G-Quadruplex-Modulated Fluorescence Detection of Potassium in the Presence of a 3500-Fold Excess of Sodium Ions

Haixia Qin,[†] Jiangtao Ren,[†] Jiahai Wang,*[,†] Nathan W. Luedtke,[‡] and Erkang Wang*[,†]

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin, 130022, China and Graduate School of the Chinese Academy of Sciences, Beijing, 100039, China, and Institute of Organic Chemistry, University of Zürich, Winterthurerstrasse 190, Zurich, CH-8057, Switzerland

A label-free detection of K^+ was developed using G-quadruplex DNA (c-Myc) modulated fluorescence enhancement of tetrakis-(diisopropylguanidino) zinc phthalocyanine (Zn-DIGP). Upon the addition of increasing concentrations of potassium, a detection limit of 0.8 μM for K^+ was easily achieved. Comparative titrations using sodium, lithium, ammonium, transition metal, or alkali earth salts revealed that the fluorescence enhancement was highly specific for potassium ions. This system has, for the first time, provided a means for detecting 40 μM of K^+ even in the presence of a 3500-fold excess of Na^+ ions.

It is well-known that potassium (K^+) plays an important role in living organisms, such as reducing the risk of high blood pressure and stoke, maintaining muscular strength, balancing the pH, etc.[1] Therefore, numerous studies have been reported that focus on K^+ binding assays.[1–10] Despite such notable progress, it is still difficult to selectively determine extracellular K^+ concentrations, due to the large excess of Na^+ and other cations present in physiological conditions. To address this challenge, several techniques have been developed for the selective detection of K^+.[7–17] Recently G-quadruplex DNAs have been reported as potential sensing elements for K^+ detection.[2–11] G-quadruplexes are four-stranded structures derived from G-rich sequences.[2] G-quadruplex folding is promoted by monovalent cations, especially by K^+ partly due to the complementary size and charge of K^+ ion as compared to the cavities within G-quadruplexes.[3,18,19] Thereby, it provides a chance to design a selective sensor for K^+ based on some G-quadruplexes. Recently, fluorescent "aptasensors" for K^+ ion have been developed using G-quadruplex DNAs.[5–8] In most such cases, however, these methods required an additional DNA tagging process or sophisticated experimental techniques which make the experiments relatively complicated and expensive to conduct. Furthermore, DNA labeling with different fluorescent and quenching molecules can even influence the properties of the target binding aptamers.[16] These studies have inspired our efforts to develop a label-free method for K^+ detection based on fluorescent G-quadruplex ligands.

G-quadruplex ligands can selectively bind to and stabilize G-quadruplex structures. Features shared by many of these ligands include a large flat aromatic surface and presence of cationic charges complementary with G-quadruplex DNAs.[20,21] A range of G-quadruplex ligands with fluorescent properties have been shown to bind quadruplexes selectively in vitro.[22−24] For example, porphyrin, phthalocyanine, and triphenylmethane-based probes can become highly fluorescent in the presence of G-quadruplex, and this fluorescence intensity was expected to be

* To whom correspondence should be addressed. Phone: (+86) 431-85262003. Fax: (+86) 431-85689711. E-mail: jhwang@ciac.jl.cn (J.W.); ekwang@ciac.jl.cn (E.W.).

† Chinese Academy of Sciences, Changchun, and Graduate School of the Chinese Academy of Sciences, Beijing.

‡ University of Zürich.

