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Sensitive detection of maltose and glucose based on dual enzyme-displayed bacteria electrochemical biosensor

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ABSTRACT

Glucoamylase-displayed bacteria (GA-bacteria) and glucose dehydrogenase-displayed bacteria (GDH-bacteria) were co-immobilized on multi-walled carbon nanotubes (MWNTs) modified glassy carbon electrode (GCE) to construct GA-bacteria/GDH-bacteria/MWNTs/GCE biosensor. The biosensor was developed by optimizing the loading amount and the ratio of GA-bacteria to GDH-bacteria. The as-prepared biosensor exhibited a wide dynamic range of 0.2–10 mM and a low detection limit of 0.1 mM maltose (S/N=3). The biosensor also had a linear response to glucose in the range of 0.1–2.0 mM and a low detection limit of 0.04 mM glucose (S/N=3). Interestingly, at the same concentration, glucose was 3.75-fold sensitive than that of maltose at the proposed biosensor. No interferences were observed for other possible mono- and disaccharides. The biosensor also demonstrated good long-term storage stability and repeatability. Further, using both GDH-bacteria/MWNTs/GCE biosensor and GA-bacteria/GDH-bacteria/MWNTs/GCE biosensor, glucose and maltose in real samples can be detected. Therefore, the proposed biosensor is capable of monitoring the food manufacturing and fermentation process.

1. Introduction

Maltose is an important disaccharide with properties of sweetness, high thermostability, low hygroscopy and viscosity, as well as absence of crystallization during storage (Pyeshkova et al., 2009). Compared with other small molecular carbohydrates, maltose could prevent saccharose crystallization more effectively, so it is applied in the production of wide range of confectionery, such as caramel, frozen dairy produce, jellies, canned fruits, dietetic and sports foodstuff, bread, preserves, beverages, etc. Maltose is also of significance in brewage because its content would affect the quality of final products, particularly for beer and kvass (Soldatkin et al., 2013). Thus, determination of maltose is essential in monitoring the processes of food manufacturing and brewing industry (Nielsen, 2003; Serna Cock et al., 2009). Chromatography has been widely used to detect maltose with high precision, however, which requires complex and expensive equipment operated by professional technicians (Filipiak et al., 1996; Grembecka et al., 2014; Morales et al., 2004; Pyeshkova et al., 2009). Chemical titration and colorimetric detection of maltose was also reported for its simpleness and rapidnes, nevertheless, it was hindered by the interference from either other reduced monosugars or solution turbidity (de Cortes Sánchez-Mata et al., 2002; Moody and Purdy, 1971; Qu et al., 1995). Electrochemical biosensing technologies with such advantages as fast procedure, low cost, high sensitivity and selectivity, are promising to meet the challenges for maltose analysis (Rana et al., 2010). To date, many kinds of electrochemical maltose biosensors based on free enzyme immobilization at electrodes have been developed (Ge et al., 1998a; Gondo et al., 1997; Qu et al., 1995; Sun et al., 1996; Váradi et al., 1993; Zajoncová et al., 2004). However, the stability of maltose biosensors need to be further improved. Additionally, maltose commonly co-exists with glucose and other sugars in food, beer and juice (Qu et al., 1995). Therefore, the selective determination of maltose and glucose has remarkably practical significance especially in brewage and industrial fermentation.

Bi-enzyme based biosensors were often used in the determination of disaccharides (Ge et al., 1998a; Hamid et al., 1990; Odaci et al., 2010; Qu et al., 1995), starch (Hamid et al., 1990; Lang et al., 2014) or cholesterol (Motonaka and Faulkner, 1993). The performance of the dual enzyme biosensors is affected by several

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important factors including the amount of enzyme uploading, ratio and distribution control of two enzymes as well as the immobilization methods (Zhou et al., 2001). Compared with single enzyme based biosensors, the stability and sensitivity of the bi-enzyme based biosensors were unsatisfactory, which might be contributed from the complexity in enzyme membrane preparation (Zhou et al., 2001).

