Glucose-specific Sensing with Boronic Acid Utilizing Enzymatic Oxidation

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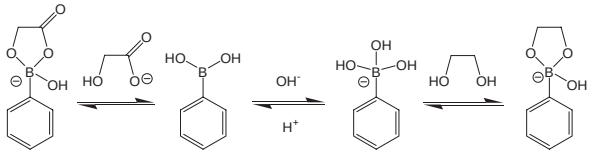
A novel sensing system for glucose-specific detection has been established based on a combination of a boronic acid and enzymes. To overcome the inherently low biding affinity of glucose toward boronic acids, glucose is converted into gluconic acid by the enzymatic reaction using glucose oxidase (GOx), and then complexed with a fluorescent boronic acid through the α-hydroxycarboxylate moiety. According to the present strategy, glucose concentration is exclusively determined among other saccharides in aqueous solutions.

Growing number of diabetic prompts researchers to develop glucose-specific sensing systems for the diagnosis and treatment of patients. Since 1990's, boronic acid-based sensory system for saccharides has been extensively studied by the group of Shinkai and others.[1] These research have proven that boronic acid is a powerful tool for the molecular recognition of saccharides in aqueous systems. However, significant drawbacks exist in the boronic acid-based system, that is, 1; binding affinity toward glucose is the lowest among major saccharides and 2; complexation with glucose scarcely takes place at physiological pH. Actually, the stabilities of phenylboronic acid–saccharide complexes are in the order: fructose $\gg$ galactose $>$ mannose $>$ glucose (Supporting Information, Table S1).[2] Since glucose is the most important saccharide in biological systems as an energy source and hence the primary target for saccharide sensing, the above mentioned drawbacks greatly limit the usefulness of boronic acid-based glucose sensing system. To enhance the binding affinity toward glucose, a series of diboronic acid receptors have been synthesized. Since glucose has a unique tendency to form 1:2 complexes in which one glucose molecule is bound by two boronic acid moieties,[3] the diboronic acid receptors show fairly good binding selectivity toward glucose.[4]

We here report an alternative strategy for a glucose-specific boronic acid-based sensing system utilizing an enzymatic oxidation reaction of glucose. As illustrated in Scheme 1, boronic acids reversibly react with diols to form cyclic esters, and the reaction takes place preferentially at alkaline pH where free boronic acids take the anionic boronate form. Since pK_a values of boronic acids are usually around 9.0, glucose shows very weak or no response against boronic acids when pH is equal to or lower than 7.0. It is noteworthy that boronic acids can bind α-hydroxycarboxylic acids under physiological and weakly



Scheme 1. Complex formation equilibria of boronic acids with diols and α-hydroxycarboxylic acids.[5]

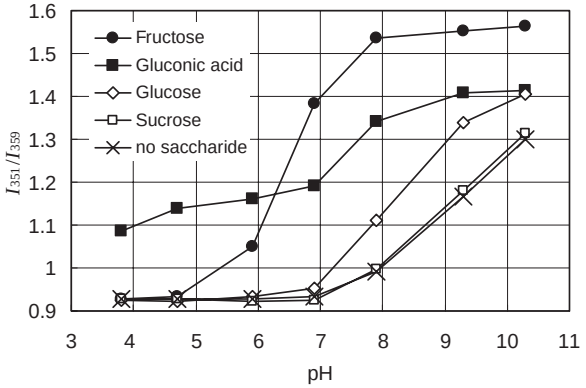
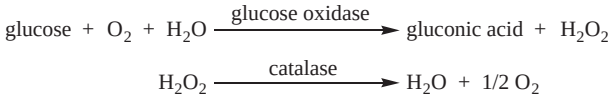


Figure 1. Plots of I_{351}/I_{359} vs pH for **1** at 25 °C; [**1**] = 1 μM, [saccharide] = 10 mM, λ_{ex}: 298 nm.

acidic conditions where pH value is between pK_a of α-hydroxycarboxylic acids (typically 2.0–4.0) and that of boronic acids.[6] It occurred to us that the reaction between boronic acids and α-hydroxycarboxylic acids would be useful for glucose-specific sensing by combining with a selective oxidation process of glucose converting into gluconic acid, an α-hydroxycarboxylic acid.

Figure 1 shows the pH profile of the complexation between 9-phenanthreneboronic acid (**1**) and saccharides in aqueous solutions. Fluorescence spectrum of **1** is significantly changed upon addition of saccharides, which is attributable to complexation with saccharides (Supporting Information, Figure S1). Since the increase in fluorescence intensity at 351 nm is much larger than that at 359 nm, one can accurately evaluate the complex formation by ratiometric measurements (I_{351}/I_{359}).[7] We chose excitation wavelength at 298 nm in order to avoid interferences by the absorption in shorter wavelength arising from enzymes (Supporting Information, Figure S2). It is seen from Figure 1 that the complexation behavior of glucose and gluconic acid are totally different from each other: glucose reacts with **1** only at alkaline pH, whereas gluconic acid reacts throughout the pH range. This observation clearly indicates that the binding mode between **1** and saccharides changes depending on pH. In alkaline solutions, diol unit in both glucose and gluconic acid is responsible for the binding. In contrast, α-hydroxycarboxylate unit in gluconic acid should be the binding site under acidic conditions. Fructose, not having α-hydroxycarboxylic acid unit, shows some response even at neutral pH due to its higher binding affinity toward boronic acids, but it looses responsiveness at acidic pH. Sucrose, a disaccharide composed of glucose and fructose, shows no response even at alkaline pH because it has no *cis*-diol unit suitable for complexation. Thus, the result shown in Figure 1 suggests the possibility of glucose-specific detection by selectively converting glucose into gluconic acid. The titration curve of **1** with gluconic acid at pH 4.7 shows a saturation type



Scheme 2. Enzymatic reaction pathways.

