Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1019–1022

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Novel Type of Fluorescent Boronic Acid That Shows Large Fluorescence Intensity Changes Upon Binding with a Carbohydrate in Aqueous Solution at Physiological pH

Wenqian Yang, Jun Yan, Greg Springsteen, Susan Deeter and Binghe Wang*

Department of Chemistry, North Carolina State University, Raleigh, NC 27695-8204, USA

Received 24 October 2002; revised 10 January 2003; accepted 22 January 2003

Abstract—In this paper we report 8-quinolineboronic acid as a novel type of fluorescent probe for carbohydrates. This boronic acid responds to the binding of a carbohydrate with over 40-fold increases in fluorescence intensity and shows optimal fluorescence change at physiological pH in aqueous solution.

Due to its high affinity for diols,[1–3] boronic acid has generated much interest as the recognition motif for the synthesis of fluorescent sensors for carbohydrates.[4–20] Critical to this effort is the availability of practical fluorescent reporters that respond to the binding event with significant fluorescence intensity changes under physiological conditions. During the last decade, there has been a great deal of progress made in the construction of boronic acid-based sensors for carbohydrates and other diol-containing compounds.[4–20] 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.[13,21] Our group has also applied the Shinkai system for the preparation of sensors for mono- and oligosaccharides.[17–20] However, the anthracene-based fluorescent reporter has many undesirable properties such as low water solubility and poor photochemical stability. Furthermore, the fluorescence intensity of the anthracene fluorophore can be affected by minor changes in the environment such as temperature and oxygen concentration. All these affect the reproducibility and application both in vitro and in vivo. Therefore, there has been much interest in search for new reporter compounds that change spectroscopic properties upon boronic acid binding with a carbohydrate with limited success.[22–33] Our group has been interested in the development of fluorescent sensors for cell-surface carbohydrates as biomarkers.[19] In such an effort, we desire a fluorescent reporter boronic acid compound that shows large fluorescence intensity changes upon binding, is water soluble, gives stable and reproducible readings, and is functional at physiological pH. Herein we report 8-quinolineboronic acid (8-QBA) as a novel fluorescent sensor for carbohydrates, that (**1**) responds to the binding of a carbohydrate with more than 40-fold increases in fluorescence intensity; (**2**) is soluble in aqueous solution; (**3**) shows optimal fluorescence intensity changes upon binding at physiological pH; and (**4**) has stable fluorescence readings that are not affected by minor environmental changes. All these properties make such a system ideal for the construction of fluorescent sensors for carbohydrates. This basic building block or its slightly modified analogues can be used for the synthesis of diboronic acid receptors for the selective recognition and detection of certain carbohydrates.[19,20]

8-QBA itself is essentially non-fluorescent at pH above 5 and weakly fluorescent at lower pH in aqueous solution.[34] However, upon addition of D-fructose, the fluorescence intensity increased dramatically in a concentration-dependent manner (Fig. 1). In an effort to examine the generality of this phenomenon, a few other sugars were tested. Figure 2 shows the concentration profiles of fructose, tagatose, galactose, and arabinose with binding constants of $108\,M^{-1}$, $62\,M^{-1}$, $7.5\,M^{-1}$, and $1.1\,M^{-1}$, respectively. It is interesting to point out that

*Corresponding author. Tel.: +1-919-515-2948; fax. +1-919-515-3757; e-mail: binghe_wang@nscu.edu

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00086-6

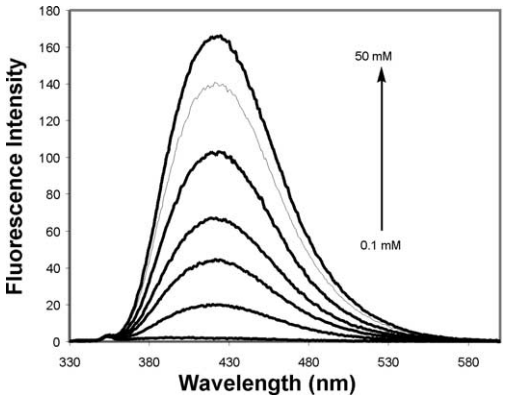


Figure 1. Fluorescence response of 8-QBA (6.3×10^{-5} M) in 0.10 M phosphate buffer at pH 7.4 in the presence of D-fructose (0.1, 1.0, 2.5, 5.0, 10, 25, 50 mM): $\lambda_{ex}=314$ nm.

