A Novel Biosensor Based on Terminal Protection and Fluorescent Copper Nanoparticles for Detecting Potassium Ion

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A novel biosensor for sensitively detecting potassium ion (K^+) has been developed based on fluorescent copper nanoparticles (Cu NPs). In our design, we employ a label-free single-strand DNA (ssDNA) that contains two parts. One is 3'-terminus structure-switching aptamers (SSAs) that can fold into G-quadruplex after binding with its target K^+ . The other is 5'-terminus poly thymine (polyT) which works as a template to construct fluorescent Cu NPs. After incubating with K^+ , the part SSAs go through target-induced conformational changes. Benefiting from the exceptional digestion ability of exonuclease I (Exo I), the G-quadruplexes display effective resistance to nuclease digestion, so that 5'-terminus polyT remains and the *in situ* formation of Cu NPs provides a turn-on fluorescent signal that is used to evaluate the concentration of K^+ . The recovery of the fluorescence intensity is linearly correlated with the K^+ concentration in the range of 0.05 to 1 mM with a detection limit of 0.05 mM. Compared with some methods, this assay is cost-effective and facile with high specificity. Meanwhile, this excellent strategy shows a great potentiality in other sensing approaches that can study the interaction between similar SSAs and different specific targets.

Keywords Fluorescent copper nanoparticles, potassium ion, Exo I, G-quadruplex, terminal protection

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Introduction

Potassium ion (K^+) is one of the most necessary cations in the human body, since it has a great influence on many regulating physiological functions, including hyperpolarization in the signal transduction of neurons, cellular osmotic pressure, apoptosis, pH balance, muscular strength, and renal function.¹⁻⁵ Therefore, it is still highly essential and urgent to develop novel K^+ probes or efficient detecting approaches with high specificity and sensitivity.

Up to now, many K^+ probes, such as nucleic acid-based aptamers, have been reported.⁶⁻⁸ Nucleic acid-based aptamers that are generated by a systematic evolution of ligands with an exponential enrichment (SELEX) process and obtained through selections,⁹ are artificial single-stranded DNA (ssDNA) or RNA sequences that can recognize specific biological molecules with a wide range, such as small molecules,¹⁰ organic compound,¹¹ proteins¹² or even whole cells.¹³ With outstanding stability, high affinity, excellent selectivity, easy modification, as well as low immunogenesis, nucleic acid-based aptamers have successfully attracted the increasing attention of researchers, and have been widely used in food safety, diagnosis, cell imaging and drug delivery.¹⁴⁻¹⁸

Among nucleic acid-based aptamers, different from normal affinity adsorption, a kind of aptamers, named structure-switching aptamers (SSAs), which are able to experience

conformational changes and to fold into secondary structures, like a G-quadruplex or a Y-shape structure when binding with the specific targets, have been extensively utilized to construct various biosensors.^{19,20} If the secondary structures happen to be at the end of the ssDNA, they can effectively resist degradation from the 3'-termini specific exonuclease I (Exo I). Based on the terminal protection, there is a growing number of studies concerning the interaction between nuclear acids and specific targets. For example, Jiang *et al.* reported on the quantitative analysis of the interaction of folate with a tumor biomarker of the folate receptor.²¹ Wei *et al.* developed a novel method to sensitively detect adenosine triphosphate (ATP).²² All of the studies indicate that terminal protection is a desirable strategy to fabricate new biosensors, and are prospectively used in more sensing platforms.

Herein, inspired and encouraged by the structure-sensitive terminal protection strategy, we combine SSAs with metal nanoparticles. Metal nanoparticles have attracted increasing attention due to their desirable properties, including easy synthesis, low toxicity, and sequence dependence. Recently, nanoparticle-based analytical methods were explored in many sensing detecting platforms, such as heavy metal ions,²³ antibodies,²⁴ and enzymes.²⁵ In our study, we form poly thymine (polyT)-templated fluorescent Cu NPs to work as the fluorescent signal, since Qing *et al.* proved that single-stranded polyT could be applied as an excellent template to form fluorescent Cu NPs.²⁶ Compared with other metal nanoparticles, Cu NPs are low-cost and easy to synthesize. Additionally, the polyT-templated fluorescence produced is highly efficient and accomplish within a few minutes after the start of construction under suitable conditions.^{27,28} In this study, we explore a simple

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Fig. 1 (A) TEM image of Cu NPs. Scale bar, 10 nm. (B) The maximum excitation and emission spectra of Cu NPs: excitation, 340 nm; emission, 602 nm. (C) Fluorescence intensity of 4 μM ssDNA and 20 U Exo I under a different reaction system: black, with 5 mM K⁺; red, with 2 mM Mg²⁺; green, without K⁺. (D) Agarose gel electrophoresis images of in the absence or presence of Exo I and K⁺; lane 1, 4 μM ssDNA; lane 2, 4 μM ssDNA pre-incubated with 2 mM K⁺ then added 20 U Exo I incubated for 90 min; lane 3, 4 μM ssDNA incubated with 20 U Exo I for 90 min.

