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Synthesis, characterization, and sol-gel entrapment of a crown ether-styryl fluoroionophore

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Abstract

The synthesis and initial evaluation of a new dye-functionalized crown-ether, 2-[2-(2,3,5,6,8,9,11,12,14,15-decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin)ethenyl]-3-methyl benzothiazolium iodide (denoted BSD), is reported. This molecule contains a benzyl 18-crown-6 moiety as the ionophore and a benzothiazolium to spectrally transduce ion binding. Binding of K⁺ to BSD in methanol causes shifts in the both absorbance and fluorescence emission maxima, as well as changes in the molar absorptivity and the emission intensity. Apparent dissociation constants (K_d) in the range of 30 – 65 μ M were measured. In water and neutral buffer, K_d values were approximately 1 mM. BSD was entrapped in sol-gel films composed of methyltriethoxysilane (MTES) and tetraethylorthosilicate (TEOS) with retention of its spectral properties and minimal leaching. K⁺ binding to BSD in sol-gels films immersed in pH 7.4 buffer causes significant fluorescence quenching, with an apparent response time of approximately 2 min and an apparent K_d of 1.5 mM.

Introduction

Potassium is an important analyte in many types of media, particularly clinical samples. Analysis using an ion selective electrode based on a plasticized polymer membrane that contains a potassium ionophore is the most common approach. Optical methods provide an alternative that is potentially more sensitive and selective, and thus their development has been the focus of considerable research efforts (1–3). Although many types of chromoionophores have been synthesized and investigated as K⁺-sensing molecules, many of these absorb in the UV spectral region, such as the commercially available PBF1 (4). Furthermore, although they may exhibit high binding affinity for K⁺ in polar organic solvents (5–7), in aqueous solution these properties are frequently less optimal. Consequently, there is a continuing need for development of K⁺-sensing molecules that absorb/fluoresce in the visible spectrum and exhibit high sensitivity and selectivity in aqueous solution (8–10). Recent work has produced fluoroionophores that exhibit these properties but their synthesis is complex and of low yield (9, 11).

The metal ion binding properties of a family of styryl dyes conjugated to crown ethers have been extensively studied, mainly in the form of molecular aggregates in Langmuir-Blodgett films (12–16). These dyes generally have absorption maxima in the visible range. Here we report the synthesis and initial evaluation of a new dye-functionalized crown-ether, 2-[2-(2,3,5,6,8,9,11,12,14,15-decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin)ethenyl]-3-methyl benzothiazolium iodide (**6** in Figure 1; hereafter referred to as BSD), which was designed based on the aforementioned styryl dyes. 18-Crown-6 was chosen as the

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ionophore, with the benzothiazolium moiety acting as the signal transducer to convert ion binding into an optically detectable signal. The positive charge on the amine makes the molecule water-soluble. The absorbance and fluorescence properties of this molecule were characterized in methanol, aqueous solution, and entrapped in porous sol-gel glass films.

Materials and Methods

Synthesis

BSD was synthesized in the Chemical Synthesis Facility, University of Arizona. The scheme is outlined in Figure 1. Briefly, reaction of 2-methylbenzothiazole (**1**) with iodomethane in hot ethanol afforded 2,3-dimethyl benzothiazolium iodide (17, 18) (**2**) in 65 % yield (eq 1). The 4'-formyl benzo 18-crown-6 ether **5** was prepared (19–21) by the Williamson reaction of 3,4-dihydroxybenzaldehyde (**3**) with 1,14-dichloro-3,6,9,12-tetraoxatetradecane(21) (**4**) in the presence of sodium hydroxide in hot 1-butanol in 37 % yield (eq 2). The crown ether **5** was used in the next reaction as crude material. Another method of the synthesis of 4'-formylbenzo-18-crown-6 ether proved to be unsuccessful in our hands (22). Condensation of the benzothiazolium salt **2** with the aldehyde moiety of crown ether **5** afforded **6** (BSD) in 45 % yield after recrystallization from methanol (eq 3). A more detailed synthetic procedure along with MS and NMR spectral data are given in Appendix A.