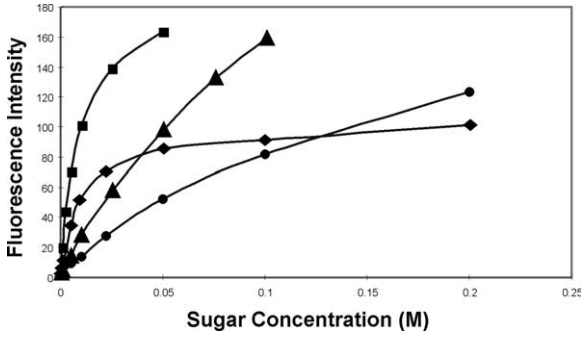


Figure 2. Fluorescence intensity of 8-QBA (6.3×10^{-5} M) in 0.10 M phosphate buffer at pH 7.4 in the presence of D-fructose (■), D-galactose (▲), D-tagatose (◆), and L-arabinose (●): $\lambda_{ex}=314$ nm, $\lambda_{em}=417$ nm.

(**1**) glucose only showed a maximum of 3-fold increase in fluorescence intensity and (**2**) the fluorescence intensity increases of 8-QBA in the presence of various sugars at the lower concentration range (<25 mM) seem to correlate with the intrinsic binding affinity of different sugars for 8-QBA (Fig. 2).

Since the fluorescence intensity increase upon binding with a sugar seems to be a general phenomenon, next we were interested in examining the pH profile of the fluorescence intensity changes. For this, we chose a fixed sugar concentration of 0.5 M. The fluorescence intensity increase is observable between pH 2–10 with the maximum between pH 4.5–7.5 (Fig. 3). This is true with the two out of three sugars tested. The fluorescence intensity change at pH 7 and 7.5 (phosphate buffer) were about 42- and 47-fold, respectively, in the presence of 0.5 M fructose. Just as important is the feature that this sensor is functional at physiological pH without the need to add an organic co-solvent, which is required by the anthracene-based boronic acid sensors.

Aimed at examining how 8-QBA functions as a fluorescent probe for diols, we studied the UV pH profiles of both 8-QBA alone and 8-QBA in the presence of D-fructose (1.0 M). 8-QBA was found to have two p*K*a's (Fig. 4), one at about 4 and the other at about 10. In order to be able to assign each p*K*a, we recorded the ^{11}B

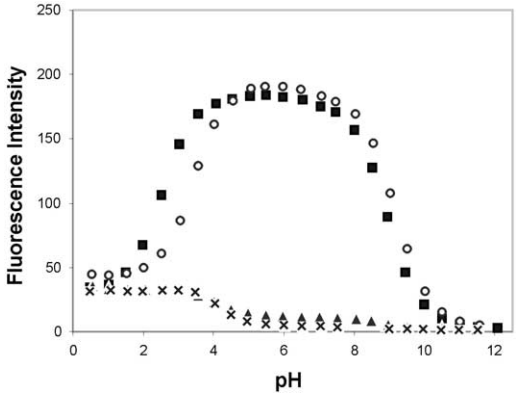


Figure 3. Fluorescence intensity pH profile of 8-QBA (6.3×10^{-5} M) in 0.10 M phosphate buffer: [saccharide] = 0.5 M, $\lambda_{ex}=314$ nm, $\lambda_{em}=417$ nm. × blank, ■ D-fructose, ▲ D-glucose, ○ D-galactose.

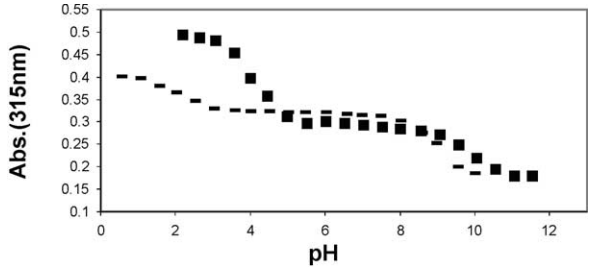
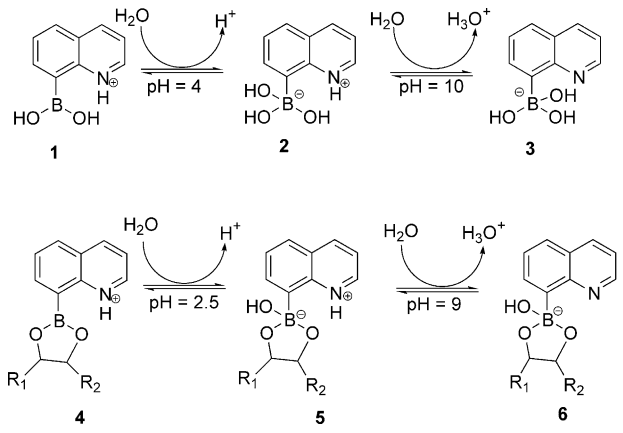


Figure 4. Absorbance intensity pH profile of 8-QBA (6.3×10^{-5} M) in 0.10 M phosphate buffer. ■ 8-QBA, -8-QBA + 1.0 M D-fructose.

