Short Communication

A Simple and Rapid Method for Probing of Isomerization of Glucose to Fructose with Ferroceneboronic Acid

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Isomerization of glucose to fructose is used in a variety of industrially relevant processes and in glycolysis. Currently used methods for probing of the isomerization need complicated instruments and are time-consuming. In this work, we reported a simple and rapid method for probing of the isomerization with electrochemically active ferroceneboronic acid (FBA) probe. Specifically, fructose resulting from the isomerization of glucose induced the shift of the FBA potential through the formation of FBA-fructose complex. To demonstrate the feasibility and application, the isomerization mediated by glucose isomerase (GI) was investigated. The effect of experimental environmental (e. g. pH, reaction time and temperature, enzyme concentration) on the conversion ratio of glucose to fructose was demonstrated. We believe that the method will find many applications in both food industry and laboratory research for probing of the activity of enzymes (e.g. isomerase, phosphatase and kinase) and screening of new catalysts for isomerization of sugars.

1. INTRODUCTION

The transformations of biomass into a wide variety of products have attracted extensive attention in chemical and food industry. Isomerization of glucose to fructose is of commercial importance in food industry, such as the production of high-fructose corn syrup (HFCS), since fructose is the most sweet natural sugar and glucose is the cheapest and the only abundant monosaccharide available in nature [1]. In the present commercial process, the isomerization is carried out by glucose isomerase (GI, EC 5.3.1.5), one of the three highest tonnage value enzymes (GI, amylase and protease). Very recently, metalloenzyme-like chemical catalysts have been shown to be promising

alternative to GI in the production of fructose in view of some drawbacks of GI-mediated isomerization, such as narrow pH operation window, requirement of Co^{2+} for the enzyme activity, inhibition by some essential elements (e.g. Ca and Zn) and low efficiency from an economic point of view [2-7]. Activity of enzymes and chemical catalysts is the most important factor in evaluation of their suitability for industrial application. Currently used methods for monitoring the isomerization activity is to determine the amount of produced fructose with high-performance liquid chromatography (HPLC), gas chromatography (GC) and Seliwanoff's test (Scheme 1) [7,8]. However, these methods need complicated instruments and are time-consuming. For example, in the classical Seliwanoff's test, fructose produced from glucose is first conversed into the form of ketose with concentrated acid under heating for reaction with resorcinol, which makes the experiments complex and needs the use of hazardous chemical. In addition, a variety of optical and electrochemical techniques have also been reported for the selective detection of fructose [9-11]. However, these methods need the use of expensive and less-stable enzymes [12]. Therefore, there remains significant room for the development of a theoretically and technically simple approach for probing of the isomerization of glucose to fructose.

It is well known that boronic acids can form boronate ester covalent bonds with cis-diols. Based on this interaction, extensive efforts have been focused on the development of colorimetric, fluorometric and electrochemical detection sensors for determination of diol-containing compounds (e.g. sugars, glycoproteins, RNA, catechol) [13-15]. The principle of the recognition is the formation of five- and six-membered cyclic diesters between boronic acids and 1,2- or 1,3-diols. Note that, the diols must be in the form of cis-configuration, and the molecule of diols should possess syn-periplanar torsion angle between the neighboring hydroxyls for effective complexation interaction [16]. Ferroceneboronic acid (FBA), an electrochemically active ferrocene derivative, can bind to diol-containing compounds and have been used for design of electrochemical sensors because of its well-defined redox reaction [17-26]. For example, Scheller's group reported an amperometric biosensor for the determination of glycated hemoglobin in human with FBA [19,27]. Li et al. investigated the activity of the enzymes (tyrosinase and thrombin) based on the interaction of FBA and enzyme-generated catechol ligand on electrode [21]. Because of the difference in the structure of fructose verse glucose, the binding affinity of fructose to boronic acid is 1~2 orders higher than that of glucose [18,28]. In the present work, we distinguished glucose and fructose using FBA probe, and reported a simple and rapid electrochemical method for probing of the isomerization of glucose to fructose.

