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An enzyme-free glucose sensor based on a difunctional diboronic acid for molecular recognition and potentiometric transduction†

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On the basis of the unusual anionic potential response of a diboronic acid and its ability to specifically recognize glucose, a highly selective enzyme-free potentiometric glucose sensor was developed.

Glucose is a basic necessity of living organisms. The normal human blood glucose levels for a fasting test are within a narrow range of about 4.4 to 6.1 mmol L⁻¹ (79.2 to 110 mg dL⁻¹). An abnormal glucose level is a warning signal of metabolic disorders and can be regarded as an important marker for various diseases such as diabetes, cardiovascular diseases, and obesity.¹ In the past decades, many efforts have been devoted to developing enzyme sensors for blood glucose detection using various readout strategies such as SPR,² fluorometry,³ amperometry⁴ and potentiometry.⁵ However, the instabilities originating from the intrinsic variable features of the enzymes may prevent these enzyme-based sensors from being widely applied.^{6,7} It has been reported that the activities of glucose oxidase widely used in the glucose sensor construction can be easily affected by the environmental conditions such as temperature, pH and humidity.⁸ Therefore, enzyme-free sensors are highly desired for glucose monitoring.

The high-affinity and reversible bindings of boronic acids to *cis*-1,2- or 1,3-diols to form five- or six-membered cyclic boronate esters have attracted much attention in recent years.^{9,10} Boronic acids have been employed for many saccharide sensors based on UV-vis absorption,¹¹ fluorescence,¹² plasmonics,¹³ SPR¹⁴ and potentiometry.^{15–17} It should be noted that monoboronic acids

bind more strongly to fructose than to glucose.¹⁸ In contrast to monoboronic acids, a pair of boronic acid groups in one structure display a higher binding constant to glucose than to other monosaccharides *via* formation of a 1 : 1 stoichiometry boronate ester complex.¹⁹ Many researches have been done on designing and synthesizing diboronic acid (DBA) derivatives with the capacities to bind glucose selectively, and fluorescence methods are usually employed.^{20–23} It should be noted that in most of these methods the boronic acid groups are only used for molecular recognition, while signal transduction is implemented by other groups modified onto them.

Potentiometry with polymeric liquid membrane electrodes (PLMEs) represents a powerful electrochemical approach for trace analysis, due to its reliability, simplicity and low cost.²⁴ Recently, our group reported the potential responses of a class of monoboronic acid compounds on quaternary ammonium salt-doped PLMEs and applied such unusual potential responses to saccharide detection.¹⁷ However, the approach cannot be used for glucose detection owing to the above mentioned stronger binding affinities of monoboronic acids to fructose.¹⁸ To achieve a higher binding affinity to glucose than to its stereoisomers, two boronic acid groups (*i.e.*, DBA) can be arranged face to face in one structure.²⁵ In this paper, a PLME for highly selective enzyme-free glucose detection based on a DBA is described. The DBA probe is used not only for molecular recognition but also for potentiometric transduction.

The proposed response mechanism is illustrated in Scheme 1.¹⁷ The lipophilic and water “coordinated” DBA²⁶ can be extracted spontaneously from the aqueous phase into the polymeric membrane phase according to the partition equilibrium, where the dissociation of the DBA leaves the nucleophilic OH⁻ coordinating to the central boron atom to form a sp³-hybridized tetrahedral anion. The quaternary ammonium cations assist this process by forming ion pairs with the tetrahedral boron anions. In this case, hydrophilic H⁺ and its counterion X⁻ can be co-extracted into the aqueous phase from the organic polymeric membrane phase. The anionic potential response to the DBA may be due to the local pH change in the

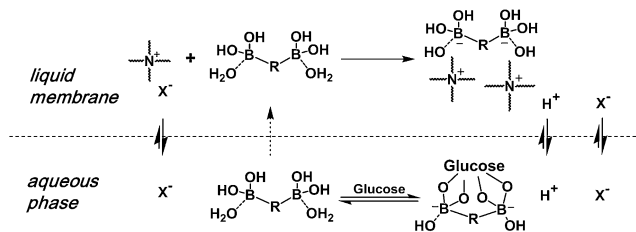
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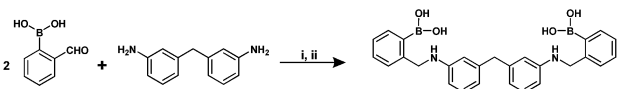
Scheme 1 Response mechanism of the proposed enzyme-free potentiometric glucose sensor.

membrane. After introducing glucose into the aqueous solution, the formed hydrophilic 1 : 1 stoichiometry boronate ester complex (an anion) shifts the partition and dissociation equilibria of the DBA in the two immiscible phases to the opposite direction, and therefore a cationic potential response can be observed. In this work, both the glucose recognition and signal transduction are implemented by one diboronic acid group, which could dramatically simplify the probe preparation process.