Microbial surface display could eliminate onerous purification processes of enzyme by fusing them with appropriate anchoring motifs such as ice nucleation protein (INP) (Liang et al., 2012, 2013b). The resultant whole cell has been widely used in construction of biosensors (Li et al., 2012; Liang et al., 2013a, 2015; Liu et al., 2016; Tang et al., 2014; Wang et al., 2013) and biofuel cells (Fan et al., 2015; Feng et al., 2016; Xia et al., 2013), benefiting from the excellent stability and simple preparation process. Glucoamylase (GA, EC 3.2.1.3) is capable of catalyzing the hydrolysis of maltose to result in glucose. This important enzyme in fermentation has been displayed on yeast's cell wall by using either agglutinin (Murai et al., 1997; Schreuder et al., 1993). Glucose dehydrogenase (GDH, EC 1.1.1.47) catalyzes oxidation of the first hydroxyl group of glucose, utilizing the cofactor nicotine adenine dinucleotide (NAD^+) as the primary electron acceptor (Ferri et al., 2011). *Eschericia coli* surface-displayed GDH or GDH mutants using INP as an anchor motif had been constructed and used in the preparation of glucose biosensor in our previous studies (Liang et al., 2013b). The immobilization of bacteria was much easier and more stable in the modification of electrodes (Liang et al., 2013b). Thus the bacterial surface displayed GDH based glucose biosensor exhibited good properties (Liang et al., 2013b).

In this paper, by co-immobilization of GA-displayed bacteria (GA-bacteria) and GDH-displayed bacteria (GDH-bacteria) instead of free enzymes as biocatalysts, we first constructed a sensitive electrochemical biosensor for both maltose and glucose. By combining with GDH-bacteria based glucose biosensor we proposed before (Liang et al., 2013b), we developed a approach capable of determination of maltose and glucose sensitively and rapidly, which is also applicable to real samples.

2. Experimental

2.1. Reagents and materials

Maltose, glucose and other sugars, poly(acrylic acid) (PAA) and glutaraldehyde (25% solution) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Bovine serum albumin (BSA) was purchased from F. Hoffmann-La Roche, Ltd (Basel, Swiss). Nafion (perfluorinated ion-exchange resin, 5 wt% solution in a mixture of lower aliphatic alcohols and water) was purchased from Aldrich. Multiwalled carbon nanotubes (MWNTs) were kindly presented from Dr. Gebo Pan in Suzhou Institute of Nan-Tech and Nano-Biomics, Chinese Academy of Sciences. All other reagents were of analytical grade. Aqueous solutions were prepared with deionized water (18 MΩ cm).

2.2. Preparation of GA-displayed- and GDH-displayed bacteria

The detail for the construction of plasmid harboring *gdh* has been described in our previous work (Liang et al., 2013b). GA encoding gene was cloned into plasmid pTInaPb-N to generated pTInaPb-N-ga (Liang et al., 2012). *E. coli* BL21 (DE3) harboring the expression plasmids were grown in Luria-Bertani media and the expression of GA and GDH were induced with isopropyl-β-D-thiogalactopyranoside at a final concentration of 1.0 mM at 25 °C overnight. GA- and GDH-displayed cells were harvested and washed with acetate buffer solution (pH 5.6), respectively. For determining enzymatic activity of GDH, 5 mM D-glucose and 2 mM NAD^+ were added in recombinant strain suspension. The reaction was carried out at 30 °C for 10 min. Activity was expressed in units (1 mmol NADH generated per min) under assay conditions. GA activity test was performed in acetate buffer solution (pH 5.6) containing 30 mM maltose at 70 °C for 15 min. The production of glucose was detected using D-glucose detection kit (Jiancheng, China). One unit of GA enzymatic activity is defined as the amount of enzyme that liberates 1 μmol of glucose per min under assay conditions.

2.3. Fabrication of the biosensors

A glassy carbon electrode (GCE, 3-mm in diameter) was prepared by polishing successively with 1.5-, 0.5- and 0.05-μm alumina powders to obtain a mirror finish, followed by sonicating in sequence in anhydrous ethanol and deionized water for 5 min and dried with a nitrogen gas stream. 2 mg MWNTs were dispersed in 1 ml deionized water containing 2.0% PAA with sonication processing for 20 min until a homogeneous aqueous dispersion obtained (Liu et al., 2006). Then the aqueous dispersion was washed twice with deionized water to remove excess PAA. Nafion was diluted with anhydrous ethanol to a 0.05 wt% solution. In preparation of GA-bacteria/GDH-bacteria/MWNTs/GCE, 5 μL of MWNTs dispersion was dripped on the inverted GCE surface and dried in air, followed by adding 10.24 μU GA-bacteria and 6.40 μU GDH-bacteria (corresponding to 8 μL GA-bacteria and 5 μL GDH-bacteria dispersion) onto electrode surface and dried overnight in 4 °C in refrigerator. The resultant modified electrode was marked as GA-bacteria/GDH-bacteria/MWNTs/GCE. Finally, 5 μL of Nafion solution (0.05 wt%) was syringed to the electrode surface. Before use, the modified electrodes were washed repeatedly with deionized water to remove the loosely combined modifiers. Similarly, a glucose biosensor marked as GDH-bacteria/MWNTs/GCE was also prepared with our previous work (Liang et al., 2013b). For comparison, electrodes modified with counterpart free enzymes were also prepared. After 5 μL of MWNTs dispersion was dripped on the inverted GCE surface and dried, a mixture of 10.24 μU free GA solution, 6.40 μU free GDH solution, 2 μL of BSA (1% w/w) and 5 μL of glutaraldehyde (1% w/w) were dripped, then dried overnight in 4 °C in refrigerator. The as-modified electrode was marked as GA/GDH/MWNTs/GCE.

