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ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · JANUARY 2013

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Target-Responsive "Sweet" Hydrogel with Glucometer Readout for Portable and Quantitative Detection of Non-Glucose Targets

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Supporting Information

ABSTRACT: Portable devices with the advantages of rapid, on-site, user-friendly, and cost-effective assessment are widely applied in daily life. However, only a limited number of quantitative portable devices are commercially available, among which the personal glucose meter (PGM) is the most successful example and has been the most widely used. However, PGMs can detect only blood glucose as the unique target. Here we describe a novel design that combines a glucoamylase-trapped aptamer-cross-linked hydrogel with a PGM for portable and quantitative detection of non-glucose targets. Upon target introduction, the hydrogel collapses to release glucoamylase, which catalyzes the hydrolysis of amylose to produce a large amount of glucose for quantitative readout by the PGM. With the advantages of low cost, rapidity, portability, and ease of use, the method reported here has the potential to be used by the public for portable and quantitative detection of a wide range of non-glucose targets.

For modern analytical systems, it is highly desired to make detection more precise, accurate, selective, sensitive, and reliable and, from the practical point of view, to reduce the cost, enhance the speed, simplify the procedure, and enable multiplexing. However, considering the difficulty of meeting all these criteria, compromises always exist. For instance, although many well-developed methods and techniques, such as mass spectrometry,[1] chromatography,[2] spectroscopy,[3] and enzyme-linked immunosorbent assay (ELISA),[4] can achieve highly accurate and sensitive detection, most of them are expensive and require complicated instruments and sophisticated operations involving professional personnel in laboratories. However, as proposed by the World Health Organization, the ASSURED[5] standard requires the diagnostic techniques used in developing countries to be affordable, sensitive, specific, user-friendly, fast, robust, equipment-free, and deliverable to end users. Point-of-care testing (POCT) is considered one of techniques to satisfy these requirements for applications in the field by first responders in disaster situations, in home healthcare, for medical testing facilities in rural areas, and for environmental monitoring in field work.[6] In recent decades, considerable effort has been devoted to the development of POC devices, such as commercial pregnancy tests, patterned paper devices,[5,7] visual detection platforms,[8] microfluidic systems,[9] and other novel sensors.[10]

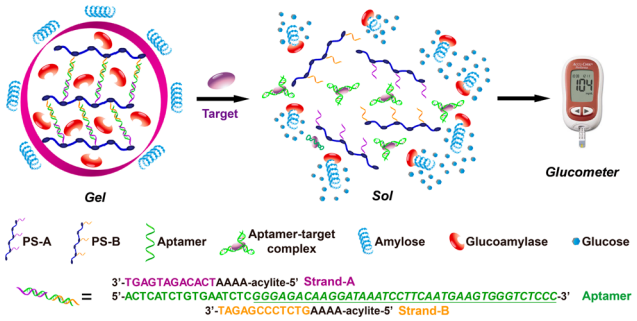
In spite of substantial progress, only a limited number of quantitative POC devices are commercially available, most notably personal glucose meters (PGMs), which are widely used because of their portable size, easy operation, low cost, and reliable quantitative results. However, PGMs can detect only glucose as the target. Recently, Lu's group reported an elegant method that combines functional DNA probes with a PGM for multitarget analysis,[11] enabling a number of non-glucose targets to be detected quantitatively. However, the method requires the conjugation of DNA to invertase through several chemical reactions that might affect the structure and function of the enzyme to a certain extent. New signal transduction strategies that provide simple sample processing without enzyme structure modification while still allowing efficient conversion of the target recognition event into a cascaded glucose production reaction are thus highly desirable. Herein we present a simple and general method based on a target-responsive "sweet" hydrogel combined with a PGM (SH-PGM) for the detection of a wide range of non-glucose targets. The term "sweet" refers to the generation of glucose upon target recognition.