(1) Teresa, M.; Gomes, S. R.; Tavares, K. S.; Oliveira, J. *Analyst* **2000**, *125*, 1983–1986.
(2) Li, T.; Wang, E.; Dong, S. J. *Chem. Commun.* **2009**, 580–582.
(3) Huang, C. C.; Chang, H. T. *Chem. Commun.* **2008**, 1461–1463.
(4) Kong, D. M.; Guo, J. H.; Yang, W.; Ma, Y. E.; Shen, H. X. *Biosens. Bioelectron.* **2009**, *25*, 88–93.
(5) Shi, C.; Gu, H. X.; Ma, C. P. *Anal. Biochem.* **2010**, *400*, 99–102.
(6) Nagatoishi, S.; Nojima, T.; Juskowiak, B.; Takenaka, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 5067–5070.
(7) Nagatoishi, S.; Nojima, T.; Galezowska, E.; Gluszynska, A.; Juskowiak, B.; Takenaka, S. *Anal. Chim. Acta* **2007**, *581*, 125–131.
(8) Ueyama, H.; Takagi, M.; Takenaka, S. *J. Am. Chem. Soc.* **2002**, *124*, 14286–14287.
(9) He, F.; Tang, Y. L.; Wang, S.; Li, Y. L.; Zhu, D. B. *J. Am. Chem. Soc.* **2005**, *127*, 12343–12346.
(10) Radi, A. E.; O'Sullivan, C. K. *Chem. Commun.* **2006**, 3432–3434.
(11) Wang, L. H.; Liu, X. F.; Hu, X. F.; Song, S. P.; Fan, C. H. *Chem. Commun.* **2006**, 3780–3782.
(12) Xia, W. S.; Schmehl, R. H.; Li, C. J. *J. Am. Chem. Soc.* **1999**, *121*, 5599–5600.
(13) Chen, H. X.; Gal, Y. S.; Kim, S. H.; Choi, H. J.; Oh, M. C.; Lee, J.; Koh, K. *Sens. Actuators, B: Chem.* **2008**, *133*, 577–581.
(14) Lee, J.; Kim, H. J.; Kim, J. *J. Am. Chem. Soc.* **2008**, *130*, 5010–5011.
(15) Wu, Z. S.; Chen, C. R.; Shen, G. L.; Yu, R. Q. *Biomaterials* **2008**, *29*, 2689–2696.
(16) Choi, M. S.; Yoon, M.; Baeg, J. O.; Kim, J. *Chem. Commun.* **2009**, 7419–7421.
(17) Yang, X.; Li, T.; Li, B. L.; Wang, E. K. *Analyst* **2010**, *135*, 71–75.
(18) Walmsley, J. A.; Burnett, J. F. *Biochemistry* **1999**, *38*, 14063–14068.
(19) Sundquist, W. I.; Klug, A. *Nature* **1989**, *342*, 825–829.
(20) De Cian, A.; Lacroix, L.; Douarre, C.; Temime-Smaali, N.; Trentesaux, C.; Riou, J. F.; Mergny, J. L. *Biochimie* **2008**, *90*, 131–155.
(21) Luedtke, N. W. *Chimia* **2009**, *63*, 134–139.
(22) Yang, P.; De Cian, A.; Teulade-Fichou, M. P.; Mergny, J. L.; Monchaud, D. *Angew. Chem., Int. Ed.* **2009**, *48*, 2188–2191.
(23) Ma, D. L.; Che, C. M.; Yan, S. C. *J. Am. Chem. Soc.* **2009**, *131*, 1835–1846.
(24) Alzeer, J.; Vummidi, B. R.; Roth, P. J. C.; Luedtke, N. W. *Angew. Chem., Int. Ed.* **2009**, *48*, 9362–9365.

10.1021/ac101894b
Published on Web 08/30/2010

highly potassium-dependent due to a high selectivity of such compounds for folded G-quadruplex versus unstructured DNAs.[24–26] Fluorescent G-quaduplex ligands therefore provide an opportunity to utilize fluorescence readout for indirect detection of potassium ions.

