CHEMISTRY
A EUROPEAN JOURNAL

COMMUNICATION

DOI: 10.1002/chem.201202925

A New Indicator for Potassium Ions at Physiological pH by Using a Macrocyclic Luminescent Metal Complex

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Potassium ions [potassium(I)] play an important role in biological systems. They are not only involved in the maintenance of extracellular osmolarity and proper pH balance, but also associated with the regulation of blood pressure and concentration of other ions in living cells, such as calcium and chloride ions, which are transported across the plasma membrane.[1] Moreover, its importance for regulating heartbeat has already been reported.[2] An unbalance of potassium(I) concentration can lead to several human diseases, such as stroke, seizures, hypertension, myasthenia and renal disease.[3] Therefore, a delicate balance of potassium is decisive between beneficial and harmful roles, and the sensitive and selective detection of potassium(I) concentration in human body fluids is crucial to biomedical diagnosis. Although a number of potassium(I) probes have been proposed, none of them present adequate sensitivity and selectivity for practical imaging applications.[4] Selectively and accurately measuring the extracellular concentration of potassium(I) is challenging owing to the presence of the large excess of sodium in the medium. In the blood, the normal concentration of potassium(I) is 3.5–5.3 mM, whereas that of sodium(I) is about 135–148 mM.[5] Obviously, an effective probe for potassium(I) must possess great selectivity so as not to be impacted by the large excess of sodium(I). To meet this challenge, many methods[6] have been developed for the exclusive detection of potassium(I) concentration, of which fluorescence resonance energy transfer (FRET) is the most common. Background luminescence from biological media can also interfere with accurate detection of potassium(I) concentration; this results in much inconvenience in clinical diagnosis. Time-gated luminescence imaging presents an elegant solution to the problem of background luminescence by setting a time delay between the excitation pulse and luminescence detection; this allows the luminescence of the media to decay before that of the probe is measured. However, this technique requires chemical probes with luminescence lifetimes significantly longer than that of the biological medium. In previous work we found that terbium complexes, with extremely long luminescence lifetimes in the millisecond range, were ideally suited for such applications. Herein we present a new terbium-based luminescent sensor for the time-gated detection of potassium(I) concentration with enhanced selectivity and sensitivity through change of the conformation of terbium complex (Tb-**L**).

In our design, the Tb-**L** is based on sensitized luminescence because of its considerable advantages over the use of existing fluorescent probes for the detection of potassium(I) concentration. Firstly, terbium ions are photochemically inert because their f–f transitions are Laporte forbidden, and thus they have negligible molar extinction coefficients.[7] Secondly, the forbidden nature of the f–f transition also results in extremely long luminescence lifetimes, up to a millisecond, which can be utilized to increase detection sensitivity by eliminating the short-lived background fluorescence of biological samples by using a time delay set between the excitation pulse and the detection window.[8] Lastly, owing to the shielding effect of the outer electron shell, the emission spectra of the terbium are line-like and insensitive to environmental changes.[9] Although the terbium ion exhibits weak luminescence in the aqueous solution because of the negligible absorption, the problem can be overcome by grafting an antenna onto the ligand complexing the metal. The antenna absorbs energy from UV–visible radiation and subsequently transfers it to the terbium ion upon irradiation. The metal ion then emits its characteristic light.[10] For the terbium ion, the distinctive luminescence range from 480 to 630 nm consists of four emission bands, assigned to the respective transitions from $^5D_4$ state to the ground state ^7F_J ($J=3$, 4, 5, 6).[11] In terms of antenna selection, benzophenone (BP) was chosen as the antenna since it was previously proven to be an efficient sensitizer of terbium ion luminescence.[12] A diaza-18-crown-6 receptor was also used to distinguish potassium(I) from other metal ions as reported before.[13] However, the difference is that the high selectivity and sensitivity of the Tb-**L** observed did not result only from selective binding of potassium(I) by the diazacrown. We reasoned that our probe could enhance selectivity and sensitivity because of the cation–π interaction[14] that occurs between the cation added and the arene in our Tb-**L** complex.

