J|A|C|S
COMMUNICATIONS

Published on Web 01/14/2003

A Fluorescent Sensor with High Selectivity and Sensitivity for Potassium in Water

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The construction of a fluorescent potassium sensor (fluoroionophore) that functions in water, with the high selectivity against sodium and pH necessary for clinical diagnostics, has proven to be highly desired by scientists working on the design and syntheses of artificial receptors.[1−8] The principle challenges in the design of a sensor for the measurement of extracellular potassium (in whole blood or serum) are (a) strong aqueous binding properties, with a potassium K_d around 5 mM; (b) good selectivity against sodium and other extracellular cations; (c) no pH interference in the physiological pH range; (d) large fluorescent signal response; and (e) excitation (>400 nm) and emission (>500 nm) wavelengths that minimize the effects of the background fluorescence of biological fluids.

One of the earliest and best known potassium fluoroionophores designed for clinical applications is PBFI,[1] which uses a diaza-18-crown-6 as an ion receptor (ionophore) and substituted benzofurans as fluorophores. Unfortunately, PBFI is not suitable for extracellular potassium sensing because of insufficient potassium binding strength and interference from sodium. However, this indicator possesses some attractive design features such as low pH interference and large fluorescence changes. To increase the binding strength, several groups have used cryptands as potassium ionophores and coupled them to a fluorophore such that the nitrogen of the cryptand acts as a trigger for fluorescent signaling. The first one reported by Masilamani and co-workers[2] was constructed by fusing a [222] cryptand[3] with a coumarin dye. While this indicator did meet the potassium binding requirements, it suffered from the disadvantages of strong pH interference, resulting from the protonation of the two aliphatic nitrogens at physiological pHs, and interference from sodium. Another [222] cryptand reported first by A. P. de Silva[4] and later by Sammes and co-workers[5] attempted to overcome the pH sensitivity issue by aromatizing the two nitrogens of the cryptand. This design minimized the pH interference but also restricted the rotation of the bond between the trigger nitrogen and carbon. That rotation is responsible for large signal changes that occur upon ion binding.[9] Those sensors suffered from the disadvantages of sodium interference, small relative signal change, and excitation with UV light.

Photoinduced electron transfer (PET)[10,11] type fluoroionophores have proven highly successful as direct fluorescent cation sensing molecules.[12,13] In an earlier work, we constructed a practical PET type fluoroionophore for extracellular sodium measurements using a 4-aminonaphthalimide fluorophore, an aniline-type PET donor, and an aza-15-crown-5 ion receptor.[9] We have maintained the same motif in the construction of a new PET type fluorophore for extracellular potassium measurements. Compound **1** is constructed by building a cryptand around an *o*-alkoxyaniline PET donor such that potassium binding into the cryptand interrupts PET fluorescence quenching. This ionophore is joined to the 4-aminonaphthalimide fluorophore by an ethylene group. The benzoic acid moiety is used for covalently linking the fluoroionophore onto a solid support. Sensor **1** meets all above-mentioned requirements and is used for the measurement of potassium in whole blood and serum samples in the Roche OPTI CCA, a portable blood/serum analyzer using optical sensor technologies. The instrument uses blue-LED as the excitation source and photodiode as the emission detector. The measuring parameters include pH, CO_2, O_2, sodium, potassium, calcium, chloride, glucose, total hemoglobin, and oxygen saturation.