In this study, we develop a novel and label-free DNA-ligand sensor for detecting K^+ using G-quadruplexes and the fluorescent dye tetrakis(diisopropylguanidinio) zinc phthalocyanine (Zn-DIGP). It has been reported that Zn-DIGP binds to an intramolecular G-quadruplex (c-Myc) with the highest reported affinity, and c-Myc quadruplex significantly modulates the fluorescence enhancement of Zn-DIGP which is not fluorescent in the free state.[24] Since K^+ ion can promote the formation of G-quadruplexes, the fluorescent intensity of Zn-DIGP should be K^+ ion dependent. Indeed, this approach provides a highly sensitive and selective method for detecting K^+ ion. Upon the addition of increasing concentrations of potassium, a submicromole detection limit for K^+ was easily achieved. Comparative titrations using sodium, lithium, ammonium, transition metal, or alkali earth salts revealed that the fluorescence enhancement was highly specific for potassium. This system has, for the first time, provided a means for detecting 40 μM of K^+ even in the presence of a 3500-fold excess of Na^+.[8,9] This sensor platform is even capable of quantifying potassium ion concentrations in very complex mixtures including urine samples. Previous studies,[3] in contrast, reported a 10 000-fold selectivity for potassium determined in independent fluorescence titrations rather than in a mixture. The lowest concentration of potassium detected in these previous studies was 1 mM with the selectivity of 145-fold when titrations were conducted in a mixture of potassium and 145 mM sodium (see Figure S7 of the Supporting Information in ref 3).

EXPERIMENTAL DETAILS

Materials. Tetrakis(diisopropylguanidinio)zinc phthalocyanine (Zn-DIGP) was synthesized according to published procedures.[24] Purified oligonucleotides (c-Myc, 5′-TGAGGGTGGGGAGGGTGGGGAA-3′) and tris(hydroxymethyl)-aminomethane (Tris) were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). Dimethylsulfoxide (DMSO) was purchased from Tientsin Reagent (Tientsin, China). All reagents were used as received without further purification. DNA stock solutions were prepared by dissolving DNA in Tris-HCl buffer (50 mM Tris-HCl, pH = 7.4) and diluted to required concentrations before use. The stock solution of Zn-DIGP (1 mM) was prepared in DMSO, stored in the dark at −20 °C, and diluted to the required concentration with Tris-HCl buffer. All fluorescence experiments were carried out using samples prepared in Tris-HCl buffer.

Apparatus. Cary 500 Scan UV−vis spectrophotometer (Varian) was used to quantify the oligonucleotides. Fluorescence intensities were recorded on a LS-55 luminescence spectrophotometer (Perkin-Elmer). The emission spectra were recorded in the wavelength of 655−760 nm upon excitation at 620 nm.

Fluorescent Measurements. The DNA solutions were heated at 96 °C for 5 min and gradually cooled to room temperature. An equal volume of K^+ solution was added to the DNA solutions and incubated at room temperature for 30 min. Then an equal volume of Zn-DIGP in Tris-HCl buffer was added to each G-quadruplex solution. The working solution of the fluorescence assay was Tris-HCl buffer (50 mM, pH = 7.4), which contained 200 nM (or 500 nM) DNA, 1 μM Zn-DIGP, and variable concentrations of K^+ ions. The assay procedures for lithium, sodium, ammonium, calcium, magnesium, zinc, iron, and copper ions were the same as those for K^+ ions, except that LiCl, NaCl, NH_4Cl, $CaCl_2$, $MdCl_2$, $ZnCl_2$, $FeCl_3$, and $CuCl_2$ were used instead of KCl.

Application. Urine was used to confirm the feasibility of this aptasensor for analysis of real-world sample. Urine samples were harvested from four members of our laboratory and filtered through 0.22 μM membranes. KCl (10 mM) was added into urine samples to test the recovery. Then the urine samples were diluted 100-fold with Tris-HCl buffer and analyzed in Tris-HCl buffer (50 mM, pH = 7.4), which contained 500 nM DNA and 1 μM Zn-DIGP.

RESULTS AND DISCUSSION

It is well-known that K^+ ions can effectively stabilize G-quadruplex DNA.[9] However, only a few G-quadruplex ligands are known to exhibit enhanced fluorescence properties upon binding K^+-stabilized G-quadruplexes.[22–24] Recently, a guanidinium-modified phthalocyanine (Zn-DIGP) which exhibits re-

