Journal of Molecular Catalysis B: Enzymatic 58 (2009) 194–198

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Direct electrochemistry and electrocatalysis of glucose oxidase immobilized on glassy carbon electrode modified by Nafion and ordered mesoporous silica-SBA-15

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ARTICLE INFO

Article history:
Received 27 March 2008
Received in revised form
27 November 2008
Accepted 26 December 2008
Available online 8 January 2009

Keywords:
Glucose oxidase
SBA-15
Direct electrochemistry
Electrocatalysis
Glucose

ABSTRACT

In this paper, it was found that glucose oxidase (GOD) has been stably immobilized on glassy carbon electrode modified by ordered mesoporous silica-SBA-15 and Nafion. The sorption behavior of GOD immobilized on SBA-15 matrix was characterized by transmission electron microscopy (TEM), ultraviolet–visible (UV–vis), FTIR, respectively, which demonstrated that SBA-15 can facilitate the electron exchange between the electroactive center of GOD and electrode. The direct electrochemistry and electrocatalysis behavior of GOD on modified electrode were characterized by cyclic voltammogram (CV) which indicated that GOD immobilized on Nafion and SBA-15 matrices displays direct, nearly reversible and surface-controlled redox reaction with an enhanced electron transfer rate constant of 3.89 s^{-1} in 0.1 M phosphate buffer solution (PBS) (pH 7.12). Furthermore, it was also discovered that, in the absence of O_2, GOD immobilized on Nafion and SBA-15 matrices can produce a wide linear response to glucose in the positive potential range. Thus, Nafion/GOD-SBA-15/GC electrode is hopeful to be used in the third non-mediator's glucose biosensor. In addition, GOD immobilized on SBA-15 and Nafion matrices possesses an excellent bioelectrocatalytic activity for the reduction of O_2. The Nafion/GOD-SBA-15/GC electrode can be utilized as the cathode in biofuel cell.

1. Introduction

Glucose oxidase (GOD) is an ideal model enzyme for bioelectrochemistry because of its high bioelectrocatalytic activity and biochemical stability together with inexpensive price [1]. It is a structurally rigid glycoprotein with a molecular weight of 52,000–186,000 Da, which including two identical polypeptide chains and a flavin adenine dinucleotide (FAD) redox center embedded deeply in the enzyme. Thus, realizing direct electron transfer (DET) for GOD is extremely difficult. In recent years, lots of matrices have been utilized to immobilize enzyme in order to improve electron transfer between active site and electrode. For instance, GOD has been immobilized on carbonnanotubes [2], carbonnanotubes/chisol [3] and CdS nanoparticles [4] and so on.

Now, ordered mesoporous silica materials have gathered more and more researchers' attention in various branches of materials science and bioscience [5–8]. The reason is that ordered mesoporous silica can provide a rigid, open, and uniform pore structure as well as high pore volume with large surface area [9]. Up to now, several ordered mesoporous silicas, such as MCM-41 [10], SBA-15 [11] and mesocellular foam (MCF) [12] have been synthesized and utilized for bioimmobilization [13–17].

SBA-15 is a mesoporous silica material with uniform tubular channels varying from 5 to 30 nm [18]. Its outstanding properties, such as highly ordered pore structures, large surface areas and huge pore volume, render it an ideal candidate as host for biomolecules. There have been a number of reports describing the use of SBA-15 to immobilize proteins or enzymes, such as catalase [19], lysozyme [20,21], HRP, ubtilisin [7,22], myoglobin, trypsin and bovine serum albumin [23]. However, there are few literatures reported the direct electrochemistry and electrocatalysis behavior of GOD on the electrode modified with SBA-15. In this article, direct electrochemistry and electrocatalysis of GOD immobilized on the glass carbon electrode modified with SBA-15 are realized. The Nafion/GOD-SBA-15/GC

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doi:10.1016/j.molcatb.2008.12.022

electrode with fast electron transfer rate and high bioelectrochemical activity may be used in biofuel cell and biosensors in the future.

2. Experimental

2.1. Reagent

Glucose oxidase (EC 1.1.3.4, Type X-S from *Aspergillus niger*) was purchased from Sigma Co. Glucose was obtained from Beijing Chemical Company (Beijing, China) and the stock solution of glucose was allowed to mutorotate at room temperature overnight before use. Pure SBA-15 was obtained from Jilin University and was prepared using a reported procedure [24]. Nafion (5 wt%) was from Du Pont Co. 0.1 M phosphate buffer solutions (PBSs) were employed as a supporting electrolyte. All other chemicals used were of analytical grade and were used without further purification. Water used was doubly distilled and deionized.