2.4. Electrochemical measurements

The electrochemical measurements were carried out on a CHI 660D electrochemical workstation (CH Instruments, Chenhua, Shanghai, China). A conventional three-electrode system was employed with a saturated calomel electrode (SCE) as the reference electrode, a Pt wire auxiliary electrode as the counter electrode, and the chemically modified GCEs as the working electrodes. All potentials were reported in this paper with respect to SCE. All measurements were performed at room temperature (~23 °C).

3. Results and discussion

3.1. Cell surface display of GA and GDH

Both GA and GDH encoding genes were separately ligated into plasmid pTInaPb-N, which was constructed before in our laboratory (Liang et al., 2012). Both enzymes were displayed on the surface of *E. coli* through ice nuclear protein. GA and GDH activities were assayed to be 1.26 ± 0.08 U/ml and 0.64 ± 0.06 U/ml, respectively.

3.2. Electrochemical characterization of GA-bacteria/GDH-bacteria/MWNTs/GCE

Maltose can be converted to glucolactone by using GA and GDH, which involves in two reactions, Eqs. (1) and (2). First, maltose is hydrolyzed to glucose by GA (Eq. (1)), the latter is further oxidized to glucolactone by GDH in the presence of coenzyme NAD^+, which is reduced to NADH (Eq. (2)). The resultant NADH could be electrochemically oxidized to regenerate NAD^+ (Eq. (3)).

$$\text{Maltose} \xrightarrow{GA} 2\ \text{Glucose} \quad (1)$$

$$\text{Glucose} + NAD^+ \xrightarrow{GDH} \text{Glucolactone} + NADH \quad (2)$$

$$NADH \rightarrow NAD^+ + e^- \quad (3)$$

The electrochemical properties of various modified electrodes were investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). A small anodic peak was observed at about +0.7 V for GA-bacteria/GDH-bacteria/GCE (Fig. 1A, curve a), while the anodic peak shifted 0.4 V negatively and the anodic peak current increased significantly for GA-bacteria/GDH-bacteria/MWNTs/GCE (Fig. 1A, curve d), indicating the excellent electron transfer properties and electrical conductivity of MWNTs (Musameh et al., 2002; Liu et al., 2006; Liu et al., 2007; Liu, 2008; Wooten and Gorski, 2010). There was just background current for GA-bacteria/GDH-bacteria/MWNTs/GCE in the absence of maltose (Fig. 1A, curve b). For comparison, the electrode modified with free GA and GDH enzyme was also tested. A smaller anodic peak was obtained at +0.3 V for GA/GDH/MWNTs/GCE (Fig. 1A, curve c), indicating that the catalytic efficiency of free GA and GDH were lower than the bacteria surface displayed GA and GDH (Fig. 1A, curve d). It should be mentioned here that the amount and ratio of uploading for free GA and GDH were consistent with the optimized GA-bacteria/GDH-bacteria/MWNTs/GCE based on the enzyme activity. The possible reason may be that the immobilization of free enzyme through cross-linking by glutaraldehyde could decrease catalytic activity of enzymes (Cunningham et al., 1982; Schejter and Bar-Eli, 1970). Considering that DPV has good sensitivity over CV, the DPV responses of the GA-bacteria/GDH-bacteria/MWNTs/GCE in buffer solution with varying maltose concentration were measured. Sharp anodic peak was observed for GA-bacteria/GDH-bacteria/MWNTs/GCE in maltose solution (Fig. 1B). Further, the electrocatalytic current (i_{pa}) increased with the increasing maltose concentration in the buffer solution, suggesting the possible maltose detection.