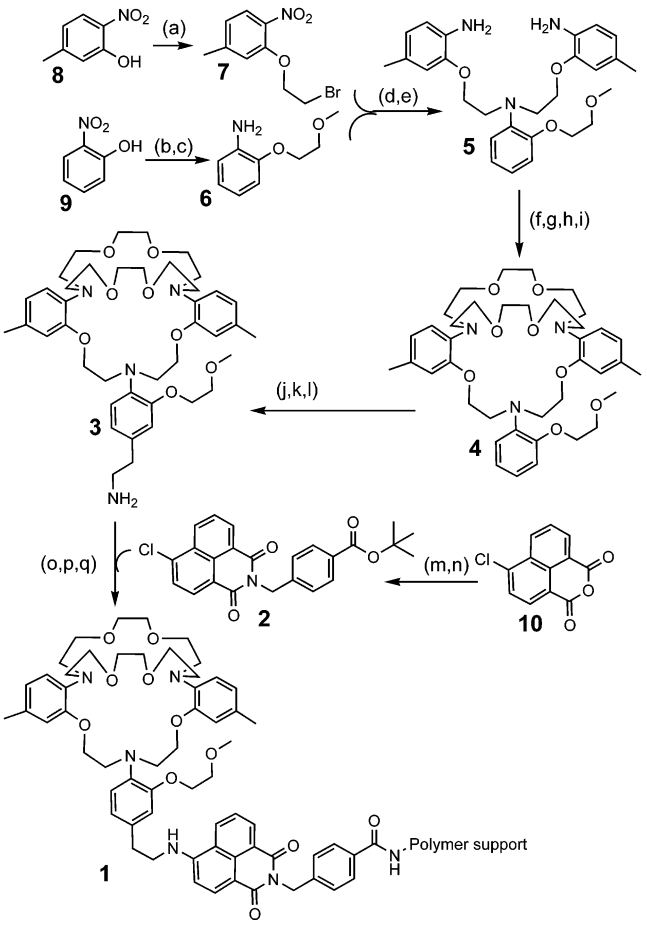

Figure 1. Synthesis of potassium fluorescence sensor **1**. (a) $BrCH_2CH_2Br$, K_2CO_3, DMF, 100 °C, 50%; (b) $CH_3OCH_2CH_2Cl$, K_2CO_3, DMF, 100 °C, 80%; (c) H_2, 5% Pd/C, EtOH, 95%; (d) K_2CO_3, KI, CH_3CN, reflux, 3 days, 55%; (e) H_2, 5% Pd/C, DMF, 95%; (f) DODC, TEA, CH_2Cl_2, 70%; (g) BH_3/THF, 60%; (h) DODC, Py, CH_2Cl_2, 50%; (i) BH_3/THF, 60%; (j) $POCl_3$, DMF, 0−25 °C, 70%; (k) CH_3NO_2, NH_4OAc, AcOH, 50 °C, 18 h, 80%; (l) LAH, THF, reflux, 5 h, 50%; (m) AMBA, DMF, room temperature, 18 h, 70%; (n) *tert*-butyl alcohol, CDI, DBU, 40 °C, 18 h, 30%; (o) NMP, DIPEA, 80 °C, 18 h, 30%; (p) TFA, CH_2Cl_2, room temperature, 1 h, 100%; (q) amino-cellulose, DCC, NHS, DMF, room temperature, 18 h.

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10.1021/ja0284761 CCC: $25.00

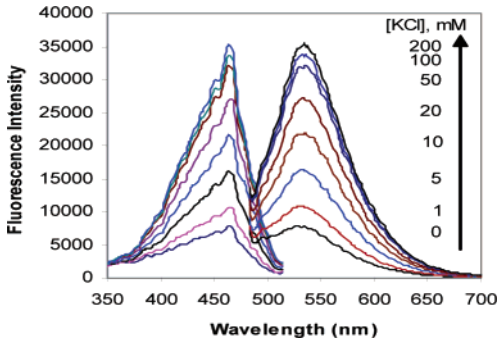

Figure 2. Excitation and emission spectra of a sensor disk exposed to different potassium chloride solutions in TRIS−HEPES buffer at pH 7.40 (left, excitation spectra, fixed emission at 540 nm; right, emission spectra, fixed excitation at 470 nm).