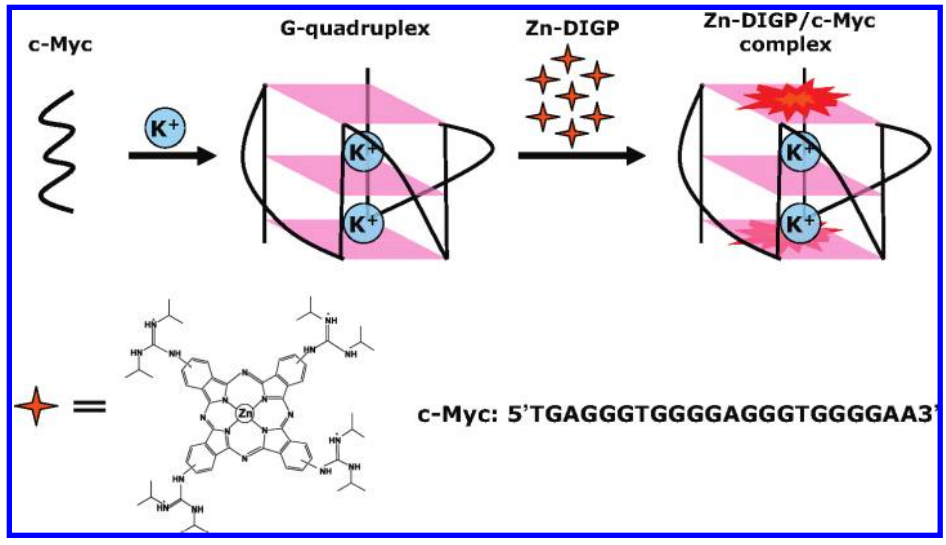

Figure 1. Schematic illustration of the label-free assay for K^+ ions.

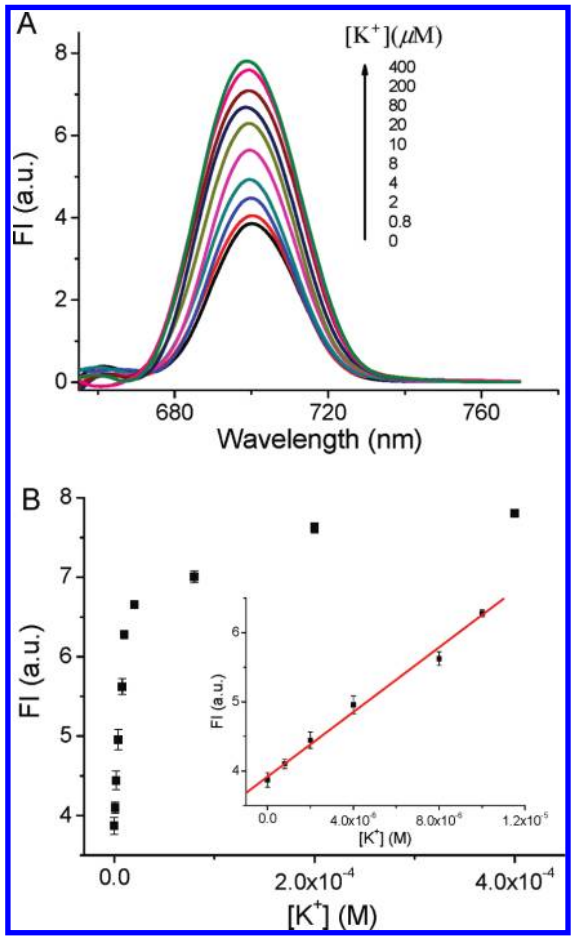

Figure 2. (A) Fluorescence response of the Zn-DIGP/c-Myc complex upon addition of different concentrations of K^+. (B) Plot of fluorescence intensity at 700 nm as a function of K^+ concentration. The inset shows a linear range from 0.8 to 10 μM. Experimental conditions: 50 mM Tris-HCl (pH 7.4) containing 200 nM c-Myc and 1 μM Zn-DIGP.

