A NEW TYPE OF WATER-SOLUBLE FLUORESCENT BORONIC ACID SUITABLE FOR CONSTRUCTION OF POLYBORONIC ACIDS FOR CARBOHYDRATE RECOGNITION

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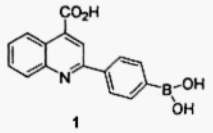
Abstract: In this paper we report boronic acid **1** with a quinoline moiety as a new type of fluorescent probe for carbohydrates, which shows significant fluorescence intensity increases upon sugar binding at physiological pH. This compound has the unique structural feature of separating the boronic acid moiety from the presumed fluorophore, and is ready for the construction of polyboronic acids through tethering to its carboxylic group for high selectivity and affinity recognition of carbohydrates of biological interest.

Introduction

The development of synthetic compounds that are capable of recognition of molecules and ions is a long-standing challenge in organic chemistry.[1] Such work is of increasing practical value in the development of chemosensors for compounds of biological and environmental importance. Recently, much attention has been paid to the development of molecular receptors for polyols, including saccharides.[2-19] Boronic acids readily and reversibly form cyclic esters with diols in aqueous media; this property makes boronic acid group an ideal recognition moiety for the development of synthetic sensors for carbohydrates.[20-23] For almost a decade, boronic acids have been used in many research groups as the recognition motif for the synthesis of fluorescent sensors for carbohydrates.[2-19] Different mechanisms have been used to induce spectroscopic changes upon binding of the boronic acid moiety with a saccharide.[3,19] Among the most important discoveries is an anthracene-based fluorescent reporter system developed by Shinkai and co-workers, which has been widely used because of its large change in fluorescence upon ester formation due to the switching of a photoelectron transfer process.[19] Our group has also applied the Shinkai system for the preparation of sensors for mono- and oligosaccharides.[24-26] However, the anthracene-based fluorescent reporter has many undesirable properties such as low water solubility. Furthermore, the fluorescence intensity of the anthracene fluorophore can be affected by minor changes in the environment such as temperature and oxygen concentration. These features affect the reproducibility and application both in vitro and in vivo. Therefore, it is desirable to find new water-soluble fluorescent boronic acid reporters that give stable and reproducible readings and are functional at physiological pH.[27,28]

Recently, we reported 8-quinolineboronic acid (8-QBA) as a fluorescent reporter with an unusual fluorescent change mechanism that could be used to make fluorescent sensors for cell-surface polysaccharides for in vivo applications.[27] The ultimate goal is the incorporation of such reporter units into di- or polyboronic acid sensors for

specific and high affinity recognition of saccharides of biological interest.[24, 25] For this application, we were interested in developing fluorescent boronic acids that have a "handle" allowing for ready tethering to other scaffolds. Along this line, we report boronic acid **1** that has two unique properties. First, unlike in 8-QBA, the quinoline moiety in **1**, the presumed fluorophore, is not directly attached to boronic acid group. Second, **1** has a free carboxyl group for the ready tethering of other structural moieties for sensor construction. This boronic acid responds to the binding of a carbohydrate with a large fluorescence intensity change at physiological pH in aqueous solution.



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Results and Discussion

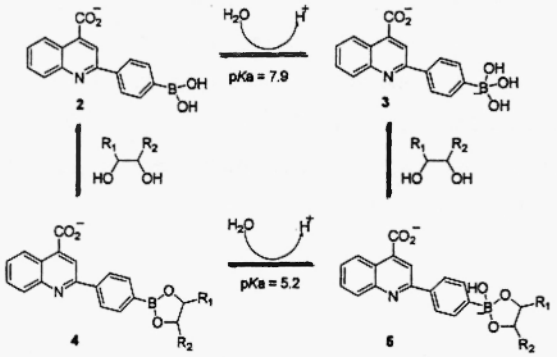
The effect of different sugars on the fluorescent properties of compound **1** was determined in phosphate buffer at pH 7.4. The fluorescence spectral changes of **1** with fructose at different concentrations are shown in Figure 1. A 25-fold emission intensity increase was observed in the presence of 25 mM fructose. This was accompanied by a 24 nm shift in the emission λ_{max}. In an effort to examine the generality of this phenomenon, other sugars were tested (Figure 2). All sugars tested were found to cause significant fluorescence intensity increases at physiological pH with varying magnitude. Fructose induced the largest fluorescence intensity changes, about 25-fold, at a concentration of 25 mM. Glucose, on the other hand, induced a maximum of 6-fold fluorescence intensity increase at a higher concentration of 0.2 M. The binding constants (*K*a) between compound **1** and the four sugars were further determined assuming the formation of a 1:1 complex. As expected,[22] the affinity trend with **1** followed that of simple phenylboronic acid in the order of fructose > mannose ≈ galactose > glucose (Table 1).

