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An extremely sensitive monoboronic acid based fluorescent sensor for glucose

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Abstract

An extremely sensitive monoboronic acid based fluorescent sensor for glucose was developed. This was carried out by assembling a fluorescent monoboronic acid, 3-aminophenylboronic acid (PBA) indirectly onto gold surface via its electrostatic interaction with cysteine (Cys) that was directly assembled on the gold surface. The formation of self-assembled bilayers (SAB) was confirmed and primarily characterized by cyclic voltammetry and X-ray photoelectron spectra (XPS). The SAB containing PBA was found fluorescent and its fluorescence showed an extremely high sensitivity to the presence of glucose and other monosaccharides such as galactose and fructose with quenching constants at $10^8\ M^{-1}$ order of magnitude compared to those at $10^2\ M^{-1}$ in bulk solutions. The quenching constants were found to vary in the order of D-glucose > D-galactose > D-fructose > D-mannose that is different from that in bulk solution which shows the highest binding affinity toward D-fructose and very low sensitivity toward glucose. The reported monoboronic acid based SAB fluorescent sensor showed the highest sensitivity towards glucose with the capacity of detecting saccharides of concentration down to nanomolar level. It was also demonstrated that the fluorescence from PBA/Cys/Au can be easily recovered after each measurement event and therefore also represents a new reusable method for immobilizing reagent in fabricating chemosensors.

Keywords: Self-assembled bilayer; Fluorescent sensor; Monoboronic acid; Glucose; Saccharide

1. Introduction

Development of synthetic molecular receptors for neutral organic species of important biological relevance such as saccharides has been a subject of intensive recent interest. Boronic acid has been an important structural unit for constructing receptors for saccharides [1–5] because of its ability of feasible covalent binding of saccharides in aqueous alkaline media. The reported monoboronic acid based receptors show selective recognition for D-fructose with binding constants at $10^2\ M^{-1}$ orders of magnitude and low sensitivity for glucose detection. However, detection and monitoring of glucose is particularly important because of the rapidly increasing number of diabetics and the results that a tight control of the blood glucose levels in diabetics reduces the risk of long terms complications [6,7].

It was shown that receptors with multiple boronic acid binding sites in good arrangement could show enhanced binding affinity towards glucose [1,2], although sophisticated organic synthesis is required. This provides a hint of constructing efficient boronic acid based receptors highly selective for glucose by making boronic acid groups in good "proximity". Self-assembled monolayers (SAMs) are stable monolayers in which the forming molecules are arranged in a well-defined relationship. It appears to us that by employing SAMs it would be possible to construct monoboronic acid based sensors good for glucose. Actually SAMs have been extensively applied to chemo- and bio-sensing in which the signaling mechanisms hitherto employed are mainly electrochemical [8,9]. SAMs based fluorescent sensors development, however, has encountered difficulties in ensuring fluorescence emission, likely because of an efficient fluorescence quenching on metal surfaces [10]. Although recent

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reports showed that SAMs based fluorescent sensors could indeed be constructed in cases where structurally modified alkylthiols were employed or mixed SAMs were involved [11,12], complicated organic syntheses were needed. In the present report we show that indirectly assembling a fluorophore, 3-aminophenylboronic acid (PBA), onto gold surface via its electrostatic interaction with cysteine that is directly assembled on the gold surface could efficiently eliminate the fluorescence quenching by gold. The such-formed self-assembled bilayer (SAB) was found to work as a highly sensitive fluorescent sensor for saccharides in alkaline aqueous media with the highest binding affinity shown for glucose at $10^8\,M^{-1}$ order of magnitude.

2. Experimental

2.1. Apparatus

Electrochemical measurements were carried out on a BAS-100B electrochemical analyzer with a conventional three-electrode system using bare or modified Au as working electrode, platinum wire as counter electrode, and saturated calomel electrode (SCE) as reference electrode. The solutions were bubbled with N_2 for 10 min prior to and during the application of potential.