The DBA ([methylene bis(3,1-phenyleneiminomethylene-2,1-phenylene)]diboronic acid) was synthesized according to Scheme 2,²⁵ and its ¹H NMR and ¹³C NMR are shown in Fig. S1 and S2† respectively. The DBA can induce significant anionic potential responses on the PLME, which is in agreement with our previous report (see the ESI, Fig. S3†).¹⁷ It should be noted that the DBA ($pK_a = 10.88 \pm 0.02$) is dominantly nonionic (>99%) at pH 8.0, and this confirms our proposed response mechanism based on a neutral boronic acid.¹⁷

The sensitivity of a boronic acid based glucose sensor is pH-dependent.²⁷ As shown in Fig. 1A, good selectivity and linearity can be obtained at pH 8.0 and the potential change to glucose is about 20 mV higher than that to fructose. The pH-dependent behaviors of the DBA based glucose sensor may be due to the fact that the sample pH determines the equilibrium between the neutral trigonal and anionic tetrahedral forms of the boronic acid groups, which induces a significant effect on binding to glucose.^{18,27} The concentration of the DBA also affects the sensitivity of the proposed potentiometric glucose sensor. Fig. 1B shows the ratios of the potential responses in the presence and absence of glucose at different concentrations of the DBA probe. It can be seen that the highest sensitivity can be obtained by using 10^{-5} M DBA.

Under the optimized conditions, potential responses to glucose at different concentrations on the TDDA⁺Cl⁻-doped PLME were measured. As shown in Fig. 2, glucose induces a potential reversal which is due to the fact that the boronate ester complex generated from the esterification reaction



Scheme 2 Synthesis path of the DBA. Reagents and conditions: (i) MeOH; (ii) MeOH, NaBH₄. See the ESI† for details.

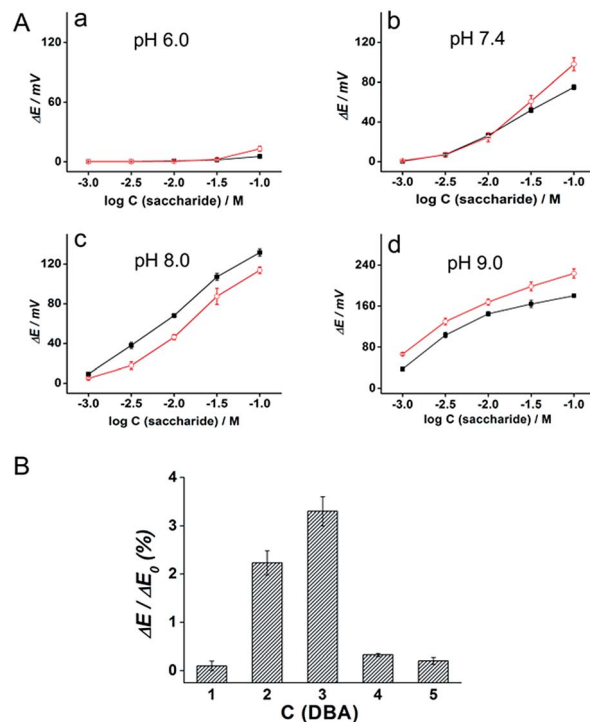


Fig. 1 (A) Potential (ΔE)–concentration curves of glucose (■) and fructose (○) obtained by the TDDA⁺Cl⁻-doped PLME in 50 mM phosphate buffer with pH values of 6.0 (a), 7.4 (b), 8.0 (c) and 9.0 (d) in the presence of 10^{-5} M DBA. (B) Ratios of the potential responses to the DBA at different concentrations on the TDDA⁺Cl⁻-doped PLME with the presence (ΔE) and absence of 1 mM glucose (ΔE_0) in 50 mM phosphate buffer (pH 8.0). Numbers 1–5: the DBA concentrations of 1, 5, 10, 50 and 100 μ M, respectively. Each error bar represents one standard deviation for three measurements.

between the DBA and glucose is less lipophilic than the parent DBA, and thus cannot be extracted easily into the polymeric membrane phase. The potential difference (ΔE)

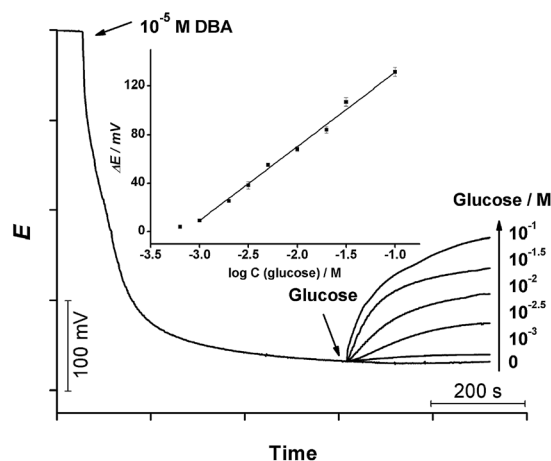


Fig. 2 Potential responses to glucose at different concentrations on the TDDA⁺Cl⁻-doped PLME in 50 mM phosphate buffer (pH 8.0). Inset shows the calibration curve. Each error bar represents one standard deviation for three measurements.

