Biosensors and Bioelectronics 70 (2015) 455–461

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A glucose oxidase-coupled DNAzyme sensor for glucose detection in tears and saliva

Chengcheng Liu [a], Yongjie Sheng [a], Yanhong Sun [a], Junkui Feng [a], Shijin Wang [a], Jin Zhang [a,*], Jiacui Xu [b,**], Dazhi Jiang [a,*]

[a] Key Lab for Molecular Enzymology & Engineering of the Ministry of Education, School of Life Science, Jilin University, Qianjin Street 2699, Changchun 130012, China

[b] Department of Biochemistry, University of Wisconsin, Madison, WI 53706, USA

ARTICLE INFO

Article history:
Received 5 December 2014
Received in revised form
15 March 2015
Accepted 27 March 2015
Available online 30 March 2015

Keywords:
Pistol-like DNAzyme
Glucose oxidase
Glucose
Tear
Saliva
Non-invasive technology

ABSTRACT

Biosensors have been widely investigated and utilized in a variety of fields ranging from environmental monitoring to clinical diagnostics. Glucose biosensors have triggered great interest and have been widely exploited since glucose determination is essential for diabetes diagnosis. In here, we designed a novel dual-enzyme biosensor composed of glucose oxidase (GOx) and pistol-like DNAzyme (PLDz) to detect glucose levels in tears and saliva. First, GOx, as a molecular recognition element, catalyzes the oxidation of glucose forming H_2O_2; then PLDz recognizes the produced H_2O_2 as a secondary signal and performs a self-cleavage reaction promoted by Mn^{2+}, Co^{2+} and Cu^{2+}. Thus, detection of glucose could be realized by monitoring the cleavage rate of PLDz. The slope of the cleavage rate of PLDz versus glucose concentration curve was fitted with a Double Boltzmann equation, with a range of glucose from 100 nM to 10 mM and a detection limit of 5 μM. We further applied the GOx–PLDz 1.0 biosensor for glucose detection in tears and saliva, glucose levels in which are 720 ± 81 μM and 405 ± 56 μM respectively. Therefore, the GOx–PLDz 1.0 biosensor is able to determine glucose levels in tears and saliva as a non-invasive glucose biosensor, which is important for diabetic patients with frequent/continuous glucose monitoring requirements. In addition, induction of DNAzyme provides a new approach in the development of glucose biosensors.

1. Introduction

Diabetes is a metabolic disease around the world, which can cause serious health complications including blindness, heart disease, kidney failure (Calcutt et al., 2009). Diagnosis and treatment of diabetes require tight monitoring glucose levels, thus a simple, sensitive and efficient approach for glucose measurement is mandatory. For this purpose, many efforts have been devoted to develop glucose biosensors. So far, many biosensors for glucose monitoring have been developed and classified into the following categories based on detection methods: visible absorption spectrum, infrared spectrum, fluorescence spectrum, electrochemistry and so on (Yoo and Lee, 2010; Gifford, 2013; Gruhl et al., 2013). In recent years, researchers further developed various novel glucose biosensors based on gold/platinum nanoparticles, graphene, carbon nanotube and gold nanoclusters (Scognamiglio, 2013; Lin et al., 2014a; Lawal, 2015). For instance, Chaturvedi et al. (2014) developed an electrochemical biosensor based on nanoceria-platinum-graphene nanocomposite and glucose oxidase (GOx); Cui et al. (2014) obtained a nano-based electrochemical biosensor by integration of a highly ordered gold nanowires array with glucose oxidase; Leng et al. (2014) constructed PtAu bimetallic nanoparticle/graphene nanocomposites for application in glucose level detection; Khan and Park (2014) designed a proton sensitive glucose biosensor based on liquid crystal; He et al. (2014) developed a highly sensitive and specific fluorescent biosensor for blood glucose monitoring based on hemin-functionalized graphene quantum dots (GQDs) and GOx system; Gao et al. (2014) reported a novel biosensor by chemiluminescence assay for the detection of glucose based on GOx functionalized graphene oxide (GO)/lucigenin nanocomposite. Based upon the above biosensors, an essential approach to design novel biosensors for glucose detection is to use GOx as a signal transducer turning glucose into H_2O_2 and combine a new 'material' for H_2O_2 recognition and signal output.

In this study, we take DNAzyme as a new 'material' and apply it

Abbreviations: FAD, flavin adenine dinucleotide; GOx, glucose oxidase; PLDz, pistol-like DNAzyme

* Corresponding authors. Fax: +86 431 85155210.
** Corresponding author. Fax: +1 608 262 4693.
E-mail addresses: zhangjin@jlu.edu.cn (J. Zhang), jiacui2000@gmail.com (J. Xu), jiangdz@jlu.edu.cn (D. Jiang).

http://dx.doi.org/10.1016/j.bios.2015.03.070
0956-5663/

for construction of glucose biosensor. DNAzyme (deoxyribozyme, catalytic DNA, DNA enzyme) is a DNA that has the capability of performing catalytic functions. The first DNAzyme was isolated through *in vitro* selection (Breaker and Joyce, 1994). So far, a variety of DNAzymes have been identified (Höbartner and Silverman, 2007). Most DNAzymes require cofactors for their activities, and some even show cofactor sensitivity and specificity, which allow DNAzymes to be widely used for cofactor sensing (Lu and Liu, 2006; Liu et al., 2009; Zhang et al., 2011). Among DNAzymes, pistol-like DNAzyme (PLDz) is an oxidative DNA-cleaving DNAzyme, which requires either H_2O_2/Cu^{2+} or ascorbic acid/Cu^{2+} as cofactors (Carmi et al., 1998; Carmi and Breaker, 2001). Based on PLDz, researchers have developed DNA molecular logic gates, a dual-catalytic allosteric DNAzyme (Chen et al., 2006; Jiang et al., 2010). In addition, PLDz has been further engineered into biosensors for detection of its specific cofactors either Cu^{2+} or ascorbic acid (Miao et al., 2012; Malashikhina and Pavlov, 2012). Here, we take advantage of PLDz to recognize its specific cofactor—H_2O_2, which happens to be a by-product of glucose oxidation catalyzed by GOx. Therefore, glucose triggered cascade reactions which are linked by H_2O_2 can be sequentially performed by GOx and PLDz. In the following studies, we utilize GOx and PLDz for construction of a dual-enzyme cascade biosensor for glucose detection in tears and saliva to achieve non-invasive glucose testing.

