Detection of anions using a fluorescent alizarin–phenylboronic acid ensemble†

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Received (in Cambridge, UK) 9th March 2005, Accepted 6th April 2005
First published as an Advance Article on the web 21st April 2005
DOI: 10.1039/b503588k

Selective anion-induced organization of phenylboronic acids and alizarin results in a new TURN-ON fluorescent sensor for anions in MeOH.

In the field of molecular recognition, the sensing of anions has attracted growing attention because of its important role in numerous biological processes.[1] Both colorimetric and fluorometric anion sensors have been reported. Where the strategy involves the covalent linking of a reporter fragment (chromophore or fluorophore) to the receptor, the approach is often limited by the synthetic complexity of the receptor molecules. In contrast, self-organized receptor–reporter systems represent a powerful and simple way of analyte detection. The most successful example is the displacement assay approach,[2] pioneered by Ansyln, in which a receptor–reporter ensemble is selectively dissociated by the addition of an appropriate anion, accompanied by a detectable response of the reporter. It occurred to us an alternative approach could be envisioned where the anion-induced receptor–reporter organization could be utilized for the assay of anions. Towards this strategy our attention focused on phenylboronic acids (PBAs) which not only serve as a Lewis acid metal center[3] but also rapidly and reversibly form cyclic esters with diols.[4] Utilizing these properties, Paugam and Smith have shown the F^- accelerated saccharide transport by PBA at neutral pH. The tetrahedral fluoroboronate anion afforded by the reaction of PBA and F^- enhances the formation of cyclic boronate esters with diols.[5] Our idea was to use this property to develop new sensor systems; if one employs a diol-containing reporter, such as alizarin,[6] then the formation of the receptor (PBA)–reporter (alizarin) ensemble would be enhanced by an appropriate anion (*e.g.*, F^-), resulting in a change in the optical properties of the system.[7]

In this communication, we report the behaviour of PBAs and alizarin in MeOH solution. As described below in detail, it was found that F^-- (or AcO^--) induced alizarin–PBA conjugation produced a significant change in the fluorescence intensity of alizarin. Although Shinkai *et al.* have previously reported a colour change in a system involving the redox couple of ferrocene boronic acid and dye molecules,[8] the system represented here is the first example where the fluorescent detection of anions using an anion-induced receptor–reporter ensemble has been attained, making it of potential use as a new sensing system.

Fig. 1 shows the titration results for monitoring the fluorescence intensity change of alizarin (λ_{ex} = 420 nm) upon the addition of PBA in the absence and presence of an excess amount of KF in MeOH. Under F^--free conditions, alizarin shows only small changes in fluorescence when excited at 420 nm (a slightly enhanced emission was observed at low concentrations of PBA, attributable to an increased acidity caused by adding the PBA to methanol).[9] This result suggests that alizarin hardly binds to PBA in MeOH solution. In the presence of KF (40 equiv.) a significant fluorescence enhancement was obtained by adding 3-nitrophenylboronic acid (NPBA) (Fig. 1 (●)). This fluorescence response is influenced by the acidity of PBA. Indeed, by using phenylboronic acid (PBA) the sensitivity is somewhat diminished with a lower saturation intensity in the fluorescence spectra.

F^--induced fluorescence changes were also elucidated from a solution containing NPBA and alizarin: Fig. 2(a) shows the fluorescence change when KF was added to alizarin (50 μM) and NPBA (2 mM) in MeOH. Under F^--free conditions, the fluorescence intensity was low, and consistent with that in Fig. 1. However, the addition of KF produced an increase in the fluorescence intensity of alizarin. The above results allow us to consider that the anion-induced fluorescence enhancement process would take place according to Scheme 1. The equilibria were investigated in a CD_3OD solution by ^1H and ^{11}B NMR spectroscopy. Fig. 3 shows the ^1H NMR spectra (400 MHz), ranging from δ 6.8 to 8.8 ppm, of alizarin (a), alizarin plus NPBA (b) and alizarin plus NPBA upon adding F^- (c) in CD_3OD at room temperature. No perturbation of the chemical shifts for