3.3. Optimization of conditions for the construction of GA-bacteria/GDH-bacteria/MWNTs/GCE

The buffer pH, enzyme loading amount and dual enzymes loading ratios will affect the performance of the biosensor. In order to obtain the maltose biosensor with best performance, the effect of buffer pH, recombinant bacteria loading as well as ratios of GA-bacteria to GDH-bacteria on the i_{pa} were investigated. Considering that the enzyme activity of GA was relatively low when the pH was out of 4.5–6.0, the dual enzymes activity of pH ranging from 4.5 to 6.0 was studied. As shown in Fig. 2A, the i_{pa} values were depended on the buffer solution pH. Interestingly, two peaks appeared within pH 4.5–6.0, one peak at pH 4.8, which might be corresponding to the optimal pH value for GA (Zheng et al., 2010). However, the highest i_{pa} value was obtained in pH 5.6 buffer solution, which was different from the optimal pH value of 8.0 for GDH (Liang et al., 2013a, 2013b). The exact reason for this is unclear for the moment, which may be originating from the integration of catalytic reactions of the two enzymes involved. Thus

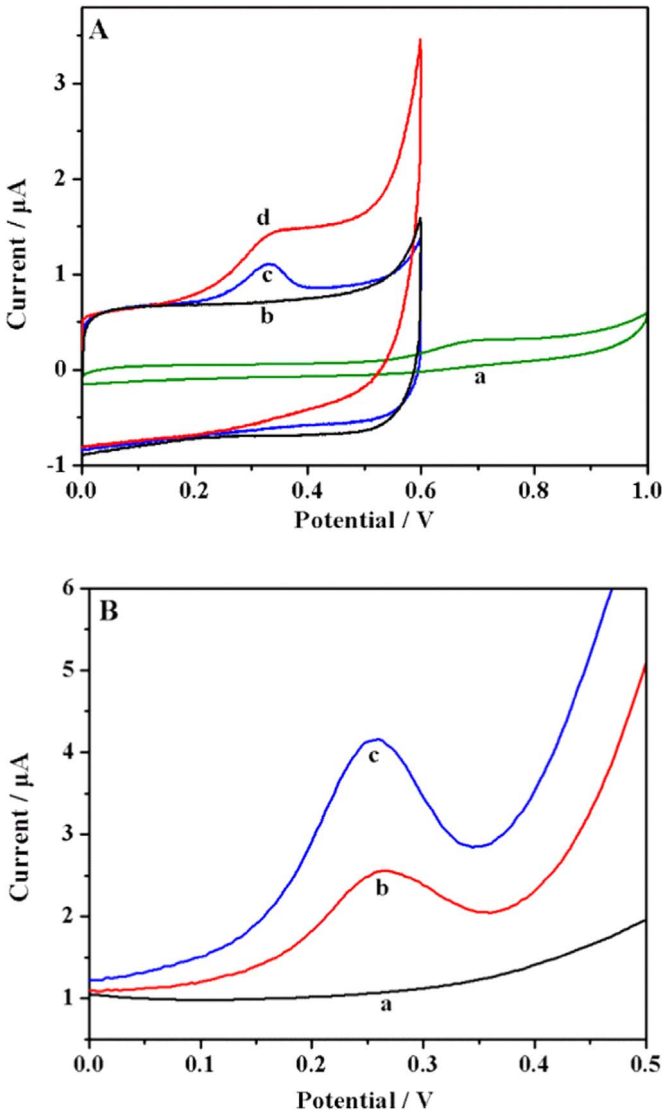

Fig. 1. A) CVs of GA-bacteria/GDH-bacteria/GCE in 5 mM maltose (a); CVs of GA-bacteria/GDH-bacteria/MWNTs/GCE in the presence of 0 (b) and 5 mM maltose (d); CV of GA/GDH/MWNTs/GCE in 5 mM maltose (c). CVs of the different bioelectrodes were measured in 0.1 M acetate buffer (pH 5.6) containing 2 mM NAD^+ and maltose with varying concentration. Scan rate, 50 mV s^{-1}. B) Differential pulse voltammograms of GA-bacteria/GDH-bacteria/MWNTs/GCE in 0.1 M acetate buffer (pH 5.6) containing 2 mM NAD^+ with varying maltose concentrations: 0 (a), 5 mM (b), and 10 mM (c). The DPV conditions: amplitude, 0.05 V; pulse width, 0.2 s; sampling width, 0.0167; pulse period, 0.5 s.

the pH 5.6 buffer solution was used for the following tests. On the other hand, different bioelectrodes were prepared with various ratios and loading amount of GA-bacteria and GDH-bacteria. The dependence of i_{pa} value on loading is shown in Fig. 2B. Generally, the i_{pa} value increased with the increase of uploading amount of GA-bacteria and GDH-bacteria. However, when the uploading of GA-bacteria and GDH-bacteria exceeded 10.24 and 6.40 μU, respectively, the i_{pa} began to decrease. It is obvious that the best current response was reached when 10.24 μU GA-bacteria and 6.40 μU GDH-bacteria were immobilized on the electrodes. By varying GDH-bacteria loading, the ratio of uploading amount of GA-bacteria to uploading amount of GDH-bacteria on the current was also investigated. The i_{pa} values were increased gradually with the ratio increasing from 1:2, 1.2:1, 1.6:1 (Fig. 2B). Apparently, when the loading amount of GA-bacteria was more than that of GDH-bacteria, the i_{pa} values increased. The reason might be that the hydrolysis of maltose to glucoses by GA is a rate-limiting step