2. EXPERIMENTAL

2.1 Chemicals and reagents

Ferroceneboronic acid (FBA) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were obtained from Sigma–Aldrich. Glucose, fructose, mannose, xylose, maltose, sucrose and lactose were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). GI (HR7-100, Hampton) from Streptomyces rubiginosus was obtained from Seebio (Shanghai, China), which was used as received. All other reagents are of the AR grade. FBA stock solution was prepared with

phosphate-buffered saline solution (PBS buffer, 200 mM, pH 7.0) containing 1% DMSO. All aqueous solutions were prepared with a Millipore system (Simplicity Plus, Millipore Corp.).

2.2 Electrochemical measurements

The glass carbon (GC) electrode with a diameter of 3 mm was polished with alumina power, and then sonicated in ethanol and water. Voltammetric determination in phosphate-buffered saline solution (PBS buffer, 200 mM) was performed on a uECS-PRO electrochemical workstation (Changchun Institute of Applied Chemistry Chinese Academy of Science, China) using a homemade plastic electrochemical cell room temperature. A platinum wire and a Ag/AgCl electrode were used as the auxiliary and the reference electrodes, respectively. For the assay of enzyme activity, GI was incubated with 1.8 M glucose in 50 mM HEPES solution in the presence of 0.5 mM Co^{2+}. Then, 10 μL of the GI/glucose mixed solution was mixed with 3 mL FBA solution for 30 s before the electrochemical measurement.

3. RESULTS AND DISCUSSION

3.1 Feasibility for probing of isomerization of glucose to fructose with FBA probe
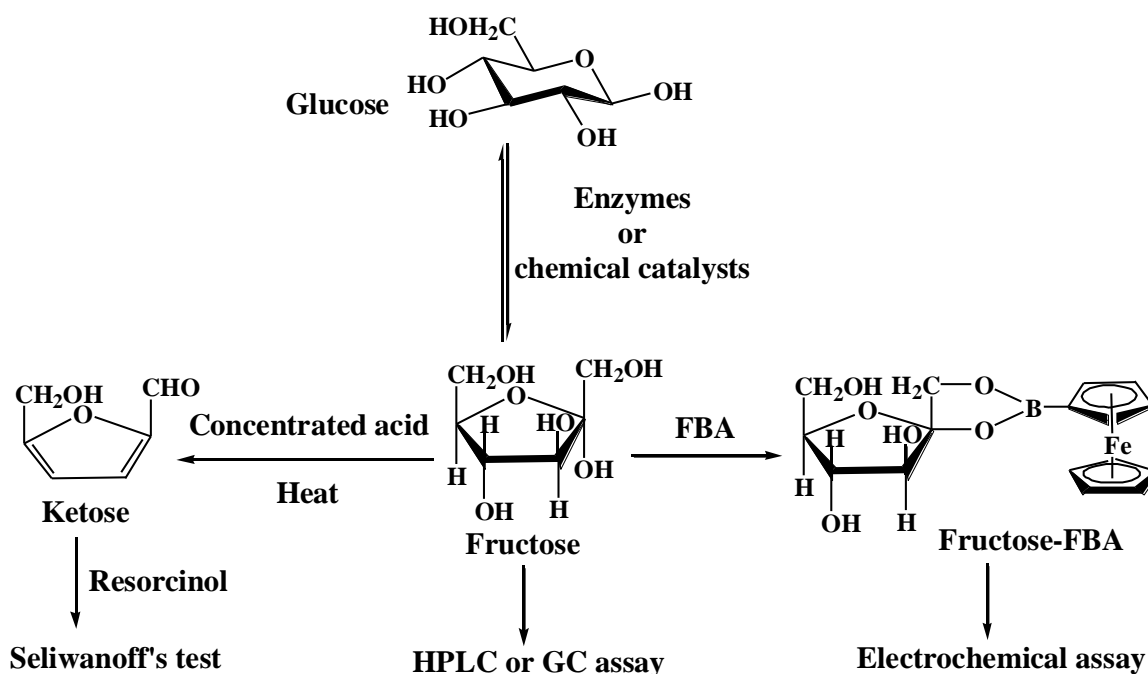
Scheme 1. Assay methods for probing of the isomerization of glucose to fructose and the activity of enzymes and chemical catalysts.