2. Materials and methods

2.1. Reagents and chemicals

All DNA oligonucleotides were purchased from Sangon Biotech Co., Ltd. (Shanghai, China) and purified by denaturing PAGE (*cis*-PLDz: 5′-GAG ATC TTT CTA ATA CGA CTC AGA ATG AGT CTG GGC CTC TTT CTT TTA GAA AGA AC-3′, *tran*-PLDz: 5′-TGA GTC TGG GCC TCT TTC TTT TAG AAA GAA C-3′, substrate of *tran*-PLDz: 5′-GAC TTC TAA TGA AAT TAG AAG TCA TCG GAG ATC TTT CTA ATA CGA CTC AGA A-3′). GOx was purchased from Sigma-Aldrich (Spain). GelRed™ was purchased from Biotium Inc. Metal ions, glucose and its analogs were of analytical reagent grade. Ultrapure water from Milli-Q (Merck Millipore) was used throughout the experiments.

2.2. Glucose detection by the GOx–PLDz 1.0 biosensor

Glucose detection was tested as follows: 0.5 μM PLDz, 0.1 U/μl GOx, 50 μM Co^{2+}, 200 mM HAc–NaOAc (pH 5.4), 300 mM NaCl and 0.1 μM–10 mM glucose in 100 μl reaction solution. The reaction mixture was incubated at 37 °C for 1 h and stopped by adding precipitants (200 μl of 100% ethanol, 20 μl of 3 M NaOAc (pH 5.2), 1 μl of 10 mg/ml glycogen) for precipitation. The dried samples were dissolved in 20 μl denaturing loading buffer (4 M urea, 10 mM EDTA, 25 mM Tris–HCl pH 7.5, 0.125‰ xylene cyanol FF, 0.125‰ bromophenol blue) and separated by electrophoresis in denatured 20% polyacrylamide gel. Gel was stained with GelRed dye for 10 min and visualized by UV trans-illumination.

2.3. Double Boltzmann equation

$$y = 1.01678 + 61.51923 \times (0.44565/(1+\exp((x-5.33383)/(-0.55135))) + 0.55435/(1+\exp((x-5.83542)/(-0.10648))))$$

where y is the cleavage rate of PLDz and x is the log (glucose concentration (nM)).

2.4. Glucose detection in real samples

Tears and saliva samples were provided by Dr. Jiang. Methods of sampling: Tears were obtained by inducing tearing with onion and saliva was collected by psychological stimulation at two hours after eating. Methods of sample treatment: obtained samples were boiled for 10 min and centrifuged at 14,000 rpm for 10 min. The supernatant was collected for analysis. 100 μl of reaction mixture contains 30 μl saliva or tear, 0.5 μM PLDz, 0.1 U/μl GOx, 50 μM Co^{2+}, 200 mM HAc–NaOAc (pH 5.4) and 300 mM NaCl. Samples were separated on a denaturing polyacrylamide gel and stained with GelRed dye for visualization under UV light. The cleavage ratio was determined using quantitative scanning of corresponding gel bands and calculated using the equation cleaved fragments/(cleaved fragments+uncleaved fragment) and glucose concentrations were determined by Double Boltzmann curve.

3. Results

3.1. Design of the GOx–PLDz 1.0 biosensor

We designed a dual-enzyme cascade biosensor (GOx–PLDz 1.0) for glucose detection based on glucose oxidase (GOx) and pistol-like DNAzyme (PLDz) (Fig. 1) (Carmi et al., 1998; Bankar et al., 2009). Its working principle is: β-D-glucose in samples as a first signal can be recognized and catalyzed by GOx forming gluconic acid and H_2O_2. The generated H_2O_2 as a second signal can be further identified by PLDz, which performs a self-cleavage reaction in the presence of H_2O_2 and divalent metal ions. A mathematic relationship exists between the cleavage rate of PLDz and the

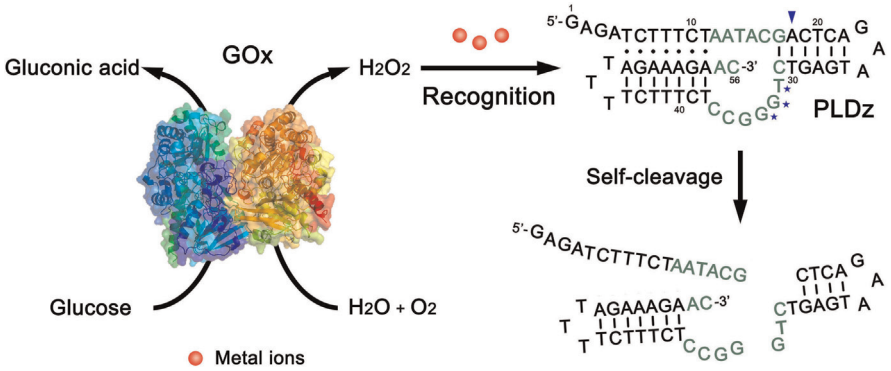

Fig. 1. Schematic illustration of the glucose biosensor (GOx–PLDz 1.0) based on glucose oxidase (GOx) and a pistol-like DNAzyme (PLDz). The left part indicates 3D structure of GOx (Wohlfahrt et al., 1999), and the right part indicates the sequence and secondary structure of a 56-nucleotide self-cleaving PLDz. PLDz consists of a central catalytic core (the green letters), flanked with substrate-binding arms as a triple helix (black dots) and double stranded helix (black lines). The blue arrowhead and asterisks, respectively, indicate the major and minor sites of DNA cleavage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

concentration of glucose in samples. Thus, GOx and PLDz are integrated in the cascade by H_2O_2 and PLDz plays a dual role in both secondary signal recognition and signal output.

