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A dual role of phenylboronic acid as a receptor for carbohydrates as well as a quencher for neighboring pyrene fluorophore

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ABSTRACT

A simple amino acid based compound (**1**) containing a phenyl boronic group and pyrene fluorophore showed an enhanced fluorescence in aqueous solutions at physiological pH through suppression of the photoinduced electron transfer from pyrene to boronic acid on carbohydrate binding. The compound exhibited an interesting fluorescence change depending on pH with decreased emission intensity at acidic pH but enhanced emission intensity at basic pH unlike the fluorescent carbohydrate chemosensors using a PET process with amine and aryl-boronic acid. We have characterized a dual role of phenylboronic acid as a receptor for carbohydrates as well as a quencher for the fluorescence of pyrene fluorophore.

1. Introduction

Detection methods for carbohydrates have been of great interest because carbohydrates play important roles in many biological processes such as nutrition, metabolism, and cell structure.[1] During the last decade, there have been many advances in the development of fluorescent chemosensors for carbohydrate using aryl-boronic acid as a receptor part because the aryl-boronic acid rapidly formed reversible covalent bonding with carbohydrates in aqueous solutions.[2,3] Aryl-boronic acid has been also used as a receptor part in the chemosensor for anions, diol compounds, serine, and glycoproteins as well as a building block of self-assembled molecules.[2,3]

As it was considered that the covalent interactions of aryl-boronic acid with carbohydrates did not considerably change the fluorescence of the neighboring fluorophore, the fluorescent chemosensors based on the aryl-boronic acid have required certain processes for the change of fluorescence depending on the boronic acid–carbohydrate interactions such as an internal charge-transfer (ICT) process and a photoinduced electron transfer (PET) process.[2–4] Fluorescent chemosensors for carbohydrates with ICT process have received attention because of their sensitive response and ratiometric detection of sugars with fluorescent shift of the complex. However, this kind of chemosensors required a sophisticated design and difficult synthesis of the fluorophores containing both electron-donating group and boronic acid group for carbohydrate binding.

Shinkai et al. have reported pioneer works for fluorescent carbohydrate chemosensors using a PET process with tertiary amine and aryl-boronic acid.[5] In general, this type of fluorescent chemosensors consists of an electron-rich group (e.g., tertiary amine and tetrathiafulvalene group), a fluorophore, and an aryl-boronic acid group (Scheme 1). Before carbohydrate binding, electron-rich amine group quenches fluorescence from the adjacent fluorophore by PET (fluorophore as the acceptor of ET). When the boronic acid group forms cyclic boronate esters with carbohydrates, the acidity of the boronic acid increases and therefore the acid–base interaction between the boronic acid and the amine group increases. As a result, PET from amine to the fluorophore is inhibited and the fluorescence from the fluorophore increases. This kind of PET process with amine and aryl-boronic acid has been widely used for detecting carbohydrates and diol compounds because of sensitive change of emission intensity upon formation of cyclic boronate esters between boronic acid and carbohydrates.[2–4,6]

In recent years, there have been many efforts to synthesize chemosensors based on the scaffold of amino acids and peptides because of their compatibility with aqueous media and biological compatibility, and high binding affinity for specific metal ions and biomolecules.[7] These chemosensors based on the scaffold of amino acids and peptides have shown highly sensitive responses to specific analytes in aqueous solutions. In accordance with this trend, as an effort to search for sensitive and water-soluble fluorescent chemosensor for carbohydrates, we synthesized compounds by

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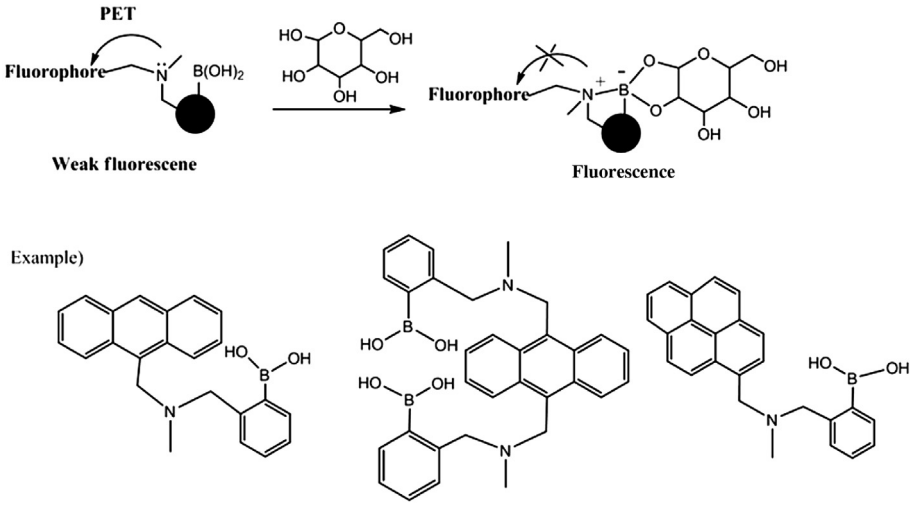