2.2. Apparatus and measurements

Transmission electron microscopy (TEM) analysis was carried out with a JEOL2010 microscope operated at 200 kV with nominal resolution. The IR spectra (range: 4000–400 cm^{-1}, resolution: 2 cm^{-1}) were recorded with a Shimadzu 4200 FTIR spectrometer at 22 ± 2 °C. Ultraviolet–visible (UV–vis) absorption spectra were recorded with a Cary 50 Scan UV–vis spectrophotometer (Varian). The electrochemical experiments were carried out with a CHI 660A electrochemical workstation with a conventional three-electrode cell. The Pt wire electrode was used as the counter electrode. The Ag/AgCl electrode was used as the reference electrode and all the potentials were quoted with respect to the Ag/AgCl electrode. All solutions were purged with high purity nitrogen for at least 20 min prior to experiments and a nitrogen environment was then kept over the solution in the cell. All experiments were performed at a temperature of 25 ± 1 °C.

2.3. Preparation of Nafion/GOD-SBA-15/GC electrode

The GC electrode (3 mm in diameter) was successively polished with the slurry of 1.0, 0.5 and 0.03 μm alumina. Then, it was sonicated and washed with water. GOD was dissolved in 1 mL of 0.1 M PBS (pH 7.12). 10 mg of SBA-15 was dispersed into 5 mL of an aqueous solution. Then, the mixture solution of GOD and SBA-15 was stirred in the ice bath for 3 h to obtain a suspension. 100 μL of the GOD-SAB-15 suspension was mixed with 10 μL of 5 wt% Nafion solution to produce Nafion/GOD-SBA-15 suspension. 6 μL of Nafion/GOD-SBA-15 suspension was dropped on the surface of the GC electrode. After the electrode was dried at the room temperature and rinsed with water, the Nafion/GOD-SBA-15/GC electrode was obtained. The electrode was stored in refrigerator at 4 °C before use. The surface coverage of GOD on Nafion/GOD-SBA-15/GC electrode was calculated by equation $\Gamma = Q/nFA$ [25] in which the surface coverage of GOD is Γ, Q is the charge obtained from integrals of the anodic peak, n is the number of electrons per GOD molecule, F is Faraday constant and A is the electrode surface area. The surface coverage of GOD immobilized on Nafion/GOD-SBA-15/GC electrode is 5.23×10^{-12} mol/cm^2. If we suppose that the surface coverage of a saturated monolayer of GOD is 1.7×10^{-12} mol/cm^2 [26], the surface coverage of GOD on the Nafion/GOD-SBA-15/GC electrode is about three times of a monolayer because of the larger specific surface area of the SBA-15 which can absorb more GOD.

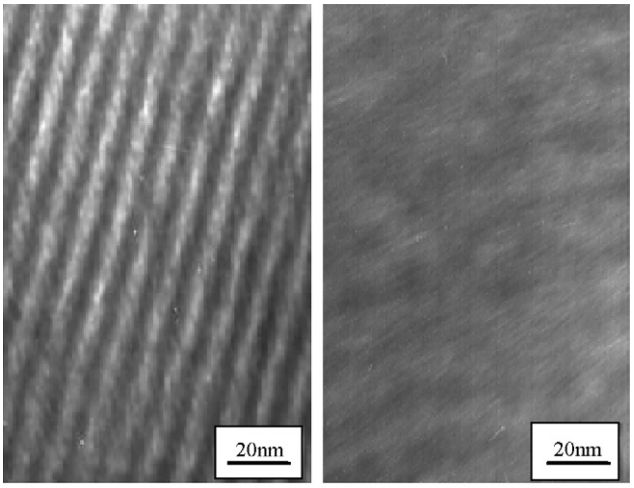

Fig. 1. TEM images of (A) SBA-15 and (B) GOD/SBA-15 films.