3.2. Glucose assay simulation by external H_2O_2

We characterized the GOx–PLDz 1.0 biosensor in the presence of external H_2O_2 to mimic GOx-generated H_2O_2. To investigate the cooperative roles of various metal ions with H_2O_2 on the cleavage activities of PLDz, we performed DNA cleavage experiments in the presence of H_2O_2 and divalent metal ions (Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ba^{2+}, Hg^{2+} and Pb^{2+}) respectively. As demonstrated in Figs. 2A and S1, PLDz showed significant cleavage activities in the presence of H_2O_2/Cu^{2+}, H_2O_2/Mn^{2+} and H_2O_2/Co^{2+}, higher than that of H_2O_2 treatment alone. This result is consistent with the findings in our previous studies (Sun et al., in preparation).

The amount of PLDz that was self cleaved was linear over a concentration range of 10 μM–100 mM H_2O_2 (Fig. 2B), but with relatively low cleavage rates. Different metal ions showed different effects on the cleavage activities of PLDz, the order of activation by divalent metal ions: $Cu^{2+} > Co^{2+} > Mn^{2+}$ (Fig. 2C). The catalytic activity of PLDz exhibited a bell-shaped curve depending on the concentration of Cu^{2+}, with the highest activity found at 100–500 μM; The cleavage rate of PLDz increased linearly with the concentration of Co^{2+} from 10 to 100 μM, reaching a plateau over 100 μM Co^{2+} ions; The concentration of Mn^{2+} from 10 μM to 10 mM did not seem to have a significant impact on the cleavage rate of PLDz.

3.3. Selection of metal ions for the GOx–PLDz 1.0 biosensor

We further replaced external H_2O_2 with GOx-generated H_2O_2 to detect glucose in reaction systems. In theory, GOx catalyzes glucose forming equal amount of H_2O_2, which induces self-cleavage of PLDz as a cofactor. At the concentration of glucose from 1 μM to 10 mM, PLDz demonstrated a significant cleavage rate at a concentration of 500 μM (Figs. 3A and S2). Since metal ions and H_2O_2 cooperate in promoting efficient PLDz cleavage, metal ions were added into the reaction system to improve the detection sensitivity. Based on Fig. 2A, we chose Cu^{2+}, Co^{2+} and Mn^{2+} for tests and their concentrations used in experiments were 500, 100 and 50 μM respectively, according to Fig. 2C. Data showed that Cu^{2+} alone led to background level signal, and combination of Cu^{2+} with GOx resulted in a much more significant cleavage of PLDz. Such a high background signal interferes with the detection of glucose, making Cu^{2+} cofactors not suitable for glucose biosensor construction (Fig. S3). We thought that significant DNA cleavage caused by Cu^{2+} and GOx is due to the cofactor of GOx, flavin adenine dinucleotide (FAD), which non-covalently binds with GOx. Trace amount of FAD dissociates from GOx and participates in the cleavage reaction in the presence of Cu^{2+}, resulting in a higher cleavage rate of PLDz than that of Cu^{2+} treatment alone. DNA self-cleavage caused by combination of Cu^{2+} and FAD is higher than using Cu^{2+} alone. This hypothesis was proven by a cleavage reaction in the presence of Cu^{2+} and FAD (Fig. S4). Meanwhile, we found that smeared bands appeared with elevated concentrations of glucose and DNA was completely degraded in the presence of 10 mM glucose. A possible reason is that higher concentration of glucose generates higher H_2O_2 levels; excess H_2O_2 and Cu^{2+} release high amounts of superoxide anion; excess H_2O_2 and superoxide anion form hydroxyl radicals, leading to oxidative DNA damage (Haber–Weiss cycle, $HO^{\bullet}+H_2O_2 \rightarrow H_2O+O_2^{\bullet-}+H^+$ (1), $O_2^{\bullet-}+H^++H_2O_2 \rightarrow O_2+HO^{\bullet}+H_2O$ (2) (Koppenol, 2001). A simulation experiment using H_2O_2 and Cu^{2+} proved our hypothesis (Fig. S5).

PLDz showed no detectable activity in the presence of either Co^{2+} or Mn^{2+} alone and only exhibited very weak signal with additional GOx, which make Co^{2+} or Mn^{2+} good options for the GOx–PLDz 1.0 biosensor system preparation in the presence of 500 μM Co^{2+}, the detection limit of the GOx–PLDz 1.0 biosensor for glucose is about 50 μM (Fig. S6). Since Co^{2+} has an inhibitory effect on GOx performance (Guascito et al., 2008), the concentration of Co^{2+} was reduced to 100 or 50 μM. Under these conditions, glucose detection limits are still about 50 μM. But the cleavage rate of PLDz showed a better relationship with glucose concentration in the presence of 50 μM of Co^{2+}. Similarly, the detection limits of glucose are all 100 μM upon the addition of 500, 100 and 50 μM of Mn^{2+} respectively. However, the catalytic activity of PLDz has no significant increase with increased concentrations of glucose, indicating that Mn^{2+} has a weak effect on the self-cleavage rate of PLDz (Fig. S7). Above experiments showed that 50 μM Co^{2+} was the best metal condition for PLDz catalysis and chosen for the preparation of the PLDz biosensor system.