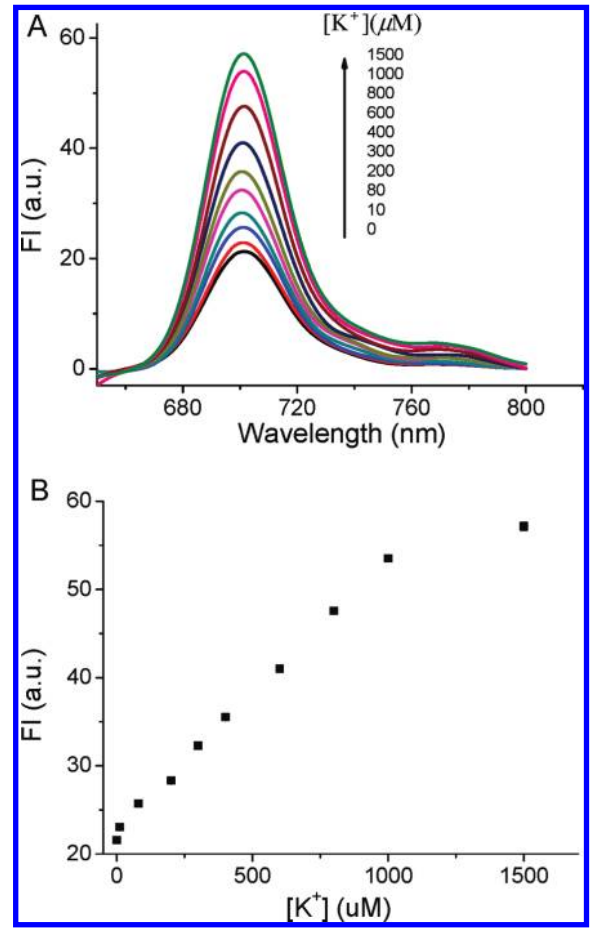

Figure 3. (A) Fluorescence response of the Zn-DIGP/c-Myc complex upon addition of different concentrations of K^+. (B) Plot of fluorescence intensity at 700 nm as a function of K^+ concentration. Experimental conditions: 50 mM Tris-HCl (pH 7.4) containing 500 nM c-Myc and 1 μM Zn-DIGP.

markable "turn-on" fluorescence upon binding G-quadruplex (c-Myc) was reported by Luedtke and co-workers.[24] Potassium-dependent fluorescence enhancement assays using Zn-DIGP have not been reported previously. In this study, we conducted a series of titrations to test the ability of a complex composed of Zn-DIGP and c-Myc to detect potassium ion even in the presence of a large excess of Na^+ ions.

Fluorescent Measurements for K^+ Ions. Figure 1 outlines the sensing mechanism that we employed in this study. In the presence of K^+, the conformation of c-Myc changes from a random coil to a "parallel" G-quadruplex structure[27,28] which can bind Zn-DIGP with high affinity and the resulting Zn-DIGP/c-Myc complex exhibits much greater fluorescence as compared to Zn-DIGP. The presence of K^+ ion is therefore detected indirectly by fluorescence enhancement. The analytic protocol based on the Zn-DIGP/c-Myc complex was assessed by fluorimetric titration with KCl as described in the Experimental Section. As shown in Figure 2A, the observed fluorescence intensity enhanced with increasing concentrations of K^+ ions. In the absence of K^+, the fluorescence spectrum exhibited a weak fluorescence emission peak at 700 nm. With the increase of K^+ ions concentrations, the resulting emission intensity gradually increased owing to the potassium inducing ability to form and stabilize G-quadruplex. Moreover, we also observed that at the lower concentrations of potassium, the fluctuation of the emission peak of Zn-DIGP at 700 nm was much bigger than at a higher concentration of potassium, which may be ascribed to the lower binding affinity to the random DNA coil and unstable DNA conformation. Once the stable conformation of the G-quadruplex at a higher concentration of potassium was formed, much steadier, higher emission peaks appeared, which was consistent with the result reported previously.[24]

Sensitivity and Selectivity of K^+ Ions Detection. As shown in Figure 2A, a distinct increase in the emission spectrum is observed upon addition of as little as 800 nM of K^+, indicating that a much lower detection limit can be reached than previously reported methods.[5–11,29] Figure 2B illustrates the relationship between the K^+ concentrations and fluorescence intensity (FI) at 700 nm, the maximal emission wavelength of the Zn-DIGP/c-Myc complex. The fluorescence intensity increased as the K^+ concentration was increased from 0.8 to 400 μM and then saturated. The inset of Figure 2B shows the calibration curve for K^+ ions and reveals a linear relationship of fluorescent intensity vs potassium concentration from 0.8

(25) Arthanari, H.; Basu, S.; Kawano, T. L.; Bolton, P. H. *Nucleic Acids Res.* **1998**, *26*, 3724–3728.
(26) Kong, D. M.; Ma, Y. E.; Wu, J.; Shen, H. X. *Chem.—Eur. J.* **2009**, *15*, 901–909.
(27) Qin, Y.; Hurley, L. H. *Biochimie* **2008**, *90*, 1149–1171.