NMR spectra of 8-QBA in a mixed deuterated methanol–water (1:1) solvent at different pH. Methanol was used so that the concentration of 8-QBA can be increased to 44 mM so as to allow for a meaningful NMR determination within a reasonable period of time. The pH titration studies of 8-QBA in a mixed methanol–water (1:1) solution showed that both the fluorescence and UV profiles were nearly identical as that in 100% water solution (data not shown), and it is known that the addition of 50% methanol to water solution results in minimal changes of the solution pH.[35] Therefore, we measured the ^{11}B NMR in the mixed aqueous solution to investigate the boron hybridization state. The boron signal of 8-QBA appeared at 24.3 ppm at pH 1.5 and shifted to 8.3 ppm at pH 6.5 and 6.5 ppm at pH 11.5, respectively. These results indicated that the boron changed hybridization from sp^2 to sp^3 between pH 1.5 to 6.5.[11] This means that 8-QBA exists at pH 7.4 predominantly as the zwitterionic quinolinium boronate form (**2**) (Scheme 1).[36,37] This allowed us to assign the first pKa at 4 of 8-QBA to the boronic acid group and the second p*K*a at 10 to the quinolinium nitrogen. Since the formation of a boronic ester with a diol further lowers the p*K*a of the boron,[1] the same assignments for 8-QBA esters should also be correct. This indicates that the fructose ester (**5**) is the fluorescence species. The quantum yield of **5** (fructose ester) at pH 7.4 in phosphate buffer was determined to be 0.28.

Past work in searching for new boronic acid-based fluorescent reporter compounds has been focused on exploring the inductive effect of boronic ester formation



Scheme 1. The ionization steps of 8-QBA and its esters.

on a conjugated π chromophoric system[22–31] and/or the utilization of B–N bond formation.[23,25,32,33] In this study, there is no B–N bond formation expected due to the large angle strain if such a B–N bond were to form. Furthermore, the boron atom in both 8-QBA and its esters are in the tetrahedral forms at pH 7.4 indicating that the fluorescence intensity changes are not due to the change in hybridization state of the boron. This is further substantiated by the fact that with the pH increase from 2 to 7 the fluorescence intensity of 8-QBA decreases, but those of 8-QBA esters increase (Fig. 3). The environmental change of quinoline ring of 8-QBA upon binding with a sugar is a possible reason that causes the fluorescence intensity changes. It is known that the fluorescence of quinoline-type compounds could be switched 'on' if the $n\pi^*$ state is perturbed such that the lowest energy singlet excited state is of the $\pi\pi^*$ state.[38] For example, quinoline is itself nonfluorescent in hydrocarbon solvent. However, it does exhibit fluorescence in hydroxylic media due to the perturbation from hydrogen bond formation. It is possible that 8-QBA work the same way due to the perturbation from the attached diols.[34,39,40] However, further study is needed in order to understand the mechanism through which 8-QBA changes its fluorescence upon binding with a diol.

The availability of 8-QBA-based fluorescent reporter compounds will be very useful to the effort of making fluorescent sensor for cell-surface carbohydrates for in vivo applications.[19] In our earlier efforts of making such cell-surface carbohydrate sensors using the anthracene-based fluorophore, it was always necessary to add some organic co-solvent (commonly methanol) for the cell-labeling studies due to their poor water solubility. The need for organic co-solvent can be tolerated in an in vitro experiment, but not in an in vivo experiment. The availability of water-soluble fluorescent reporter compounds such as 8-QBA will significantly help the effort of making biocompatible fluorescent sensors for cell-surface carbohydrates as biomarkers.

In conclusion, 8-QBA was found to be a fluorescent reporter compound with many desirable properties for biosensor preparation. Such properties include (1) large fluorescence intensity changes upon binding and (2) being functional in aqueous solution at physiological pH. Work is underway to better understand the fluorescence change mechanism and use this new type of fluorescent boronic acid compounds for the synthesis of diboronic acid compounds for high selectivity and affinity recognition of carbohydrates of biological interest.[19]

Acknowledgements

Financial support from the National Institutes of Health (NO1-CO-27184 and CA88343) and the North Carolina Biotechnology Center (2001ARG0016) is gratefully acknowledged. We also thank Professor Jon Lindsey for his help with the quantum yield determination, Dr. Sabapathy Sankar for his help with the ^{11}B NMR experiments, and Dr. Tony Czarnik for helpful discussions.