3.4. Optimization of the assay conditions

Several experimental parameters were systematically investigated to establish optimal conditions for the proposed GOx–PLDz 1.0 biosensor, including the pH values of the buffer, the concentration of GOx and incubation time. Since PLDz has optimal catalytic activity at pH 7.0 and GOx exhibits optimal activity at pH 5.0–6.0 (Bankar et al., 2009), the designed biosensor could be pH-dependent. Therefore, we investigated the effect of pH on the

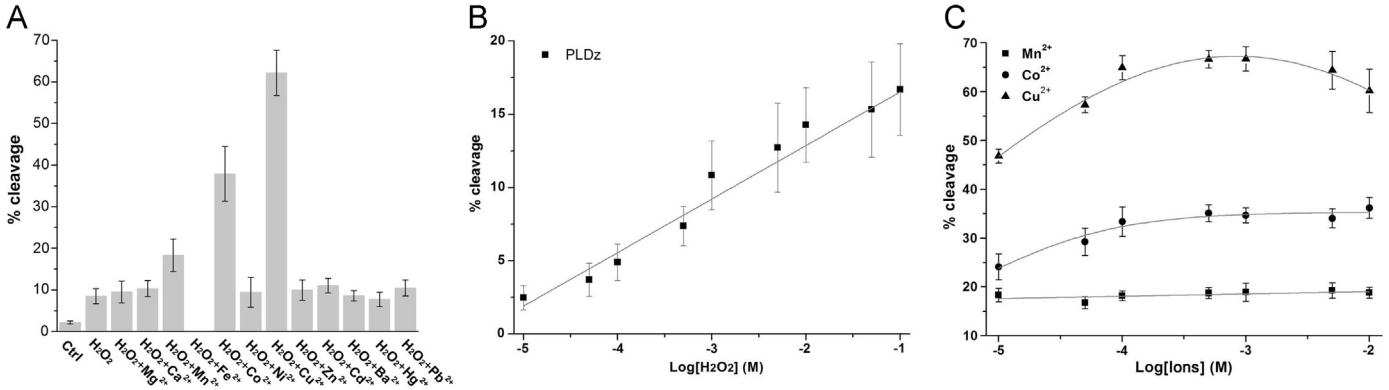

Fig. 2. (A) Effects of metal ions and H_2O_2 on the cleavage rates of PLDz. All experiments were conducted in the presence of 0.5 μM PLDz, 500 μM H_2O_2, 300 mM NaCl, 50 mM Tris–HCl (pH 7.0), with 500 μM metal ions, at 37 °C for 2 h. (B) The relationship between the cleavage rates of PLDz and the concentrations of H_2O_2 in the presence of 0.5 μM PLDz, 300 mM NaCl, 50 mM Tris–HCl (pH 7.0). (C) The relationships between the cleavage rates of PLDz and various amounts of metal ions in the presence of 500 μM H_2O_2. The error bars represented the standard deviation for a series of three measurements.