3. Results and discussion

3.1. Morphologies of SBA-15 and GOD/SBA-15 films

The morphologies of SBA-15 and GOD/SBA-15 films were characterized by transmission electron microscopy. Fig. 1A shows the TEM image of the pure SBA-15. The TEM image shows that the cylindrical pores are arranged in an ordered hexagonal array. The characteristics of cylindrical channels are in accordance with the reported mesostructure of SBA-15 [11]. Fig. 1B shows that the cylindrical channels of SBA-15 seen in Fig. 1A cannot be seen clearly now because of the complete covering of GOD on them.

3.2. FTIR spectra

Fig. 2a shows a typical FTIR spectra of the SBA-15 in which the Si–OH stretching vibration peak is presented in 3446 cm^{-1}, the Si–O–Si asymmetrical stretching vibration peak is appeared in 1087 cm^{-1} and the Si–O–Si symmetrical stretching vibration peak is presented in 464 cm^{-1} [27]. Fig. 2c is a FTIR spectra of pure GOD in which characteristic absorption peak of amide I (1700–1600 cm^{-1}) and amide II (1600–1500 cm^{-1}) is located at 1655 and 1545 cm^{-1} separately [28]. The FTIR spectra of the GOD/SBA-15 shown in Fig. 2b not only exhibit all the characteristic absorption peak of SBA-15,

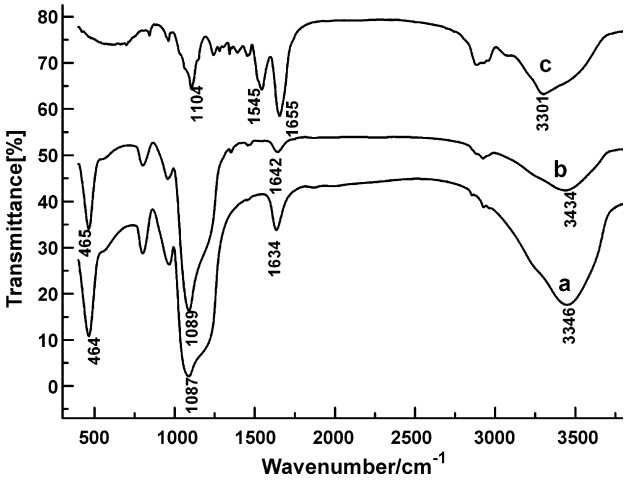

Fig. 2. FTIR spectra of (a) SBA-15, (b) GOD absorption onto SBA-15, and (c) pure GOD.

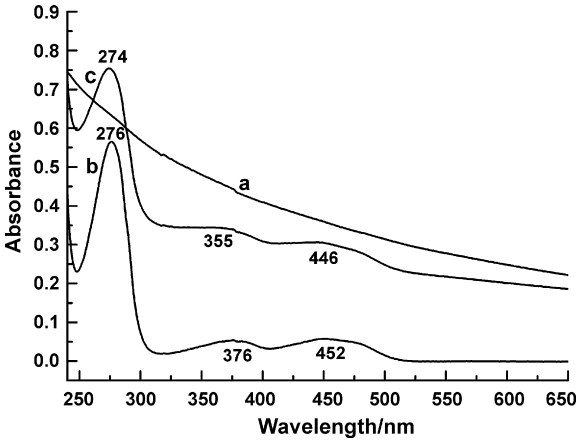

Fig. 3. UV–vis absorbent spectra of (a) SBA-15, (b) pure GOD and (c) GOD + SBA-15.

but also show that the characteristic absorption peak of amide I (1655 cm^{-1}) is shifted to 1642 cm^{-1} and the characteristic absorption peak of amide II (1545 cm^{-1}) disappeared. It is indicated that spatial structure of the GOD on the SBA-15 surface has been changed and mesoporous structure of the SBA-15 has been completely maintained.

3.3. UV–vis absorbent spectra

UV–vis spectrum is a useful conformational probe for monitoring the possible conformational changes in the protein secondary structure [29], thus we measured the UV–vis absorbent spectra of pure solution of GOD, the solution of SBA-15 and the mixture solution of GOD and SBA-15. The UV–vis spectra of the SBA-15 shown in Fig. 3a no any absorption peak is observed in the range of 250–650 nm. Fig. 3b shows a UV–vis absorbent spectra of the pure GOD solution in which characteristic absorption peak of amino acid residues in the native GOD is located at 276 nm and characteristic absorption peak of the oxidized (FAD) form of native GOD is located at 376 and 452 nm separately [30]. Fig. 3c is the UV–vis spectra of the mixture solution of GOD and SBA-15 in which characteristic absorption peak of amino acid residues in the immobilized GOD is blue shifted to 274 nm and characteristic absorption peak of the oxidized (FAD) form of the immobilized GOD is also blue shifted to 355 and 446 nm separately. All these also shown that spatial structure of the GOD immobilized on the SBA-15 surface has been changed.