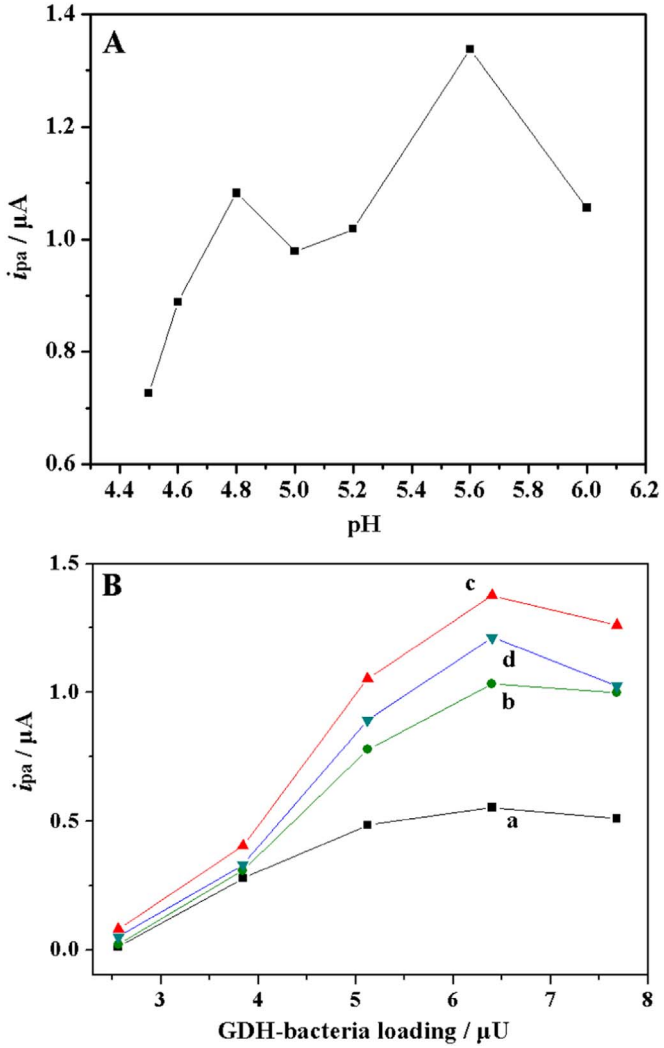

Fig. 2. A) The dependence of i_{pa} on buffer pH. DPVs of the GA-bacteria/GDH-bacteria/MWNTs/GCE were recorded in 0.1 M acetate buffer containing 2 mM NAD^+ and 5 mM maltose with varying pH. B) The dependence of i_{pa} on loading amount of GDH-bacteria with different GA-bacteria/GDH-bacteria ratios of 1:2 (a), 1.2:1 (b), 1.6:1 (c) and 2:1 (d). DPVs of the GA-bacteria/GDH-bacteria/MWNTs/GCE were recorded in 0.1 M acetate buffer (pH 5.6) containing 2 mM NAD^+ and 5 mM maltose.