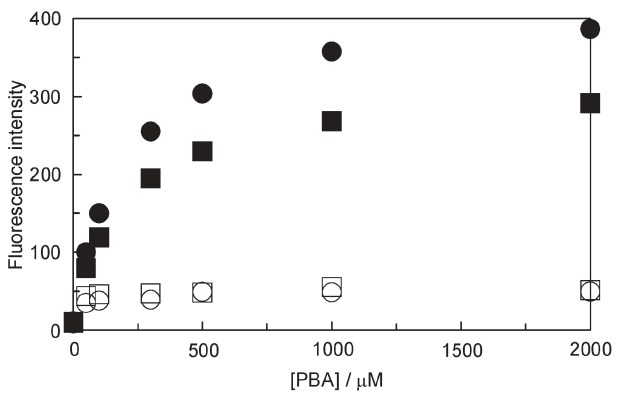

Fig. 1 Plots of the fluorescence intensity of alizarin with an incremental amount of NPBA or PBA in the absence and presence of KF at 25 °C; NPBA plus KF at 586 nm (●); NPBA at 586 nm (○); PBA plus KF at 600 nm (■); PBA at 600 nm (□). [Alizarin] = 50 μM, [KF] = 2 mM, λ_{ex} = 420 nm.

† Electronic supplementary information (ESI) available: ^{11}B NMR, FAB MS spectra, fluorescence titrations of NPBA with KF, ^1H NMR for NPBA upon adding $(n\text{-Bu})_4NF$, ^1H NMR for alizarin plus NPBA upon adding $(n\text{-Bu})_4NOAc$. See http://www.rsc.org/suppdata/cc/b5/b503588k/

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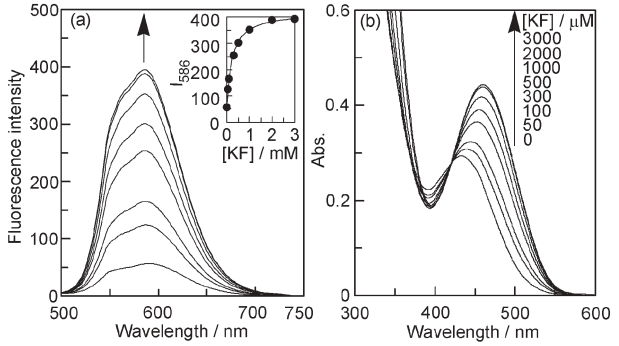
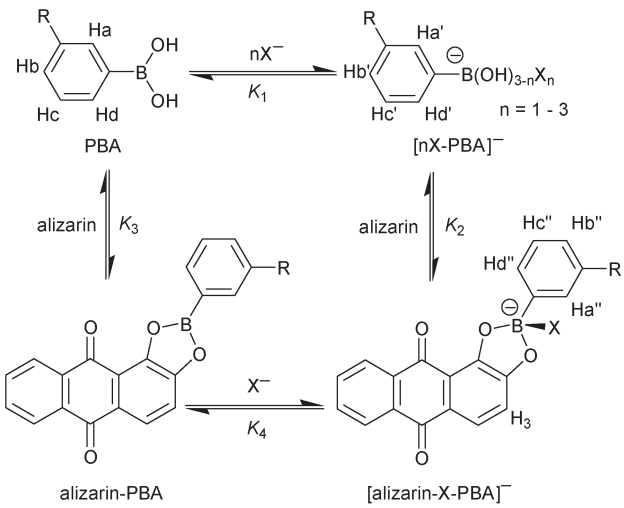


Fig. 2 Change in fluorescence spectra (λ_{ex} = 420 nm) (a) and UV/vis spectra (b) for alizarin (50 μM) upon the addition of KF in the presence of NPBA (2 mM) in MeOH at 25 °C. Inset: representative titration curve and fitting based on a 1 : 1 binding model.



Scheme 1

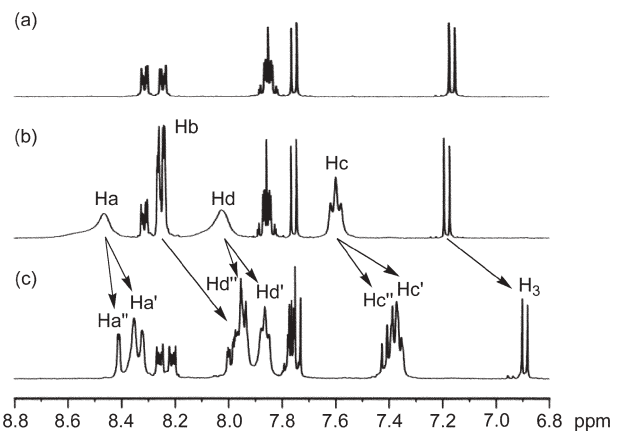


Fig. 3 ^{1}H NMR spectra (400 MHz, CD_3OD, room temperature) of alizarin (a), alizarin plus NPBA (b) and alizarin plus NPBA upon adding (n-Bu)$_4$NF (c). [Alizarin] = 3.8 mM, [NPBA] = 20 mM, [(n-Bu)$_4$NF] = 20 mM.