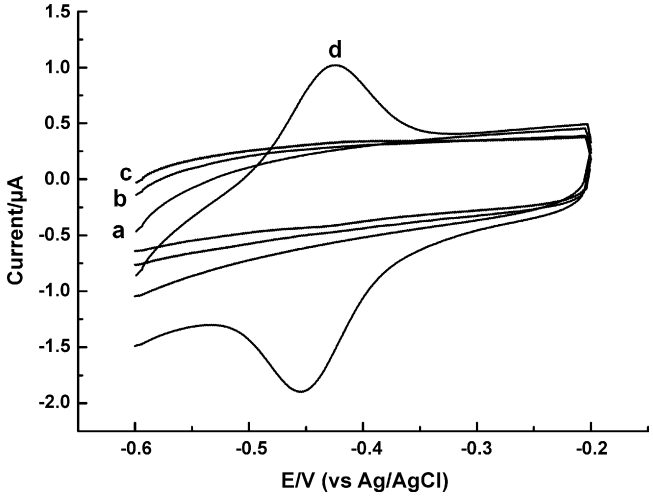

Fig. 4. Cyclic voltammograms of the bare GC (a), Nafion/SBA-15/GC (b), Nafion/GOD/GC (c) and Nafion/GOD-SBA-15/GC (d) electrodes in 0.1 M PBS (pH 7.12), at scan rate of 100 mV/s.

3.4. Direct electron transfer of Nafion/GOD-SBA-15/GC electrode

Fig. 4 shows the cyclic voltammograms of bare GC electrode, Nafion/SBA-15/GC electrode, Nafion/GOD/GC electrode and Nafion/GOD-SBA-15/GC electrode at scan rate of 100 mV/s in 0.1 M PBS (pH 7.12). In Fig. 4, curve a, the bare GC electrode has not response in 0.1 M PBS (pH 7.12) solution. At the same time, also no peak was observable at Nafion/SBA-15/GC electrode (Fig. 4, curve b), which indicates SBA-15 and Nafion film is electroinactive in the potential window. While there is only very small response on the GC electrode modified by GOD/Nafion film (Fig. 4, curve c), this response is very weak. However, a pair of well-defined redox peaks can be observed on Nafion/GOD-SBA-15/GC electrode in 0.1 M PBS (pH 7.12) (Fig. 4, curve d). The anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}) are located at −0.420 and −0.453 V, at scan rate of 100 mV/s, respectively. The separation of peak potentials (ΔE_p) is 0.033 V. The anodic peak current is almost equal to the cathodic peak current. The formal potential ($E^{\circ\prime}$) of the modified electrode, calculated from the average value of the anodic and cathodic peak potentials, is −0.436 V, which is close to the formal potential of −0.430 V (vs. Ag/AgCl) for FAD/$FADH_2$ at pH 7.0 [31]. So, a direct electrochemical reaction of nearly reversible and two electrons is attributed to GOD immobilized on Nafion/GOD-SBA-15/GC electrode which indicates SBA-15 plays an important role in facilitating the electron exchange between the electroactive center of GOD and GC electrode. It is so because of the following factors: the first is that the SBA-15 provides a large surface area which enables GOD to obtain powerful immobility so that it is difficult for GOD to run into the test solution. The high stability of GOD can obviously enhance current response [32]. The second is the orientation effect. When GOD molecule is adsorbed on the surface of SBA-15, its structure is very easy to be changed. Hydrophobic SBA-15 and hydrophobic part of GOD absorb mutually which causes hydrophilic active center FAD in GOD to face the environment to enhance its activity [33]. The configuration of the GOD has been changed a lot after GOD molecule orientates which accords with the FTIR spectra and the UV–vis absorbent spectra. Hoshi et al. [34] and Cohen [35] have also proved that the orientated GOD can produce more current response.