References and Notes

1. Springsteen, G.; Wang, B. *Tetrahedron* **2002**, *58*, 5291.
2. Sugihara, J. M.; Bowman, C. M. *J. Am. Chem. Soc.* **1958**, *80*, 2443.
3. Lorand, J. P.; Edwards, J. O. *J. Org. Chem.* **1959**, *24*, 769.
4. Yoon, J.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 5874.
5. James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Nature (London)* **1995**, *374*, 345.
6. Eggert, H.; Frederiksen, J.; Morin, C.; Norrild, J. C. *J. Org. Chem.* **1999**, *64*, 3846.
7. Norrild, J. C.; Eggert, H. *J. Am. Chem. Soc.* **1995**, *117*, 1479.
8. Sandanayake, K. R. A. S.; Nakashima, K.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1994**, 1621.
9. Norrild, J. C.; Eggert, H. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2583.
10. Shinkai, S.; Takeuchi, M. *Trends in Anal. Chem.* **1996**, *15*, 188.
11. Wiskur, S. L.; Lavigne, J. L.; Ait-Haddou, H.; Lynch, V.; Chiu, Y. H.; Canary, J. W.; Anslyn, E. V. *Org. Lett.* **2001**, *3*, 1311.
12. Yang, W.; He, H.; Drueckhammer, D. G. *Angew. Chem. Int. Ed.* **2001**, *40*, 1714.
13. James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Chem. Commun.* **1994**, 477.
14. Wang, W.; Gao, X.; Wang, B. *Curr. Org. Chem.* **2002**, *6*, 1285.
15. Lavigne, J. J.; Anslyn, E. V. *Angew. Chem. Int. Ed.* **1999**, *38*, 3666.
16. Cabell, L. A.; Monahan, M.-K.; Anslyn, E. V. *Tetrahedron Lett.* **1999**, *40*, 7753.
17. Gao, S.; Wang, W.; Wang, B. *Bioorg. Chem.* **2001**, *29*, 308.
18. Wang, W.; Gao, S.; Wang, B. *Org. Lett.* **1999**, *1*, 1209.
19. Yang, W.; Gao, S.; Gao, X.; Karnati, V. V. R.; Ni, W.; Wang, B.; Hooks, W. B.; Carson, J.; Weston, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2175.
20. Karnati, V. R.; Gao, X.; Gao, S.; Yang, W.; Ni, W.; Sankar, S.; Wang, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3373.
21. James, T. D.; Sandanayake, K. R. A. S.; Iguchi, R.; Shinkai, S. *J. Am. Chem. Soc.* **1995**, *117*, 8982.
22. Adhikiri, D. P.; Heagy, M. D. *Tetrahedron Lett.* **1999**, *40*, 7893.
23. Ward, C. J.; Patel, P.; Ashton, P. R.; James, T. D. *Chem. Commun.* **2000**, 229.

24. DiCesare, N.; Lakowicz, J. R. *Org. Lett.* **2001**, *3*, 3891.
25. Ward, C. J.; Patel, P.; James, T. D. *Org. Lett.* **2002**, *4*, 477.
26. Cao, H.; Diaz, D. I.; DiCesare, D.; Lakowicz, J. R.; Heagy, M. D. *Org. Lett.* **2002**, *4*, 1503.
27. DiCesare, N.; Lakowicz, J. R. *Tetrahedron Lett.* **2002**, *43*, 2615.
28. DiCesare, N.; Lakowicz, J. R. *Tetrahedron Lett.* **2001**, *42*, 9105.
29. DiCesare, N.; Lakowicz, J. R. *Chem. Commun.* **2001**, 2022.
30. DiCesare, N.; Lakowicz, J. R. *J. Photochem. Photobiol. A-Chem.* **2001**, *143*, 39.
31. DiCesare, N.; Lakowicz, J. R. *J. Phys. Chem. A* **2001**, *105*, 6834.
32. Ward, C. J.; Patel, P.; James, T. D. *J. Chem. Soc., Perkin Trans. 1* **2002**, 462.
33. Arimori, S.; Bosch, L. I.; Ward, C. J.; James, T. D. *Tetrahedron Lett.* **2001**, *42*, 4553.
34. Goldman, M.; Wehry, E. L. *Anal. Chem.* **1970**, *42*, 1186.
35. Bates, R. G. *Determination of pH*; John Wiley & Sons: London, 1964.
36. Mohler, L. K.; Czarnik, A. W. *J. Am. Chem. Soc.* **1993**, *115*, 2998.
37. Fisher, F. C.; Havinga, E. *Recl. Trav. Chim. Pays.-Bas* **1974**, *93*, 21.
38. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.
39. Morrison, J. D.; Letsinger, R. L. *J. Org. Chem.* **1964**, *29*, 3405.
40. Wulff, G.; Lauer, M.; Böhnke, H. *Angew. Chem. Int. Ed.* **1984**, *23*, 741.