As shown in Fig. 1C, in the presence of K⁺ (5 mM), the system showed strong fluorescence emission at a wavelength of about 602 nm when excited at a wavelength of 340 nm (curve a), while in the absence of K⁺, the signal was very weak (curve c). We then tested the influence of Mg²⁺, which had the same concentration as it was in the binding reaction buffer, what we observed was that the fluorescence was as weak as the system without K⁺ (curve b). These results revealed that the presence of K⁺ was a vital element to produce fluorescence. We also proved efficient terminal protection by the gel electrophoresis experiment (Fig. 1D). In the absence of K⁺, there was no band in lane 3 after introducing Exo I. Only when we added K⁺, could the substrate ssDNA remain, and show a band in lane 2, which was consistent with reports before that the SSAs parts only specifically recognized K⁺ and then folded into G-quadruplexes to prevent the cleavage reaction of Exo I.²⁰ On the basis of above data and images, we successfully synthesized Cu NPs and proposed a new analytical method to detect K⁺.

Optimization of experimental conditions

In order to obtain the desirable performance and optimal signal responses of the method, some vital parameters, such as the system of Cu NPs synthesis, units of Exo I, cleavage reaction time, concentration of copper ion (Cu²⁺) and ascorbate, we carried out optimization experiments. Firstly, we investigated the systems of Cu NPs synthesis among Tris-HCl, HEPES, MOPs and water (Fig. 2A). The result indicated that all of them were suitable environments to form Cu NPs. But considering

the repeatability of a previous experiment,²⁰ we chose Tris-HCl as the reaction buffer. The units of Exo I have a great effect on the fluorescent signal intensity because insufficient Exo I may not cleave the ssDNA totally, and any remaining polyT may cause a false positive signal. Considering the duration of our method, we chose 120 min as the longest reaction time we can accept, and then optimize the units of Exo I. Figure 2B indicates that in a 120-min cleavage reaction time, at least 20 U Exo I could cleave the substrate ssDNA completely. We then chose 20 U Exo I to optimize the cleavage reaction time. From Fig. 2C, we observed that the fluorescent intensity decreased from 0 to 120 min, and reached a platform when having reacted for 90 min. We chose a 90-min reaction time as the optimal condition. As the fluorescent signal, Cu NPs formation is one of the vital procedures in this method. After investigating the influences of Cu²⁺ and ascorbate, which were important conditions in the process of forming Cu NPs (Figs. 2D and 2E), the optimized concentrations of 500 μM Cu²⁺ and 2 mM ascorbate were used in subsequent research.

The detection of K⁺

The fluorescence responses toward various K⁺ concentrations, as shown in Fig. 3A, depicted that in the absence of K⁺, the fluorescence intensity was very weak. This result was ascribed to the cleavage of polyT and a failure of fluorescent Cu NPs construction. However, adding K⁺ to the reaction system and increasing its concentrations from 50 μM to 10 mM led to a gradual increase of the fluorescent signal, and eventually to a

Fig. 2 (A) Fluorescence intensity in different reaction solution (2 μ M ssDNA, 150 mM NaCl, 2 mM ascorbate, 0.45 mM CuSO_4). All of the buffer was 20 mM, pH 7.6. (B) Fluorescence intensity changes of Cu NPs in 120 min with various amount of Exo I from 5 – 30 U. (C) Fluorescence intensity changes of different Exo I cleavage time from 0 – 120 min. (D) Cu NPs in buffered solution (20 mM Tris-HCl, 2 mM MgCl_2 , 150 mM NaCl, pH 7.6). Effect of the synthetic conditions at $\lambda_{\text{em}} = 602$ nm after adding different Cu^{2+} concentration. The concentration of Cu^{2+} is 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mM, respectively. (E) Effect of the synthetic conditions at $\lambda_{\text{em}} = 602$ nm after adding different ascorbate concentration. The concentration of ascorbate is 0, 1, 2, 3, 4, 5, and 6 mM, respectively.

