J|A|C|S COMMUNICATIONS

Published on Web 12/29/2008

A Highly Selective Luminescent Sensor for the Time-Gated Detection of Potassium

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The sensitive and selective detection of potassium is essential to biomedical diagnosis since variations in serum and extracellular potassium levels have been linked to hypertension, stroke, and seizures.[1] The difficulty in measuring accurately the extracellular concentration of potassium stems from the large excess of sodium present in the medium. In blood, the typical concentration of K^+ is 3.5−5.3 mM, whereas that of Na^+ is 135−148 mM.[2] A practical probe must therefore bind K^+ with great selectivity so as not to be impacted by the large excess of Na^+ also present. Although a number of luminescent K^+ probes have been proposed, none present adequate sensitivity and selectivity for practical imaging applications.[3−5] The most promising probes demonstrate sufficient selectivity versus Na^+ but minimal increase in signal at the mM K^+ concentration that has clinical relevance.[4] Consequently, the background luminescence from the biological media may interfere with accurate measurement of K^+ concentration.

Time-gated luminescence imaging presents an elegant solution to the problem of background luminescence by setting a time delay between the excitation pulse and the luminescence detection, thereby allowing the luminescence of the media to decay before measuring that of the probe. This technique, however, requires chemical probes with luminescence lifetimes significantly longer than that of the biological medium. Lanthanide complexes, with extremely long luminescence lifetimes in the millisecond range, are ideally suited for such applications.[6] Herein we present a luminescent sensor for the time-gated detection of K^+ with enhanced selectively, likely resulting from the formation of a cation−π interaction.

The selectivity of any probe is inherently limited by that of its receptor. The poor sensitivity (detection in the M range) and selectivity toward Na^+ (4-fold) of the Tb-based K^+ probes previously reported[5] is consistent with the poor selectivity for K^+ over Na^+ (5- to 10-fold) of their diaza 18-crown-6 receptors.[7] We reasoned that the selectivity of this class of probes could be enhanced by use of the cation−π interaction.[8] Gokel and co-workers reported phenyl derivatives of diaza 18-crown-6 with apparent selectivity for K^+ over Na^+ that results from a selective cation−π interaction.[9] As demonstrated by X-ray crystallography, the structure of the receptor enables concomitant complexation of K^+ by the Lariat ether and sandwich π-type complexation with the arene. In contrast, the crystallographic structure of the receptor bound to the Na^+ revealed that although Na^+ can be complexed by the ring, its smaller size sterically prevents formation of the cation−π interaction. We reasoned that the selectivity of a sensor for K^+ could be significantly enhanced by use of this interaction.

The design of our potassium probe Tb-**1** (Figure 1) also relies on the sharp dependence of the lanthanide luminescence on the distance separating the metal center from its sensitizing antenna.[6,10] In our system, in the "off" state, the flexible structure of the ligand results in an overall large separation between the Tb ion and its sensitizing azaxanthone, resulting in weak Tb luminescence. Complexation of K^+ by the ring favors a cation−π interaction with

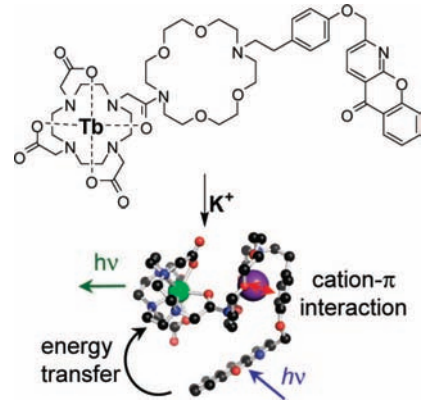


Figure 1. Chemical structure and mode of action of the K^+ sensor Tb-**1**.

the aryl ether, thereby locking the complex in a conformation where the antenna is significantly closer to the Tb center. Consequently, the efficiency of energy transfer from the azaxanthone to the Tb and the resulting luminescence from the complex are increased. Since complexation of Na^+ by the ring does not enable the cation−π interaction, it was predicted that Tb-**1** would demonstrate greater selectivity toward K^+ over Na^+ than the previously reported complexes.[5] Azaxanthone was chosen as the antenna since it was previously demonstrated to be an efficient sensitizer of Tb luminescence.[11]

The potassium sensor Tb-**1** was synthesized according to Scheme 1. A time-gated titration of Tb-**1** with potassium acetate in ethanol is shown in Figure 2. The binding affinity of Tb-**1** for K^+ of 0.33(4) μM^{-1} is well suited for the determination of K^+ concentration in the clinically important range of 0−10 mM. Moreover, the time delay of 0.2 ms ensures that any background luminescence is negligible. Under these conditions, the luminescence of Tb-**1** still

