analytical chemistry

Article

pubs.acs.org/ac

Yeast Surface Displaying Glucose Oxidase as Whole-Cell Biocatalyst: Construction, Characterization, and Its Electrochemical Glucose Sensing Application

Hongwei Wang,[†,‡,∇] Qiaolin Lang,[†,∇] Liang Li,[†] Bo Liang,[†] Xiangjiang Tang,[†] Lingrang Kong,[‡] Marco Mascini,[§] and Aihua Liu*,[†]

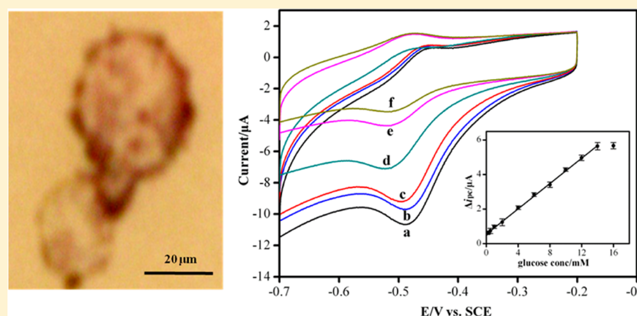
[†]Laboratory for Biosensing, Qingdao Institute of Bioenergy & Bioprocess Technology and Key Laboratory of Bioenergy, Chinese Academy of Sciences, 189 Songling Road, Qingdao 266101, People's Republic of China

[‡]State Key Laboratory of Crop Biology, College of Agronomy, Shandong Agricultural University, 61 Daizong Street, Tai'an, Shandong 271018, People's Republic of China

[§]Dipartimento di Chimica, Universita degli Studi di Firenze, Via della Lastruccia, 3 50019 Sesto Fiorentino, Italy

Supporting Information

ABSTRACT: The display of glucose oxidase (GOx) on yeast cell surface using a-agglutinin as an anchor motif was successfully developed. Both the immunochemical analysis and enzymatic assay showed that active GOx was efficiently expressed and translocated on the cell surface. Compared with conventional GOx, the yeast cell surface that displayed GOx (GOx-yeast) demonstrated excellent enzyme properties, such as good stability within a wide pH range (pH 3.5−11.5), good thermostability (retaining over 94.8% enzyme activity at 52 °C and 84.2% enzyme activity at 56 °C), and high D-glucose specificity. In addition, direct electrochemistry was achieved at a GOx-yeast/multiwalled-carbon-nanotube modified electrode, suggesting that the host cell of yeast did not have any adverse effect on the electrocatalytic property of the recombinant GOx. Thus, a novel electrochemical glucose biosensor based on this GOx-yeast was developed. The as-prepared biosensor was linear with the concentration of D-glucose within the range of 0.1−14 mM and a low detection limit of 0.05 mM (signal-to-noise ratio of S/N = 3). Moreover, the as-prepared biosensor is stable, specific, reproducible, simple, and cost-effective, which can be applicable for real sample detection. The proposed strategy to construct robust GOx-yeast may be applied to explore other oxidase-displaying-system-based whole-cell biocatalysts, which can find broad potential application in biosensors, bioenergy, and industrial catalysis.



Glucose-1-oxidase (GOx) (beta-D-glucose: oxygen-1-oxidoreductase, EC 1.1.3.4) is an important glycoprotein, consisting of two identical 80-kDa subunits with two flavin adenine dinucleotides (FAD) coenzymes bound. GOx is capable of oxidizing beta-D-glucose to D-gluconolactone and releasing hydrogen peroxide using glucose as an electron donor, while the capacity of catalyzing other substrates, such as 2-deoxy-D-glucose, 4-*O*-methyl-D-glucose, and 6-deoxy-D-glucose, were relatively poor.[1] Most enzymatic properties of GOx were from *Aspergillus niger* (*A. niger*), which showed high enzyme stability, edible safety, substrate specificity, and antimicrobe properties that were due to oxidative capacity.[2] The virtue of selective oxidation of glucose and its extreme stability, compared with other enzymes, has permitted GOx to play a leading role in enzyme electrodes.[3] However, the purified GOx is required for immobilization process, which increased the cost and has become the bottleneck in the area of immobilized enzyme-based electrode development.[4] In addition, the conventional physical or chemical immobilization approaches often result in enzyme activity loss, enzyme leakage, and mass-transfer impedance.[5]

It is noteworthy that a major advantage in the development of microbial surface display enables the direct utilization of whole cell biocatalysts with recombinant protein displayed on the cell surface, thus providing an economical alternative to traditional production of purified enzymes. Recently, robust surface display systems on bacteria such as *Escherichia coli* (*E. coli*) and *Bacillus anthracis*, as well as yeast such as *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Yarrowia lipolytica* have been developed and widely applied in live vaccine development, screening-displayed peptide libraries, antibody production, and whole-cell biocatalysts construction for biofuel cells,[6,7] while the application in biosensor design is rare.[8] We have previously constructed sensitive D-xylose or glucose