alizarin and NPBA is caused by mixing them under these conditions (Fig. 3(b)), being consistent with the result in Fig. 1 where in MeOH almost no change in the fluorescence of alizarin was observed upon adding only NPBA. When adding F^- into the solution, however, the chemical shifts altered (Fig. 3(c)): in particular, (1) for alizarin, a significant up-field shift of ArH_3 ($\Delta\delta$ = 0.28 ppm) was obtained; (2) the resonances (Ha, Hc and Hd) arising from NPBA when bound and not bound to alizarin were clearly distinguishable signals in the presence of F^-. The assignment of chemical shifts for [nF–NPBA]$^-$ (Ha′–Hd′) was done based on the spectral data which were obtained when KF (20 mM) was added to a MeOH solution of NPBA (20 mM). We note that the chemical shifts for Ha″, Hc″ and Hd″ (ppm) appeared at 8.41 (d, J = 1.8 Hz), 7.41 (t, J = 7.7 Hz) and 8.01–7.93 (a multiplet signal containing Hb′ and Hb″), respectively, can be assigned to [alizarin–NPBA–F]$^-$,[10] since the spectral behaviour as well as the up-field shift of the ArH_3 signal (*vide supra*) could be fully explained on the basis of the formation of a boronate ester between alizarin and [nF–NPBA]$^-$.

Further assessment of the F^--induced NPBA–alizarin association process came from a ^{11}B NMR (96.3 MHz, 23 °C) study. The ^{11}B NMR signal of NPBA (1 mM) in CD_3OD shows one boron signal at 28.2 ppm when boron trifluoride diethyl etherate was used as an external reference. The signal shifted to δ 5.6 ppm upon addition of 5 equiv. of F^- as a (n-Bu)$_4$N$^+$ salt, being attributable to a change from sp^2 to sp^3 boron on F^--binding. The significant shift of $\Delta\delta$ = 22.6 ppm is almost consistent with the finding of Reetz *et al.*[3] On the other hand, the addition of alizarin (1 mM) into the CD_3OD solution of NPBA caused no shift in the ^{11}B spectra. However, it is noteworthy that further addition of F^- (5 equiv.) into the solution involving alizarin and NPBA allowed us to detect two boron signals at 12.3 ppm and 4.8 ppm. The latter signal (δ 4.8 ppm) could correspond to tetrahedral [nF–NPBA]$^-$. Thus, we reason that the signal of 12.3 ppm is assigned to the ternary complex [alizarin–F–NPBA]$^-$. These results indicate that the production of sp^3-hybridized phenylfluoroboronate plays an important role in the alizarin–NPBA association which allows us to detect F^-.

As inferred from the results of Fig. 1 as well as the NMR study, the K_3 value for PBAs with the alizarin (Scheme 1) is quite small. It means that the path *via* alizarin–PBA can be ruled out. Therefore in this study, where MeOH is employed as a solvent, the path *via* [nX–PBA]$^-$ is plausible to produce [alizarin–X–PBA]$^-$. Evidence that F^- can complex with PBAs to form tetrahedral fluoroboronates was gained from fluorescence titrations of NPBA (2 mM) with KF in MeOH (λ_{ex} = 268 nm, λ_{em} = 333 nm). The experimental curve could be fitted assuming the formation of a trifluoroboronate (n = 3) (see, supplementary information†).[11] The association constant K_1 was then estimated to be (9.4 ± 4.2) × 10^9 M^{-3},[12] suggesting that PBAs can very effectively bind F^- in MeOH. Finally, K_2 is an association constant between alizarin and [nX–PBA]$^-$, the assessment coming from Fig. 2 (*vide supra*). Under conditions containing excess amounts of NPBA, the binding profile could be reproduced by a nonlinear curve fitting plot based on a 1 : 1 complex formated between alizarin and F^-, indicating that monofluoroboronate [F–NPBA]$^-$ (n = 1) binds to alizarin prior to the formation of a di(tri)fluoro tetrahedral boronate; it is plausible that the observed fluorescence change reflects the formation of an [alizarin–F–PBA]$^-$ ensemble produced by reaction of alizarin with [F–NPBA]$^-$ where the K_2 can be estimated to be 4800 ± 150 M^{-1}. These results imply that, although PBAs hardly interact with alizarin in MeOH, a tetrahedral fluoroboronate produced by adding F^- could