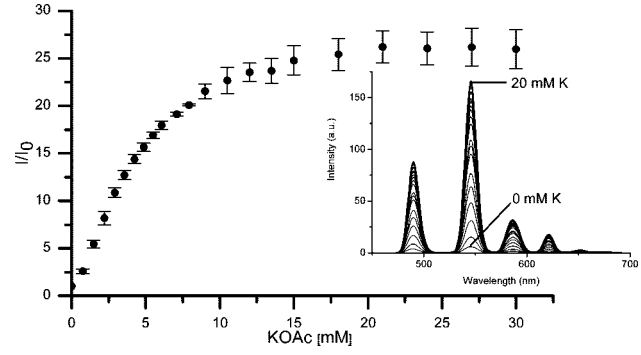
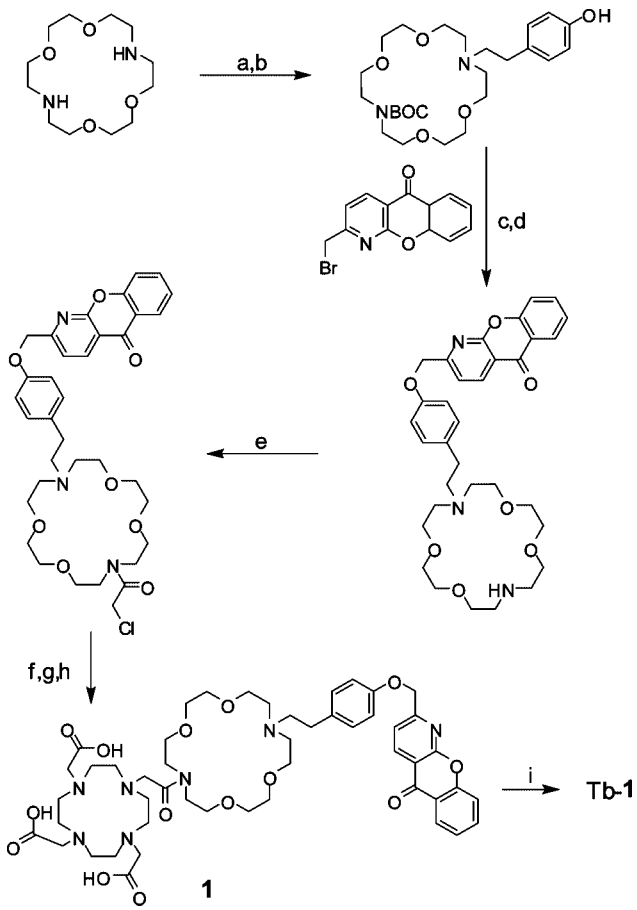


Figure 2. Relative time delayed luminescence of Tb-**1** as a function of K^+ concentration (error bars represent standard deviation (s.d.), $n = 3$). Inset, luminescence spectra of Tb-**1**·K^+ titration. Excitation at 332 nm, emission at 545 nm, time delay 0.2 ms, [Tb-**1**] = 50 μM, $T = 20$ °C.

10.1021/ja8077889 CCC: $40.75

Scheme 1. Synthesis of Tb-**1**[a]



[a] Reagents and conditions: (a) BOC_2O, dioxane, 20 °C, 16 h; (b) 4-(2-bromoethylphenol), Cs_2CO_3, MeCN, 65 °C, 18 h; (c) Cs_2CO_3, DMF, 20 °C, 1 h; (d) TFA, CH_2Cl_2, 20 °C, 1 h; (e) chloroacetyl chloride, NEt_3, CH_2Cl_2, 20 °C, 2.5 h; (f) cyclen, Cs_2CO_3, MeCN, 60 °C, 8 h; (g) *tert*-butylbromoacetate, Cs_2CO_3, MeCN, 20 °C, 16 h; (h) TFA, CH_2Cl_2, 20 °C, 16 h; (i) $TbCl_3$, NaOH, H_2O, 80 °C, 16 h. All compounds are pure and have correct analysis by MS and NMR.

increases significantly with increasing potassium concentration. Addition of 10 mM K^+ results in a 22-fold increase in Tb luminescence at 545 nm. Notably, the signal is stable over several hours.

The selectivity of Tb-**1** toward several physiological cations is shown in Figure 3. Tb-**1** detects K^+ with high selectivity: a 93-, 260-, 105-, and 61-fold selectivity over Na^+, Li^+, Mg^{2+}, and Ca^{2+} was observed, respectively. Importantly, the subsequent addition of 20 mM KOAc restores the 26-fold increase in luminescence, demonstrating that the presence of competing cations does not affect the determination of K^+ concentration.

Notably, the observed selectivity cannot result solely from selective binding of K^+ by the diaza-18-crown-6. The selectivities of the lariat ether for K^+ over Na^+ and Ca^{2+} in anhydrous alcohol are barely 5- to 10-fold.[7] Tb derivatives of these ethers also demonstrate poor selectivity (4-fold).[5] The respective 93- and 61-fold selectivities observed for Tb-**1** for K^+ over Na^+ and Ca^{2+} therefore have to include another motif. Given the selectivity of the cation$-\pi$ interaction observed in the crystal structures of the K^+ and Na^+-bound receptor,[9] we postulate that the enhanced selectivity observed is the result of this interaction.

In conclusion, a terbium complex for the time-gated luminescence detection of K^+ is presented. Tb-**1** demonstrates high sensitivity

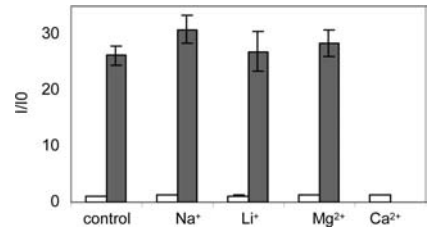


Figure 3. Selectivity of Tb-**1** to various physiological cations. White bars represent the time-delayed relative luminescence intensity after addition of an excess of the appropriate cation (20 mM for NaOAc, LiOAc, $Mg(OAc)_2$, and $Ca(NO_3)_2$). Black bars represent the time-delayed relative luminescence intensity after subsequent addition of 20 mM K^+. Excitation at 332 nm, emission at 545 nm, time delay 0.2 ms, [Tb-**1**] = 50 μM, T = 20 °C. Error bars represent s.d., n = 3.

with a 22 fold increase in luminescence intensity between 0 and 10 mM K^+. Moreover, Tb-**1** is highly selective for K^+ over other physiological cations, with a 93 fold selectivity over Na^+.

Acknowledgment. This work was supported by the University of Minnesota. We thank Hee-Yun Park for help with NMR characterization.

Supporting Information Available: Detailed experimental procedures and characterizations, excitation profile of Tb-**1**·K^+ titration. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA8077889