In general laboratory practice for monitoring the isomerization of glucose to fructose, more convenient is to measure the produced fructose by HPLC or GC assay and Seliwanoff's test (Scheme 1) [7,8]. As mentioned in Introduction, these methods are time-consuming and need complicated

instruments or use of concentrated acid. The analysis principle in the present work is based on measurements of the change of electrochemical response of FBA caused by the formation of boronate ester covalent bond between FBA and fructose. In the commercial production of HFCS, the isomerization activity is strictly dependent upon the type of organism producing the enzyme, the immobilization form of enzyme for reusability and the storage time [1]. To demonstrate the feasibility and application of our method, we tested commercial GI. We first compared the voltammetric response of FBA in the absence and presence of glucose and fructose. As shown in Fig. 1, FBA exhibits a couple of redox waves with the oxidation potential at 0.23 V and the reduction potential at 0.16 V. After the addition of fructose, the current of original reduction peak at 0.16 V decreased, which is accompanied by the appearance of a new reduction peak at around 0 V. Notice that no apparent shift in the oxidation potential of FBA was observed; this is because the reduced form of FBA has poor affinity to diol than the oxidized one [24]. Expectedly, glucose did not cause apparent change in the voltammetric response of FBA at the same condition. However, after addition of GI to the glucose solution, the electrochemical response reveals a reduction peak at 0 V, which is attributed to the complex between FBA and fructose resulting from the isomerization of glucose. We also investigated the interaction between FBA and other sugars, such as mannose, xylose, maltose, sucrose and lactose, and found that these sugars did not induce the appearance of the new peak (data not shown). The result is acceptable since these sugars have low binding affinity to boronic acids [18,28]. Thus, probing of the isomerization of glucose to fructose with FBA as redox probe is possible.
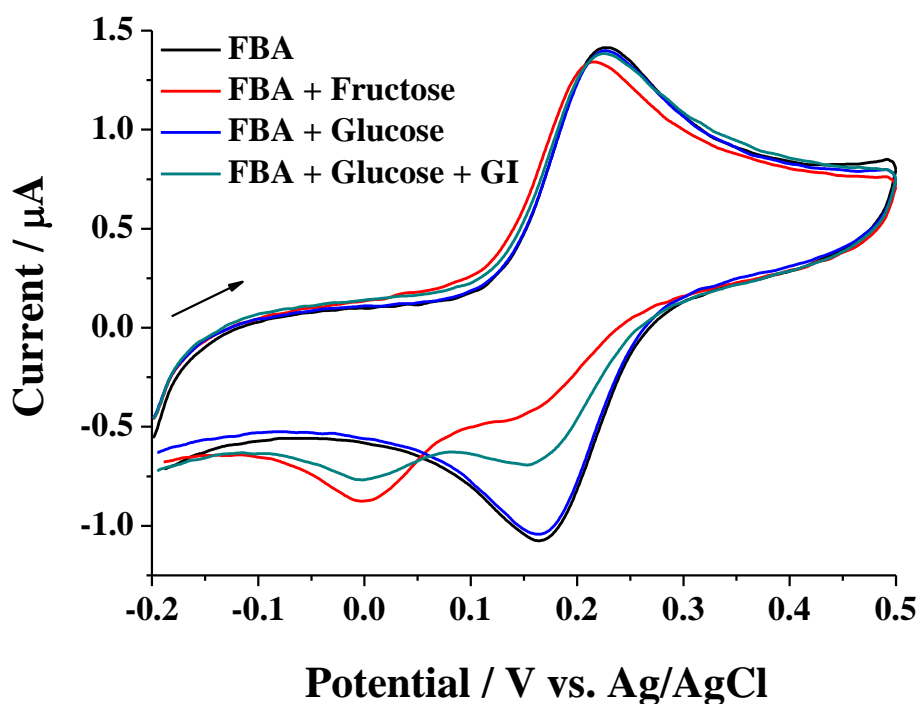

Figure 1. (A) Cyclic voltammograms (CVs) of FBA in different systems. The final concentrations of FBA, fructose, glucose and GI were 100 μM, 6 mM, 6 mM and 33 μg mL^{-1}. For the assay of isomerization, glucose was incubated with GI in pH 7.0 HEPES buffer for 30 min at 80 °C.