The basic design of the target-responsive hydrogel was adapted from our previously reported colorimetric visual detection platform based on an enzyme-encapsulated aptamer-cross-linked hydrogel.[8c] However, visual detection can provide only qualitative and semiquantitative results. Thus, to enable quantitative analysis, we propose the concept of a target-responsive "sweet" hydrogel encapsulating glucoamylase, which allows efficient conversion of the target recognition event into a cascaded glucose production reaction for subsequent PGM readout. As schematically illustrated in Scheme 1, two short DNA sequences (strands A and B) are grafted onto linear polyacrylamide polymers by copolymerization of acrylic DNA and acrylamide monomers to form polymer strands A and B (PS-A and PS-B). Strands A and B are complementary to adjacent areas of a DNA aptamer sequence. Upon the addition of aptamer, strands A and B hybridize with the aptamer to yield a three-stranded complex, thus cross-linking PS-A and PS-B into a hydrogel with glucoamylase trapped inside. In the absence of

Received: November 22, 2012

   dx.doi.org/10.1021/ja3114714 | J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

Scheme 1. Schematic Illustration of the SH-PGM Method[a]



[a]An aptamer-cross-linked hydrogel trapped with glucoamylase is formed by hybridization of the aptamer and its partially complementary DNA polymer strands (PS-A and PS-B). When target molecules are introduced, the aptamers specifically identify the targets to form target−aptamer complexes, causing breakdown of the hydrogel and release of glucoamylase, which catalyzes the hydrolysis of amylose to produce a large amount of glucose for quantitative readout by the glucometer. With cocaine aptamer as an example, the aptamer (shown in underlined italic type) binds with its complementary strand A and strand B in a sandwich structure as the cross-linker of the hydrogel.

target, the glucoamylase is stably trapped inside the gel and physically separated from its substrate, amylose, which is in the solution outside the gel. When target molecules are introduced, aptamers specifically and preferentially bind the targets to form target−aptamer complexes, leading to breakdown of the hydrogel and release of glucoamylase, which catalyzes the hydrolysis of amylose to produce a large amount of glucose for quantitative readout by the PGM. As a result, the SH-PGM method retains rapid and simple operation while realizing quantitative analysis using a simple, low-cost, user-friendly, and portable PGM. Since only DNA hybridization is needed to construct the hydrogel and no enzyme modification or special features of the aptamer are required, this strategy is generic and simple and can be applied with various aptamer sequences for portable and quantitative detection of a wide range of non-glucose targets.

To realize non-glucose target detection using a PGM, the relationship between target recognition and glucose generation must be established. In our design, target introduction decomposes the hydrogel to release the trapped enzyme, which then digests its substrate to generate glucose for PGM readout. Thus, the ideal enzyme must be large enough to be trapped inside the hydrogel but also must be quickly released upon target addition. Moreover, after release it must be able to hydrolyze amylose fully within a short time and generate glucose in high yield. Generally, there are three types of amylase (α-, β-, and γ-amylase), all of which are large macromolecules that can be trapped inside the gel.[12] We compared the amylose-hydrolyzing efficiency and glucose-producing efficiency of these three types of enzymes. A KI/I_2 solution was added to monitor the process of amylose breakdown, since the amylose−I_2 complex has a distinct blue color at 574 nm. As shown in Figure 1 (red bars), after 30 min of reaction, the hydrolyzing efficiencies were nearly 100% for γ-amylase, 90.9% for α-amylase, and only 4% for β-amylase. The glucose yields were further compared by using a PGM to measure the amount of glucose produced (Figure 1, blue bars); no detectable reading for β-amylase and average readings of 3.0 mM for α-amylase and 9.53 mM for γ-amylase were obtained. Although the hydrolysis efficiencies of α- and γ-amylase were

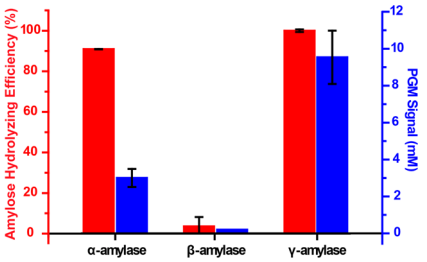


Figure 1. Comparison of the amylose-hydrolyzing efficiencies of α-, β-, and γ-amylase obtained by measuring the absorbance of amylose-KI/I_2 solutions at 547 nm (red bars, left axis) and of relative glucose-producing efficiencies of α-, β-, and γ-amylase obtained by reading glucose concentrations through the PGM (blue bars, right axis).