Sol-gel thin films

Tetraethylorthosilicate (TEOS) and methyltriethoxysilane (MTES) were obtained from Sigma-Aldrich and used as received. All aqueous solutions were prepared using deionized (DI) water obtained from a Barnstead Nanopure system with a resistivity of 18 M Ω •cm. TEOS (1.0 mL), MTES (1.0 mL), absolute ethanol (0.6 mL), DI water (0.622 mL), and 0.25 M HCl (24 μ L) were mixed and stirred for 4 hours at room temperature. Methanol containing the appropriate concentration of BSD was then added to the sol precursor solution, at a 1:1 (v/v) ratio, to make the coating solution containing 0.1 mM BSD. Dip-coating was used to deposit sol-gel films at a withdrawal speed of 10 cm/min on glass microscope slides. Slides were cleaned in Piranha solution prior to use. This procedure produced films that were 50–100 nm thick (23, 24). Sol-gel coated slides were stored at room temperature for at least 24 hours to allow the solvent to evaporate. Prior to use, the films were soaked in 10 mM BisTris buffer (Sigma-Aldrich), pH 7.4, at room temperature for an additional 24 hours.

Instrumentation and spectral measurements

Absorption spectra were obtained using a Spectral Instruments 440 UV-Vis spectrophotometer. Fluorescence excitation and emission spectra were measured using a Spex Fluorolog-3 fluorometer (HORIBA Jobin Yvon Inc., NJ). Solution-based measurements were made conventionally, with the emission collection axis at 90 degrees from the excitation beam. Fluorescence measurements on sol-gel coated slides were performed using a front-face geometry in which the emission is collected at an angle of 22.5 degrees from the excitation beam. A custom-built, liquid flow cell was used to exchange solutions in contact with the upper surface of sol-gel films. The quantum yield of BSD was estimated by comparison to a reference fluorophore (25), which was fluorescein. BSD was dissolved in methanol; fluorescein (free acid, Fluka) was dissolved in 0.1 M NaOH for which the quantum yield was assumed to be 0.95 (25). Absorbance was measured at 430 nm; this wavelength was also used to excite fluorescence.

Results and Discussion

Spectroscopic characterization of dissolved BSD

BSD is soluble in water and polar organic solvents such as acetonitrile, methanol, ethanol, chloroform, DMSO and DMF. Its absorbance maximum is dependent on the solvent, whereas its fluorescent emission maximum is largely independent of solvent. Representative excitation and emission spectra of 10 μM BSD dissolved in DI water and in methanol are shown in Figure 2. In Table 1 are listed absorbance and emission maxima and molar absorptivity values in various solvents. A quantum yield of 0.25 was measured for BSD dissolved in methanol using fluorescein as a standard.

The response of BSD to potassium ion in solution was characterized using both absorbance and fluorescence spectroscopies. The addition of concentrated KCl solution to 1.35 μM BSD in methanol shifted the fluorescence emission maximum from 535 nm (K^+ -free) to 515 nm (K^+ -saturated). A fluorometric titration of BSD with KCl was performed to determine the binding constant. The ratio of emission intensities measured at 515 nm and 535 nm is plotted as a function of molar ratio of K^+ to BSD in Figure 3. The data were fit using eq 4 (26)

$$F = F_o + (F_\infty - F_o) \frac{[\text{BSD}] + [\text{K}^+] + K_d - \sqrt{([\text{BSD}] + [\text{K}^+] + K_d)^2 - 4[\text{BSD}][\text{K}^+]}}{2[\text{BSD}]} \quad (4)$$

where F is the measured fluorescence, F_o is the fluorescence in the absence of K^+ , F_∞ is the fluorescence in the presence of a saturating concentration of K^+ , $[\text{BSD}]$ and $[\text{K}^+]$ are the total concentrations of BSD and K^+ , and K_d is the dissociation constant. A 1:1 binding stoichiometry between K^+ and BSD was assumed. The apparent K_d value recovered from the fit was 53 μM .