Received: April 3, 2013
Accepted: May 23, 2013
Published: May 23, 2013

 © 2013 American Chemical Society

biosensors based on *E. coli* surface-displayed xylose dehydrogenase and glucose dehydrogenase, respectively, using ice nucleation protein as an anchor motif.[4,9,10] However, an *E. coli* host is generally not thought to be suitable to express proteins derived from eukaryotes, because *E. coli* lack the required chaperones for correct protein folding. Therefore, it is necessary to investigate the eukaryotic cell surface display for biosensor development. As a eukaryotic system, a yeast surface display was initially constructed for antifluorescein library screening using a-agglutinin as a cell wall anchor motif.[11] This display system was expanded to be employed for cDNA library screening, vaccines, and bioenergy.[12] Although GOx was generally purified from *A. niger*, recombinant GOx was developed by yeast. Actually, active GOx could be produced by both *S. cerevisiae* and *Pichia pastoris* (*P. pastoris*) without much change in enzyme features; nevertheless, the GOx expressed by *S. cerevisiae* exhibited better pH and thermal stability, because of the high glycosylation effect.[13,14] In the present study, we systematically studied the display of GOx on the surface of *S. cerevisiae*. The developed GOx-displayed yeast (GOx-yeast) is highly specific to glucose, and stable within a wide range of pH (3.5−11.5), as well as being stable at higher temperatures (up to 56 °C). In addition, the direct electrochemistry at this newly constructed recombinant GOx/carbon-nanotube modified electrode was realized. Finally, the exploration of this interesting whole-cell biocatalyst in the electrochemical glucose biosensing application is systematically presented. To the best knowledge of the authors, this is the first report on the functional surface displaying of GOx for glucose sensing.

■ EXPERIMENTAL SECTION

Materials and Methods. *Strains, Media, and Reagents.* The *A. niger* strain for genomic DNA extraction was kindly provided by Prof. Fuli Li (Qingdao Institute of Bioenergy & Bioprocess Technology, Chinese Academy of Sciences). *E. coli* DH5α ($F^-\varphi80$ *lac*ZΔM15Δ (*lac*ZYA-*argF*) U169 *end*A1 *rec*A1 *hsd*R17 (r_k^-,m_k^+) *sup*E44λ-*thi*-1 *gyr*A96 *rel*A1*pho*A) was used for recombinant DNA manipulation and was cultured in a LB medium at 37 °C (Fisher, Pittsburgh, PA). *S. cerevisiae* EBY100 (Invitrogen, Carlsbad, CA) was used for yeast cell surface display. The wild type EBY100 was grown at 30 °C in YPD media (20 g/L glucose, 20 g/L peptone, 20 g/L yeast extract). The transformants were selected and grown at 30 °C on SD-trp plates. Galactose was used to replace glucose in SD-trp media for protein expression induction, incubated for at least 18 h at 20 °C with continuous shaking at 200 rpm. All restriction enzymes were obtained from Fermentas (MBI Fermentas, Canada). Nafion (perfluorinated ion-exchange resin, 5 wt % solution in a mixture of lower aliphatic alcohols and water) was purchased from Aldrich and used as-received. Multiwalled carbon nanotubes (MWNTs) were kindly provided by Prof. Gebo Pan (Suzhou Institute of Nan-Tech and Nano-Biomics, Chinese Academy of Sciences).

Plasmids Construction. The GOx gene was amplified from genomic DNA of *A. niger* using specific primers (GOx-F-*Bam*HI: 5′ TGGATCCATGCAGACTCTCCTTGTGAGC-TCGC 3′; GOx-R-XhoI: 5′ TCTCGAGTCACTGCATGGA-AGCATAATCTTCC 3′). PCR products were cloned in pMD18-T vector for sequence confirmation and subcloned into *BamH*I and *Xho*I sites of pYD1 vector. The GOx-pYD1 plasmid was transformed in *S. cerevisiae* EBY100, using the lithium acetate method.[15]

GOx Activity Assay. GOx activity of whole cells was determined by the coupled *o*-dianisidine peroxidase reaction as described previously.[16] One hundred microliter (100 μL) yeast cells were washed twice with 100 mM potassium phosphate buffer (pH 7.4), suspended in the same buffer, and added to 3 mL of reaction cocktail (1.72% glucose in w/v, 0.17 mM *o*-dianisidine dihydrochloride, 2 U/mL horseradish peroxidase). For the substrate specificity test, the D-glucose was replaced by a series of other substrates using yeast cells (OD_{600} = 1). The mixture was incubated at room temperature in darkness for 5 min and the absorbance at a wavelength of 500 nm was recorded. One unit of GOx activity is defined as the amount of enzyme required to oxidize 1 μmol of glucose per minute under the above assay conditions. All measurements were conducted in triplicate, and experiments were carried out at least three times with similar results.