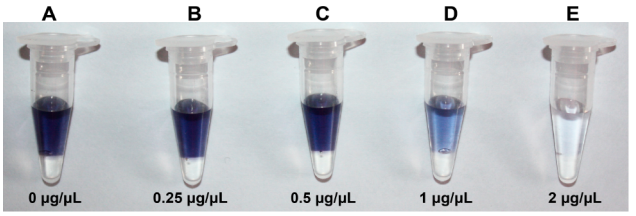


Figure 2. Study of the trapping capacity of hydrogels with cross-linking DNA concentrations of 0.7 mM. All of the experiments were performed at 25 °C with the same concentration of amylose substrate. From (A) to (E), the concentrations of glucoamylase in the hydrogel were 0, 0.25, 0.5, 1, and 2 μg/μL, respectively.

similar (>90% yield), the production of glucose by α-amylase was only one-third that of γ-amylase, leading to a lower PGM readout for α-amylase. Therefore, γ-amylase (also called glucoamylase), which can rapidly and efficiently hydrolyze amylose with a glucose yield of nearly 100%, is the ideal enzyme to use in our method.

To test the performance of the SH-PGM system, we first demonstrated quantitative detection of cocaine using a cocaine aptamer hydrogel. To prevent the abuse of cocaine, a highly addictive drug, the ability to detect small doses quantitatively is essential. The hydrogel was prepared with 4% acrylamide and cross-linking DNA (PS-A, PS-B, and aptamer) concentrations of 0.7 mM. Since the amount of trapped glucoamylase is strongly related to the sensitivity of the system, we further optimized this parameter. A greater concentration of enzyme in the gel should lead to increased glucose production to ensure highly sensitive quantitative detection. However, the loading capacity of a given hydrogel is limited. Once beyond the capacity, the redundant enzymes cannot be trapped well and will digest the substrate even in the absence of target, causing false-positive signals. Thus, the amount of glucoamylase has to be precisely controlled by trapping as much as possible but within the loading capacity of the hydrogel. To optimize the amount of gluocoamylase, KI/I_2 and amylose solutions were added to a series of hydrogels doped with different concentrations of glucoamylase (0−2 μg/μL). As shown in Figure 2, enzyme leakage became a serious problem for the two highest enzyme concentrations (1 and 2 μg/μL), both of which caused rapid color fading of the blue amylose-KI/I_2 solution. In contrast, there was no observable absorption change for the hydrogels with enzyme concentrations less than 0.5 μg/μL even after incubation for 1 h. To avoid enzyme leakage and trap as much enzyme as possible in our system, we chose to use an enzyme concentration of 0.5 μg/μL in subsequent experiments.

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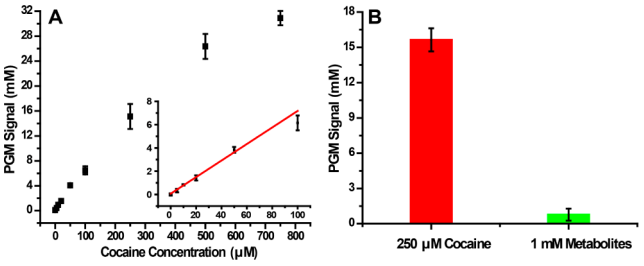

Figure 3. (A) Performance of the SH-PGM system for cocaine detection. A linear standard curve from 0 to 750 μM was obtained with a detection limit of 3.8 μM (1.2 μg/mL). (B) Responses of the SH-PGM system to 250 μM cocaine and 1 mM benzoyl ecgonine and ecgonine methyl ester in SH-PGM buffer.