The visible absorbance of BSD was also found to be sensitive to K^+ . The absorbance maximum shifted from 430 nm in the absence of K^+ to 420 nm when the methanol solution was saturated with K^+ , along with an increase in the molar absorptivity. The results from a spectrophotometric titration of 13.5 μM BSD with K^+ , in which the ratio of absorbances at 420 nm and 430 nm was determined as a function of the K^+ :BSD molar ratio, is plotted in Figure 4. The data were fit to eq 4, from which an apparent K_d value of 65 μM was recovered. When the wavelength of maximum absorbance or maximum emission was plotted as a function of the K^+ :BSD molar ratio, similar K_d values were obtained (44 μM from the fluorescence measurements and 30 μM from the absorbance measurements; data not shown). The apparent K_d values of 30 – 65 μM measured here are in good agreement with published data. For example, for K^+ binding to benzo-18-crown-6 in methanol, the reported K_d is 8.9 μM (27).

The spectroscopic properties of BSD and its response to potassium ion in aqueous solutions were also investigated. Both the molar absorptivity and the fluorescent emission intensity of BSD were found to be dependent on pH; both showed increases of in the range of 5–30% above pH 6 relative to more acidic solutions (data not shown). However, the wavelengths of maximum absorbance and maximum emission did not vary with pH.

No significant spectroscopic changes were observed when K^+ was added to BSD solutions at pH values less than 6. When the pH was greater than 6.5, fluorescence quenching was observed. However no changes in the wavelength of maximum emission occurred (data not shown). Fluorometric titrations of BSD with KCl were performed in 10 mM BisTris buffer,

pH 7.4, and in DI water. Typical results are plotted in Figures 5 and 6, respectively. In both cases the data were fit to eq 4, from which apparent K_d values of 1.3 mM and 1.5 mM were recovered for binding in water and BisTris buffer, respectively. These values are in good agreement with published data (27). The higher K_d values in aqueous solutions relative to methanol are expected based on the known affinity of hydronium ions for crown ethers (27). The mechanism of quenching was not investigated in detail; however based on similarities in the structure of BSD and its response to K^+ relative to previously described fluorescent benzyl crowns, photoinduced charge transfer is the most likely mechanism (1). In addition to potassium, it is well known other monovalent ions bind to the benzyl 18-crown-6 moiety (27). Here binding of sodium to BSD dissolved in water was confirmed by measuring fluorescence quenching similar to that observed for potassium (data not shown), confirming that BSD is not selective for potassium.

Characterization of BSD entrapped in sol-gel films

Porous sol-gel materials doped with indicator chromophores have been widely researched and developed as sensing materials (28–30). Silica-based sol-gel glasses are optically transparent in the visible and near-UV spectral regions, are easily prepared at room temperature, are chemically and mechanically stable, and can be cast into bulk objects or coated as thin films on a variety of different substrate materials (31). Furthermore the physical and chemical properties of the pores can be tuned by varying the precursors and processing conditions (32). Here a series of sol-gels of varying composition were prepared with entrapped BSD and evaluated as optically-based K^+ -sensing materials.

TEOS and MTES are commonly used as sol-gel precursors in thin film sensors (23, 24, 32). MTES, having one non-hydrolyzable methyl group, creates materials of lesser hydrophilicity and lower cross-link density than TEOS. Thus varying the mole ratio in MTES/TEOS formulations is expected to generate gels that vary in hydrophilicity and cross-link density. Less cross-linked materials are more porous, which is an important consideration with respect to indicator accessibility and sensor response time (32).

Precursor formulations having MTES volume fractions of 0%, 10%, 20%, 50%, and 100% were prepared and sol-gel films with entrapped BSD were fabricated by dip-coating. The films composed of 0 – 50% MTES still exhibited significant fluorescence after soaking for 24 hr in 10 mM BisTris buffer, pH 7.4; thus leaching of BSD from these films was insignificant under these conditions. However, films composed of 0 – 20% MTES exhibited minimal fluorescence quenching when immersed in buffer containing 100 mM KCl and low reversibility (< 50% fluorescence signal recovery when re-immersed in K^+ -free buffer). The most likely cause for this behavior is that these sol-gels, composed predominately of TEOS, are not sufficiently porous, which restricts diffusion of dissolved K^+ to and from entrapped BSD.