Fig. 3 (A) Fluorescence spectra of the sensing system in the presence of different concentrations of K^+ . (B) Fluorescence spectra response at 602 nm versus the K^+ concentration. Inset showed the linear response of the assay to K^+ . Error bars represented standard deviations from three repeated experiments.

platform. This observation demonstrated that relying on the exceptional structure-sensitivity capability of Exo I, the SSAs could prevent the cleavage of Exo I after incubating with K^+ . These findings suggested that this method indeed offers a specific and sensitive platform for K^+ detection under the best-case conditions.

The strong fluorescent peak at 602 nm linearly correlated with the K^+ concentration in the range from 0.05 to 1 mM, as shown in Fig. 3B. The calibration equation was $F = 994.275c + 416.614$ with a regression coefficient of $R^2 = 0.991$, where c represents the K^+ concentration (mM). The limit of detection

(LOD) was estimated to be 0.05 mM according to the 3σ rule. The sensitivity of this method is lower than those previously reported (Table 1), suggesting that a feasible and potential biosensor for sensitive K^+ assays has been successfully demonstrated.

The specificity and selectivity

To verify the specificity of the method, a selectivity experiment was investigated under the same conditions by examining the fluorescence response to Na^+ , Mg^{2+} , Ca^{2+} , NH_4^+ , Li^+ and a mixture of Na^+ and K^+ . As shown in Fig. 4, an obvious

Table 1 Comparison of the proposed method with other methods for K⁺ detection

Method	Technique	Detection limit/mM
Optical	Colorimetric ³⁰	1
Electrochemical	Potentiometric ³¹	0.0727
Optical	Fluorescence ³²	0.4
Optical	Colorimetric ³³	0.1
Optical	Fluorescence ³⁴	0.1
Optical	Fluorescence ³⁵	0.5
Optical	Fluorescence (this work)	0.05

Fig. 4 Changes of fluorescence intensity in the presence of 0.5 mM K⁺ and 5 mM of other ions. "Mix" is a mixture of 0.5 mM K⁺ and 1.5 mM Na⁺.Table 2 Detection of the K⁺ in real samples

Sample	ICPMS/mM	Found/mM	Ratio, %
1	0.08	0.065	81.25
2	0.26	0.209	80.28
3	0.83	0.706	85.06

fluorescence enhancement was observed only when K⁺ was present, while other ions showed no significant fluorescence intensity increase, since other ions could not trigger folding of the SSAs parts into G-quadruplexes to resist Exo I digestion. This result revealed the excellent specificity of the method for detecting K⁺ against other metal ions.

Detecting K⁺ in human serum

Clinically, detecting K⁺ in a biological sample is important for disease diagnostics. It is reported that to keep the normal biological environment, the level of K⁺ in serum must be 3.5 – 5.5 mM.²⁰ We then applied this method to detect K⁺ in diluted human serum. As can be seen in Table 2, the desirable result revealed that this biosensor had great feasibility in real biological systems.

Conclusions

In summary, a specific biosensor based on a terminal protection strategy and fluorescent Cu NPs was demonstrated for K⁺ detection. Compared with other assays, this label-free method eliminates the need for using the traditional organic dye and labeling of ssDNA, which greatly reduces the cost. As far as we know, this work is the first K⁺ detecting method that combines

exceptional structural sensitivity of Exo I with fluorescent Cu NPs. Additionally, the principle can be employed to the detection of other targets, like small molecules, proteins, and to further study the interaction between aptamers and biological targets.