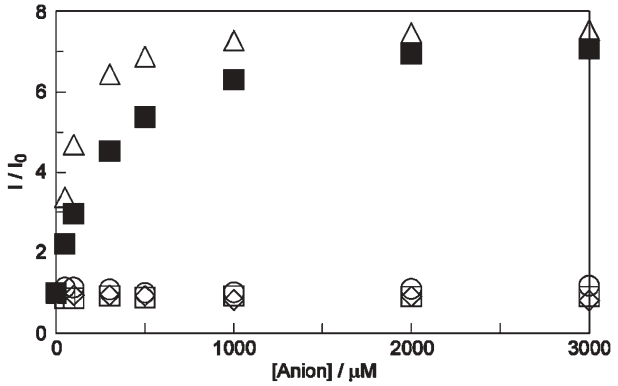


Fig. 4 Plots of the fluorescence intensity at 586 nm of alizarin (50 μM) and NPBA (2 mM) in MeOH at 25 °C excited at 420 nm as a function of anion concentration: F^- (■), Cl^- (◇), Br^- (□), I^- (○) and AcO^- (Δ) as potassium salts.

strengthen the binding of the boronic acid to the diol moiety of alizarin and enhance the fluorescence intensity.

Anion selectivity of the represented fluorescence system was investigated using F^-, Cl^-, Br^-, I^- and AcO^-[13] because these represent families of biologically important anions; Fig. 4 shows the resulting titration curves for the fluorescence intensity when these anions were added to a MeOH solution of alizarin in the presence of 40 equiv. of NPBA. The presence of Cl^-, Br^- and I^- induced no response of the fluorescence properties of alizarin, whereas in the case of AcO^- the fluorescence intensity was enhanced up to 750%.[14] From these results, the apparent association constants K_2 (M^{-1}), defined in Scheme 1, were calculated to be 4800 ± 150 for F^- (*vide supra*) and 17 000 ± 700 for AcO^-,[12] respectively. The fact that the system shows 3.5-fold higher affinity for AcO^- than for F^- is not surprising because the high Lewis basicity of AcO^- enables it to strongly bind to the boronic acid, resulting in a remarkable enhancement of the fluorescence. The order of the sensitivity observed is clearly correlated with the basicity of the added anions.

In conclusion, the results described here lead us to suggest a new approach for the generation of an easy-to-prepare sensing ensemble; anion-induced alizarin (reporter)–PBA (receptor) association results in a fluorescent sensor system showing a specific and sensitive detection for anions containing the most electronegative atoms such as fluorine (F^-)[15] and oxygen (AcO^-).[16] We believe that the results described here are an important step in the development of a simple-to-use detection tool for anions in a variety of industrial or medical applications.[17] We acknowledge a Japan–UK Joint Research Cooperative Program (Joint Project), Japan Society for the Promotion of Science, for financial support. This research has also been supported in part by a Grand-in-Aid for Scientific Research (C) from Education, Science, Sport and Culture of Japan (No. 16550119).