Effect of pH and Temperature on the Stability of Cell-Displayed GOx. The enzyme stability toward pH was investigated by incubating cells at room temperature in buffer (pH 3.0−11.5) for 1 h. To test the thermostability, the yeast cells were incubated in 0.1 M PBS buffer (pH 7.4) at different temperature for 1 h. The residual activity was then measured under the above assay condition.

Preparation of GOx-Yeast Modified Electrode. The bare glassy carbon electrode (GCE, 3 mm in diameter) was polished carefully with 1.0-, 0.3-, and 0.05-μm alumina slurries, and it then was sonicated in anhydrous ethanol and distilled deionized water, respectively. After successive sonication in anhydrous ethanol and ultrapure water, the electrode was rinsed with ultrapure water and allowed to dry at room temperature. Then, 10 μL of MWNTs-Nafion suspension (2 mg MWNTs powder was dispersed in 1 mL of 0.05 wt % Nafion with ultrasonication) was deposited dropwise on the surface of GCE and dried in air. Next, 10 μL of GOx-yeast aqueous dispersion was deposited dropwise on the inverted GCE and dried overnight at 4 °C in a refrigerator. Then, 10 μL of Nafion solution (0.05 wt %) was syringed to the electrode surface. Finally, the modified GCE was immersed in PBS to remove the loosely adsorbed GOx-yeast and was stored at 4 °C in a refrigerator under dry conditions when not in use.

Apparatus and Measurements. The absorbance at 500 nm was measured using a spectrophotometer (Model DU 800 UV/vis, Beckman Coulter, Inc., USA). The yeast cells treated with antihis and antimouse IgG, which was conjugated with alkaline phosphatase, were observed under a fluorescence inverted microscope system (Carl Zeiss, Germany) and photographed, respectively. Electrochemical measurements were performed using a potentiostat (Model CHI660D, CH Instruments, Chenhua, Shanghai, PRC). The electrochemical response was measured in a conventional three-electrode system using a chemically modified GCE as the working electrode, a platinum wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. All potentials were reported in this context, with respect to this reference. All measurements were performed at room temperature (∼23 °C).

■ RESULTS AND DISCUSSION

Displaying GOx on Surface of *S. cerevisiae*. In order to display GOx on the cell surface, the GOx gene (1815 bp) was amplified from *A. niger* genomic DNA, which is 99% identical to that from CBS 513.88. The GOx fragment was cloned into pYD1 vector and transformed in yeast strain EBY100, which had been developed for cell surface expression of protein

employing a-agglutinin receptor as an anchor motif.[11] The displayed enzyme was fused with polyhistidine tag on c-termini and thus detected using antimouse alkaline phosphatase under microscope. Compared with the wild type strain, apparent stained protein was detected on cell surface of recombinant strain (Figure 1A, image b), demonstrating the successful translocation of fusion protein on the cell wall. Similar results were also revealed in previous studies using an a-agglutinin system that Aga2-GOx fusion protein should be secreted out of the cell and bound to Aga1 partner through two disulfide bonds, which was covalently bound at beta-glucan on the cell wall.[11,12]

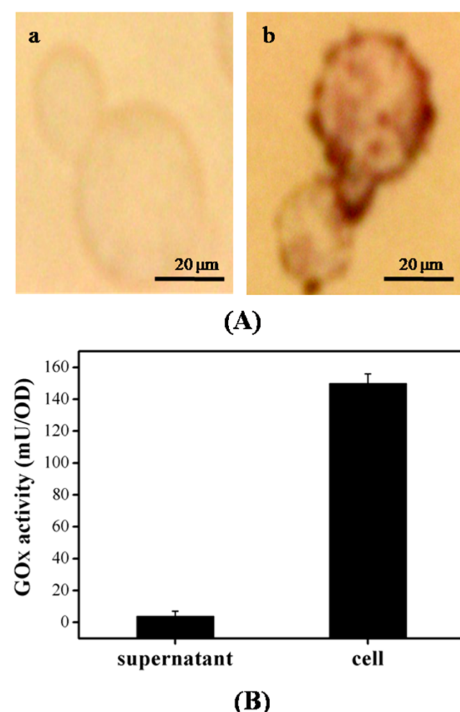


Figure 1. (A) Photograph of (a) yeast cell control and (b) GOx surface-displayed yeast. Cells was stained with antihis antibody, followed by alkaline phosphatase staining. (B) GOx activity of the surface-displayed yeast cells.

GOx Activity of the Recombinant Strain. The GOx activity was determined by a standard coupled *o*-dianisidine peroxidase reaction using intact cells. Most enzyme activity was detected on whole yeast cells while little existed in the supernatant of the cell culture (Figure 1B), further supporting that active recombinant GOx was expressed on the cell surface but not secreted in the culture.[17] When the protein expression was induced for ~30 h, using 20% galactose in an induction medium, the optimal protein display was achieved with enzyme activity as 0.15 U/OD (Figure 1B). Previously, Ko et al. obtained secretary GOx expression of 0.78 U/OD in *S. cerevisiae*, which was much less than that from *P. pastoris*,[14,18] indicating low efficient secretion capability of *S. cerevisiae*. It is supposed that the amount of the displayed GOx is attributed to both the anchor points of beta-glucan on cell wall and secretion capability of *S. cerevisiae* cell itself. An improved enzyme display system with high protein amount might be developed from the host cell of *P. pastoris*.