Scheme 1. Fluorescent carbohydrate chemosensors using a PET process with tertiary amine and aryl-boronic acid.[5]

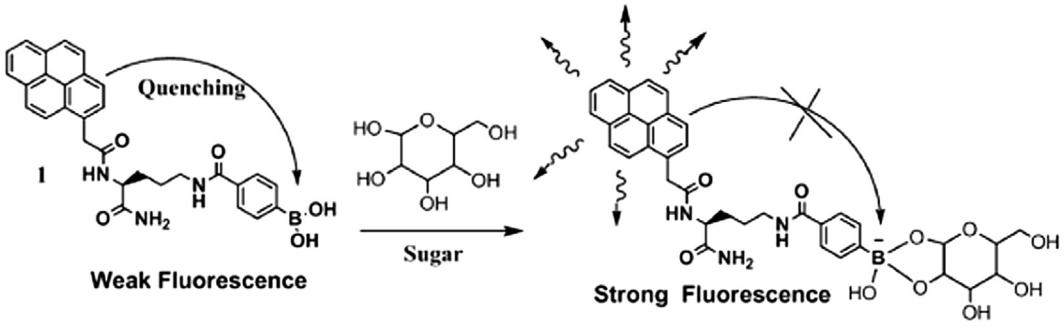
incorporating fluorophores and phenylboronic acid into an ornithine amino acid (see compound **1** in Scheme 2). Surprisingly, although compound **1** did not contain amine as an electron-rich group unlike the PET process proposed by Shinkai et al., it showed turn-on response (ca. 5.8-fold enhancement to fructose) to carbohydrate in aqueous solutions at physiological pH. The emission intensity of **1** increased as the boronate anion form of **1** increased. The pH titration experiment revealed that **1** showed weak fluorescence at neutral pH but strong fluorescence at basic pH. From further experiments, we confirmed that phenylboronic acid served as a quencher for the neighboring pyrene fluorophore, (fluorophore as the donor of the ET). When fructose interacted with the boronic acid to form boronate ester, the pK_a value of the boronic acid decreased. As a result, PET from pyrene to boronic acid might be inhibited and the fluorescence intensity from the pyrene fluorophore increased. Therefore, boronic acid uniquely serves as a carbohydrate receptor as well as a quencher for the pyrene fluorophore in aqueous solutions. This indicates that the electronic change of phenylboronic acid by covalent interaction with carbohydrates is enough to change the fluorescent of the adjacent pyrene fluorophore. In addition, the new role of phenylboronic acid can make it possible to develop new carbohydrate chemosensor with new fluorescence mechanism using the quenching effect of phenylboronic acid.

change of Orn acid on the resin. Fmoc group was removed in the basic condition and then, pyrene fluorophore was conjugated. The successful synthesis and high purity (>98%) of **1** was confirmed by using HPLC, ESI mass spectrum, and NMR spectrum (Supplementary data, Figs. S2–S7).

2.2. Fluorescence response of compound 1 to D-fructose

As **1** is highly soluble in water, all photochemical experiments were carried out in aqueous solution containing 1% DMSO. The fluorescent response of **1** was measured with varying amounts of fructose in 50 mM phosphate buffer solution at pH 7.4 (Fig. 1). Compound **1** exhibited a turn-on response to fructose in aqueous solution at physiological pH. About 8 mM of fructose was enough for the saturation of the emission intensity change. Upon the addition of fructose at a saturation amount, the maximum emission intensity of **1** exhibited a ca. 5.8-fold enhancement compared to the emission intensity without any fructose. The fluorescent chemosensors using a PET process with amine and aryl-boronic acid were reported to exhibit 3–7 fold enhancement in the presence of fructose.[3,4,6]

The UV–vis spectra of **1** are quite sharp both in the presence and absence of fructose and did not change in the presence of milli-



Scheme 2. PET process for carbohydrate sensing and the structure of **1**.

2. Results and discussion

2.1. Solid-phase synthesis of compound 1

Compound **1** was easily synthesized in solid-phase synthesis (Supplementary data, Fig. S1). Fmoc-Orn(Alloc)-OH was attached to the Rink amide resin. After deprotection of alloc group, phenylboronic group was conjugated into the amine group of the side

molar concentration of fructose (Supplementary data, Fig. S8), which indicates that **1** dissolved well in aqueous solution and did not aggregate in the presence of fructose.[8]

2.3. Fluorescence response of 1 to D-fructose at different pH

To investigate the role of boronic acid for the fluorescence change, the fluorescence spectrum of **1** was measured in the