Table 1. Performance Comparison of This Work with Other Homogeneous K^+ Sensors

type	detection limit	assay time	probe synthesis	ref
G-quadruplex ligands	0.8 μM	rapid (minutes)	convenient (label-free)	TW[a]
crystal violet−G-quadruplex complexes	~mM	slow (hours)	convenient (label-free)	4
pyrene-labeled aptamer	0.4 mM	rapid (minutes)	expensive (dual labeling)	5
pyrene-labeled G-quadruplex	~mM	NR[b]	expensive (dual labeling)	6
fluorescein-labeled G-quadruplex	~mM	NR[b]	expensive (dual labeling)	7
synthetic oligonucleotid derivative	~mM	NR[b]	expensive (dual labeling)	8
fluorescein-labeled G-quadruplex	~mM	slow (hours)	expensive (single labeling)	9
aptamer conformational switch	0.015 mM	rapid	expensive (dual labeling)	10
gold nanoparticles colorimetric probe	~1 mM	rapid (minutes)	convenient (label-free)	11
hemin-G-quadruplex DNAzyme	2 μM	slow (hours)	convenient (label-free)	17

[a] TW stands for this work. [b] NR stands for not reported.

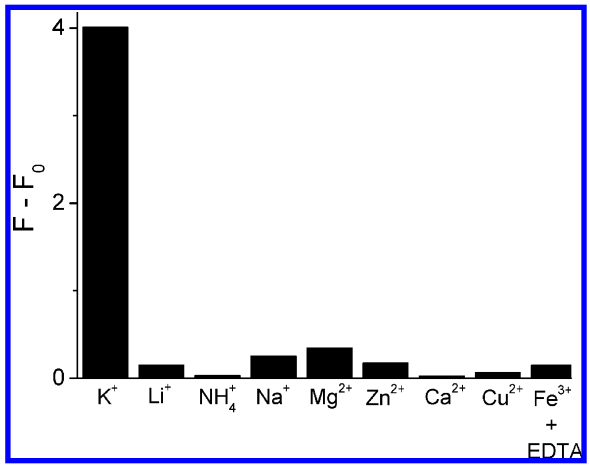

Figure 4. Selectivity assays based on the Zn-DIGP/c-Myc complex. All the metal ions were used at the concentration of 100 μM (F_0 and F stand for the fluorescent intensity in the absence and presence of metal ions), 1 mM EDTA was used as masking agent. Experimental conditions: 50 mM Tris-HCl (pH 7.4) containing 200 nM c-Myc and 1 μM Zn-DIGP.

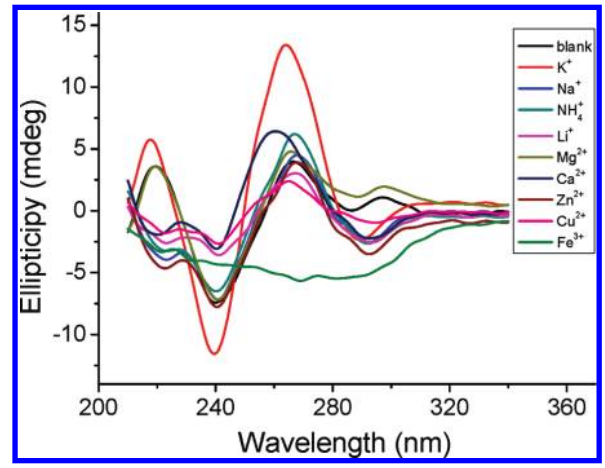

Figure 5. CD spectra of c-Myc (6 μM in 50 mM Tris-HCl, pH 7.4) upon addition of different metal ions (10 mM).