The Specificity of Recombinant GOx. The specificity of the displayed GOx was tested against different substrates (saccharides) (see Table 1). The constructed whole cell catalyst presented the highest substrate affinity toward D-glucose. A 100-fold excess of other saccharides such as D-xylose, D-fructose, D-ribose, D-galactose, D-mannose, L-arabinose, and cellobiose exhibited no absorbance at the same wavelength of 500 nm tested (see Table 1), suggesting their noninterferences to the detection of glucose. However, D-maltose and D-sucrose showed obvious interference at their high concentrations. For example, 5 mM (50-fold), 1 mM (10-fold), and 0.5 mM (5-fold) excess D-maltose had the signal accounted for 19.2%, 2.6%, 0.5% of those values for 0.1 mM glucose, separately. Ten millimolar (10 mM) (100-fold), 5 mM (50-fold), and 1 mM (10-fold) excess D-sucrose had the signal accounted for 15.3%, 6%, 0% of those values for 0.1 mM glucose, separately. Nevertheless, the absorbance for D-maltose and D-sucrose was almost nondetectable when their concentrations were <1 mM (Table 1). Thus, the recombinant GOx had good specificity to glucose. The good specificity of displayed GOx toward D-glucose suggests its potential application in developing highly selective glucose biosensors. The enzyme activity toward D-maltose and D-sucrose should be attributed to the fact that both of these sugars are composed of glucose chains.

Table 1. Substrate Specificity of Yeast Cell Surface Displayed GOx

substrate	concentration (mM)	absorbance at 500 nm
D-glucose	0.1	0.390 ± 0.01[a]
D-maltose	5	0.075 ± 0.005[a]
D-maltose	1	0.010 ± 0.001[a]
D-maltose	0.5	0.002 ± 0.0005[a]
D-sucrose	10	0.060 ± 0.005[a]
D-sucrose	5	0.024 ± 0.002[a]
D-sucrose	1	0
D-xylose	10	0
D-fructose	10	0
D-ribose	10	0
D-galactose	10	0
D-mannose	10	0
L-arabinose	10	0
cellobiose	10	0

[a] All values were obtained as the average of three repetitive measurements, plus/minus the standard deviation.

Stability of Cell Displayed GOx. In order to investigate the stability, the displayed GOx was characterized against different pH and temperature. Approximately 53% of enzyme activity was lost when the pH was <3.0, whereas over 83% of enzyme activity was maintained within pH 3.5−11.5 (Figure 2A). Previously, researchers had reported the pH stability of GOx from *A. niger*, most of which was stable within pH 4−8.[14,19,20] To investigate the possible reasons for the better stability toward pH, the pYD1-GOx plasmid was transformed in the *S. cerevisiae* strain of INVSCI to result the secretion of

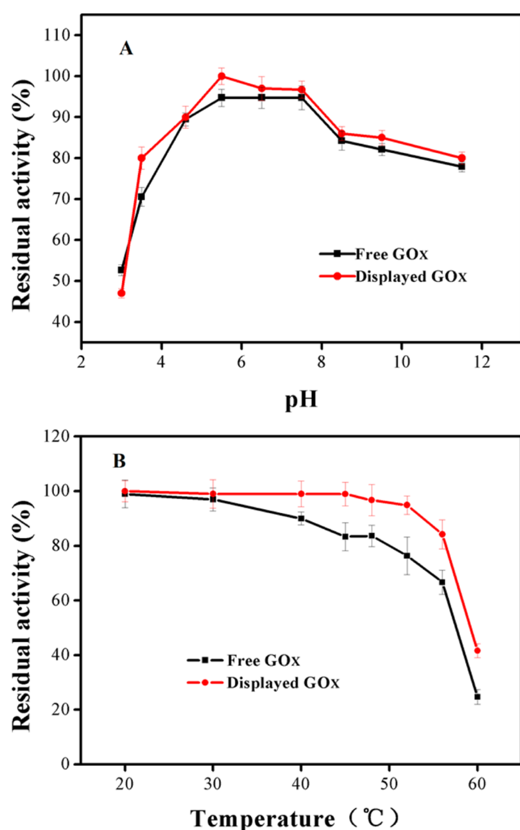


Figure 2. Effect of (A) pH and (B) temperature on GOx-displayed yeast.