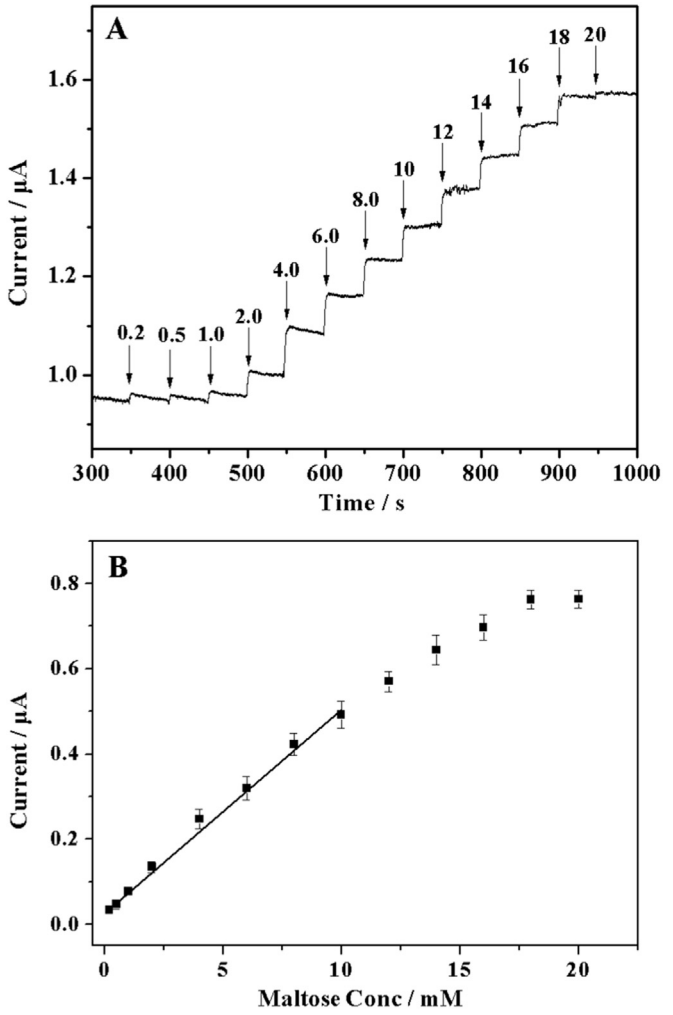

Fig. 3. A), Current-time curve obtained at the GA-bacteria/GDH-bacteria/MWNTs/GCE on the successive addition of maltose in stirring 0.1 M acetate buffer (pH 5.6) containing 2 mM NAD^+, on which the maltose concentration labeled is the final concentration (mM). Applied potential, +0.35 V vs. SCE. B), Calibration curve for maltose.

(Baik et al., 2005; Zeng et al., 2010; Zheng et al., 2010). A maximal i_{pa} was reached at ratio of 1.6:1. After that, the i_{pa} value decreased, which might result from the excessive loading bacteria on the electrode blocking the transfer of electrons (Li et al., 2012).

3.4. Amperometric determination of maltose or glucose at GA-bacteria/GDH-bacteria/MWNTs/GCE

The further characterization of responses to maltose at GA-bacteria/GDH-bacteria/MWNTs/GCE was carried out with amperometry. With the addition of maltose, the curve displayed a distinct signal in the form of current steps and reached 95% steady-state value within 5 s (Fig. 3A). The calibration curve for maltose is shown in Fig. 3B. The current increased linearly with the addition of maltose in the concentration of 0.2–10 mM (Fig. 3B), with the linear regression equation of $y=0.034+0.04x$ and correlation coefficient (R) of 0.995. The limit of detection (LOD) was calculated to be 0.1 mM maltose (S/N=3), which was lower than graphene/enzyme multilayer film-based maltose biosensor (1.37 mM) (Zeng et al., 2010), ferricyanide mediated disposable biosensor (5 mM) (Ge et al., 1998b) and α-glucosidase/pyranose-oxidase based electrochemical biosensor (0.25 mM) (Odaci et al., 2010). Thus, the proposed biosensor exhibited good analytical performance over other biosensors reported so far.

The responses of glucose at GA-bacteria/GDH-bacteria/MWNTs/GCE were also investigated with amperometric method. 95% of the steady-state current could be reached within 5 s after the addition of glucose (Fig. 4A), reflecting the fast response of sensor to glucose. Fig. 4B depicts the calibration curve, a well-defined typical behavior of an enzymatic reaction with a linear range from 0.1 to 2.0 mM. The linear regression equation was $y=0.01+0.19x$ with R of 0.991. The LOD value was calculated to be 0.04 mM glucose (S/N=3). We previously developed GDH-bacteria/MWNTs/GCE biosensor, which exhibited linear range of 50–800 μM and LOD of 4 μM D-glucose (Liang et al., 2013b). Here the sensitivity of the GA-bacteria/GDH-bacteria/MWNTs/GCE to glucose was lower than that of GDH-bacteria/MWNTs/GCE biosensor, possibly originating from the electron transfer barrier of a thicker layer of GA-bacteria on the electrode surface.

3.5. Selectivity of GA-bacteria/GDH-bacteria/MWNTs/GCE

The selectivity of the biosensor is an essential characteristics to practical applications (Turner, 2013). The i-t curve of GA-bacteria/GDH-bacteria/MWNTs/GCE was recorded upon addition of a series