3.2 Dependence on fructose concentration

Differential pulse voltammetry can decrease the background charging currents and in turn increase the detection sensitivity. The dependence of the current change upon fructose concentration was evaluated with differential pulse voltammetry. Fig. 2A depicts the voltammetric responses in FBA solutions containing different concentrations of fructose. Because glucose at the concentration above 6 mM would induce the appearance of peak II, the total concentration of glucose and fructose in the assay kept at 6 mM. I_{II}/I_I, the ratio of the reduction current of peak II (I_{II}) to that of peak I (I_I), was used to evaluate the performance of the sensor. As show in Fig. 2B, the I_{II}/I_I ratio increases linearly with the increase of fructose concentration from 0.25 to 6 mM, which can be expressed using $I_{II}/I_I = 0.141$ [Fructose] $+ 0.002$ ($R^2 = 0.99$).
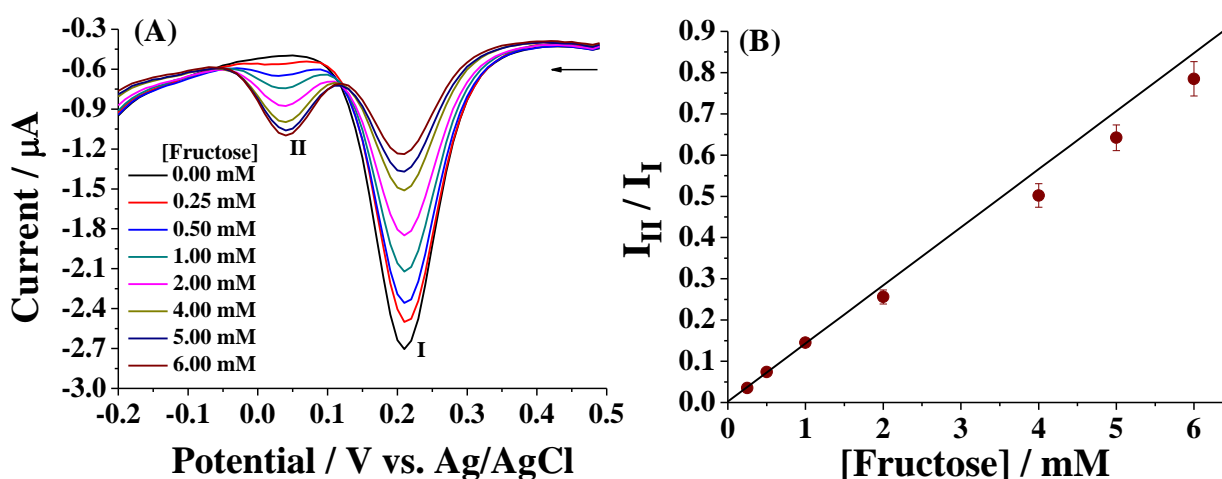

Figure 2. (A) Differential pulse voltammograms (DPVs) acquired in FBA solutions containing different concentrations of fructose and glucose. The arrow indicates the scan direction. The total concentration of glucose and fructose kept at 6 mM. (B) Plots of the I_{II}/I_I ratio against the fructose concentration. Each point was averaged from at least three replicates, and the relative standard deviations (RSDs) are shown as the error bars.

3.3 Effect of experimental environmental on the conversion of glucose to fructose

The activity of enzyme is commonly affected by temperature and chemical environmental (e. g. pH). To further demonstrate the amenability of our method for monitoring the isomerization reaction, we investigated the effect of reaction temperature and time, pH and enzyme concentration on the conversion ratio of glucose and fructose. Fig. 3A shows the effect of reaction temperature on the I_{II}/I_I ratio. The value increases with the elevation of temperature and reaches to the maximum at 70 °C. The slight decrease at the temperature above 70 °C is probably due to the instability of the enzyme at higher temperature. The effect of pH was carried out at pH varying from 5.5 to 8.5 (Fig. 3B). The optimal pH for GI activity was found to be in the range of 7 to 8. This is understandable since GI is unstable and denatured at low and high pH [1]. Furthermore, the relationship of I_{II}/I_I and incubation