Films composed of 100% MTES were found to leach most of the entrapped BSD during the initial 24 hr soak in buffer, as evidenced by a lack of detectable BSD fluorescence. Pure MTES films should be the least cross-linked and therefore the most porous of the sol-gel formulations studied; however, it was hypothesized that electrostatic attraction of the positively charged BSD molecules to the negatively charged silanols on the walls of the pores would cause the dopant to be largely retained. Clearly this was not the case. Since no significant BSD leaching was observed from films composed of 0 – 50% MTES, we conjecture that the density of sterically accessible silanols in these films is greater than that in pure MTES films and sufficient to retain the entrapped BSD via predominately electrostatic interactions. A smaller pore size distribution, correlated with greater TEOS content, should also contribute to BSD retention.

50% MTES was the only composition found to be effective for K^+ sensing. Films were mounted in a liquid flow cell which allowed various concentrations of KCl dissolved in buffer to be introduced to the sensor surface. Fluorescence emission from films was measured in a front face geometry. A typical set of responses is shown in Figure 7. When buffer containing 10 mM KCl was injected, the emission intensity dropped rapidly, due to K^+ binding to and quenching the fluorescence of entrapped BSD. The forward response time (that required to reach 90% of full response) was approximately 1.5 min. When KCl-free buffer was injected, the intensity recovered with an apparent response time of approximately 2.5 min. These times reflect a slow rate of fluid injection into flow cell and inefficient mixing; the response times for diffusion of K^+ into and out of sensing film should be faster (24). The overall signal level exhibited a consistent decline during extended repetition of this experiment. This was due to photobleaching of BSD as the film was continuously illuminated during the measurements.

Binding curves were acquired by recording emission as a function of K^+ concentration. In these experiments, the sample was not continuously illuminated so photobleaching was minimized. A set of binding data along with a pair of typical spectra are shown in Figure 8. No shifts in emission maxima in response to K^+ were observed. The binding data were fit using eq 5

$$F = F_o + (F_{\infty} - F_o) \frac{[K^+]}{[K^+] + K_d} \quad (5)$$

in which the parameters have the same definition as in eq 4. Eq 5 is a simplified version of eq 4, derived by assuming that the concentration of K^+ bound to BSD is small relative to sum of the concentrations of bound and free K^+ (33). In this case, the free K^+ concentration is approximately equal to the total concentration. The apparent K_d obtained from fitting the data in Figure 8 is 1.5 mM.

These data show that entrapping BSD in 1:1 MTES:TEOS sol-gel creates a microenvironment in which binding of K^+ causes substantial quenching of BSD fluorescence. At saturation, the degree of quenching is about 40% percent. The binding affinity is comparable to that measured for BSD dissolved in water and in pH 7.4 buffer. This is somewhat surprising given that the pH inside of the pores of the sol-gel should be less than 7.4 due to the high concentration of silanols (31, 34), which should result in lower the binding affinity. However, this factor may be somewhat offset by using in the sol-gel formulation. The non-hydrolyzable Si—CH₃ moiety imparts a moderately nonpolar character to the sol-gel material which should increase the K^+ -BSD binding affinity. However, the pores in a MTES:TEOS sol-gel clearly do not create a “methanol-like” environment for K^+ -BSD binding because the apparent K_d is about 20-fold lower in the porous glass. Finally, it should be noted that sol-gel glasses can be fabricated from a wide variety of precursor materials, which provides an opportunity to “tune” the chemical environment of the pores (28–32). Thus it may be possible to create sol-gels with “methanol-like” pores in which K^+ -BSD binding should occur with much higher affinity.