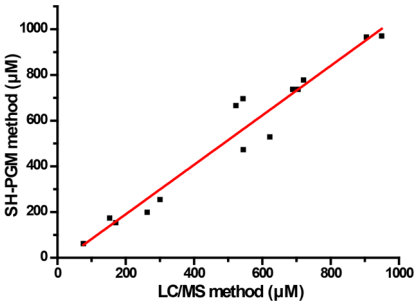

Figure 4. Comparison of the SH-PGM method with the standard LC/MS method based on 14 samples in urine.

After system optimization, the SH-PGM method was applied to quantify the concentration of cocaine. Cocaine hydrochloride standards with different concentrations were prepared. Reactions were carried out in Eppendorf tubes with 15 μL of glucoamylase-doped hydrogel and 20 μL of the solution phase containing amylose and cocaine. The tubes were shaken occasionally during the reaction at 25 °C for 1 h, after which a 0.6 μL aliquot of the supernatant was removed for glucose measurement using the PGM. Target (cocaine) concentrations of 0−750 μM were tested with three measurements each in parallel. As shown in Figure 3A, samples containing cocaine were able to produce PGM signals, confirming that the hydrogel could respond to cocaine to trigger glucose generation for PGM readout. More importantly, the PGM signal was proportional to the concentration of cocaine up to ~750 μM, establishing the quantitative detection capability of the SH-PGM platform. A detection limit of 3.8 μM (1.2 μg/mL) cocaine was observed, based on $3\sigma_b$/slope, where σ_b was the standard deviation of blank samples. Therefore, our system has a sensitivity comparable to those of commercial cocaine test kits, such as the Instant-View Cocaine Urine Dip-Strip Test (0.3 μg/mL). The gel system was further optimized by prolonging the reaction time to 2 h to reduce the detection limit down to 1.6 μM, which corresponds to <0.5 μg/mL (Figure S3 in the Supporting Information). To demonstrate the selectivity of the SH-PGM system, the cocaine metabolites benzoyl ecgonine and ecgonine methyl ester were applied as negative controls. Upon addition of 1 mM cocaine metabolites, a negligible signal was observed. In contrast, cocaine at only 250 μM produced a PGM signal that was almost 20-fold stronger than that of 1 mM cocaine metabolites (Figure 3B). This result indicates our SH-PGM system retained the aptamer selectivity with a highly specific response to the target, cocaine.

The urine test, one of the most popular methods for cocaine monitoring, is noninvasive and relatively inexpensive and can detect cocaine for up to 72 h after use.[13] Urine samples spiked with different concentrations of cocaine were tested with our SH-PGM method, which was able to give a quantitative response to cocaine in urine. The calculated detection limit was 4.4 μM (Figure S4A). Although a blood test is a more invasive form of testing and must be conducted in a medical laboratory, it is also the most accurate means to determine whether an individual is under the influence of cocaine. Therefore, we also quantitatively detected cocaine in 50% human plasma (Figure S4B), and the detection limit was 7.7 μM. These results obtained in both urine and plasma were close to that obtained in buffer, indicating that the other components in urine and plasma provide little or no interference with the performance of our SH-PGM method, thereby demonstrating the wide applicability of this method in complex body fluids.

Traditional cocaine detection relies primarily on large and expensive instruments, such as GC,[14] GC/MS[15] and LC/MS.[16] To verify the accuracy and reliability of our system, we compared it with the LC/MS method for cocaine detection (Figure 4).[16a] A total of 14 samples in urine were evaluated. A strong positive correlation between these two methods was found, with a slope of 1.08 ± 0.07 and a correlation coefficient of 0.97, demonstrating that the results from the two methods matched within the experimental error. These results strongly suggest that the accuracy of the SH-PGM method is as good as that of the standard cocaine detection method, indicating the suitability and reliability of the SH-PGM as an alternative test method that is inexpensive, rapid, portable, and user-friendly.