to 10 μM ($R = 0.998$). It is reasonable to expect that a linear response to potassium ion over 10 μM might be obtained by further augmenting the concentration of G-quadruplex DNA. Indeed, Figure 3 demonstrates that when the concentration of c-Myc was increased to 500 nM, the linear detection range can be adjusted between 10 and 1000 μM. These results compare very favorably with other reported methods for K^+ detection (Table 1). The Zn-DIGP/c-Myc complex is highly sensitive to the presence of K^+ and therefore provided an excellent sensor platform.

To test the specificity of this new sensor, metal ion selectivity assays were conducted by measuring different fluorescent responses of the Zn-DIGP/c-Myc complex toward 100 μM solutions of various metal ions. As shown in Figure 4, none of the tested metal ions, except for K^+, caused a significant increase in the fluorescence intensity at 700 nm, suggesting that only the K^+-stabilized c-Myc quadruplexes bind with Zn-DIGP and enhance its fluorescence intensity efficiently. CD spectra experiments were also conducted. It has been reported that CD spectra of a "parallel" G-quadruplex structure has a positive peak near 260 nm and a negative peak around 240 nm.[28] The CD spectrum of c-Myc in no-metal condition (Figure 5, black curve) agrees with a "parallel" G-quadruplex structure. Upon the addition of K^+ ion, both the intensity of positive and negative peaks of the CD spectra increased correspondingly, indicating the promotion and stabilization of the G-quadruplex structure by K^+ ion, which was not observed by other metal ions (Figure 5). These results suggest that the presence of NH_4^+, Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, and Cu^{2+} were unable to fold c-Myc from the random coil to parallel G-quadruplex conformation.[5,9] Interestingly the trivalent cation (Fe^{3+}) quenches the fluorescence of the Zn-DIGP/c-Myc complex which can be ascribed to the disruption of the G-quadruplex by Fe^{3+} (Figure 5, green curve), but this interference can be removed by using EDTA as a masking agent (Figure 4). This sensor platform therefore offers very high sensitivity and selectivity for potassium ions.

It is well-known that low potassium and high sodium concentrations up to 140 mM coexist in extracellular environments such as serum and tissue fluid. It is therefore very important to find a facile method that distinguishes between potassium and sodium ions. While many previous studies regarding K^+ detection have been reported, most do not perform sensitive K^+ detection in the presence of high concentrations of Na^+.[5–9] To study the influence of Na^+ ions on our system, the fluorescence intensities of the Zn-DIGP/c-Myc complex were examined at different concentrations of K^+ in the presence of 140 mM Na^+, similar to the extracellular environment. As Figure 6A shows, the fluorescence intensity gradually enhanced as the concentration

(28) Monchaud, D.; Yang, P.; Lacroix, L.; Teulade-Fichou, M. P.; Mergny, J. L. *Angew. Chem., Int. Ed.* **2008**, *47*, 4858–4861.
(29) Thibon, A.; Pierre, V. C. *J. Am. Chem. Soc.* **2009**, *131*, 434–435.