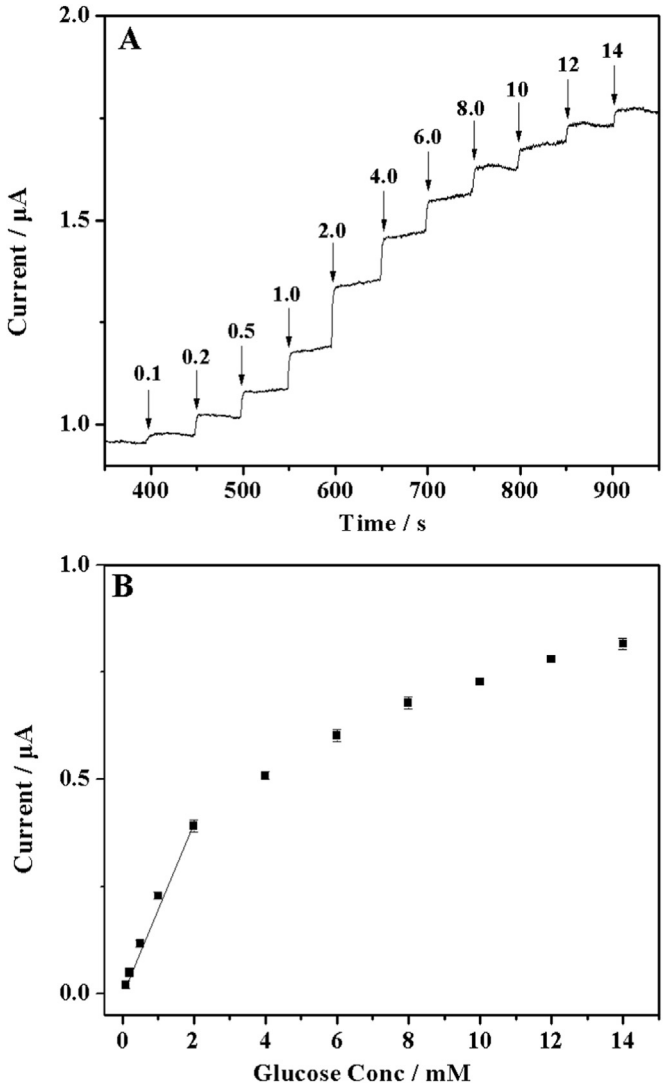

Fig. 4. A) Current-time curve obtained at the GA-bacteria/GDH-bacteria/MWNTs/GCE on the successive addition of glucose in stirring 0.1 M acetate buffer (pH 5.6) containing 2 mM NAD^+, on which the glucose concentration labeled is the final concentration (mM) in buffer solution. Applied potential, +0.35 V vs. SCE. B) calibration curve for glucose.

of mono- and disaccharides. As shown in Fig. 5, both maltose (arrow a) and glucose (arrow b) showed rapid current response. Further, for the same concentration (1 mM) of maltose and glucose, the current for glucose (arrow b) was 4.75-fold of that value for maltose (arrow a), which was in good agreement with the slope ratio of the corresponding linear regression equations for glucose and maltose (0.19/0.04=4.75). Similar result was reported that the response of maltose was much lower than that of glucose at commercial enzymes of GA/GDH modified electrode (Zeng et al., 2010). The possible reason may be ascribed to the fact that the hydrolysis of GA was slower than dehydrogenation of glucose by GDH (Baik et al., 2005; Zheng et al., 2010). On the other hand, the injection of 50-fold (compared with glucose) or 10-fold (compared with maltose) excess of other mono-saccharides including xylose, lactose, galactose, mannose, arabinose, and disaccharides such as sucrose and sorbose just showed background current, which demonstrated excellent selectivity of GA-bacteria/GDH-bacteria/MWNTs/GCE to maltose and glucose.

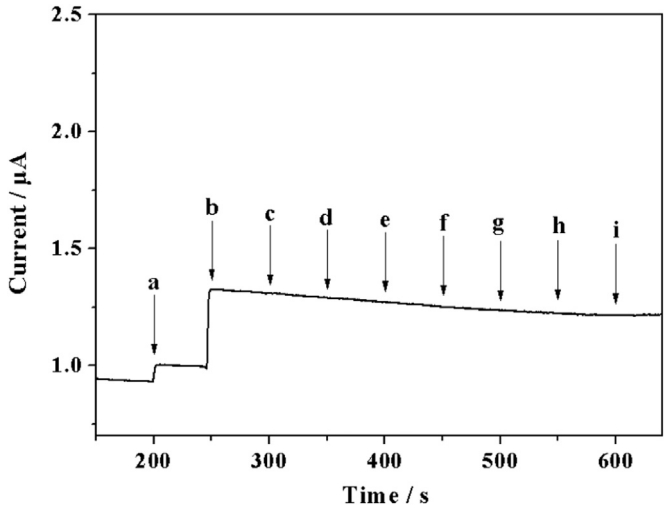

Fig. 5. Current-time curve obtained for the GA-bacteria/GDH-bacteria/MWNTs/GCE on the successive addition of 1 mM maltose (a); 1 mM glucose (b); 5 mM sucrose (c); 50 mM xylose (d); 50 mM lactose (e); 50 mM galactose (f); 50 mM mannose (g); 50 mM arabinose (h); 50 mM sorbose (i) in 0.1 M acetate buffer (pH 5.6) containing 2 mM NAD^+.