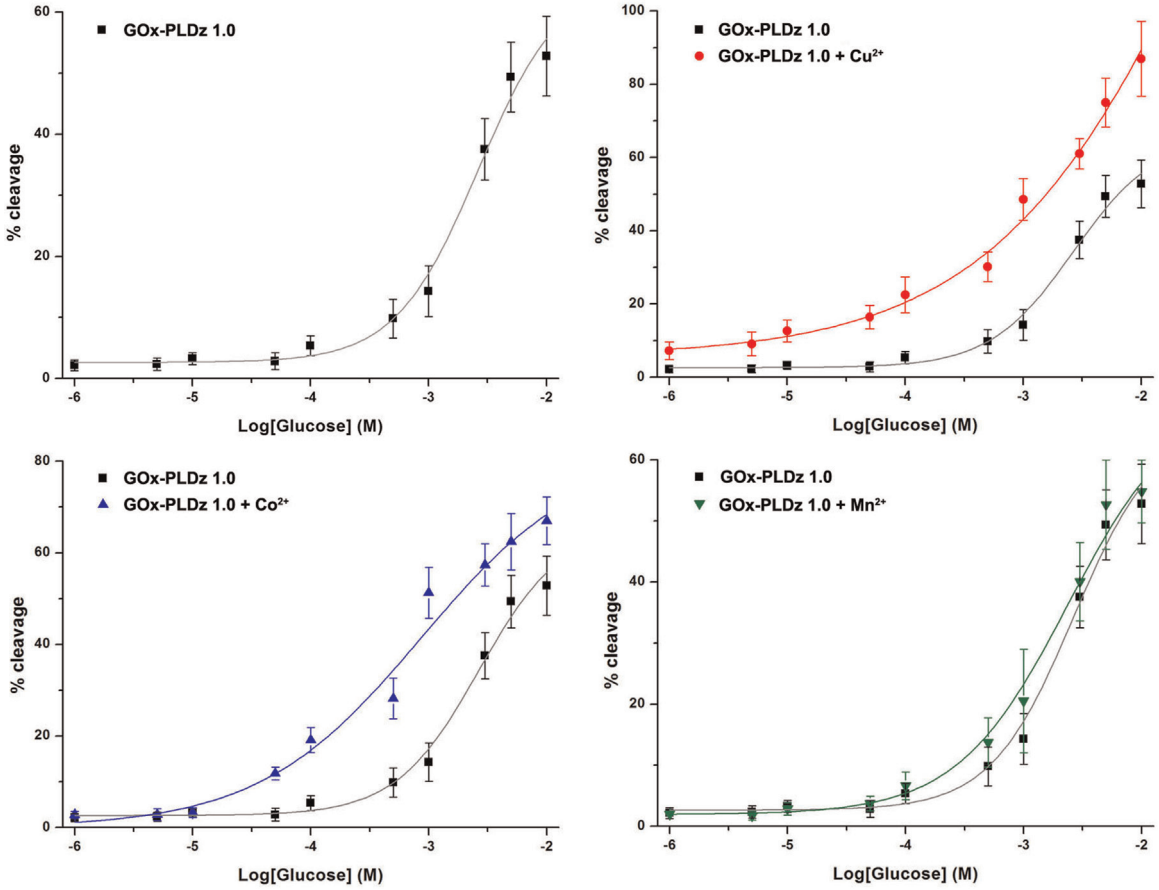

Fig. 3. Selection of metal ions for the GOx–PLDz 1.0 biosensor. (A) Cleavage rates of PLDz in the absence of metal ion. (B–D) Cleavage rates of PLDz in the presence of Cu^{2+}, Co^{2+} and Mn^{2+}, respectively. All experiments were conducted in the presence of 0.5 μM PLDz, 0.1 U/μl GOx, none or 50 μM metal ions, 300 mM NaCl, 50 mM Tris–HCl (pH 7.0), and 0.001–10 mM glucose, at 37 °C for 2 h.

biosensor system from 4.0 to 9.0. The experimental results showed that the maximal signal of the biosensor was obtained at the acidic condition from pH 4.0 to 6.0. The cleavage rate of PLDz decreased sharply from pH 6.4 to 7.4 and kept lower level over pH 8.0 (Figs. 4A and S8A). We further investigated the effect of different concentrations of GOx (0.001–0.3 U/μl) on the biosensor system (Figs. 4B and S8B). The experimental results showed that GOx has little effect on the cleavage rate of PLDz. Thus 0.1 U/μl GOx was adopted in the subsequent work. We optimized the incubation time of the sensing system, and the cleavage rate of the system reached a maximum in 60 min (Figs. 4C and S8C). Hence, incubation time of 60 min was chosen for subsequent experiments. Therefore, the optimal reaction condition is 0.5 μM PLDz, 0.1 U/μl GOx, 50 μM Co^{2+}, 200 mM HAc–NaOAc pH 5.4 and 300 mM NaCl.

3.5. Sensitivity and specificity of the assay

The sensitivity and specificity of the Co^{2+}-dependent GOx–PLDz 1.0 biosensor for glucose was evaluated before its application in a real sample. The detection limit of glucose was determined to be ~5 μM by glucose concentration-dependent assay (Figs. 5A

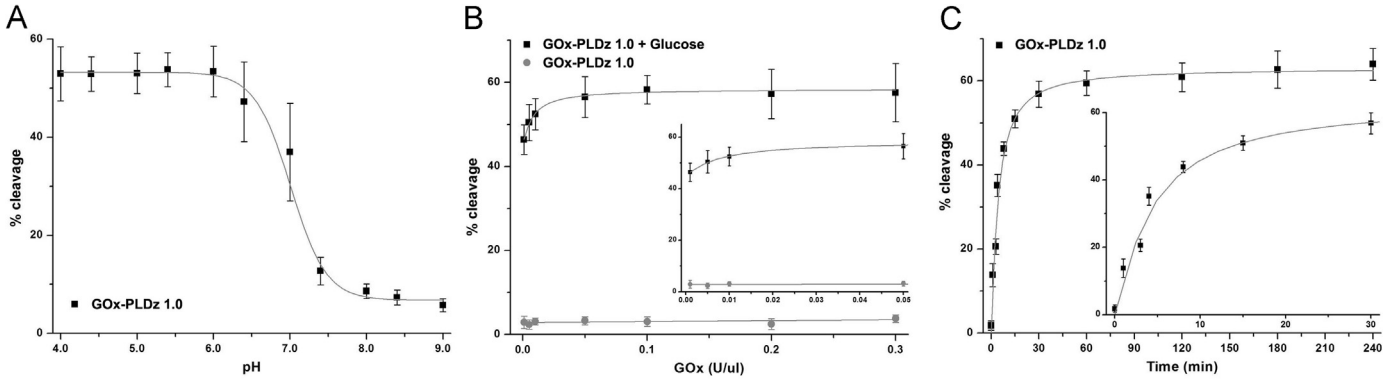

Fig. 4. Optimization of the assay conditions. (A) Effects of pH on the cleavage rate of PLDz. pH buffers: 200 mM HAc–NaOAc (4.0, 4.4, 5.0, 5.4), 50 mM Bis–Tris (6.0, 6.4) and 50 mM Tris–HCl (7.0, 7.4, 8.0, 8.4, 9.0). Experiments were conducted in the presence of 1 mM glucose, 0.5 μM PLDz, 0.1 U/μl GOx, 50 μM Co^{2+}, 300 mM NaCl at 37 °C for 2 h. (B) Effects of GOx concentration on the cleavage rate of PLDz. GOx concentrations was from 0.001 to 0.3 U/μl (200 mM HAc–NaOAc pH 5.4). Inset: rates at the low GOx region. (C) Effects of incubation time on the cleavage rate of PLDz. Incubation time was from 0 to 240 min (0.1 U/μl GOx, 200 mM HAc–NaOAc pH 5.4). Inset: rates at the early time region.