The design of our potassium probe Tb-**L** also depends on the distance between the terbium ion and the antenna, BP. Figure 1 outlines the assay process for detecting potas-

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201202925.

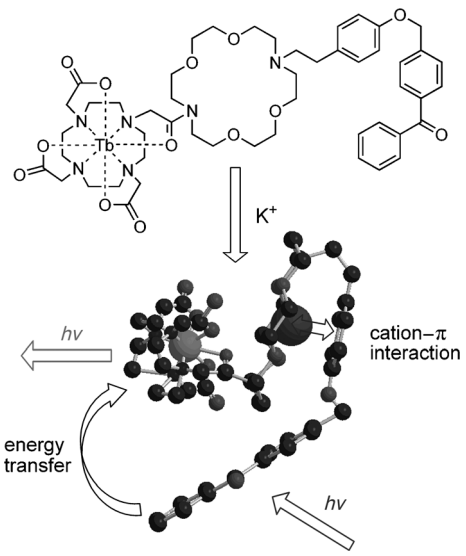


Figure 1. Chemical structure and mode of action of the potassium(I) sensor Tb-**L**. The sensor is extended in the absence of potassium(I). The flexible structure of the ligand results in an overall large separation between the Tb center and its sensitizing antenna, BP, resulting in weak Tb luminescence. However, in the presence of potassium(I) the Tb-**L** binds potassium(I) and forms a π-type sandwich complex with the arene by cation–π interaction. The locking conformation causes the antenna to be significantly closer to the Tb center. Consequently, the efficiency of energy transfer from BP to Tb and the luminescence from the complex are increased, which can be used to determine potassium(I) concentration in a complex biological medium.

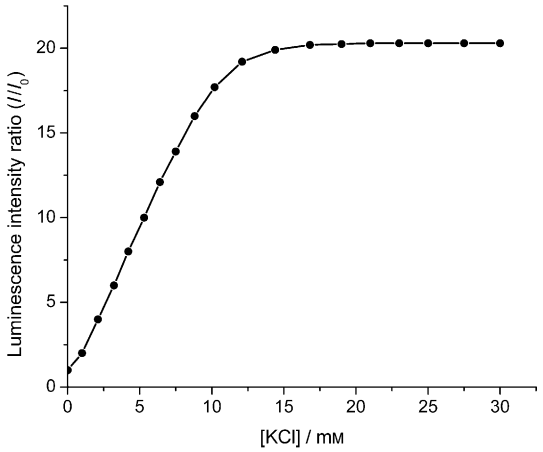


Figure 2. Relative time-delayed luminescence of Tb-**L** as a function of potassium(I) concentration. Excitation at 232 nm, emission at 545 nm, time delay 0.2 ms, [Tb-**L**] = 80 μM, T = 25 °C. All measurements were acquired in triplicate and are shown as an average. I and I_0 are the luminescence intensity of the system at 545 nm in the presence and absence of potassium(I), respectively.

sium(I). In the absence of potassium(I), the complex Tb-**L** adopts a random coil conformation in which the sensitizing antenna BP is far away from the metal center because of the flexibility of the probe; this results in weak Tb luminescence. In the presence of potassium(I), complexation of potassium(I) by the ring favors a cation–π interaction with the aryl ether and induces the probe to change from a random coil conformation to a cation–π-type sandwich conformation, which keeps the antenna BP in close spatial proximity to the terbium center; this allows for the occurrence of sensitized emission. Consequently, the resulting luminescence from the probe is increased. As a result of the smaller size of sodium ions its complexation by the ring does not enable the cation–π interaction, and sensitized emission does not occur. Therefore, the complex Tb-**L** shows much higher selectivity toward potassium(I) over sodium(I) than the previously reported complexes.[15]

A time-gated titration curve of Tb-**L** with potassium chloride in water is shown in Figure 2 (detailed measurements are given in the Supporting Information). The time delay was set at 0.2 ms; this ensures that any background luminescence in the system is negligible. From Figure 2, it can be indicated that the luminescence intensity of the Tb-**L** increases significantly with increasing potassium(I) concentration. Addition of 12 mM potassium(I) results in a 19-fold increase in Tb luminescence at 545 nm. The property is well-suited for the determination of potassium(I) concentration in the clinically important range of 0–10 mM. The Tb-**L** probe was synthesized according to Scheme 1.