recombinant GOx in culture. The crude free enzyme in supernatant showed similar pH stability with the displayed GOx (Figure 2A), indicating that the immobilization of GOx on yeast cell wall did not significantly alter the pH stability. The excellent performance toward different pH condition might be due to the heterologous expression in *S. cerevisiae*, as the expressed protein tended to be glycosylated.[21] To test the thermostability, the displayed GOx was treated at temperatures of 20−60 °C. As shown in Figure 2B, the displayed enzyme was very stable below 52 °C, and retained over 84.2% of its activity at 56 °C, while 41% enzyme activity maintained at 60 °C for 60 min (Figure 2B). Usually, the GOx from *A. niger* generally exhibited good activity below 50 °C; however, it showed poor thermostability over 50 °C.[2,14,16] In our study, the excellent thermostability should be attributed to the immobilization on cell wall, because the free enzyme showed relatively lower thermostability than the displayed GOx (Figure 2B). Similar to the effect on pH stability, the intensive glycosylation of expressed protein by *S. cerevisiae* might also be responsible for this, because the glycosylation often affects enzyme thermostability.[14,21,22]

In addition, the activity of the displayed GOx was measured everyday at 4 °C. No obvious enzyme activity loss was observed during 2 months of storage in both PBS buffer and dry condition, suggesting the good storage stability of the GOx-yeast (see Figure S1 in the Supporting Information).

Direct Electrochemistry of GOx-Yeast Immobilized on MWNT Modified Electrode. The cyclic voltammograms (CVs) of various modified electrodes were measured in N_2-saturated 0.1 M PBS (pH 7.4) (Figure 3). Nafion/GOx-yeast/GCE did not show any peaks (Figure 3, curve a). No redox

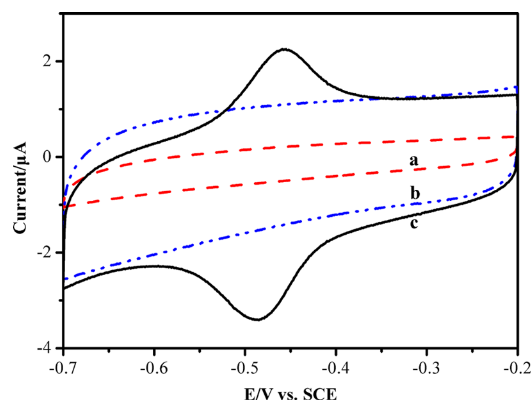
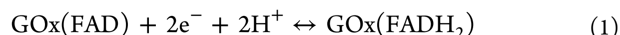


Figure 3. CVs of (a) Nafion/GOx-yeast/GCE, (b) Nafion/MWNTs/GCE, and (c) Nafion/GOx-yeast/MWNTs/GCE in N_2-saturated PBS solution (pH 7.4). Scan rate = 50 mV s^{-1}.

peaks were observed with Nafion/yeast/MWNTs/GCE either (Figure 3, curve b). However, a well-defined redox pair with a peak-to-peak separation (ΔE_p) of 29 mV was observed at the Nafion/GOx-yeast/MWNTs/GCE, where ΔE_p is defined as the difference between the anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}). The lower ΔE_p value indicates a fast electron transfer process. The formal potential ($E_p^{o\prime}$) was −0.489 V, which was close to the $E_p^{o\prime}$ values reported earlier for commercial GOx,[23,24] confirming the facile direct electron transfer from the redox site of the enzyme (FAD/$FADH_2$ redox center) to the electrode surface. Here, $E_p^{o\prime}$ is defined as the average of the E_{pa} and E_{pc} values. The influence of scan rates on the cathodic and anodic peaks of the Nafion/GOx-yeast/MWNTs/GCE is shown in Figure S2A in the Supporting Information. Both the anodic peak current (i_{pa}) and cathodic peak current (i_{pc}) increased linearly with the increase in scan rates ranging within 10−300 mV s^{-1} (see Figure S2B in the Supporting Information), which is characteristic of a surface-confined electrode process.[23,25,26] Taken together, the bioactivity of GOx anchored on the yeast cell wall was maintained and the direct electron transfer of recombinant GOx was achieved at carbon-nanotube-modified electrodes. In other words, the host cell of yeast did not have any adverse effect on the electrocatalytic property of the recombinant GOx.

On the other hand, it is well-known that the direct electron transfer of GOx is a two-electron, along with two-proton, reaction that undergoes a redox reaction,[27] as shown in reaction 1:



$$GOx(FAD) + 2e^- + 2H^+ \leftrightarrow GOx(FADH_2) \quad (1)$$

Therefore, the solution pH may have an effect on the electrochemical behavior of GOx-yeast on the Nafion/MWNTs film. As shown in Figure S3A in the Supporting Information, a negative shift of both the cathodic and anodic peak occurred when the buffer solution pH was increased. The redox potential $E_p^{o\prime}$ decreased linearly with the solution pH ranging from 5.4 to 9.4, with a slope of −53 mV/pH and a correlation coefficient of R = 0.999 (see Figure S3B in the Supporting Information). This slope was very close to the theoretical value of −58.6 mV/pH at 23 °C, according to reaction 1 for a reversible reaction,[28] indicating that two protons and two electrons are involved in the electron transfer process.