Corrected fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer with excitation wavelength of 320 nm and monochromator slits of 5 nm. The angle between gold plane and incident excitation light was set at 50° to ensure maximum efficiency of collecting emission light while avoiding reflection light interference.

X-ray photoelectron spectra (XPS) were obtained with a PHI quantum 2000 scanning ESCA microprobe spectrometer (USA) that focuses monochromatic Al posode X-rays onto samples. The pass energy was 187.85 eV for wide scans and 46.95 eV for narrow scans.

2.2. Reagents

3-Aminophenylboronc acid hemisulfate was purchased from Acros Organics. Other chemicals were of analytical grade or above. Organic solvents used for electrochemical and spectral measurements were purified before use that no fluorescent impurity was detected at the used excitation wavelength. Aqueous solutions were prepared using water obtained from a Millipore Milli-Q system (18 MΩ cm).

2.3. Fabrication of self-assembled bilayer

Gold wafer (3 mm × 4 mm) was first polished on microcloth pad to a mirror-like finish with 1.0, 0.3 and 0.05 μm alumina slurries. After removal of the trace alumina from gold surface, the gold wafer was rinsed in water and briefly cleaned in an ultrasonic bath, and finally cleaned by cycling between −0.5 and +1.5 V (versus SCE) in 1 M H_2SO_4 at a scan rate of $100\,mV\,s^{-1}$. Finally the gold wafer was thoroughly rinsed in ultra-pure water (Milli-Q) before thiol chemisorption.

Adsorption of cysteine was carried out by dipping gold wafer in 0.01 M cysteine solution of pH 11.7 for 3 h. The wafer was then rinsed thoroughly in ultra-pure water and dried with N_2. Under these conditions cysteine was adsorbed on gold surface in the form of $–SCH_2CH(NH_2)CO_2^-$ [14].

PBA/Cys/Au was fabricated by immersing the cysteine modified gold in 0.1 M NaOH for 10 min, and then into 0.01 M PBA solution of pH 4 for 1 h. In this way PBA was electrostatically bound to cysteine modified gold surface and fluorescent self-assembled bilayer was obtained (Fig. 1).

3. Results and discussion

3.1. Optimal conditions for preparation of PBA/Cys/Au self-assembled bilayer

Cysteine is known to adsorb on gold surface from its dilute solution [13]. Cysteine adsorption on gold surface was carried out following a reported procedure by dipping clean gold wafer in 0.01 M cysteine solution of pH 11.7 for 3 h [14].

pH of PBA solution was varied to adjust the charge state of PBA molecule in order to enhance its electrostatic interaction with cysteine that has already been assembled on gold surface. Fig. 2 shows the fluorescence spectra of the self-assembled bilayer obtained by dipping Cys/Au in PBA solutions of different pH. Results showed that a stable fluorescent self-assembled bilayer on gold surface (PBA/Cys/Au) was formed by dipping Cys/Au in 0.01 M PBA solution of pH 4 for 1 h.

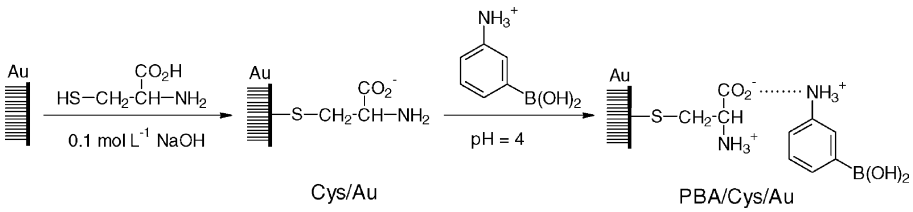

Fig. 1. Schematic diagram of self-assembling of the bilayer containing PBA.