3.6. Repeatability and stability of the biosensor

To study the repeatability of the GA-bacteria/GDH-bacteria/MWNTs/GCE biosensor, current responses of the same electrode in maltose concentration at 0.5, 1, 2, 4, 8 mM were tested 6 times, for which the coefficients of variation were calculated to be within 4.0–6.0%. Further, the responses of 6 different preparations of electrodes in 1 mM maltose solution were also tested, the repeatability was 6%. The long-term stability of the GA-bacteria/GDH-bacteria/MWNTs/GCE was examined by the measurement of CV peak current in 0.1 M acetate buffer (pH 5.6) containing 2 mM NAD^+ and 5 mM maltose every two days. The biosensor was stored under 4 °C when it was not used. The responses of peak current remained 86.4% of the initial response after 1 month (Fig. S1, Supplementary material), indicating the favorably storage stability of the GA-bacteria/GDH-bacteria/MWNTs/GCE.

3.7. Analysis of real samples

As addressed above, glucose exhibited 3.75-times higher sensitivity than maltose at GA-bacteria/GDH-bacteria/MWNTs/GCE. We initially valued the possibility to use the dual recombinant strains based electrode to check its response to different mixtures of standard glucose and maltose with known concentrations. We found that the detected glucose and maltose concentration matched well with their known concentrations (Table 1). Subsequently, we detected maltose and glucose in real samples. By

Table 1
The determination of glucose and maltose mixtures with known concentrations by using GDH-bacteria/MWNTs/GCE and GA-bacteria/GDH-bacteria/MWNTs/GCE.

Mixtures of standard solution of glucose and maltose		Detected glucose (mM)[a]	Detected maltose (mM)[a]
Glucose (mM)	Maltose (mM)		
0	2.00	–	2.01 ± 0.08
1.00	0	1.00 ± 0.04	–
1.00	2.00	1.02 ± 0.05	1.98 ± 0.09
0.50	2.00	0.49 ± 0.02	2.01 ± 0.07
2.00	0.50	1.97 ± 0.09	0.50 ± 0.02

[a] The data were shown as mean(±SD) for three separate measurements, where SD stands for standard deviation.

doing so, we prepared two biosensors, that is, GDH-bacteria/MWNTs/GCE and GA-bacteria/GDH-bacteria/MWNTs/GCE. The GDH-bacteria/MWNTs/GCE biosensor prepared based on our previous method (Liang et al., 2013b), was used to detect the glucose, while the GA-bacteria/GDH-bacteria/MWNTs/GCE was used for detecting the total current contributing from both maltose and glucose. Then the maltose concentration could be calculated based on their sensitivity ratio. Before measurement, the samples were firstly filtered through a 0.22 μm membrane to obtain the filtrates. The filtrates were accurately diluted with 0.1 M acetate buffer (pH 5.6) containing 2 mM NAD^+ to fit into the linear range of calibration curves. For each sample, three separate detections were conducted and the concentrations were calculated based on the calibration curve by multiplying the dilution ratios (Table S1, Supplementary material). In additional experiment, aliquots of 6 filtrate were measured using the standard-addition method. The recoveries were between 99.2% and 108.9%, which were acceptable in the analysis of glucose and maltose in real samples such as beer and peach juice. In the context, pyranose oxidase/gold-nanoparticle/polyaniline/AgCl/gelatin nanocomposite-modified bioelectrode was developed for the glucose detection in peach juice (Ozdemir et al., 2010).

4. Conclusions

This work reported the use of two recombinant strains displayed GA and GDH for the preparation of maltose biosensor. Compared with free enzymes based biosensor, dual recombinant strains modified electrodes exhibited better performance. The GA-bacteria/GDH-bacteria/MWNTs/GCE provided a rapid response, a low detection limit (0.1 mM) and a broad linear range (0.2–10 mM) in the determination of maltose. The proposed maltose biosensor was sensitive to maltose and glucose and insensitive to other mono- and disaccharides. The biosensor also exhibited good reproducibility and long-term stability. Finally, the detection of glucose and maltose in real samples was established by using both GDH-bacteria/MWNTs/GCE biosensor and GA-bacteria/GDH-bacteria/MWNTs/GCE biosensor.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.07.050.

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