To further investigate whether the different metal ions that could be present inside cells and human body fluids, such as sodium(I), lithium(I), cadmium(II), calcium(II), magnesium(II), cobalt(II), mercury(II), lead(II), manganese(II), iron(II), nickel(II), zinc(II) and copper(II), interfere with potassium(I) detection, potassium(I)-binding-induced luminescence changes were measured in the presence of an equal amount of other metal ions (see the Supporting Information). Firstly, the signal after the addition of other metal ions (20 mM) to Tb-**L** was measured, followed by the addition of an equal amount of potassium(I) in the tested system. The luminescence intensity change in response to the other metals was shown by setting the 0 mM potassium(I) and 0 mM other metals sample at 0% and the 20 mM potassium(I) sample at 100% in the system (Figure 3). Sodium(I) at 20 mM, led to only a 3% change of the maximum signal induced by potassium(I), whereas no significant

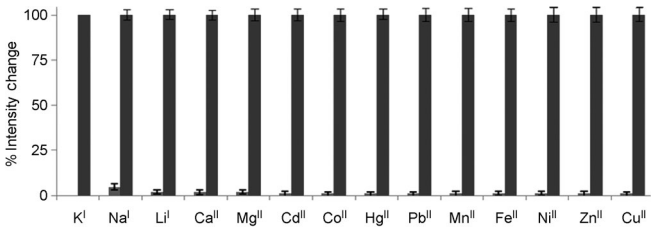
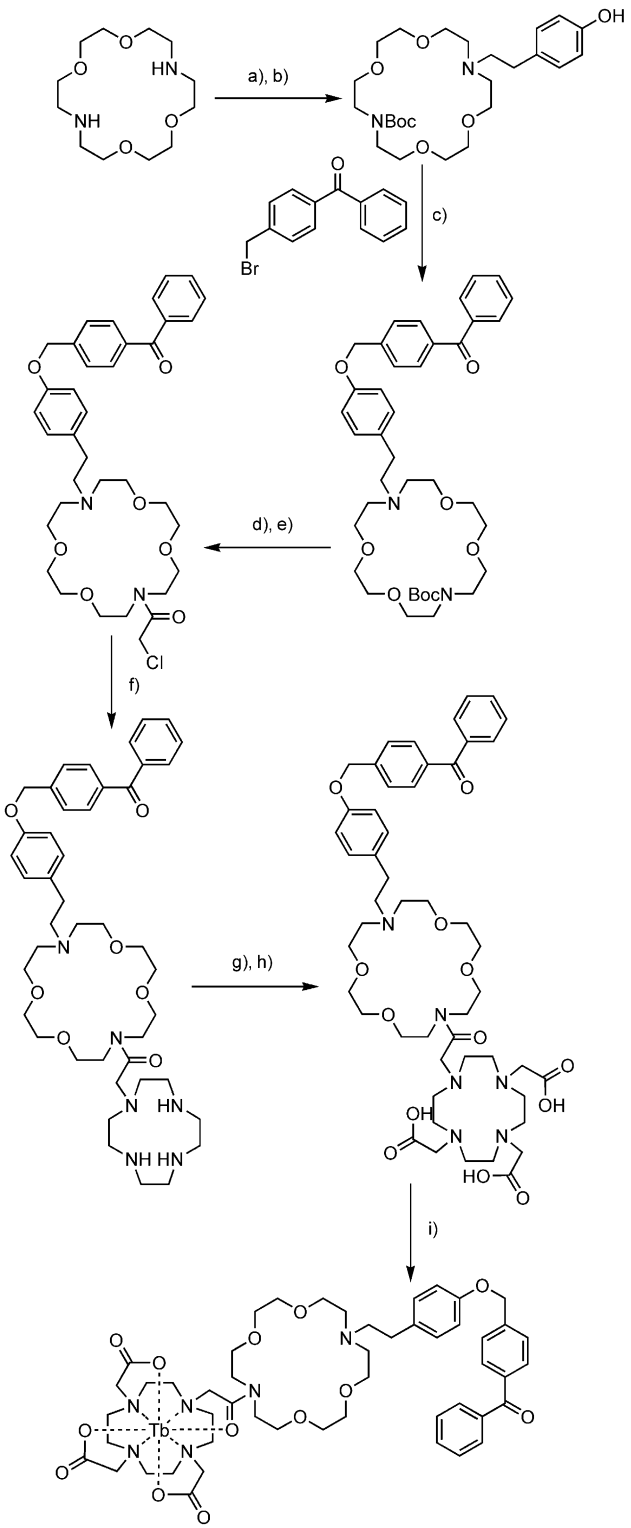