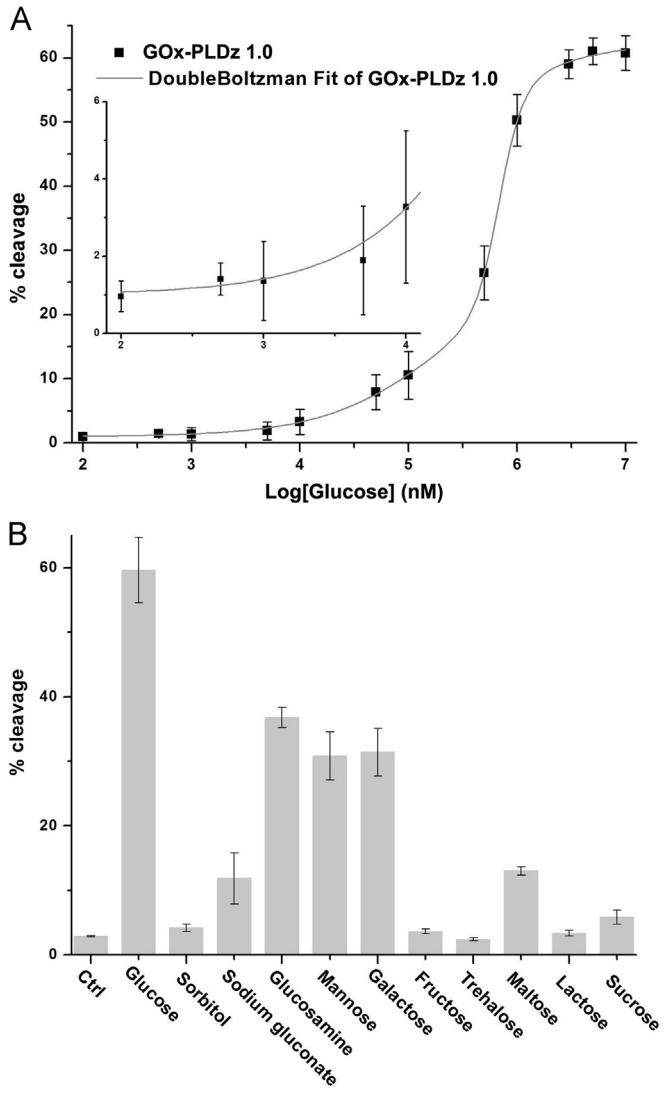

Fig. 5. Sensitivity and specificity of the GOx–PLDz 1.0 biosensor. (A) Sensitivity of the GOx–PLDz 1.0 biosensor. Glucose concentration: 0.1 μM–10 mM. Reaction condition: 0.1 U/μl GOx, 0.5 μM PLDz, 50 μM Co^{2+}, 300 mM NaCl and 200 mM HAc–NaOAc (pH 5.4), at 37 °C for 1 h. Double Boltzmann curve describes a relationship between the cleavage rate of PLDz and the concentration of glucose. Inset: rates at the low glucose region. (B) Selectivity of the GOx–PLDz 1.0 biosensor.

and S9). Data for the cleavage rate of PLDz and the concentration of glucose were used for curve fitting and data fits Double Boltzmann curve best with a $R^2=0.99$ (Section 2).

In order to demonstrate the selectivity of the GOx–PLDz 1.0 biosensor, many glucose analogs (sorbitol, sodium gluconate, glucosamine, mannose, galactose, fructose, trehalose, maltose, lactose, sucrose) were examined. The GOx–PLDz 1.0 biosensor system exhibited obvious response upon treating with mannose, galactose and glucosamine. Meanwhile, the GOx–PLDz 1.0 biosensor also has slight response on sodium gluconate and maltose (Figs. 5B and S10). This is because that GOx also recognizes β-D-glucose analogs (mannose and galactose) (Gibson et al., 1964; Leskovac et al., 2005). High-specificity of GOx means that it binds specifically to β-D-glucose and does not act on α-D-glucose.

In the above experiments, the GOx–PLDz 1.0 biosensor was constructed using *cis*-PLDz, which exhibited self-cleaving activity and led to higher background noise to interfere with the detection assay. To reduce background signal, we further designed a GOx–PLDz 1.1 biosensor based on *trans*-PLDz (Fig. S11). However, the experimental results indicated that the cleavage rate of *trans*-PLDz had decrease and its detection limit of glucose was only 50 μM (Fig. S12). A possible reason is that *trans*-PLDz could not efficiently recognize and bind with its DNA substrate and result in low catalytic rates, even with denaturation and refolding processes.

3.6. Analytical applications in saliva and tear samples

Normal glucose levels in humans are about 4–8 mM in blood, 0.1–0.6 mM in tears and 0.008–0.21 mM in saliva (Berman, 1991; Yamaguchi et al., 1998; Baca et al., 2007). Some studies indicate that there exist relationships between blood glucose levels and glucose levels in tears also saliva (Sen and Sarin, 1980; Yamaguchi et al., 1998; Baca et al., 2007; Jurysta et al., 2009). The glucose detection limit of our designed GOx–PLDz 1.0 biosensor is 5 μM, lower than the glucose levels both in saliva and tears. Thus, the GOx–PLDz 1.0 biosensor can be further applied to monitor glucose levels in saliva and tears. Onion-induced tears and psychology-stimulated saliva were collected for detection assay (Fig. S13). Samples from tears and saliva contain large amounts of proteins, which have been removed by heat denaturation and centrifugation before assay. Data showed that the GOx–PLDz 1.0 biosensor exhibited good responses on the glucose in tears and saliva. Samples from tears showed better signal than that from saliva, indicating tears contain higher glucose level than saliva. According to the Double Boltzmann equation, determined glucose levels are $720 \pm 81$and 405 ± 56 μM in tears and saliva respectively. Although the GOx–PLDz 1.0 biosensor also has responses on mannose, galactose and glucosamine, none or low levels of them in samples lead to non-interfering assay. We further investigated the effects of sample volumes on GOx–PLDz 1.0 biosensor signal. Output signal increased with increasing of sample volumes (Figs. 6B and S14). However, large sample volumes also interfere with the electrophoresis migration due to its high levels of proteins and polysaccharides in samples.