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Notes and references

1 P. D. Beer and P. A. Gale, *Angew. Chem., Int. Ed.*, 2001, **40**, 486; *Coord. Chem. Rev.*, 2003, **240**, issues 1 and 2 (Special Issues on anion receptors); R. Martínez-Máñez and F. Sancenón, *Chem. Rev.*, 2003, **103**, 4419; C. Suksai and T. Tuntulani, *Chem. Soc. Rev.*, 2003, **32**, 192.
2 S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne and E. V. Anslyn, *Acc. Chem. Res.*, 2001, **34**, 963; S. L. Wiskur, J. J. Lavigne, A. Metzger, S. L. Tobey, V. Lynch and E. V. Anslyn, *Chem. Eur. J.*, 2004, **10**, 3792.
3 M. T. Reetz, C. M. Niemeyer and K. Harms, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1472.
4 T. D. James, P. Linnane and S. Shinkai, *Chem. Commun.*, 1996, 281.
5 M.-F. Paugam and B. D. Smith, *Tetrahedron Lett.*, 1993, **34**, 3723.
6 Alizarin Red S has been used for a competitive assay of sugars, see: (*a*) G. Springsteen and B. Wang, *Chem. Commun.*, 2001, 1608; (*b*) S. Arimori, C. J. Ward and T. D. James, *Tetrahedron Lett.*, 2002, **43**, 303.
7 Colorimetric anion detection using alizarin in CH_2Cl_2 has been reported, see: H. Miyaji and J. L. Sessler, *Angew. Chem., Int. Ed.*, 2001, **40**, 154.
8 H. Yamanoto, A. Ori, K. Ueda, C. Dusemund and S. Shinkai, *Chem. Commun.*, 1996, 407.
9 The fluorescence intensity of Alizarin Red S was reported to be slightly increased as the solution was acidified. See ref. 6*a*.
10 FAB mass spectroscopy as a direct method for the detection was carried out. When 20 mM of $(n\text{-Bu})_4NF$ was added to a MeOH solution containing alizarin (3.8 mM) and NPBA (20 mM), trial in a negative mode allowed us to detect a molecular peak corresponding to the formation of [alizarin–F–NPBA]$^-$ (m/z = 390) using glycerin as a matrix.
11 C. R. Cooper, N. Spencer and T. D. James, *Chem. Commun.*, 1998, 1365.
12 The K values were estimated by three separate titrations.
13 Titrations with $H_2PO_4^-$ could not be carried out because of low solubility of the potassium salt in MeOH.
14 The characterization of [alizarin–AcO–NPBA]$^-$ was conducted by ^1H NMR study in CD_3OD; see supplymentary information.
15 E. Palomares, R. Vilar, A. Green and J. R. Durrant, *Adv. Funct. Mater.*, 2004, **14**, 111; C.-F. Chen and Q.-Y. Chen, *Tetrahedron Lett.*, 2004, **45**, 3957; J. Y. Lee, E. J. Cho, S. Mukamel and K. C. Nam, *J. Org. Chem.*, 2004, **69**, 943; A. Coskun and E. U. Akkaya, *Tetrahedron Lett.*, 2004, **45**, 4947; R. Miao, Q.-Y. Zheng, C.-F. Chen and Z.-T. Huang, *Tetrahedron Lett.*, 2004, **45**, 4959; M. Vázquez, L. Fabbrizzi, A. Taglietti, R. M. Pedrido, A. M. González-Noya and M. R. Bermeijo, *Angew. Chem., Int. Ed.*, 2004, **43**, 1962; S. Solé and F. P. Gabbaï, *Chem. Commun.*, 2004, 1284; T. Gunnlaugsson, A. P. Davis, G. M. Hussey, J. Tierney and M. Glynn, *Org. Biomol. Chem.*, 2004, **2**, 1856; S. Arimori, M. G. Davidson, T. M. Fyles, T. G. Hibbert, T. D. James and G. I. Kociok-Köhn, *Chem. Commun.*, 2004, 1640; A. Saxena, M. Fujiki, R. Rai, S.-Y. Kim and G. Kwak, *Macromol. Rapid Commun.*, 2004, **25**, 1771; L.-H. Wei, Y.-B. He, J.-L. Wu, X.-J. Wu, L.-Z. Meng and X. Yang, *Supramol. Chem.*, 2004, **16**, 561; Z.-C. Wen and Y.-B. Jiang, *Tetrahedron*, 2004, **60**, 11109; M. Boiocchi, L. D. Boca, D. E. Gómez, L. Fabbrizzi, M. Licchelli and E. Monzani, *J. Am. Chem. Soc.*, 2004, **126**, 16507.
16 R. J. Fitzmaurice, G. M. Kyne, D. Douheret and J. D. Killburn, *J. Chem. Soc., Perkin Trans. 1*, 2002, 841; J.-L. Wu, Y.-B. He, L.-H. Wei, S.-Y. Liu, L.-Z. Meng and L. Hu, *Supramol. Chem.*, 2004, **16**, 353; Q.-Y. Chen and C.-F. Chen, *Tetrahedron Lett.*, 2004, **45**, 6493; B. García-Acosta, X. Albiach-Martí, E. García, L. Gil, R. Martínez-Máñez, K. Rurack, F. Sancenón and J. Soto, *Chem. Commun.*, 2004, 774; L. Nie, Z. Li, J. Han, X. Zhang, R. Yang, W.-X. Liu, F.-Y. Wu, J.-W. Xie, Y.-F. Zhao and Y.-B. Jiang, *J. Org. Chem.*, 2004, **69**, 6449; A. Kovalchuk, J. L. Bricks, G. Reck, K. Rurack, B. Schulz, A. Szumna and H. Weißhoff, *Chem. Commun.*, 2004, **17**, 1946; T. Gunnlaugsson, A. P. Davis, J. E. O'Brien and M. Glynn, *Org. Biomol. Chem.*, 2005, **3**, 48–56.
17 Quite recently we have found that it is possible to read out the anion detection through liquid (alizarin, PBA, 18-crown-6, in CH_2ClCH_2Cl)–liquid (anions as potassium salts, in water, 50 mM MES buffer; pH 5.5) two-phase extraction. The details will be reported elsewhere.