time was investigated. As shown in Fig. 3C, I_{II}/I_I increases with the increase of the incubation time, and begins to level off beyond 40 min, indicating that GI-catalyzed isomerization reaction is completed within 40 min. Moreover, we investigated the dependence of I_{II}/I_I upon GI concentration under the optimal conditions. As shown in Fig. 3D, I_{II}/I_I increases with the increase of GI concentration. In practice, the conversion ratios of glucose to fructose by GI depend on the type and immobilization form of the enzyme. The reported value varies in the range of 26 to 59 % [1]. Our results indicate that the equilibrium conversion of glucose to fructose under the optimal conditions is around 45 %. Moreover, we also demonstrated that Co^{2+} is required for the enzyme activity in this process (Fig. 4A), and found that metal ions, such as Ca^{2+}, Ni^{2+}, Zn^{2+} and Cu^{2+}, inhibited the isomerization activity to some extent (Fig. 4B). The results, in agreement with that reported previously [1], further indicating that the method is applicable to monitor the isomerization process and probe the enzyme activity.
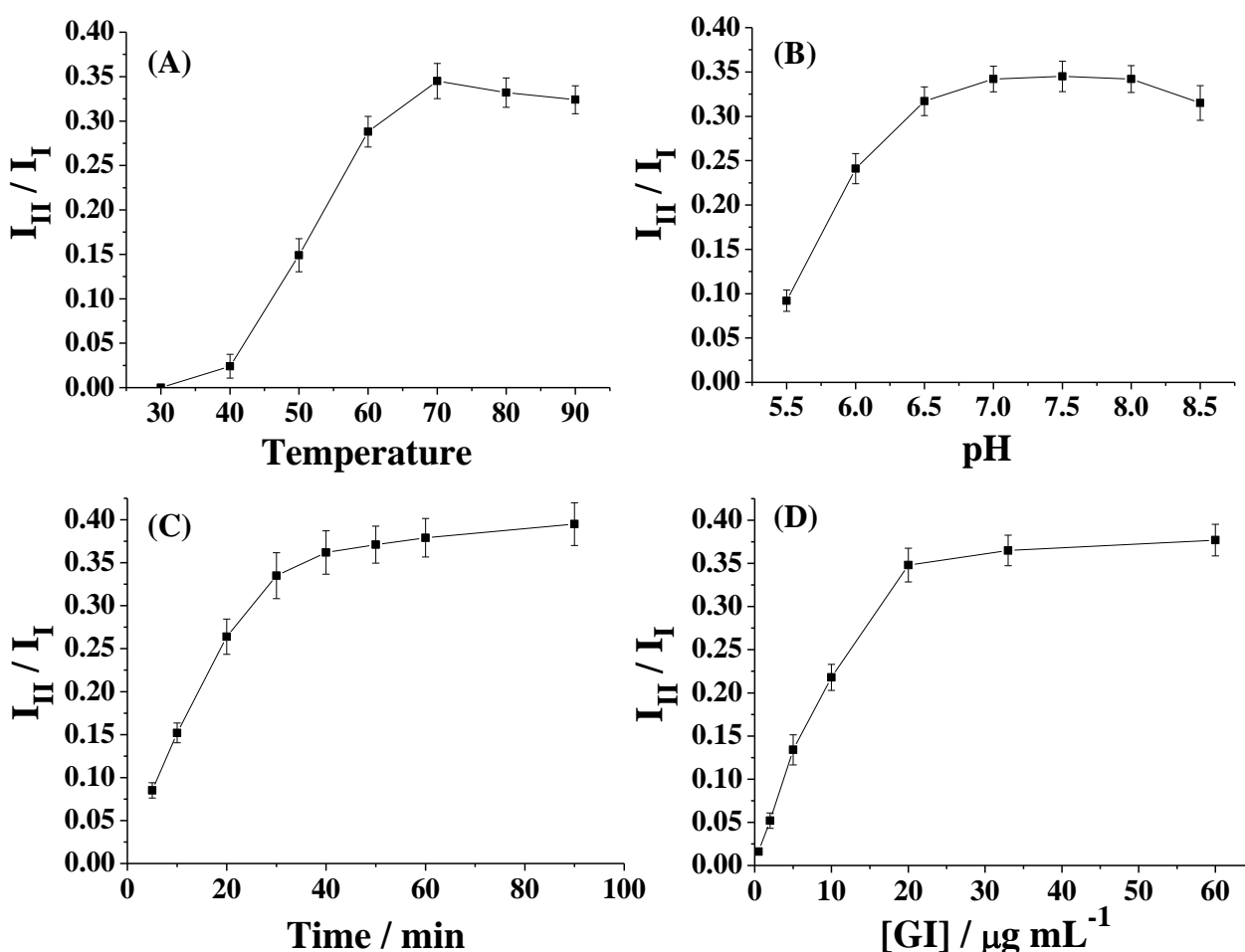