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References

1. H. He, M. A. Mortellaro, M. J. Leiner, R. J. Fraatz, and J. K. Tusa, *J. Am. Chem. Soc.*, **2003**, *125*, 1468.
2. P. Kofuji and E. A. Newman, *Neuroscience*, **2004**, *129*, 1045.
3. H. C. Kuo, C. F. Cheng, R. B. Clark, J. J. Lin, J. L. Lin, M. Hoshijima, V. T. Nguyễntrân, Y. Gu, Y. Ikeda, and P. H. Chu, *Cell*, **2001**, *107*, 801.
4. S. Takenaka and B. Juskowiak, *Anal. Sci.*, **2011**, *27*, 1167.
5. S. P. Yu, L. M. Canzoniero, and D. W. Choi, *Curr. Opin. Cell Biol.*, **2001**, *13*, 405.
6. M. Tanha, S. K. Chakraborty, B. Gabris, A. S. Waggoner, G. Salama, and D. Yaron, *J. Phys. Chem. A*, **2014**, *118*, 9837.
7. T. T. Ruckh, C. G. Skipwith, W. Chang, A. W. Senko, V. Bulovic, P. O. Anikeeva, and H. A. Clark, *ACS Nano*, **2016**, *10*, 4020.
8. B. Kim, I. H. Jung, M. Kang, H. K. Shim, and H. Y. Woo, *J. Am. Chem. Soc.*, **2012**, *134*, 3133.
9. A. D. Ellington and J. W. Szostak, *Nature*, **1990**, *346*, 818.
10. T. Zhao, R. Liu, X. Ding, J. Zhao, H. Yu, L. Wang, Q. Xu, X. Wang, X. Lou, and M. He, *Anal. Chem.*, **2015**, *87*, 7712.
11. S. Eissa and M. Zourob, *Anal. Chem.*, **2017**, *89*, 3138.
12. Z. Wu, H. Li, and Z. Liu, *Sens. Actuators, B*, **2015**, *206*, 531.
13. D. Shangguan, Y. Li, Z. Tang, Z. C. Cao, H. W. Chen, P. Mallikaratchy, K. Sefah, C. J. Yang, and W. Tan, *Proc. Natl. Acad. Sci. U. S. A.*, **2006**, *103*, 11838.
14. Y. Yang, Y. Cen, W. J. Deng, R. Q. Yu, T. T. Chen, and X. Chu, *Anal. Methods*, **2016**, *8*, 7199.
15. Z. Wu, E. Xu, M. F. Chughtai, Z. Jin, and J. Irudayaraj, *Food Chem.*, **2017**, *230*, 673.
16. J. L. Yao, X. Gao, W. Sun, X. Z. Fan, S. Shi, and T. M. Yao, *Inorg. Chem.*, **2012**, *51*, 12591.
17. G. Zhu, H. Zhang, O. Jacobson, Z. Wang, H. Chen, X. Yang, G. Niu, and X. Chen, *Bioconjugate Chem.*, **2017**, *28*, 1068.
18. F. Atabi, S. L. M. Gargari, M. Hashemi, and P. Yaghmaei, *Iran J. Pharm. Res.*, **2017**, *16*, 35.
19. A. Vallée-Bélisle and K. W. Plaxco, *Curr. Opin. Struc. Biol.*, **2010**, *20*, 518.
20. D. Zheng, R. Zou, and X. Lou, *Anal. Chem.*, **2012**, *84*, 3554.
21. Z. Wu, Z. Zhen, J. H. Jiang, G. L. Shen, and R. Q. Yu, *J. Am. Chem. Soc.*, **2009**, *131*, 12325.
22. Y. Wei, Y. Chen, H. Li, S. Shuang, C. Dong, and G. Wang, *Biosens. Bioelectron.*, **2015**, *63*, 311.
23. M. L. Firdaus, I. Fitriani, S. Wyantuti, Y. W. Hartati, R. Khaydarov, J. A. Mcalister, H. Obata, and T. Gamo, *Anal. Sci.*, **2017**, *33*, 831.
24. M. Okochi, T. Kamiya, T. Omasa, M. Tanaka, and

- H. Honda, *Anal. Sci.*, **2016**, 32, 93.
25. J. Chen, X. Ji, and Z. He, *Anal. Chem.*, **2017**, 89, 3988.
26. Z. Qing, X. He, D. He, K. Wang, F. Xu, T. Qing, and X. Yang, *Angew. Chem.*, **2013**, 52, 9719.
27. H. B. Wang, H. D. Zhang, Y. Chen, K. J. Huang, and Y. M. Liu, *Sens. Actuators, B*, **2015**, 220, 146.
28. T. Qing, X. He, D. He, X. Ye, J. Shangguan, J. Liu, B. Yuan, and K. Wang, *Biosens. Bioelectron.*, **2017**, 94, 456.
29. P. Ni, Y. Sun, S. Jiang, W. Lu, Y. Wang, Z. Li, and Z. Li, *Sens. Actuators, B*, **2017**, 240, 651.
30. L. Wang, X. Liu, X. Hu, S. Song, and C. Fan, *Chem. Commun.*, **2006**, 3780.
31. M. F. S. Teixeira, B. H. Freitas, P. M. Seraphim, L. O. Salmazo, M. A. Nobre, and S. Lanfredi, *Pro. Chem.*, **2009**, 1, 293.
32. C. Shi, H. Gu, and C. Ma, *Anal. Biochem.*, **2010**, 400, 99.
33. X. Zhu, J. Zhao, Y. Wu, Z. Shen, G. Li, and A. Chem, *Anal. Chem.*, **2011**, 83, 4085.
34. J. Lee, H. J. Kim, and J. Kim, *J. Am. Chem. Soc.*, **2008**, 130, 5010.
35. T. Li, E. Wang, and S. Dong, *Anal. Chem.*, **2010**, 82, 7576.
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