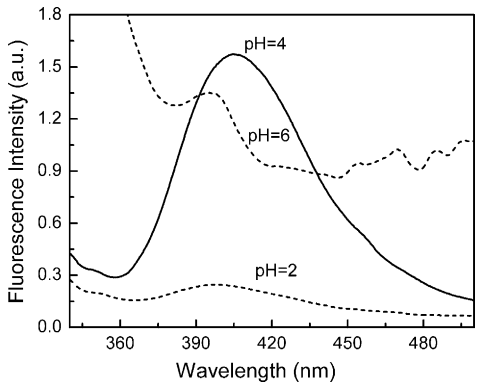

Fig. 2. Fluorescence spectra of PBA/Cys/Au obtained by dipping Cys/Au in 0.01 M PBA solutions of different pH.

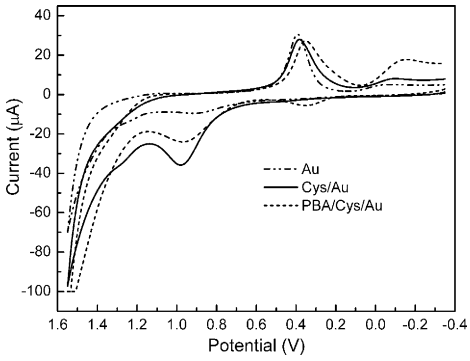

Fig. 3. Cyclic voltammograms of bare Au, Cys/Au and PBA/Cys/Au electrodes in 0.05 M $Na_2B_4O_7$–NaOH–CH_3CHO solution of pH 9.6. Scan rate was 100 mV s^{-1}.

3.2. Electrochemical characterization of self-assembled monolayer and bilayer

Assembling cysteine and PBA on gold surface was first examined by cyclic voltammetry. In 0.05 M $Na_2B_4O_7$–NaOH–CH_3CHO solution of pH 9.6, the cyclic voltammogram (CV) of the Au electrode assembled with cysteine (Cys/Au) showed an irreversible oxidation peak at +0.92 V (versus SCE) (Fig. 3). The peak current increased with increasing cysteine concentration, which indicates that cysteine has assembled on the gold surface. It was also found that, when PBA was assembled onto the surface of Cys/Au, this oxidation peak became smaller while the oxidation potential hardly changed, suggesting that PBA inhibited the oxidation of cysteine on gold electrode. In 0.5 M KOH solution, the potential of the cathodic stripping peak of PBA/Cys/Au differs, if any, not much from that of Cys/Au, which means that the assembling of PBA does not affect the S-Au interaction in the Cys/Au self-assembled monolayer [15]. Compared to those of Cys/Au electrode and bare Au electrode, the CV of PBA/Cys/Au electrode in 0.1 M $NaNO_3$ solution of pH 2.6 showed an irreversible reduction peak at −0.32 V (versus SCE) (Fig. 4). It was found that the current of this reduction peak increased with increasing PBA concentration in solution, suggesting that this peak originated from the reduction of PBA and PBA has indeed been assembled on the surface of Cys/Au leading to the formation of PBA/Cys/Au bilayer.

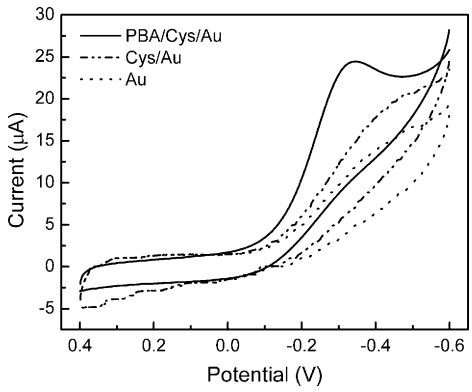

Fig. 4. Cyclic voltammograms of PBA/Cys/Au, Cys/Au and bare Au electrodes in 0.1 M $NaNO_3$ solution of pH 2.6. Scan rate was 100 mV s^{-1}.