Figure 3. Effect of temperature (A), pH (B), incubation time (C) and final GI concentration (D) on the I_{II}/I_I ratio. The incubate time was 30 min in panels A and B. The pH value was 7.0 in panels A and C. The incubation temperature was 80 °C in panels B and C. The concentrations of GI and glucose used were 1 mg mL^{-1} and 1.8 M, respectively. In panel D, GI was incubated with glucose (pH 7.0) at 70 °C for 40 min.
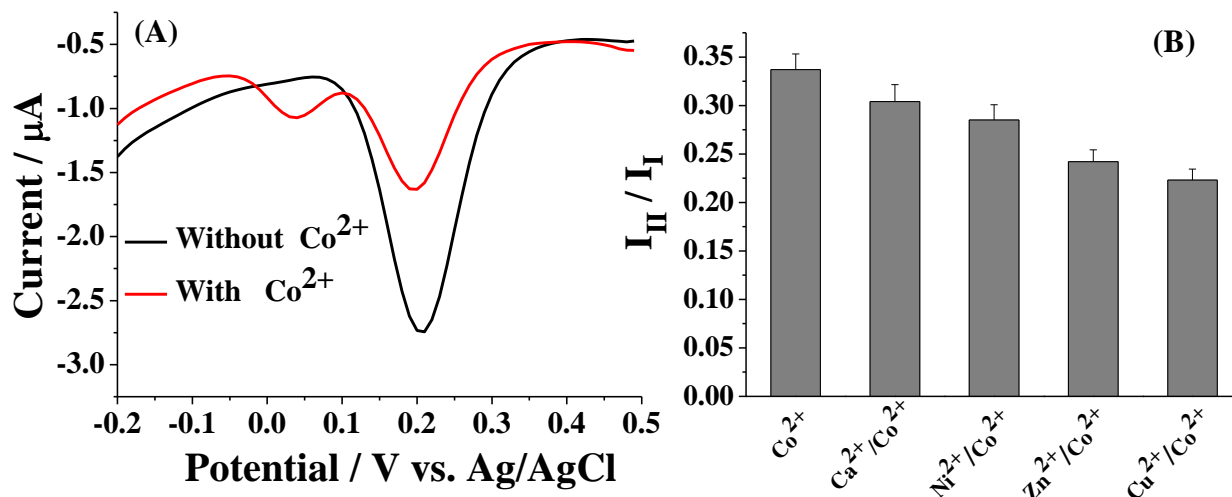
Figure 4. (A) DPVs acquired in FBA/glucose/GI mixed solutions with and without addition of Co^{2+}. (B) Effect of metal ions on the enzyme activity. The concentrations of GI and metal ions used were 33 µg mL^{-1} and 0.5 mM, respectively. The other experimental conditions are the same as those in Figure 3D.

4. CONCLUSION

In summary, we reported a simple and rapid electrochemical method for probing of the isomerization of glucose to fructose. Compared with the currently used methods, our method requires very simple and security sample-handling-procedure and minimum instrumental investment, obviates the use of expensive and less-stable enzymes, and can be conducted in the field with portable devices. Moreover, this is the first report in which the activity of isomerases was investigated based on the interaction of ferrocene boronic acids and sugars. We believe that the method will find many applications in both food industry and laboratory research for probing of the activity of enzymes (e.g. isomerase, phosphatase and kinase) and screening of new catalysts for isomerization of sugars.

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