GOx-Yeast Based Glucose Biosensor. CVs of the Nafion/GOx-yeast/MWNTs/GCE in a PBS buffer solution

containing different concentrations of glucose under the ambient-air condition were measured to investigate the change of the i_{pc} values of approximately −0.5 V from the CVs. The reduction peak decreased as the glucose concentration increased (Figure 4), indicating the consumption of oxy-

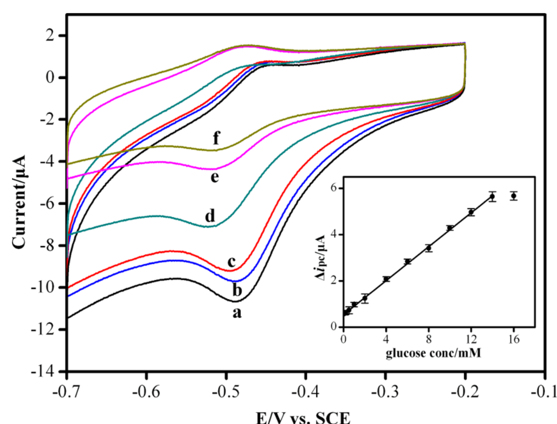
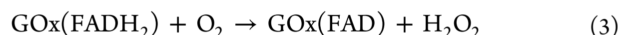


Figure 4. CVs of Nafion/GOx-yeast/MWNTs/GCE in PBS buffer (pH 7.4) containing different concentrations of glucose: 0.0 mM (trace a), 0.1 mM (trace b), 0.5 mM (trace c), 2.0 mM (trace d), 8.0 mM (trace e), and 12.0 mM (trace f). Scan rate = 50 mV s^{-1}. Inset shows a typical calibration graph of the glucose biosensor.

gen.[23,29] It was found that the Δi_{pc} value linearly increased as the glucose concentration increased, ranging within 0.1−14 mM glucose (inset of Figure 4). The limit of detection of the biosensor was estimated to be 0.05 mM glucose (signal-to-noise ratio of S/N = 3). Here, the Δi_{pc} parameter is defined as the difference of the i_{pc} from CVs of the modified electrode in the presence of glucose and in the presence of a bare buffer solution. In this context, dynamic detection ranges were reported to be 0−7.8 mM for the GOx/MWNTs/chitosan-modified electrode,[30] 0.08−0.28 mM for the GOx/colloidal gold-modified electrode,[31] 0.5−11.1 mM for the GOx/CdS-modified electrode,[32] and 2−14 mM for the GOx/graphene-modified electrode.[33] Therefore, the analytical performance of the Nafion/GOx-yeast/MWNTs/GCE is superior to other commercial GOx-nanostructure-composite-modified electrodes. Furthermore, as shown in an earlier section, compared with conventional GOx, the GOx-yeast demonstrated excellent enzyme properties, such as good stability within a wide pH range (pH 3.5−11.5), good thermostability up to 56 °C, and high D-glucose specificity, which makes the cell-based glucose biosensor unique and attractive.

Interestingly, the reduction peak potential was systematically negatively shifted as the glucose concentration increased (see Figure 4). The exact reason for this is unclear at the moment. It is most probably due to the fact that the oxygen consumption would increase rapidly as the glucose concentration increases; thus, the oxygen remaining in the solution would decrease. Based on reactions 2 and 3, the reduction peak current of GOx decreased with the increase of glucose concentration (see Figure 4), which would, in turn, induce the *IR* drop (where *I* represents the current and *R* represents the resistance of the solution), that is, a systematic shift in potential drop.

$$\text{Glucose} + \text{GOx(FAD)} \rightarrow \text{Gluconolactone} + \text{GOx(FADH}_2\text{)} \quad (2)$$



$$\text{GOx(FADH}_2\text{)} + \text{O}_2 \rightarrow \text{GOx(FAD)} + \text{H}_2\text{O}_2 \quad (3)$$

Selectivity of the Biosensor. As shown earlier, the GOx-yeast exhibited high specificity to D-glucose in comparison with other saccharides. Here, we further investigated other common electroactive species on the performance of glucose biosensor. The addition of acetaminophen, ascorbic acid, and uric acid (each 10 mM) did not change the current signal for glucose, suggesting that, at a 10-times excess, these common interfering species had no interference to the detection of 1 mM glucose. Therefore, this sensor can be used to selectively detect D-glucose without interference from other sugars and common interfering species.