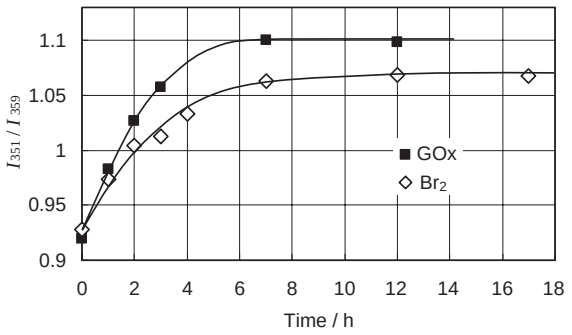


Figure 2. Time-course of glucose oxidation by Br_2 and GOx at 25 °C in aqueous solutions.[9]

of change from which the binding constant between **1** and gluconic acid ($\log K$) was determined to be 1.71 by the Benesi–Hildebrand's plot (Supporting Information, Figures S3 and S4).

For the selective oxidation of glucose, we utilized glucose oxidase (GOx)[8] or bromine (Br_2). The reaction of glucose with oxygen is catalyzed by GOx to form gluconic acid and hydrogen peroxide (H_2O_2) as shown in Scheme 2. Catalase[8] was also used for decomposing H_2O_2. The time-course of the glucose oxidation was followed by measuring I_{351}/I_{359} after adding **1** into the reaction mixtures (Figure 2).[9] In the case of GOx oxidation, I_{351}/I_{359} increases with time and saturated at the value of 1.10. Glucose seems to be nearly quantitatively converted into gluconic acid within 7 h since I_{351}/I_{359} value measured with the same concentration of gluconic acid is 1.11.[10] When Br_2 is used instead of GOx, I_{351}/I_{359} is saturated at the smaller value (1.07) than the case of GOx. This result indicates the occurrence of some side reaction on gluconic acid, which is supported by the observation that I_{351}/I_{359} value of the gluconic acid solution decreases from 1.11 to 1.08 after treating with Br_2.

To assess the sensing selectivity of the present system, we measured responsiveness against various saccharides. After the oxidation reactions for 12 h, increases of I_{351}/I_{359} values compared to that measured in the absence of saccharides were determined (ΔI). The result shown in Figure 3 clearly demonstrates the glucose-specific nature of the system using GOx. Since the enzymatic oxidation is specific for glucose and **1** selectively reacts with the resultant gluconic acid at pH 4.7, other saccharides are not responsive at all. On the other hand, oxidation by Br_2 takes place not only for glucose but also for other aldoses such as galactose.[11] Galactonic acid, an oxidation product of galactose, also has α-hydroxycarboxylate moiety through which **1** can be bound, so that some response is observed against galactose. Despite the limitation as above mentioned, Br_2 oxidation still have some selectivity because Br_2 does not oxidize ketoses (e.g. fructose) in addition to non-reducing sugars (e.g. sucrose). For the case of GOx oxidation, the response against glucose increases with increasing glucose concentration (Supporting Information, Figure S5). Thus, one can specifically sense glucose concentration without interference from other saccharides by using the present methodology.

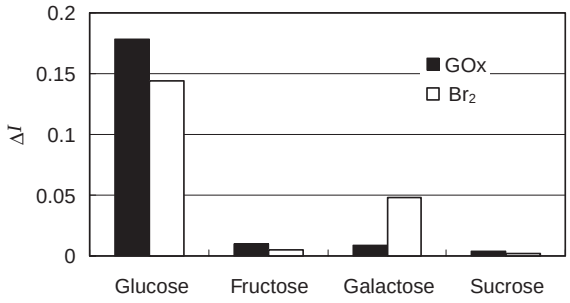


Figure 3. Comparison of responses against 10 mM of saccharides; ΔI is defined as the difference of I_{351}/I_{359} in the presence and absence of saccharides.

In conclusion, we have established a novel glucose-specific sensing system by the combination of a boronic acid and enzymes. To overcome the inherently low binding affinity of glucose toward boronic acids, glucose is enzymatically converted into gluconic acid then reacted with a fluorescent boronic acid. Since boronic acids can bind gluconic acid through the α-hydroxycarboxylate moiety in weakly acidic aqueous solutions where *cis*-diol moieties cannot form complexes, one can specifically sense the concentration of glucose. The present strategy provides an alternative way for boronic acid-based sensing systems having glucose specificity without necessity of costly and time-consuming organic synthesis. As an extension of the present methodology, we are now developing glucose-responsive materials utilizing the combination of boronic acids and enzymes.

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References and Notes

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5 Water is omitted in the Scheme for simplicity.
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7 The ratiometric measurement is advantageous over the measurement at a single wavelength since the influence of error-causing factors (e.g. fluctuation of fluorophore concentration, absorption of excitation and emission lights by coexisting substances) is largely canceled.
8 Glucose oxidase from *Aspergillus niger* and catalase from bovine liver were purchased from Wako.
9 Experimental details are described in Supporting Information.
10 The reaction can be accelerated by raising temperature and enzyme concentrations: we confirmed that the reaction is completed within 3 h by employing 5 times concentrated enzymes at 37 °C.
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