Table 1. Association constants (*K*a) and fluorescence intensity changes ($\Delta I/I_0$) of **1** with different sugars

Sugar	*K*a (M^{-1})	$\Delta I/I_0$ (sugar concentration, M)
Fructose	544 ± 22	25 (0.025)
Galactose	46 ± 9	16 (0.025)
Mannose	48 ± 5	14 (0.025)
Glucose	5.1+. 1.0	6 (0.20)

Since the fluorescence intensity increase upon binding with a sugar seems to be a general phenomenon, next we were interested in examining the conditions under which optimal fluorescence intensity changes are observed upon addition of a sugar. For this purpose, we studied the fluorescence pH profiles of both compound **1** alone and in the presence of fructose (0.5 M). The fluorescence intensity of **1** in the absence of sugars increased by 17-fold upon changing the pH from 3 to 12 (Figure 3). An apparent p*K*a of 7.9 was observed, which is assigned to the boronic acid moiety. Since the p*K*a of phenylboronic acid is 8.8 and electron-withdrawing groups are known to lower the p*K*a of a boronic acid,[22] it is reasonable to expect that p*K*a of the boronic acid moiety in **1** to be less than 8.8. Furthermore, one would expect the p*K*a values of the other two ionizable groups, the carboxylic acid and the quinolinium groups, to be

less than 7.[29,30] Therefore, assigning the p*K*a at 7.9 to the boronic acid moiety is the most reasonable option. The fluorescence intensity of **1** in the presence of fructose increased by 35-fold upon changing the pH from 2 to 10 and a p*K*a at 5.2 was observed (Scheme 1). It is well-known that the binding of a diol to boronic acid often lowers the p*K*a of the boron species.[22] Therefore, the p*K*a, which drops from 7.9 to 5.2, can also be reasonably assigned to boron. These results indicate that the boronic acid moiety of compound **1** exists at pH 7.4 predominantly in the neutral, non-ionized form. Upon addition of fructose, the p*K*a of the boron drops to about 5.2, which ensures that most of the boron species are in the anionic tetrahedral state at physiological pH. Such titration results also mean that the ionization states of the other two ionizable functional groups, quinoline and carboxylic acid, do not seem to affect the fluorescence intensity of **1**.



Scheme 1. Equilibrium involving pH and sugars

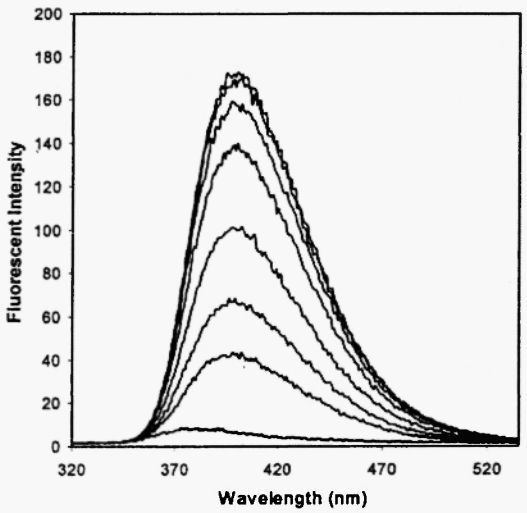


Figure 1. Fluorescence response of **1** (2×10^{-5} M) in 0.10 M phosphate buffer at pH 7.4 in the presence of D-fructose (0, 0.5, 1.0, 2.0, 5.0, 10, 20, 25 mM): $\lambda_{ex} = 270$ nm.