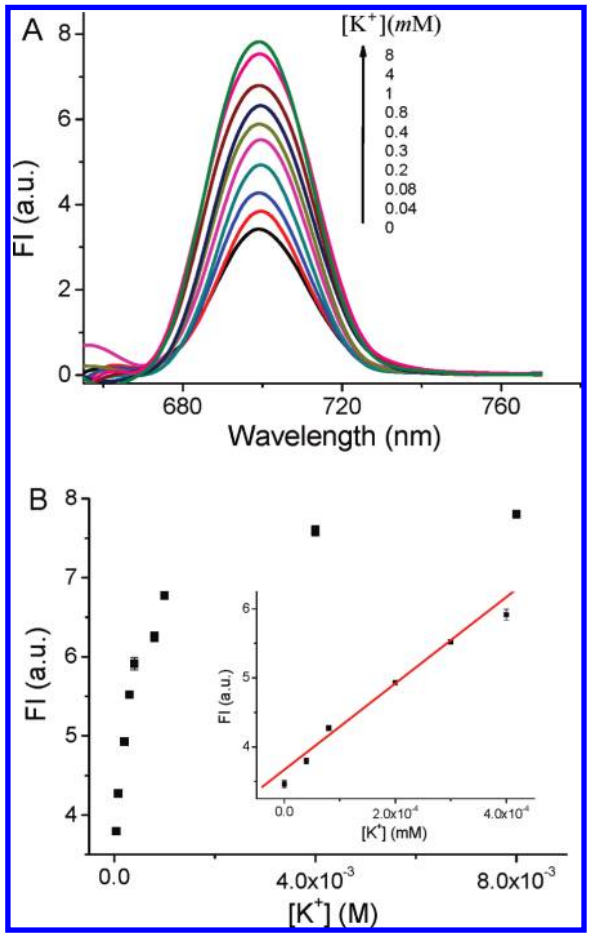

Figure 6. (A) Fluorescence spectra for different concentrations of K^+ based on the Zn-DIGP/c-Myc complex in the presence of 140 mM Na^+. (B) Plot of fluorescence intensity at 700 nm as a function of K^+ concentration. The inset shows a linear range from 0.04 to 0.4 mM. Experimental conditions: 50 mM Tris-HCl (pH 7.4) containing 200 nM c-Myc and 1 μM Zn-DIGP.

of K^+ ion increases. While the linear range of K^+ shifted to a somewhat higher concentration range because of the relatively subtle impact from Na^+ ion (Figure 6B), as little as 40 μM K^+ ion could be detected, even in coexistence with 140 mM Na^+. This sensitivity is still much higher than most previously reported detection methods.[4–10]

Application. We tested the applicability of our Zn-DIGP/c-Myc complex sensor for K^+ ion detection in urine samples. The analytical results were in good agreement with those obtained utilizing inductively coupled plasma mass spectrometry (ICPMS) (Table 2). In the sample solution, a 10 mM K^+ was added, then the K^+ content was also measured, and the recovery was in the range of 98.4−103% (Table 2). These results showed that our sensor can be used in real samples.

Table 2. Analytical Results for K^+ in Urine

sample	content (mM, n = 5)	ICPMS (mM)	added K^+ (mM)	found K^+ (mM)	recovery (%)
urine 1	12.4 ± 1.6	11.9	10.0	10.3	103
urine 2	5.92 ± 0.11	5.11	10.0	10.0	100
urine 3	28.3 ± 0.7	28.6	10.0	9.89	98.9
urine 4	42.8 ± 0.7	41.0	10.0	9.84	98.4

CONCLUSIONS

We have developed a novel and label-free fluorescence K^+ sensor based on a G-quadruplex (c-Myc) and its binding ligand (Zn-DIGP). In the presence of K^+, c-Myc is promoted to fold into the G-quadruplex structure, thus allowing Zn-DIGP to bind to the c-Myc quadruplex to form the Zn-DIGP/c-Myc complex, which can significantly enhance the fluorescence intensity of Zn-DIGP. The fluorescence intensity gradually increases along with the increasing concentrations of K^+. Without interference from any other metal ion, a potassium concentration as low as 0.8 μM is detected, indicating a high sensitivity. Furthermore, this method exhibits a good selectivity for K^+ against other metal ions. Most importantly and interestingly, even in the presence of 140 mM Na^+, 40 μM of K^+ can be detected. Compared with other assays, our method has four important characteristics: (1) this is a label-free method, which eliminates the need for covalent modification of the DNA and greatly reduces the cost; (2) this method has only three assay elements, DNA, fluorescent dye, and K^+ ions; (3) it can tolerate the presence of high concentrations of Na^+ ion; and (4) it can be used for K^+ assays in real samples.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (Grant Nos. 20905056 and 20735003), the 973 Project (Grant Nos. 2009CB930100 and 2010CB933600), and the Swiss National Science Foundation (Grant No. 116868).

Received for review July 16, 2010. Accepted August 10, 2010.

AC101894B