 mV/s was much less than those of 60 and 75 mV for HRP-*anti*-carcinoma antigen-125/titania sol–gel (Dai et al., 2003) and HRP-*anti*-hCG/titania sol–gel (Chen et al., 2005) by vapor deposition method, producing larger k_s value than those of 3.04 ± 1.21 and $1.35 \pm 0.40\ s^{-1}$ for these electrodes, respectively. The k_s value was also larger than $6.04\ s^{-1}$ of HRP immobilized on colloidal gold modified electrode (Liu and Ju, 2002). Upon the immunoreaction with hCG during incubation procedure the cyclic voltammogram of the obtained immunosensor (not shown) increased, indicating the decrease in DET.

The increasing electron transfer rate resulted from the strong interaction between HRP molecules and ormosil sol–gel particles. The small sol–gel particles could function as electron-conduction pathways between the prosthetic groups of HRP and carbon-sensing sites and therefore facilitate the electron transfer process. More importantly, the immobilized HRP showed an average formal potential of −35 mV, much more positive than those of −550 and −562 mV in titania sol–gel, indicating the reduction of immobilized HRP was easier due to the presence of amino and/or $-NH_3^+$ groups in the ormosil sol–gel matrix. These behaviors would increase the sensitivity of the formed immunosensor.

3.3. FTIR spectral analysis

Fig. 4 shows the FTIR spectra of HRP-*anti*-hCG, ormosil sol–gel and HRP-*anti*-hCG/sol–gel, respectively. The HRP-*anti*-hCG showed two peaks at 1636 and 1543 cm^{-1} (curve a) corresponding to the amide I (C=O stretching) and amide II (N–II in plane bending and C–N stretching) on the surface. The peak around 1080 cm^{-1} was ascribed to weak C–O stretching

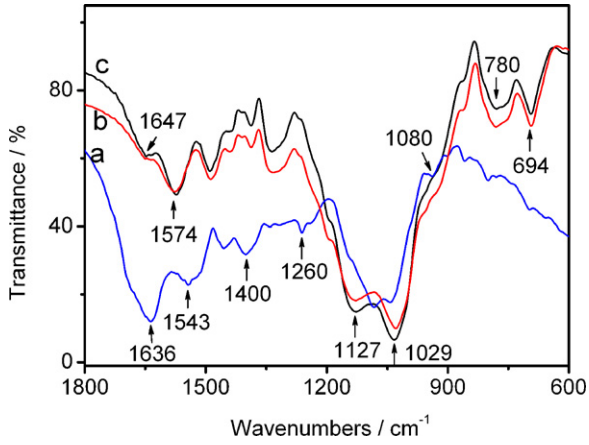

Fig. 4. FTIR spectra of HRP-*anti*-hCG (a), sol–gel (b) and HRP-*anti*-hCG/sol–gel (c) films.

vibration and the bands around 1400 and 1260 cm^{-1} was from the action of amide III (C–N) (Dong et al., 2001). Typical bands around 1127, 1029 and 780, cm^{-1} associated with the condensed silica networks of ormosil sol–gel were observed on curves b and c. The strong absorption bands in the range 1000–1200 cm^{-1} were ascribed to asymmetric stretching vibration of the Si–O–Si bond of the silica component, which was produced by in situ condensation between SiOR and/or SiOH groups. The peak at 780 cm^{-1} was associated with $SiCH_3$ rocking vibration. The presence of the weak N–H bending vibration at 694 cm^{-1} and the symmetric $-NH_3^+$ bending vibration around 1574 cm^{-1} confirmed the incorporation of amino groups. After HRP-*anti*-hCG was trapped in the ormosil sol–gel, the peak corresponding to amide II at 1543 cm^{-1} was covered by the band of symmetric $-NH_3^+$ bending vibration, while the peak corresponding to amide I shifted from 1636 to 1647 cm^{-1} and the absorption intensity decreased greatly due to the interaction between the proteins and SiOH groups in silica networks (curve c), which kept the proteins from leaking out of the thin film.

3.4. Optimal conditions for immunoassay

The factors that influenced the immunoreaction for immunoassay of analyte included the surface character and electron transfer behavior of the immunosensors, incubation temperature and incubation time in the incubation solution. The former was related to the ratio of HRP-*anti*-hCG to ormosil sol–gel used for immunosensor preparation. The effect of the volume fraction of HRP-*anti*-hCG of given concentration to ormosil sol–gel on DPV peak current at a fixed total volume was shown in Fig. 5A. With the increasing amount of HRP-*anti*-hCG, the peak current increased linearly and reached a maximum value at the volume fraction of 0.5 (1:1, v/v). More amount of the HRP-*anti*-hCG resulted in decrease of the amperometric response due to the decrease of the amount of sol–gel for protein immobilization and facilitating the electron transfer process. Thus, the immunosensor was constructed by mixing 2 μl HRP-*anti*-hCG solution and 2 μl ormosil sol–gel solution.