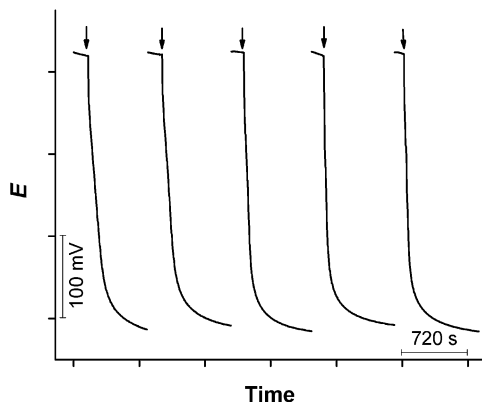


Fig. 3 Regenerability of the TDDA⁺Cl⁻-doped PLME for measuring the DBA (10^{-5} M) in 50 mM phosphate buffer (pH 8.0). After each measurement, a 2 × 3 min-procedure of washing with a 10 mM aqueous HCl solution containing 10% (v/v) ethanol was used to regenerate the electrode.

between the baseline and the potential measured at 5 minutes after glucose addition was used for quantification. The potential response is proportional to the logarithm of the concentration of glucose measured in the range of 1–100 mM ($\Delta E = 61.1 \times \log C + 192.4$, $R^2 = 0.995$, E in mV, C in M) (see the inset in Fig. 2). The detection limit is 0.2 mM (3σ), and the relative standard deviation (RSD) for 5 mM glucose is 2.7% ($n = 3$). It should be noted that the sensitivity of this method is much lower in comparison with those of the previous boronic acid-based potentiometric glucose sensors.^{15,16} Moreover, the electrode can be regenerated by washing with a 10 mM aqueous HCl solution containing 10% (v/v) ethanol and reused with a high reproducibility (Fig. 3).

The selective response to glucose over other saccharides is crucial for clinical analysis, since many saccharides are also present in blood, such as fructose and galactose (<0.1 mM).^{28,29} In order to evaluate the selectivity of this sensor, potential responses to glucose, fructose and galactose were tested, respectively. As shown in Fig. 4, these three

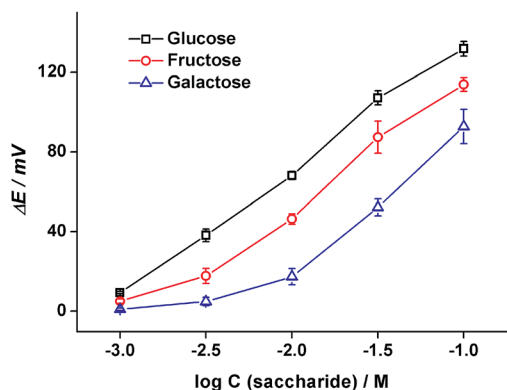


Fig. 4 Potential (ΔE)–concentration curves for three saccharides obtained by the TDDA⁺Cl⁻-doped PLME in 50 mM phosphate buffer (pH 8.0). Each error bar represents one standard deviation for three measurements.

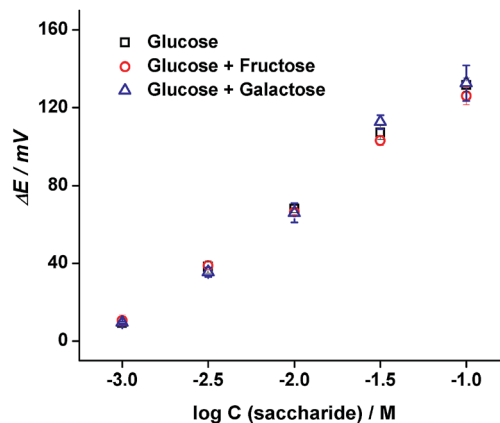


Fig. 5 Potential responses to glucose in the presence of 0.1 mM fructose or galactose on the TDDA⁺Cl⁻-doped PLME in 50 mM phosphate buffer (pH 8.0). Each error bar represents one standard deviation for three measurements.

monosaccharides induce a potential reversal in the following order: glucose > fructose > galactose. These results are consistent with the binding affinities of the DBA to these three saccharides (the stability constants for glucose, fructose, and galactose are 140, 55, and 26 dm³ mol⁻¹, respectively).²⁵ The enhanced binding affinity of the DBA to glucose is due to the formation of a 1 : 1 cyclic complex, whereas other saccharides tend to form the 2 : 1 diboronic acid ester complexes.²⁵ Moreover, the presence of these saccharides (0.1 mM) has little influence on the potential response of glucose (Fig. 5). To evaluate the reliability of this glucose sensor, some coexisting anions such as NO₃⁻, SCN⁻ and Cl⁻ ions which may interfere with the detection of glucose were tested.^{30,31} As illustrated in Fig. S4,[†] although small potential changes can be induced after additions of these ions, they would not interfere with the glucose detection.

Conclusions

In summary, a facile and enzyme-free potentiometric glucose sensor based on a difunctional diboronic acid probe has been proposed. This is the first glucose sensor where the diboronic acid groups are used for both molecule recognition and potentiometric transduction. With the advantages of excellent sensitivity, high selectivity and simplicity, this proposed enzyme-free potentiometric sensor is promising for application to blood glucose detection.

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