Fig. 5A shows the cyclic voltammograms of Nafion/GOD-SBA-15/GC electrode in 0.1 M PBS (pH 7.12) at different scan rates. With increasing scan rate, the anodic peak potential and the cathodic peak potential of GOD was nearly not shifted. Both the cathodic and anodic peaks currents was linearly proportional to the scan rate (υ) in the scan range from 20 to 400 mV/s (linear regression equations: pa—$y = -0.01024 + 0.0049x$, $r = 0.99955$; pc—$y = -0.19697 - 0.00624x$, $r = -0.99942$), as shown in Fig. 5B, which indicated a surface-controlled electrode process of GOD redox [24,36]. The rate constant (k_s) can be calculated according to the model of Laviron [36] for a surface-controlled electrode process. When the charge transfer coefficient α is 0.5, k_s calculated is 3.89 s^{-1} which is much higher than the rate of GOD adsorbed on the annealed SWNTP (1.7 s^{-1}) [37], anodized electrode (1.6 s^{-1}) [38] and Nafion-CNT/GC electrode (1.53 ± 0.45 s^{-1}) [2]. It indicated that the electron transfer rate of Nafion/GOD-SBA-15/GC electrode was very fast.

3.5. Influence of solution pH

Fig. 6A shows the cyclic voltammograms of the Nafion/GOD-SBA-15/GC electrode in 0.1 M PBS at pH 5.18 (a), 6.44 (b), 7.12 (c), and 8.00 (d) at scan rate of 100 mV/s, respectively. Obviously, a pair of stable and well-defined nearly reversible redox peaks is obtained

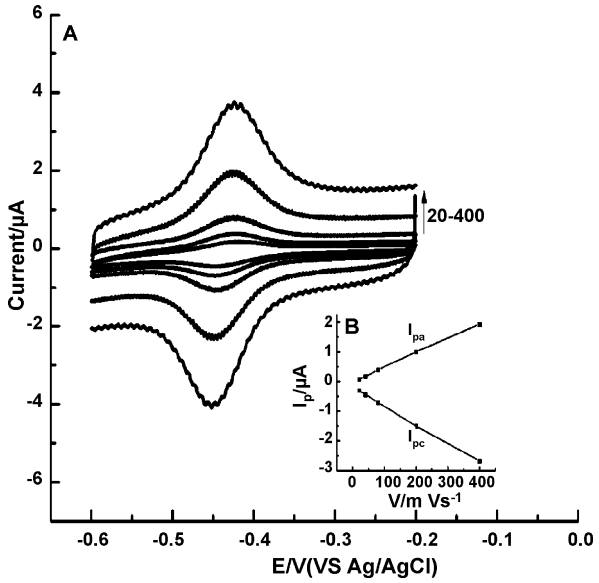

Fig. 5. (A) CVs of Nafion/GOD-SBA-15/GC electrode at different scan rate in 0.1 M PBS (pH 7.12). The scan rate is 20, 40, 80, 200 and 400 mV/s (from inner to outer). Inset (B): plots of peak currents vs. v.

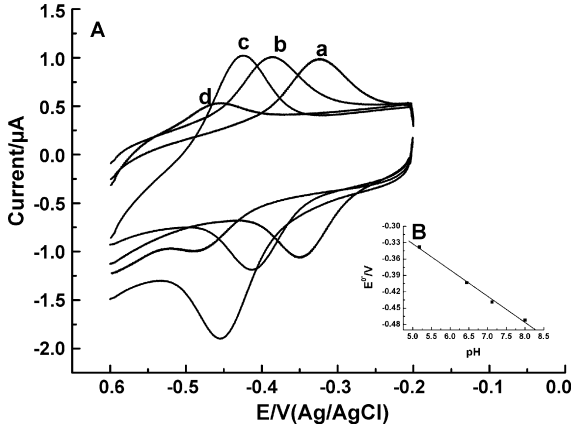

Fig. 6. (A) CVs of Nafion/GOD-SBA-15/GC electrode in 0.1 M PBS at pH 5.18 (a), 6.44 (b), 7.12 (c) and 8.00 (d), scan rate is 100 mV/s. Inset (B): plot of peak potential vs. pH.

on Nafion/GOD-SBA-15/GC electrode in different pH solution. With increasing pH from 5.18 to 8.00, the peak potentials shifted towards negative direction, which is in agreement with that obtained by Ju and Liu [39]. Furthermore, the maximum current response is occurred at pH 7.12. In addition, in Fig. 6B $E^{\circ\prime}$ has a linear relationship with pH from 5.18 to 8.00 with a slope of −48.00 mV/pH, which is close to the theoretical value of −58.50 mV/pH [39] for a reversible. It proves that two protons ($2H^+$) and two electrons ($2e^-$) maybe participate in the direct electrochemical reaction of GOD immobilized on Nafion/GOD-SBA-15/GC electrode.