The pH value of detection solution affected the DET of the immobilized HRP. The maximum DPV response of the immunosensors after incubated in a solution without presence of hCG occurred at pH 7.0. Fig. 5B shows the effect of incubation temperature on amperometric response of obtained immunocomplex. The increase of incubation temperature was favorable to the formation of the immunocomplex that increased the barrier of electron transfer between immobilized HRP and sensing surface, thus reducing the amperometric response. The amperometric response was readily decreased until a platform response was observed at an incubation temperature of 30 °C. The incubation temperatures higher than 35 °C resulted in further decrease of the response, which was possibly due to the change in architecture of HRP-*anti*-hCG/sol–gel composite and denaturation of the immobilized HRP. At the incubation temperature of 30 °C, the amperometric response of obtained immunocomplex reached a platform at 40 min (Fig. 5C), indicating the saturated formation of immunocomplex in the matrix. The optimal incubation conditions were at 30 °C for 40 min. The incubation time was much less than that reported previously (Zhang et al., 2004), resulting in short detection time.

3.5. Amperometric response of immunosensor to hCG concentration

Under optimal incubation conditions, the hCG molecules in incubation solution occupied the binding sites of the encapsulated HRP-*anti*-hCG to form immunocomplex. With an increasing hCG concentration the DPV peak current decreased (Fig. 6), which resulted from the increasing amount of formed immunocomplex and the increasing barrier to direct electron transfer. The plot of the average peak current ($n = 5$) versus hCG concentration in standard solution or original sample for preparation of incubation solution showed a linear range from 0.5 to 50.0 mIU/ml with a slope of 0.255 μA/mIU/ml and the correlation coefficient of 0.997 (inset in Fig. 6). From the slope the limit of detection was calculated to be 0.3 mIU/ml at 3σ (Chen et al., 2006; Liu and Ju, 2002), which was lower than those reported previously (Lode et al., 2003; Kerman et al., 2006). When the

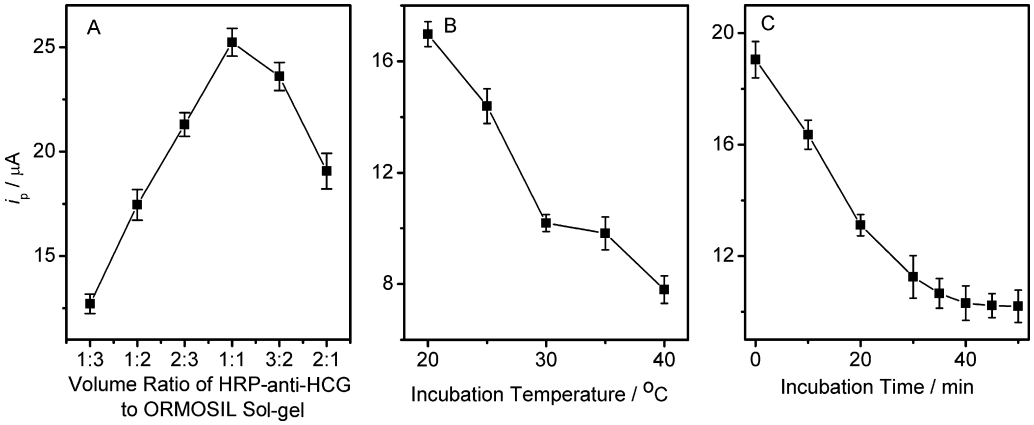

Fig. 5. Effects of the volume ratio of HRP-*anti*-hCG to ormosil sol–gel solution for immunosensor preparation (A), dependence of DPV peak current of HRP-*anti*-hCG/sol–gel/GE on incubation temperature with a incubation of 40 min (B) and incubation time at a incubation temperature of 30 °C (C) in 50 μL incubation solution containing 10 μl 20.0 mIU/ml hCG in 0.1 M pH 7.0 PBS.