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APPENDIX A. SYNTHESIS

General Experimental

Proton Nuclear Magnetic Resonance Spectra (^1H NMR) were obtained on a Bruker AM-250 MHz or Varian Inova 600 MHz instrument in the solvents deuteriochloroform (CDCl_3), acetone- d_6 (CD_3COCD_3) or dimethyl sulfoxide- d_6 ($\text{DMSO}-d_6$) where indicated employing tetramethyl silane (TMS) as an internal standard ($\delta = 0.00$ ppm). Carbon-13 Magnetic Resonance spectra (^{13}C NMR) were obtained on a Varian Inova 600 MHz instrument. The NMR deuterated solvents were obtained from Cambridge Isotope Laboratories, Inc. Mass Spectra were obtained in the University of Arizona Mass Spectrometry Facility using Electrospray Ionization (ESI).

Reactions were performed under an argon atmosphere in glassware equipped with a Teflon® coated magnetic stir bar. Sealed reactions were performed in a resealable, heavy wall glass, pressure threaded vessel sealed with an O-ring coupled with a threaded Teflon bushing.

All reagents and starting materials were of the best chemical grade, obtained commercially from VWR or Sigma-Aldrich. Chromatography solvents, extraction solvents, reaction solvents, drying agents and reagents of the best chemical grade were obtained from VWR and were used without further purification. Geduran Silica Gel 60 (SiO_2 , 200–400 mesh) for Flash Chromatography was obtained from EMD.

2,3-Dimethylbenzothiazolium iodide (2)

A solution of 2-methylbenzothiazole (**1**, 3.23 g, 21.6 mmol) in ethanol (9 mL) was treated with iodomethane (8.0 g, 56.4 mmol, 2.6 equiv). The reaction vessel was sealed under argon

and was placed into an oil bath maintained at 80 °C. After stirring for 2 h, the reaction mixture was cooled to room temperature whereupon a precipitate was formed. The solid was collected by filtration, washed with ethanol (3 × 5 mL), and dried under vacuum to afford the product as a white solid (**2**, 4.1 g, 6.3 g theore., 65 %): ¹H NMR (250 MHz, acetone-*d*₆, δ) 8.51 (1 H, d, *J* = 8.5 Hz, C4-*H*), 8.38 (1 H, d, *J* = 8.5 Hz, C7-*H*), 7.99-7.85 (2 H, m, C5-*H*, C6-*H*), 4.51 (3 H, s, *N*-CH₃), 3.43 (3 H, s, C2-CH₃); ESIMS *m/z* (49 % methanol: 49 % water: 2% acetic acid eluent) *m/z* 164 (base, M⁺ - I, C₉H₁₀NS).

1,14-Dichloro-3,6,9,12-tetraoxatetradecane (**4**)

A solution of pentaethylene glycol (25.5 g, 107 mmol) in benzene (100 mL) was treated with pyridine (20 mL, 19.6 g, 247 mmol, 2.3 equiv). A reflux condenser and a pressure-equalizing addition funnel charged with thionyl chloride (18 mL, 29.4 g, 247 mmol, 2.3 equiv) were attached and the reaction mixture was warmed at reflux. The thionyl chloride was added slowly over 1 h and the reaction mixture was warmed at reflux overnight.

The reaction mixture was cooled to room temperature and was treated with 15% hydrochloric acid (50 mL). The reaction mixture was separated and the benzene layer was dried (Na₂CO₃). Benzene was removed in vacuo to afford an oil (**4**, 22.0 g, 29.4 g theore., 75 %): ¹H NMR (250 MHz, chloroform-*d*, δ) 3.74 (t, 4 H, *J* = 5.6 Hz, C1-*H* and C14-*H*), 3.69-3.59 (m, 16 H, OCH₂); FABMS (matrix mix) *m/z* 279 (11, MH⁺ C₁₀H₂₀³⁷Cl₂O₄+H⁺), 278 (8), 277 (62, MH⁺ C₁₀H₂₀³⁷Cl³⁵ClO₄+H⁺), 276 (11), 275 (base, MH⁺ C₁₀H₂₀³⁵Cl₂O₄+H⁺), 151 (47), 107 (88), 63 (79). The material was judged to be sufficiently pure to use in the next step.