4. Discussion

Induction of DNAzyme into glucose biosensor provides a new approach in biosensor design. As a non-invasive glucose biosensor, the GOx–PLDz 1.0 is able to measure the levels of glucose in tears and saliva, which is significant for diabetic patients to frequently/continuously monitor of blood glucose (Makaram et al., 2014). However, we also noticed that there are some limitations in the current version of GOx–PLDz biosensor. For example, detection requires a relatively long time (more than one hour); gel electrophoresis is not simple and causes a larger determinable error; sample volume for analysis is large (comparing to 30 μl sample requirement for the current version of biosensor, 1 μl sample requirement would be the goal for the expected version of biosensor); onion induction causes eye discomfort, although it is efficiency to obtain tears. To solve the above problems, we would improve the current biosensor as follows: (1) Develop highly H_2O_2-sensitive DNAzyme via mutations or *in vitro* evolution to decrease detection time. (2) Induce nano-materials into the GOx–PLDz biosensor (Fig. S15A), such as gold nanoparticles (AuNPs) (Zeng et al., 2012; Liang et al., 2014; Lin et al., 2014b). Immobilize PLDz and GOx on gold nanoparticles, so that H_2O_2 generated by GOx can be recognized by nearby PLDz to improve detection sensitivity. In addition, color changes depending on the size of the gold particles would make the GOx–PLDz biosensor as a visualizing biosensor. (3) Replace gel electrophoresis assay by fluorescence spectroscopy using fluorophore labeled DNA to improve the sensitivity of GOx–PLDz biosensor (Fig. S15B) (Zadran et al. 2012). (4) Induction of polymerase chain reaction (PCR) or rolling circle amplification (RCA) into the GOx–PLDz biosensor results in

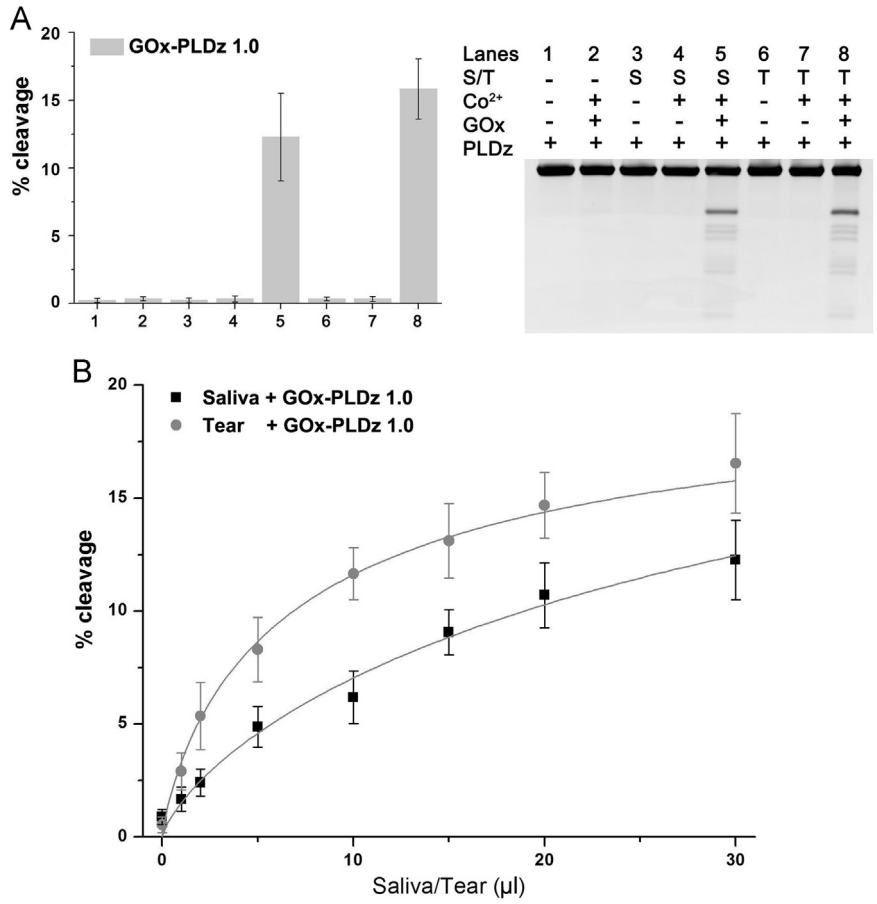

Fig. 6. Determine glucose levels in saliva and tears. (A) Detect glucose levels in saliva and tear samples. Left: column chart using data derived from the gel depicts the cleavage rates of PLDz in samples. Right: PLDz undergo self-cleavage in samples and the resulting products are separated from the precursor by 20% denaturing PAGE. S: saliva, T: tear, sample volume is 30 μl. (B) Relationships between the cleavage rates of PLDz and sample volumes. Sample volumes: 1–30 μl.

enhanced signal response (Xu et al., 2014; Zhuang et al., 2014). (5) Convert the GOx–PLDz biosensor into a monitoring platform, facilitated to detect varied target molecules (Xiang and Lu, 2011; Tram et al., 2014).

5. Conclusion

We successfully applied a DNA-cleaving DNAzyme for glucose biosensor design and generated a dual-enzyme cascade biosensor (GOx–PLDz 1.0) mediated by H_2O_2.The relationship between glucose concentration and the cleavage rates of PLDz was simulated as Double Boltzmann equation. The designed GOx–PLDz 1.0 biosensor is able to determine glucose levels both in tears and saliva as a noninvasive assay, complying with the tide of developments in biosensors.

Acknowledgements

This research was supported by the Fundamental Research Funds for the Central Universities (450060501435, 450060488087 and 450060445666) and National Natural Science Foundation of China (31100573).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.03.070.