3.3. XPS experiments on self-assembled bilayer

Fig. 5 shows the X-ray photoelectron spectra (XPS) of Cys/Au and PBA/Cys/Au. Existence in XPS of the O1s, C1s, N1s, B1s, and S2p peaks confirmed the formation of self-assembled bilayer on gold surface.

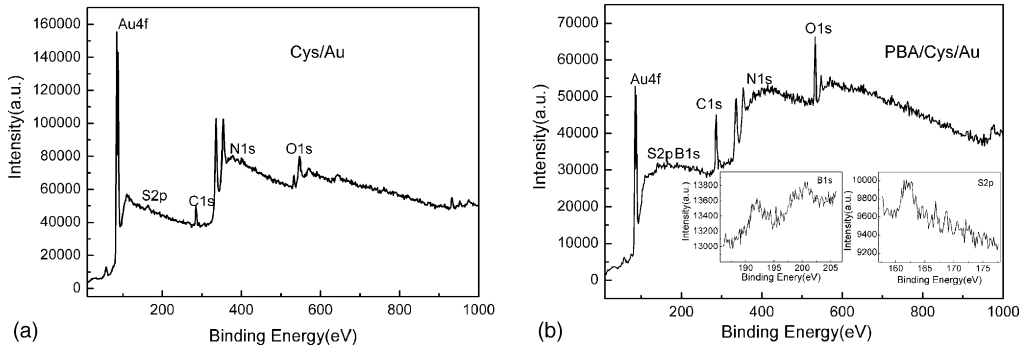

Fig. 5. XPS spectra of Cys/Au (a) and PBA/Cys/Au (b). Insets in (b) show the B1s and S2p XPS spectra.

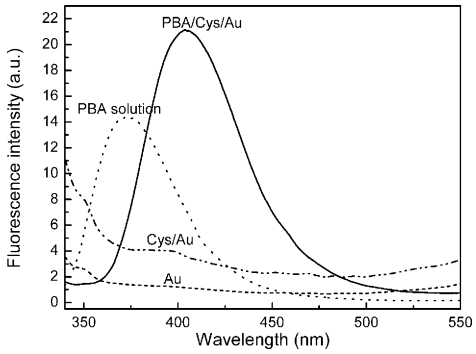

Fig. 6. Fluorescence spectra of PBA/Cys/Au, Cys/Au, bare Au wafers and PBA molecule in buffer solution of pH 8.2.

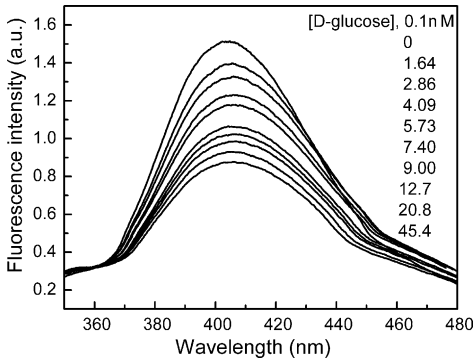

Fig. 7. Fluorescence spectra of PBA/Cys/Au in methanolic buffer solutions of pH 8.2 containing D-glucose of varied concentration.

3.4. Fluorescence properties of PBA/Cys/Au

Surface fluorescence spectra of PBA/Cys/Au, Cys/Au and bare Au wafers under the excitation of 320 nm are presented in Fig. 6. It was found that PBA/Cys/Au emitted PBA-characteristic fluorescence, whereas Cys/Au and Au without PBA were nonfluorescent, further verifying the assembling of PBA molecules on Au surface. It was also found that the 2-thiolacetamide derivative of PBA directly assembled on Au surface was nonfluorescent either. This on the other hand supported the role of cysteine self-assembled monolayer in ensuring the fluorescence emission of the PBA/Cys/Au self-assembled bilayer in which the rapid electron transfer between PBA and Au metal surface that led to fluorescence quenching [16] might be blocked.