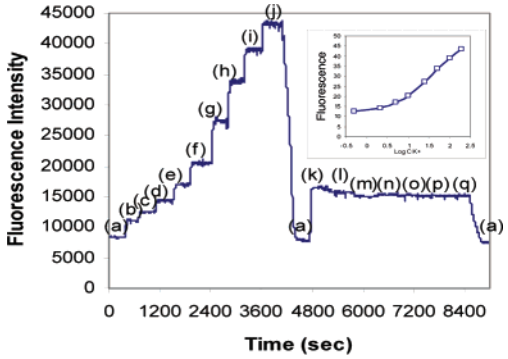

Figure 3. Response curve of **1** in TRIS−HEPES buffer at pH 7.40, excitation at 470 nm, emission at 540 nm. Potassium, sodium, and calcium concentrations are (K/Na/Ca, in mM): (a) 0/0/0; (b) 0/60/0; (c) 0/160/0; (d) 2/160/0; (e) 5/160/0; (f) 10/160/0; (g) 25/160/0; (h) 50/160/0; (i) 100/160/0; (j) 200/160/0; (k) 5/0/0; (l) 5/100/0; (m) 5/200/0; (n) 5/160/0; (o) 5/160/5; (p) 5/160/0, pH 6.8; (q) 5/160/0, pH 7.8. Insertion: Intensity of emission as a function of log [K^+].

The complete synthesis[14] of potassium sensor **1** is illustrated in Figure 1.

Figure 2 shows the fluorescence emission and excitation spectra of a sensor disk exposed to increasing concentrations of potassium chloride in pH 7.4 TRIS−HEPES buffer. The green fluorescence intensity increases substantially with increasing concentrations of potassium ion. As expected, binding of the cation to the triaza-cryptand inhibits fluorescence quenching by the anisidine donor. The fluorophore does not directly interact sterically or electronically with the cation. As a result, the excitation and emission maxima are nearly invariant with changing potassium concentrations. This is characteristic of a PET sensor and contrasts internal charge transfer (ICT) sensors in which the excitation maxima of a fluorophore change upon binding of an analyte.

Figure 3 shows the dynamic response of a potassium sensor disk to a series of sodium, potassium, and calcium containing buffers. From steps a to c, the sodium concentration is raised from 0 to 160 mM (without any potassium present), and a small increase in fluorescence is observed. The fluorescent response to potassium in the presence of 160 mM sodium is shown in steps c−j. In the clinically important concentration range between 2 and 10 mM potassium, a 5.8% signal change per millimolar of potassium is observed. This response allows the Roche OPTI CCA whole blood analyzer to measure potassium with a precision better than ±0.06 mM (1 SD). From steps k to m, the sodium concentration is increased from 0 to 200 mM, while potassium is held constant at 5 mM. The slope of sodium in the presence of potassium is −0.047% signal change per millimolar of sodium. The slight decrease in fluorescence intensity with increasing sodium can be attributed to the displacement of some bound potassium ion by sodium. The smaller sodium cation cannot fill the cryptand [223] completely and is less efficient at interrupting PET quenching by the arylamine lone pair electrons. In other words, the displacement of bound potassium by sodium causes the net loss of sum total of fluorescence enhancement, because binding of potassium gives much higher fluorescence enhancement than binding of sodium. A more detailed explanation of interference of sodium and other cations can be found in the references.[14] It is noteworthy that the sensor gives very similar response to 5 mM potassium in the presence and absence of 160 mM sodium (steps e and k). That is one of the essential requirements for a potassium sensor to be practically useful for measurements of potassium in blood or serum with very minor or even no correction for sodium. No fluorescence response to calcium (steps n and o) or pH (steps p and q) at fixed sodium and potassium concentrations is observed. One can see from the whole curve that stable signals are generally obtained in less than 1 min and the fluorescence response is fully reversible.

In conclusion, we have described a new optical sensor suitable for practical measurement of extracellular (serum or whole blood) potassium. Sensor **1** responds rapidly and reversibly to changes in potassium concentrations typical of whole blood samples. No interferences from clinical concentrations of calcium or pH are observed, and the sodium interference is very minor. Excitation and emission occur in the visible light region. This new potassium sensor is currently used in the Roche OPTI CCA, a commercially available whole blood analyzer.

Supporting Information Available: Experimental details for syntheses and characterization of **1**, sensor disk preparation, and description of Roche OPTI CCA (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0284761