It is worth mentioning that the fluorescence intensity increase of compound **1** upon binding with sugars is observable in the whole range of pH above 5.0. This suggests that **1** could be used for monitoring sugar at any pH value

in the range. Particularly important to our work is that **1** shows close to optimal fluorescence intensity changes at physiological pH. As with the free boronic acid, the most fluorescent form in the presence of fructose is the form when the boron is in the anionic tetrahedral form. However, it is interesting to note that the boronate form **5** for the sugar-boronic acid complex is far more fluorescent than the corresponding boronate form **3** of the free acid (Scheme 1), which forms the functional basis for this sensor. These facts also indicate that the fluorescence intensity increase of compound **1** at physiological pH in the presence of the sugar is not entirely due to the ionization state change of the boron, but also due to diol-binding's perturbation as in 8-QBA.[27] Therefore, both boron hydridization state change and complexation perturbation are critical factors that make **5** strongly fluorescent. The exact mechanism through which this fluorescence intensity change occurs needs to be examined further.

The finding of compound **1** as a new fluorescent sensor with a boronic acid group that is not directly attached to the quinoline moiety suggests that the quinoline moiety could be used as a general fluorescent reporter for carbohydrate sensor development. The numerous descriptions of substituted quinolines available in the literature could lead to long-wavelength and conjugatable fluorescence probes for sugars. Furthermore, **1** has a free carboxylic acid, which can be used as a handle for tethering other functional groups. Such a "handle" is critical for the future construction or di- or polyboronic acid sensors that have the desired specificity and affinity for the intended analyte. Preliminary results in our lab show that the amide coupling can be easily achieved with this free carboxylic acid under standard amide synthesis conditions, and further fluorescence studies demonstrated that amide formation does not change the fluorescent properties of **1**.

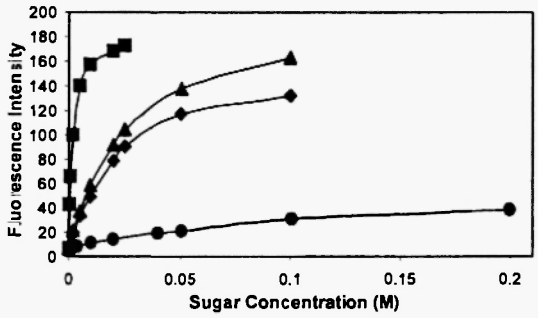


Figure 2. Fluorescence intensity of **1** (2×10^{-5} M) in 0.10 M phosphate buffer at pH 7.4 in the presence of D-fructose (•), D-galactose (•), D-mannose (♦), and D-glucose (•): λ_{ex} = 270 nm, λ_{em} = 400 nm.

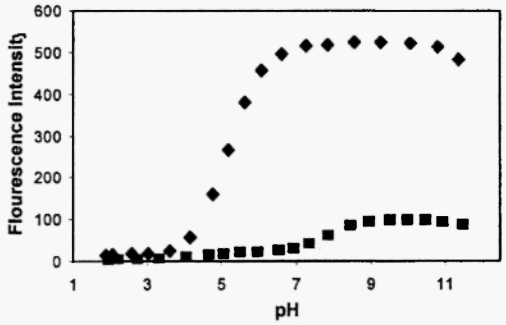


Figure 3. Fluorescence intensity pH profile of **1** (2×10^{-5} M) in 0.10 M phosphate buffer: [saccharide] = 0.5 M, λ_{ex} = 270 nm, λ_{em} = 400 nm. • **1**, ♦ **1** + 0.5 M D-fructose.

Conclusions

In conclusion, compound **1** was found to be a new type of fluorescent reporter compound with the boronic acid moiety separated away from the presumed fluorophore. It shows a large fluorescence intensity changes upon binding with sugars in aqueous solution at physiological pH. Moreover, it shows an interesting fluorescence change mechanism that combines both the boron hybridization state change and diols perturbation as a way to modulate the fluorescence. To the best of our knowledge, this is the first example of this kind. The availability of the free carboxylic acid also makes it easy to prepare the diboronic acid sensors for improved specificity and affinity.[18, 24-26] Work is underway to use this new fluorescent reporter compound for the synthesis of di- and polyboronic acid sensors for high selectivity identification and detection of carbohydrates of biological interest.[24, 25]

Acknowledgements

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