Fluorescence spectrum of PBA/Cys/Au immersed in buffer solution of pH 8.2 was found peaked at 406 nm which is red shifted by 31 nm from that of PBA in bulk solution buffered at pH 8.2 (Fig. 6). This indicated that the microenvironment of PBA in self-assembled bilayer on gold surface was different from that in the bulk solution [17].

PBA/Cys/Au thus prepared was found to be highly stable that practically no fluorescence signal could be detected from the solution phase after 1 day of immersing the self-assembled bilayer in it and the fluorescence of PBA/Cys/Au bilayer itself did not change.

3.5. Fluorescent sensing of PBA/Cys/Au for saccharides

The fluorescence emission of PBA/Cys/Au immersed in methanol–aqueous buffer solution (30% methanol (v/v) plus 0.025 M KH_2PO_4–0.025 M Na_2HPO_4 buffer of pH 8.2 based on reported data [5–7] and our pH titration) was found quenched by saccharides such as glucose, fructose, galactose and mannose with extremely high sensitivity. The solution ionic strength was maintained by the buffer solution at 0.2. Trace of the fluorescence spectra of PBA/Cys/Au in the presence of D-glucose is shown in Fig. 7, in which it is seen that glucose of down to nano molar concentration leads to substantial quenching of the fluorescence. Plots of the fluorescence intensity of PBA/Cys/Au as a function of saccharide concentration are shown in Fig. 8. At lower saccharide concentration portion, the quenching follows Stern–Volmer theory. The quenching constants with relative standard deviation of 7.7% were obtained on three PBA/Cys/Au bilayer sensors and were in the order of D-glucose ($4.53 \times 10^8\ M^{-1}$) > D-galactose ($4.21 \times 10^8\ M^{-1}$) > D-fructose ($2.71 \times 10^8\ M^{-1}$) > D-mannose. This order is obviously different from that observed in the bulk solution with monoboronic acid based saccharide sensor [2].

In order to demonstrate the advantages of the self-assembled bilayer fluorescent sensor, the fluorescence of PBA in the same bulk buffer solution was examined in the presence of saccharides. As in self-assembled bilayer sensor, the fluorescence of PBA in bulk solution was also found quenched by saccharides in a Stern–Volmer manner (Fig. 9). The quenching constants varied in the order of D-fructose ($6.26 \times 10^2\ M^{-1}$) > D-mannose ($0.96 \times 10^2\ M^{-1}$) > D-galactose ($0.50 \times 10^2\ M^{-1}$) > D-glucose ($0.26 \times 10^2\ M^{-1}$), the same as that observed with reported other monoboronic acid based sensors. It was found that the quenching constants in the self-assembled bilayer sensor were 10^6 orders of magnitude higher than those in the solution phase sensor, demonstrating that not only the sensitivity order towards saccharide species changed, the response sensitivity was

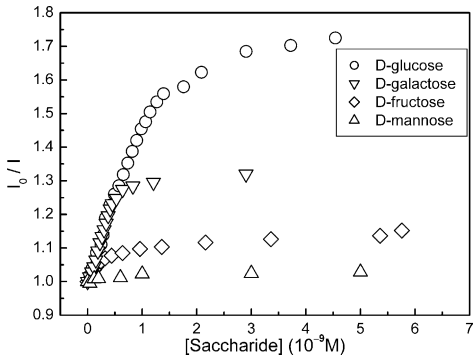

Fig. 8. Plots of the relative fluorescence intensity of PBA/Cys/Au vs. saccharide concentration in methanolic buffer solutions of pH 8.2. I_0 and I represent fluorescence intensity in the absence and presence of saccharide, respectively.