2,3,5,6,8,9,11,12,14,15-Decahydro-1,4,7,10,13,16-benzohexaoxacyclootadecin-18-carboxaldehyde. [4'-Formyl benzo-18-crown-6 (**5**)]

A mixture of 3,4-dihydroxybenzaldehyde (**3**, 5.54 g, 40.1 mmol) in 1-butanol (degassed with argon, 150 mL) was treated with a solution of sodium hydroxide (3.5 g, 87.5 mmol) dissolved into water (10 mL). The heterogeneous mixture was treated with 1,14-dichloro-3,6,9,12-tetraoxatetradecane (**4**, 11 g, 40 mmol) dropwise. The reaction mixture was placed into a hot oil bath and was warmed at reflux for 20 h. The oil bath was cooled to 60 °C and stirred an additional 48 h. The reaction mixture was cooled to room temperature, acidified with 15% aqueous HCl, filtered and the resultant solids were washed with methanol (6 × 20 mL). The filtrate and washing were combined and concentrated in vacuo. Flash chromatography (SiO₂, 3.5 cm bore column, 3 × 100 g) afforded the product (5.0 g, 13.6 g theore., 37 %) as a white solid. ¹H NMR (250 MHz, CDCl₃, δ) 9.25 (s, 1 H, Formyl-*H*), 7.39-7.31 (m, 2 H, aromatic *H*), 6.90 (m, *J* = 8 Hz, 1 H, aromatic *H*), 4.18-4.12 (m, 2 H, aromatic C4-OCH₂), 3.90-3.84 (m, 2 H, aromatic C3-OCH₂), 3.3.73-3.60 (m, 8 H, OCH₂); ESIMS (acetonitrile eluant) *m/z* 379 (29, MK⁺: C₁₇H₂₄O₇ + K⁺), 363 (94, MNa⁺ C₁₇H₂₄O₇ + Na⁺); FABMS (matrix mix) *m/z* 341 (MH⁺: C₁₇H₂₄O₇ + H⁺). The chromatography was insufficient to remove all impurities; however, the material was judged by the ¹H NMR data and MS data to be sufficiently pure to carry onto the next step.

2-[2-(2,3,5,6,8,9,11,12,14,15-decahydro-1,4,7,10,13,16-benzohexaoxacyclooctadecin)ethenyl]-3-methyl benzothiazolium iodide. (**6**)

A solution of the formyl benzocrown ether **5** (720 mg, 2.12 mmol) in ethanol (20 mL) was treated with the benzothiazolium iodide salt **2** (561 mg, 1.93 mmol). The reaction mixture was treated with pyridine (0.25 mL) and was stirred for 10 min at room temperature. The

pressure vessel was sealed and placed into a hot oil bath (80 °C) and stirred. After 18 h, an orange precipitate formed. The reaction mixture was cooled to room temperature and was filtered. The solid was collected and recrystallized from methanol to afford the product **6** (536 mg, 1.18 g theoreti, 45 %) as an orange solid: mp 193–195 °C; ^{13}C NMR (150 MHz, DMSO- d_6) δ 172.02, 152.45, 149.08, 148.45, 141.98, 129.20, 128.14, 127.49, 126.87, 125.98, 124.09, 116.61, 112.60, 112.34, 111.14, 69.93, 69.89, 69.78, 69.74, 69.66, 68.54, 68.46, 68.41, 68.37, 36.27; ^1H NMR (600 MHz, DMSO- d_6) δ 8.40 (dd, 1H, J = 8.1, 1.6 Hz), 8.21 (d, 1H, J = 8.5 Hz), 8.14 (d, 1H, J = 15.7 Hz); 7.87 (d, 1H, J = 15.7 Hz); 7.85 (ddd, 1H, J = 8.5, 7.3, 1.2 Hz), 7.77 (ddd, 1H, J = 8.2, 7.3, 1.0 Hz); 7.68 (d, 1H, J = 2.1 Hz), 7.61 (dd, 1H, J = 8.5, 2.1 Hz), 7.14 (d, 1H, J = 8.5 Hz), 4.31 (s, 3H), 4.22 (AB, 4H, OCH_2), 3.79 (AB, 4H, OCH_2), 3.60 (m, 12H, OCH_2); ESIMS (49 % methanol, 49 % water, 2 % acetic acid eluant; $\text{M}^+ = \text{C}_{26}\text{H}_{32}\text{NO}_6\text{S}$) m/z (rel. abundance) 486 (M^+ , 40), 442 (40), 398 (base), 354 (13), 310 (38); ESIHRMS m/z 486.1951 ($\text{C}_{26}\text{H}_{32}\text{NO}_6\text{S}$ requires 486.1950).