In certain cases, endogenous glucose in samples may interfere with the final results. To circumvent this problem, the glucose concentration in an unknown sample must be measured using the PGM prior to target detection. If a small detectable background signal is obtained, it can be subtracted from the signal obtained in the subsequent actual test. However, once the background signal plus the signal generated by the target exceeds the upper limit of the PGM readout, the background subtraction method would be invalid. To overcome high glucose backgrounds, glucose oxidase can be introduced prior to target detection to oxidize the pre-existing β-D-glucose to PGM-inert D-gluconolactone,[17] thus eliminating the background signal. Subsequently, the glucose oxidase can be deactivated by heating. As shown in Figure S5, the results of cocaine detection in buffers containing different concentrations of glucose after both treatments were comparable to those in the glucose-free buffer. Thus, both sample pretreatment methods are reliable for successfully eliminating glucose interference, which may occur in samples from hyperglycemia patients.

Another consideration, especially for public or in-field use, is the acceptable shelf life of the hydrogel. After refrigerated storage for 3 months, our hydrogel performed as well as freshly prepared material (Figure S6), establishing that the hydrogel is robust, stable, and reliable.

Since DNA hybridization is the only requirement of the aptamer-hydrogel design, our SH-PGM method should have the versatility to detect other targets when other aptamer sequences are used. To verify such generality, we designed an ATP sensor based on the same principle by using ATP aptamer and its partially complementary strands to cross-link the hydrogel.[18] This ATP SH-PGM method allowed the quantitative analysis of ATP with a linear range from 0 to 1 mM (Figure 5A). Meanwhile, control experiments with ATP analogues such as UTP, CTP, and GTP did not produce enhanced PGM readings, indicating good

dx.doi.org/10.1021/ja3114714 | J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

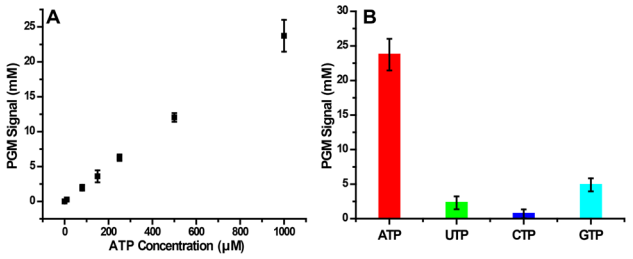

Figure 5. (A) Detection of ATP with the SH-PGM. (B) Selectivity of the SH-PGM system with 1 mM ATP, UTP, CTP, and GTP.

selectivity for this ATP SH-PGM system (Figure 5B). Therefore, this strategy is a generic approach that can be modified with different aptamer sequences for portable and quantitative detection of a wide range of non-glucose targets.

In summary, we have designed a simple and general method based on a target-responsive "sweet" hydrogel and a personal glucose meter for inexpensive, rapid, portable, user-friendly, and quantitative detection of a wide range of non-glucose targets. The SH-PGM method offers several advantages. First of all, the method is designed to meet the ASSURED standard[5] and is sensitive, selective, and reliable compared with standard instrumental analysis methods. Second, the physical trapping of glucoamylase inside the hydrogel provides a quantitative relationship between the target concentration and PGM readout without any troublesome chemical modification of the enzyme, which otherwise may decrease the enzyme activity. Moreover, as a variety of aptamers binding a broad range of targets are either available or can be obtained through systematic evolution of ligands by exponential enrichment (SELEX), the method developed here can be used as a powerful tool for rapid and quantitative assessment of other targets simply by inclusion of specific aptamer sequences in the hydrogel. In view of the low cost, rapid detection, user-friendliness, and wide availability of PGMs, the SH-PGM method reported here has the potential to be used by the public for quantitative detection of a wide range of non-glucose targets.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and additional characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the National Basic Research Program of China (2010CB732402), the National Natural Science Foundation of China (21205100, 21275122 and 21075104), Natural Science Foundation of Fujian Province for Distinguished Young Scholars (2010J06004), National Instrumentation Program (2011YQ03012412), and National Fund for Fostering Talents of Basic Science (J1210014), and General Administration of Quality Supervision, Inspection and Quarantine (2010IK150, 2012JK052) for financial support. We acknowledge Dr. K. R. Williams for manuscript review.

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