References

Baca, J.T., Finegold, D.N., Asher, S.A., 2007. Clin. Sci. 5, 280–293.
Bankar, S.B., Bule, M.V., Singhal, R.S., Ananthanarayan, L., 2009. Biotechnol. Adv. 27, 489–501.
Berman, E.R., 1991. Biochemistry of the Eye, New York, pp. 70.
Breaker, R.R., Joyce, G.F., 1994. Chem. Biol. 1, 223–229.
Calcutt, N.A., Cooper, M.E., Kern, T.S., Schmidt, A.M., 2009. Nat. Rev. Drug Discov. 8, 417–429.
Carmi, N., Balkhi, S.R., Breaker, R.R., 1998. Proc. Natl. Acad. Sci. USA 95, 2233–2237.
Carmi, N., Breaker, R.R., 2001. Bioorg. Med. Chem. 9, 2589–2600.
Chaturvedi, P., Vanegas, D.C., Taguchi, M., Burrs, S.L., Sharma, P., McLamore, E.S., 2014. Biosens. Bioelectron. 58, 179–185.
Chen, X., Wang, Y., Liu, Q., Zhang, Z., Fan, C., He, L., 2006. Angew. Chem. Int. Ed. 45, 1759–1762.
Cui, J., Adeloju, S.B., Wu, Y., 2014. Anal. Chim. Acta 809, 134–140.
Gao, L., Zhang, H., Cui, H., 2014. Biosens. Bioelectron. 57, 65–70.
Gibson, Q.H., Swoboda, B.E., Massey, V., 1964. J. Biol. Chem. 239, 3927–3934.
Gifford, R., 2013. ChemPhysChem 14, 2032–2044.
Gruhl, F.J., Rapp, B.E., Länge, K., 2013. Adv. Biochem. Eng. Biotechnol. 133, 115–148.
Guascito, M.R., Malitesta, C., Mazzotta, E., Turco, A., 2008. Sens. Actuators B Chem. 131, 394–402.
He, Y., Wang, X., Sun, J., Jiao, S., Chen, H., Gao, F., Wang, L., 2014. Anal. Chim. Acta 810, 71–78.
Höbartner, C., Silverman, S.K., 2007. Recent advances in DNA catalysis. Biopolymers 87, 279–292.
Jiang, D., Xu, J., Sheng, Y., Sun, Y., Zhang, J., 2010. FEBS J. 277, 2543–2549.
Jurysta, C., Bulur, N., Oguzhan, B., Satman, I., Yilmaz, T.M., Malaisse, W.J., Sener, A., 2009. J. Biomed. Biotechnol. 2009, 430426.
Khan, M., Park, S.Y., 2014. Anal. Chem. 86, 1493–1501.
Koppenol, W.H., 2001. Redox Rep. 6, 229–234.
Lawal, A.T., 2015. Talanta 131, 424–443.
Leng, J., Wang, W.M., Lu, L.M., Bai, L., Qiu, X.L., 2014. Nanoscale Res. Lett. 9, 99.
Leskovac, V., Trivic, S., Wohlfahrt, G., Kandrac, J., Pericin, D., 2005. Int. J. Biochem. Cell Biol. 37, 731–750.
Liang, H., Zhang, X., Lv, Y., Gong, L., Wang, R., Zhu, X., Yang, R., Tan, W., 2014. Acc. Chem. Res. 47, 1891–1901.

Lin, Y., Ren, J., Qu, X., 2014a. Acc. Chem. Res. 47, 1097–1105.
Lin, Y., Ren, J., Qu, X., 2014b. Adv. Mater. 26, 4200–4217.
Liu, J., Cao, Z., Lu, Y., 2009. Chem. Rev. 109, 1948–1998.
Lu, Y., Liu, J., 2006. Curr. Opin. Biotechnol. 17, 580–588.
Makaram, P., Owens, D., Aceros, J., 2014. Diagnostics 4, 27–46.
Malashikhina, N., Pavlov, V., 2012. Biosens. Bioelectron. 33, 241–246.
Miao, X., Ling, L., Cheng, D., Shuai, X., 2012. Analyst 137, 3064–3069.
Scognamiglio, V., 2013. Biosens. Bioelectron. 47, 12–25.
Sen, D.K., Sarin, G.S., 1980. Br. J. Ophthalmol. 64, 693–695.
Sun, Y., Ma, R., Liu, C., Wang, S., Sheng, Y., Feng, J., Zhang, J., Xu, J., Jiang, D., 2015 (in preparation).
Tram, K., Kanda, P., Salena, B.J., Huan, S., Li, Y., 2014. Angew. Chem. Int. Ed. 53, 1–5.
Wohlfahrt, G., Witt, S., Hendle, J., Schomburg, D., Kalisz, H.M., Hecht, H.J., 1999. Acta Crystallogr. D Biol. Crystallogr. 55, 969–977.
Xu, J., Sun, Y., Sheng, Y., Fei, Y., Zhang, J., Jiang, D., 2014. Anal. Bioanal. Chem. 406, 3025–3029.
Xiang, Y., Lu, Y., 2011. Nat. Chem. 3, 697–703.
Yamaguchi, M., Mitsumori, M., Kano, Y., 1998. IEEE Eng. Med. Biol. Mag. 17, 59–63.
Yoo, E., Lee, S., 2010. Sensors 10, 4558–4576.
Zadran, S., Standley, S., Wong, K., Otiniano, E., Amighi, A., Baudry, M., 2012. Appl. Microbiol. Biotechnol. 96, 895–902.
Zeng, D., Luo, W., Li, J., Liu, H., Ma, H., Huang, Q., Fan, C., 2012. Analyst 137, 4435–4439.
Zhang, X., Kong, R., Lu, Y., 2011. Annu. Rev. Anal. Chem. 4, 105–128.
Zhuang, J., Lai, W., Chen, G., Tang, D., 2014. Chem. Commun. 50, 2935–2938.