Stability and Reproducibility of the Biosensor. Three different electrodes were prepared using the same procedure. Continuous CV measurements were carried out in 1 mM glucose solution to evaluate the operational stability of the as-prepared biosensor. It was found that both i_{pa} and i_{pc} retained 86% of their initial values and no obvious potential shifts were observed after 80 scans (see Figure 5), which revealed that the

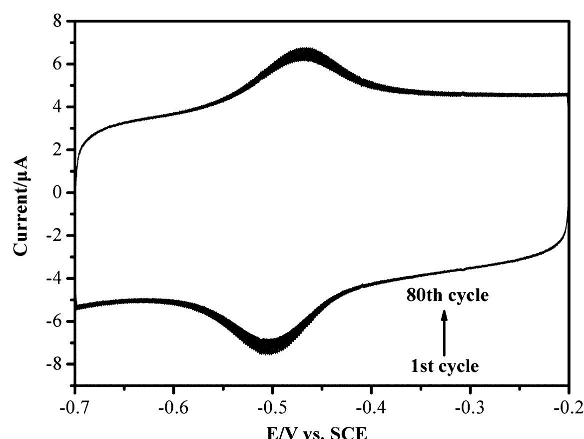


Figure 5. CVs of the Nafion/GOx-yeast/MWNTs/GCE in 0.1 M PBS (pH 7.4) containing 1 mM glucose for 80 continuous cycles. Scan rate = 100 mV s^{-1}.

modified electrode had good operational stability. The current responses of the modified electrode were measured daily in 1 mM glucose solution to examine the long-term stability of the Nafion/GOx-yeast/MWNTs/GCE. The biosensor was stored under 4 °C when it was not being used. During a one-month test, the current responses gradually decreased; however, the Δi_{pc} values still retained over 60% of the initial response after 30 days (see Figure S5 in the Supporting Information). To verify the reproducibility of this method, six bioelectrodes were fabricated with the same procedure. The relative standard deviation (RSD) of Δi_{pc} values in 1 mM glucose solution was 5.7%, indicating that the sensor exhibited good reproducibility.

Analysis of Real Samples. The Nafion/GOx-yeast/MWNTs/GCE was used to detect glucose levels in real samples such as beverages, the degradation products of agricultural wastes, and serum. The degradation product of agricultural waste sample was first filtered through a 0.22-μm membrane to collect the filtrate. It may be necessary to dilute the sample solution with PBS buffer before measurement. Based on the established method, the detection results are shown in Table S1 in the Supporting Information. The glucose levels in the samples can be calculated based on the calibration graph by multiplying the dilution ratios. To estimate the

recovery of the method over the added standard concentration, 5 μL of 1.00 M glucose standard solution was spiked into the 5.00 mL sample solution and stirred well before CV measurements were performed again. The recoveries of the added glucose standards were within 98%−105% (see Table S1 in the Supporting Information), which indicate good accuracy of the proposed method. The relative standard deviation (RSD) values were within 7%, which demonstrates that the precision and stability of the calibration graph is suitable for measuring glucose in real samples.

■ CONCLUSIONS

In this work, active GOx was successfully displayed on the surface of yeast cell. Compared with conventional immobilization approaches, the whole-cell catalyst was capable of self-amplifying easily and eliminating the expensive purification step. Moreover, it showed several advantages, such as excellent glucose specificity, wide pH range stability, and thermostability up to 56 °C. In addition, direct electrochemistry was realized at the GOx-yeast/carbon-nanotube modified electrode. Subsequently, a novel electrochemical glucose biosensor Nafion/GOx-yeast/MWNTs/GCE was constructed. The as-prepared biosensor was linear with the concentration of D-glucose within the range of 0.1−14 mM. The proposed biosensor is stable, specific, reproducible, simple, and cost-effective, which can be applicable for real sample detection. The strategy presented in this study may shed light on the development of other important oxidase (such as alcohol oxidase, lactate oxidase, and glutamate oxidase)-displaying system-based whole-cell biocatalysts, which are under way in this laboratory, holding great potential application in bioenergy, biosensors, and industrial catalysis.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures and table as mentioned in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail address: liuah@qibebt.ac.cn.

Author Contributions

[∇]These two authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by National Natural Science Foundation of China (Nos. 31200598, 31200982, 21275152, and 91227116), the Hundred-Talent-Project (No. KSCX2-YW-BR-7), and the Knowledge Innovation Project in Biotechnology (No. KSCX2-EW-J-10-6), Chinese Academy of Sciences.

■ REFERENCES

(1) Witteveen, C. F.; Veenhuis, M.; Visser, J. *Appl. Environ. Microbiol.* **1992**, *58*, 1190−1194.

(2) Wong, C. M.; Wong, K. H.; Chen, X. D. *Appl. Microbiol. Biotechnol.* **2008**, *78*, 927−938.

(3) Wang, J. *Chem. Rev.* **2008**, *108*, 814−825.

(4) Liang, B.; Li, L.; Mascin, M.; Liu, A. *Anal. Chem.* **2012**, *84*, 275−82.

(5) Hanefeld, U.; Gardossi, L.; Magner, E. *Chem. Soc. Rev.* **2009**, *38*, 453−468.

(6) Fishilevich, S.; Amir, L.; Fridman, Y.; Aharoni, A.; Alfonta, L. *J. Am. Chem. Soc.* **2009**, *131*, 12052−12053.