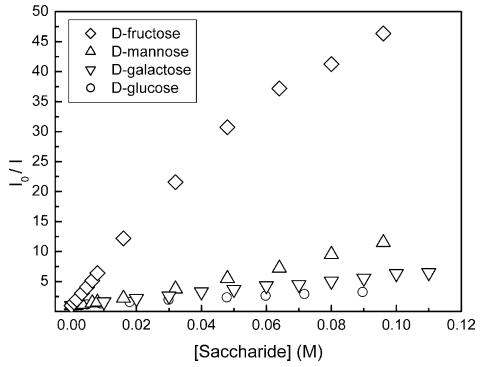

Fig. 9. Relative fluorescence intensity vs. saccharide concentration profile for PBA in methanolic buffer solution of pH 8.2. [PBA] = 4.00×10^{-4} M.

dramatically enhanced as well. It should be pointed out that, although the exact reasons for the high sensitivity of the PBA/Cys/Au bilayer fluorescence in sensing saccharides are at the moment not yet made clear, we suppose that this observation might be due to the well-ordered structure of the fluorophore-containing bilayer with substantially increased local concentration of the fluorophore at the bilayer surface. Further detailed experiments, however, should be carried out to understand the mechanism, which is currently underway.

3.6. Fluorescence reversibility

Reversibility is an important parameter of a sensor. It is desirable that the sensor-analyte complexation should be readily reversed after each analysis event so that the analyte would be released that the sensor is restored to be ready for the next measurement. We illustrated the reversibility of the PBA/Cys/Au bilayer sensor by following its fluorescence variations. It was found that the fluorescence of the PBA/Cys/Au bilayer immersed in buffer solution containing

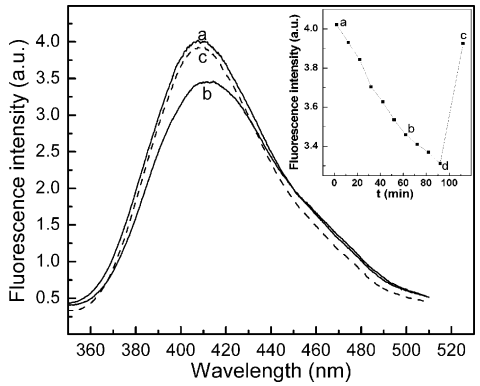

Fig. 10. Fluorescence spectra of PBA/Cys/Au (a) in buffer solution, (b) in buffer solution containing 2.24×10^{-10} M D-glucose for 1 h, and (c) washed with 0.1 M HCl and then in buffer solution. The buffer solution is methanolic phosphate solution of pH 8.2. Inset shows the profile of the fluorescence intensity of PBA/Cys/Au vs. time for PBA/Cys/Au immersed in buffer solution containing 2.24×10^{-10} M D-glucose. From (d) to (c), PBA/Cys/Au was first washed in 0.1 M HCl solution and was then put in buffer solution.

D-glucose decreased with immersion time (inset, Fig. 10). The boronic acid–sugar complex was found to be broken when the PBA/Cys/Au sensor was immersed in 0.1 M HCl solution for 30 min, since after followed rinsing in ultra-pure water its fluorescence was efficiently recovered nearly to the original intensity (Fig. 10). This demonstrated the excellent reversibility of the fluorescence of this PBA/Cys/Au bilayer sensor.

4. Conclusions

A fluorescent monoboronic acid was successfully assembled onto gold surface via its electrostatic interaction with cysteine that was directly assembled on the gold surface. The formed self-assembled bilayer was found highly fluorescent and its fluorescence showed extremely sensitive response to the presence in alkaline buffer solution of glucose and other saccharides down to nanomolar level. The surface fluorescence quenching constants at 10^8 M^{-1} order of magnitude are 10^6 times of those for bulk solution phase quenching. It is significant to point out that the monoboronic acid based self-assembled bilayer sensor, which in solution phase shows the lowest sensitivity towards glucose among common saccharides, shows the highest sensitivity towards glucose, indicating that the self-assembling method alters the fluorescence response order among saccharides. It was also found that the fluorescence of the prepared self-assembled bilayer sensor could be easily recovered after each measurement. The methodology of assembling fluorophore on gold surface, therefore, provides a new strategy for immobilizing reagent in constructing a reusable fluorescent sensor [18].

Acknowledgements

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