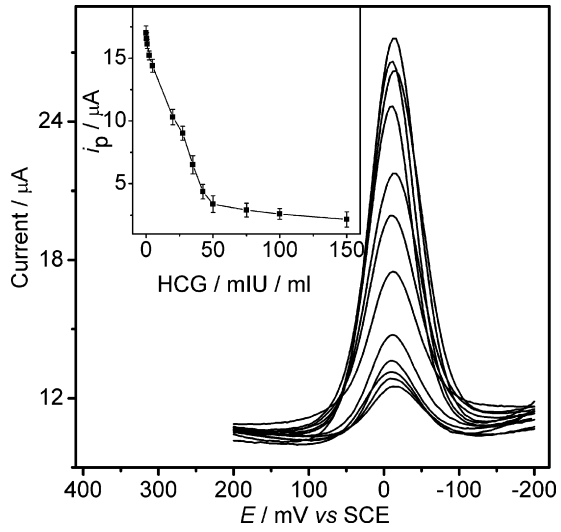

Fig. 6. DPV of hCG/HRP-*anti*-hCG/sol–gel/GE in 0.1 M pH 7.0 PBS after incubated in 50 μl solutions contanning 10 μl 0.5, 1.0, 2.5, 5.0, 20.0, 27.5, 35.0, 42.5, 50.0, 75.0, 100 and 150 mIU/ml hCG at 30 °C for 40 min (from highest to lowest peak current). *Insets*: plot of DPV peak current vs. hCG concentration in standard solution for preparation of incubation solution.

dilution fraction of 10/50 (v/v) for preparation of incubation solution was taken into account, the practical linear ranges were from 0.1 to 10.0 mIU/ml, and the limit of detection in the incubation solution was 0.06 mIU/ml. The proposed limit of detection was much lower and the linear range was also wider than those of hCG electrochemical immunosensors reported previously (Chen et al., 2005; Chen et al., 2006).

3.6. Reproducibility and stability of the hCG immunosensor

The reproducibility of the immunosensors for hCG was investigated with intra- and inter-assay precision. The intra-assay precision of the immunosensors was evaluated by assaying the hCG of three sera for five replicate measurements in the same run. The variation coefficients (CVs) of the intra-assay for this method were 6.1, 5.8, and 7.5% at hCG concentrations of 2, 10, and 35 mIU/ml, respectively. The inter-assay precision or the fabrication reproducibility was estimated by determining in duplicate the hCG level in one serum sample with three immunosensors made at the same electrode independently. The inter-assay precision of this method was 6.3% at the hCG concentration of 10 mIU/ml, showing acceptable fabrication reproducibility. The performance stability of the biosensor was examined by storage in air at 4 °C, it retained 90% of its initial current after a storage period of 30 days. This indicated that ormosil sol–gel prepared by a mild process provided a biocompatible microenvironment around the antibody molecules. The large quantities of hydroxyl groups in the sol–gel hybrid material could form strong hydrogen bonds. These hydrogen bonds and the intermolecular interactions between antibody molecules and specific sites of ormosil sol–gel prevented the immobilized antibody from leaking out of the film. On the other hand, the ormosil sol–gel could retain its porous structure upon the storage, which made the antigen able to bind with the immobilized HRP-labeled antibody.

3.7. Specifity, accuracy and clinical application

Using an incubation solution of 10 mIU/ml hCG containing 10 mIU/ml carbohydrate antigen 19-9 (CA19-9) or 30 ng/ml carcinoembryonic antigen (CEA), the specifity of the proposed hCG immunosensor was examined by detecting the amperometric responses. No difference of currents was observable in comparison with the result obtained in presence of only hCG. The increase of CA19-9 or CEA concentration to some extent did not lead to a significant current change. Thus, the immunosensor had a good specifity to hCG. The accuracy of hCG determination was examined by comparing the results obtained from three sera with this method and the commercial electrochemiluminescent immunoassay method. The hCG concentrations determined with this method were 0.4, 0.7 and 7.4 mIU/ml, while the values obtained from a recognized and commercial electrochemiluminescent analysis performed with Elecsys 2010 analyzer (Roche) for clinical diagnosis were 0.3, 0.5 and 6.8 mIU/ml, respectively. These results indicated the present method was in good agreement with the electrochemiluminescent method. Thus, the present method could satisfy the need of immunoassays of hCG in clinical diagnosis.

4. Conclusions

A uniform three-dimentsional macroporous ormosil sol–gel film can be synthesized via traditional sol–gel processing. This film provides high mechanical stability, large area and biocompatible microenvironment for immobilization of proteins and retaining their biological activity, particularly necessary conduction pathway to assist the direct electron transfer of the immobilized enzyme. These advantages make the construction of biosensors and/or immunosensors simpler and the cost lower, and lead to fast direct electron transfer behavior of the immobilized proteins and excellent performance of immunoassays. Using hCG, usually detected in urine or serum samples for pregnancy tests or monitoring trophoblastic diseases, as model this work provides a convenient and low-cost reagentless and mediatorless immunoassay method for the determination of hCG. Thus, this strategy could be readily extended toward the preparation of other amperometric immunosensors and the detection of other clinically important antigens.

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