(7) Xia, L.; Liang, B.; Li, L.; Tang, X.; Palchetti, I.; Mascini, M.; Liu, A. *Biosens. Bioelectron.* **2013**, *44*, 160−163.

(8) Lee, S. Y.; Choi, J. H.; Xu, Z. *Trends Biotechnol.* **2003**, *21*, 45−52.

(9) Li, L.; Liang, B.; Shi, J.; Li, F.; Mascini, M.; Liu, A. *Biosens. Bioelectron.* **2012**, *33*, 100−105.

(10) Liang, B.; Li, L.; Tang, X.; Lang, Q.; Wang, H.; Li, F.; Shi, J.; Shen, W.; Palchetti, I.; Mascini, M.; Liu, A. *Biosens. Bioelectron.* **2013**, *45*, 19−24.

(11) Georgiou, G.; Stathopoulos, C.; Daugherty, P. S.; Nayak, A. R.; Iverson, B. L.; Curtiss, R., III. *Nat. Biotechnol.* **1997**, *15*, 29−34.

(12) Liang, P. *Biotechniques* **2002**, *33*, 338−344, 346.

(13) Malherbe, D. F.; du Toit, M.; Cordero Otero, R. R.; van Rensburg, P.; Pretorius, I. S. *Appl. Microbiol. Biotechnol.* **2003**, *61*, 502−511.

(14) Guo, Y.; Lu, F.; Zhao, H.; Tang, Y.; Lu, Z. *Appl. Biochem. Biotechnol.* **2010**, *162*, 498−509.

(15) Gietz, R. D.; Schiestl, R. H. *Nat. Protocols* **2007**, *2*, 38−41.

(16) Gouda, M. D.; Singh, S. A.; Rao, A. G.; Thakur, M. S.; Karanth, N. G. *J. Biol. Chem.* **2003**, *278*, 24324−24333.

(17) Bergmeyer, H. U.; Gawehn, K.; Grassl, M. *Enzymes as Biochemical Reagents. Glucose Oxidase*; Academic Press: New York, 1974; Vol. *1*, pp 457−458.

(18) Ko, J. H.; Hahm, M. S.; Kang, H. A.; Nam, S. W.; Chung, B. H. *Protein. Expression Purif.* **2002**, *25*, 488−493.

(19) Altikatoglu, M.; Basaran, Y.; Arioz, C.; Ogan, A.; Kuzu, H. *Appl. Biochem. Biotechnol.* **2010**, *160*, 2187−2197.

(20) Jan, U.; Khan, A.; Husain, Q. *World J. Microbiol. Biotechnol.* **2006**, *22*, 1033−1039.

(21) Innis, M. A.; Holland, M. J.; McCabe, P. C.; Cole, G. E.; Wittman, V. P.; Tal, R.; Watt, K. W.; Gelfand, D. H.; Holland, J. P.; Meade, J. H. *Science* **1985**, *228*, 21−26.

(22) Bhatti, H. N.; Madeeha, M.; Asgher, M.; Batool, N. *Can. J. Microbiol.* **2006**, *52*, 519−524.

(23) Shan, C.; Yang, H.; Song, J.; Han, D.; Ivaska, A.; Niu, L. *Anal. Chem.* **2009**, *81*, 2378−2382.

(24) Zafar, M. N.; Wang, X.; Sygmund, C.; Ludwig, R.; Leech, D.; Gorton, L. *Anal. Chem.* **2012**, *84*, 334−341.

(25) Zhao, X.; Mai, Z.; Kang, X.; Zou, X. *Biosens. Bioelectron.* **2008**, *23*, 1032−1038.

(26) Wang, Z.; Liu, S.; Wu, P.; Cai, C. *Anal. Chem.* **2009**, *81*, 1638−1645.

(27) Liu, S.; Ju, H. *Biosens. Bioelectron.* **2003**, *19*, 177−183.

(28) Liu, Q.; Lu, X.; Li, J.; Yao, X.; Li, J. *Biosens. Bioelectron.* **2007**, *22*, 3203−3209.

(29) Chen, W.; Ding, Y.; Akhigbe, J.; Brückner, C.; Li, C. M.; Lei, Y. *Biosens. Bioelectron.* **2010**, *26*, 504−510.

(30) Yu, P.; Zhou, H.; Cheng, H.; Qian, Q.; Mao, L. *Anal. Chem.* **2011**, *83*, 5715−5720.

(31) Wang, Z.; Etienne, M.; Quilès, F.; Kohring, G.-W.; Walcarius, A. *Biosens. Bioelectron.* **2012**, *32*, 111−117.

(32) Matsushika, A.; Inoue, H.; Kodaki, T.; Sawayama, S. *Appl. Microbiol. Biotechnol.* **2009**, *84*, 37−53.

(33) Shan, C. S.; Yang, H. F.; Song, J. F.; Han, D. X.; Ivaska, A.; Niu, L. *Anal. Chem.* **2009**, *81*, 2378−2382.