Figure 1.
Scheme for synthesis of BSD (6).

Figure 2. Fluorescent excitation and emission spectra of 10 μ M BSD in DI water (light curves) and in methanol (dark curves).

Figure 3.

Fluorimetric titration of 1.35 μM BSD with KCl in methanol. The solid diamonds are ratios of emission intensities at 515 nm to 535 nm (excited at 415 nm) vs. molar ratio of KCl:BSD; the solid line is the fit of the data to eq 4 ($K_d = 53 \mu\text{M}$). Data were corrected for dilution during titration.

Figure 4.

Spectrophotometric titration of 13.5 μM BSD with KCl in methanol. The solid diamonds are ratios of absorbance values at 420 nm to 430 nm vs. molar ratio of KCl:BSD; the solid line is the fit of the data to eq 4 ($K_d = 65 \mu\text{M}$). Data were corrected for dilution during titration. Inset: Absorbance spectra of BSD before mixing with KCl (A) and after saturation with KCl (B).

Figure 5. Spectrophotometric titration of 10 μ M BSD with KCl in 10 mM bis-Tris buffer, pH 7.4. The solid diamonds represent emission intensity excited at 415 nm and measured at 525 nm; the solid line is the fit of the data to eq 4 ($K_d = 1.3$ mM). Data were corrected for dilution during titration.

Figure 6.

Fluorometric titration of 10 μ M BSD with KCl in DI water. The solid diamonds indicate emission intensity excited at 415 nm and measured at 525 nm; the solid line is the fit of the data to eq 4 ($K_d = 1.5$ mM). Data were corrected for dilution during titration. Inset: Fluorescence spectra of BSD before mixing with KCl (A) and after saturation with KCl (B).

Figure 7.

Fluorescence intensity (excited at 415 nm and measured at 510 nm) measured versus time for a sol-gel film composed of 1:1 (v/v) MTES/TEOS and containing approximately 0.1 mM BSD. The film was mounted in a liquid flow cell. The solid line arrows indicate when 10 mM, BisTris buffer, pH 7.4, containing 10 mM KCl was injected. The dashed line arrows indicate when buffer lacking KCl was injected.

Figure 8.

Fluorimetric titration of a sol-gel thin film composed of 1:1 (v/v) MTES/TEOS and containing approximately 0.1 mM BSD. The film was mounted in a liquid flow cell and increasing concentrations of KCl dissolved in 10 mM BisTris buffer, pH 7.4, were sequentially injected. Fluorescence intensities (excited at 415 nm and measured at 510 nm) were measured 10 min after each injection. The solid line is the fit of the data to eq 5 ($K_d = 1.5$ mM). Inset: Spectra of the film in potassium-free buffer (A) and in buffer containing 100 mM KCl (B).

Table 1

Spectroscopic properties of BSD in different solvents.

Solvent	Absorption Max (nm)	Emission Max (nm)	Molar absorptivity ($M^{-1}\cdot cm^{-1}$)
Water	415	530	3.3×10^4
Methanol	430	535	4.0×10^4
Ethanol	430	535	
DMF	430	535	
DMSO	430	535	
Acetonitrile	430	535	
Chloroform	452	535	