3.6. Enzymatic activity of immobilized GOD

The results of above experiments indicate the GOD immobilized on the Nafion/GOD-SBA-15/GC electrode can retain its functional activity. However, the ultimate test that the immobilized enzyme retains its enzymatic activity is to carry out the actual catalysis of its substrate. The GOD induces electrocatalysis of glucose can be carried out by increasing the potential in the positive polarity to about 0.6 V in the absence of oxygen [40]. Thus, in absence of oxygen, Fig. 7A shows the response of the Nafion/GOD-SBA-15/GC electrode

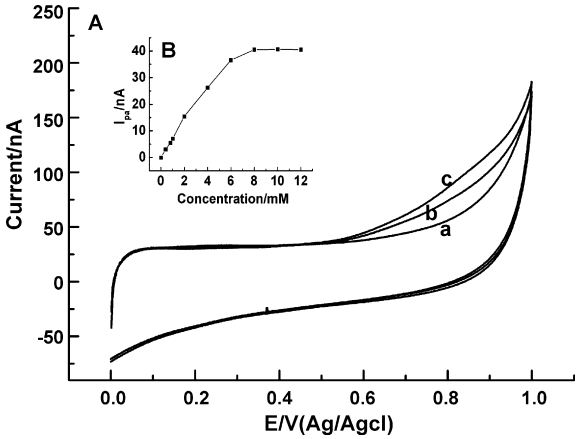

Fig. 7. (A) CVs of the Nafion/GOD-SBA-15/GC electrode in nitrogen-saturated 0.1 M PBS (pH 7.12) without glucose (a), with 4.0 mM glucose (b) and 6.0 mM glucose (c). Inset (B): plot of electrocatalytic oxidation current (I_{pa}) vs. glucose concentration at 0.8 V.

to glucose in the positive potential range (0.0–1.0 V). When glucose was added to pH 7.12 PBS, the catalytic oxidation current vs. glucose began to appear at about 0.6 V and increased with glucose concentration (Fig. 7A, curves b and c). Inset of Fig. 7B shows the catalytic oxidation current vs. glucose concentration at 0.8 V (vs. Ag/AgCl). It showed that the catalytic oxidation current had a linear response with glucose concentration from 0 to 6.0 mM with a detection limit of 0.6 mM ($N = 7$; $R = 0.996$) at a signal-to-noise ratio of 3, thus, the Nafion/GOD-SBA-15/GC electrode not only may be fabricated non-mediator's glucose biosensor in the positive potential range but also may be used to detective blood sugar of human in daily life, the physiological level glucose concentration range of human blood is 3–6 mM [41]. The non-mediator's glucose biosensor has successfully been fabricated by the Nafion/GOD-SBA-15/GC electrode, which completely should be attributed to the unique property of the SBA-15 adsorbed on GC electrode surface. In addition, the material disturbance experiments also have been done in the positive potential range. While 0.06 mM ascorbic acid and 0.3 mM p-acetaminophenol were joined to 4 mM glucose solution, the disturbance of 0.06 mM ascorbic acid could neglect nearly, but 0.3 mM p-acetaminophenol caused the response current to increase 15.7%, it indicated that the non-mediator's glucose biosensor fabricated by the Nafion/GOD-SBA-15/GC electrode has been able to

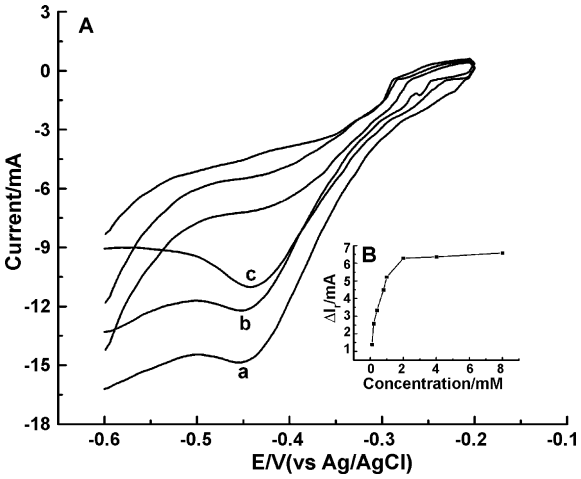

Fig. 8. (A) CVs of the Nafion/GOD-SBA-15/GC electrode in oxygen-saturated 0.1 M PBS (pH 7.12) without glucose (a), with 1.0 mM glucose (b) and 2.0 mM glucose (c). Inset B: plot of electrocatalytic current ΔI_r vs. glucose concentration.

resist completely the disturbance of the ascorbic acid, but the influence of the p-acetaminophenol would still maintain.