Figure 3. Selectivity of Tb-**L** towards potassium(I), sodium(I), lithium(I), cadmium(II), calcium(II), magnesium(II), cobalt(II), mercury(II), lead(II), manganese(II), iron(II), nickel(II), zinc(II), and copper(II). Tb-**L** (50 μM) with 20 mM of the respective metal ions is shown by the left-hand columns, and addition of 20 mM potassium(I) to the sample is shown by the right-hand columns. Excitation at 232 nm, emission at 545 nm, time delay 0.2 ms, T = 25 °C. All measurements were acquired in triplicate and are shown as an average.



Scheme 1. Synthesis of Tb-**L**. Reagents and conditions: a) Boc_2O, dioxane, 25 °C, 24 h; b) 4-(2-bromoethyl)phenol, Cs_2CO_3, MeCN, 65 °C, 24 h; c) Cs_2CO_3, DMF, 25 °C, 1.5 h; d) TFA, CH_2Cl_2, 25 °C, 1 h; e) chloroacetyl chloride, NEt_3, CH_2Cl_2, 25 °C, 2.5 h; f) cyclen, Cs_2CO_3, MeCN, 60 °C, 8 h; g) *tert*-butylbromoacetate, Cs_2CO_3, MeCN, 25 °C, 20 h; h) TFA, CH_2Cl_2, 25 °C, 16 h, i) $TbCl_3$, NaOH, H_2O, 80 °C, 16 h. Detailed experimental procedures are described in the Supporting Information.

changes were induced by other metal ions tested. In all cases, 100 % of the signal was recovered; this shows that potassium(I)-binding-induced luminescence was much stronger compared to that of other metal ions tested, and that the presence of other metal ions did not interfere with potassium(I) binding to Tb-**L**.

Notably, the observed selectivity cannot result solely from selective binding of potassium(I) by the diaza-18-crown-6. The selectivities of the lariat ether for potassium(I) over sodium(I) and calcium(II) in solution are barely five- to tenfold.[16] Tb derivatives of these ethers also demonstrate poor selectivity (fourfold).[17] The greater selectivity observed for Tb-**L** for potassium(I) over sodium(I) therefore has to include another cause. We postulate that the enhanced selectivity observed is the result of the conformation of the complex locked by potassium–π interactions, so that the antenna is significantly closer to the Tb center.

In summary, we have developed a new terbium complex as the potassium(I) probe for complicated biological systems. This probe presents high sensitivity towards potassium(I) with a 19-fold increase in luminescence intensity between 0 and 12 mM potassium(I). Moreover, Tb-**L** is highly selective for potassium(I) with a more than 97-fold selectivity over sodium(I) and 99-fold selectivity over other cations tested. We reason that the good selectivity of the Tb-**L** is the result of potassium–π interaction. In this work, time-resolved measurements were utilized to solve the problem of background luminescence. The presented design principle can be applicable to many other chemical and biosensor developments, and allows highly selective and sensitive quantitative analysis of potassium(I) concentration in human body fluids at physiological pH.

Acknowledgements

The work was supported by the National Natural Science Foundation of China (Grant No.: 20271008).

Keywords: biosensors · luminescence · potassium · terbium · time-resolved spectroscopy

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Received: August 16, 2012
Published online: December 7, 2012