On the other hand, the electrocatalysis of the Nafion/GOD-SBA-15/GC electrode to glucose was observed by monitoring the changes in the redox peak currents in the negative potential range (−0.6 to −0.2 V) [42]. Fig. 8 shows the cyclic voltammograms in 0.1 M PBS (pH 7.12) saturated with O_2 without and with 1.0 mM and 2.0 mM of glucose. When PBS was saturated with O_2, a large reduction peak of O_2 was observed, indicating that GOD immobilized on Nafion and SBA-15 matrices can catalyze the reduction of O_2 (Fig. 8, curve a). When glucose is added into the oxygen-saturated PBS, the peak current of the reduction of O_2 decreased with increasing the glucose concentration (Fig. 8, curves b and c). The catalytic reduction peak potential of O_2 was relatively low (−0.454 V), which can completely prevent from interference of uric acid and ascorbic acid [43]. Moreover, the Nafion film existing on the modified electrode surface can also prevent from penetration of uric acid and ascorbic acid [44]. ΔI_r was defined as the difference of the peak currents of the reduction of O_2 in absence and presence of glucose, increasing linearly with increasing the glucose concentration in the range from 0.20 to 1.0 mM of glucose with a correlation coefficient of 0.9987 and a detection limit of 0.07 mM at a signal-to-noise ratio of 3 (Fig. 8, inset B). The narrow linear range is due to suppressing reaction of glucose oxidation resulted from decrease oxygen partial pressure [45]. There is a sensitivity of 43.31 $\mu A\,mM^{-1}\,cm^{-2}$ in the linear range for the Nafion/GOD-SBA-15/GC electrode, which is higher than 7.46 $\mu A\,mM^{-1}\,cm^{-2}$ produced by AMWNTs and IO_4^--oxidized GOD biosensor [46]. All these are proved in our experiments that the immobilized GOD by Nafion and SBA-15 can keep its own biocatalytic activity both in positive potential range and in negative potential range.

3.7. Stability and reproducibility of Nafion/GOD-SBA-15/GC electrode

The further measurement indicated that after 500 cycles at scan rate of 100 mV/s the anodic peak current of GOD is only decreased by 7.6%, illustrating that GOD immobilized on SBA-15 possesses the excellent stability. In negative potential range, when the glucose concentration is 0.8 mM, the relative standard deviation is only 5.4% for six successive measurements. The relative standard deviation of the peak currents of the reduction of O_2 for four different electrodes is only 5.2%, indicating the good fabrication reproducibility of the Nafion/GOD-SBA-15/GC electrode.

4. Conclusion

GOD can be immobilized on SBA-15 and Nafion matrices, and the immobilization of GOD is of the excellent stability. GOD immobilized on SBA-15 and Nafion matrices can undergo a direct and nearly reversible electrochemical reaction, containing two electrons and two protons exchange. Moreover, the formal potential of electrochemical reaction is close to that of the native GOD. GOD immobilized on SBA-15 and Nafion matrices has wide linear response to glucose in the positive potential range, so the third generation non-mediator's glucose biosensor may be successfully fabricated by ordered mesoporous silica-SBA-15 as matrix in next years. GOD immobilized on SBA-15 and Nafion matrices possesses an excellent bioelectrocatalytic activity for the reduction of O_2. Thus, in the future, ordered mesoporous silica-SBA-15 may be used as matrix of the cathodic biocatalyst in biofuel cell. In short, ordered mesoporous silica-SBA-15 provides an efficient matrix for immobilization of GOD and facilitates the electron exchange between GOD and the electrode. Therefore, as a matrix material of modified electrode, SBA-15 is hopeful to be applied in biosensor and biofuel cell in the future.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (20373068, 20703043 and key Project 20433060), and State Key Laboratory of Inorganic Synthesis and Preparative Chemistry, Jilin University.

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