Analytical Methods

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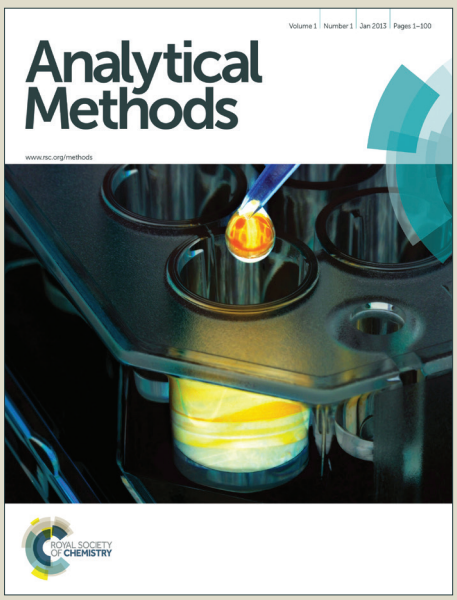

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ARTICLE TYPE

A simple colorimetric sensor for potassium ion based on DNA G-quadruplex conformation and salt-induced gold nanoparticles aggregation

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A simple colorimetric sensor for potassium ion (K^+) based on DNA G-quadruplex conformation and salt-induced gold nanoparticles (AuNPs) aggregation was developed. In the presence of K^+ and NaCl, the conformation of anti-K^+ aptamer changed from a random coil structure to a G-quadruplex one, this rigid G-quadruplex structure lost the ability of protecting AuNPs from salt-induced aggregation, and thus the color change of AuNPs from wine red to blue could be observed by the naked eyes. The aptasensor showed that the analytical linear range covered from 1 μM to 1 mM and the detection limit was 0.42 nM. The presence of other metal ions did not affect the detection of K^+, which indicated that an excellent specificity of K^+ detection could be detected. Rapidity, simplicity, high sensitivity and excellent selectivity made it suitable for practical use in determination of K^+ in real urine samples.

Introduction

The potassium ions (K^+) play an important role as a transmitter in cells and in the regulation of membrane potential, nerve stimulation, and hormone secretion.[1] Abnormal physiological K^+ concentrations may lead to several diseases. A serious shortage of K^+ in body fluids may cause a potentially fatal condition known as hypokalemia. Excess of K^+ may increase the risk of high blood pressure and stroke.[2] Thus, sensitive detection of K^+ is of great significance in both clinical diagnosis and basic research.

To date, a variety of techniques have been employed to detect K^+, such as electrochemistry,[2,3] ion chromatography,[4] surface plasmon resonance,[5] flame atomic absorption spectrometry,[6] fluorescence spectrum,[7] ion-selective electrodes,[8] and so on. These conventional methods, however, are complex and time-consuming. Therefore, it is necessary to develop new strategies for K^+ detection.

Aptamers are DNA or RNA sequences selected from combinatorial libraries through in vitro SELEX (systematic evolution of ligands by exponential enrichment). They bind to their target molecules with high affinity and specificity, including metal ions, drugs, small organic compounds, metabolites, proteins and even cells.[9-12] Recently, the appearance of G-quadruplex DNA as a functionalized nucleic acid provides another chance for K^+ detection.[13-17] However, these aptasensors also suffer from disadvantages such as time-consuming and label-requiring.

To circumvent these disadvantages, in this work, we employed colorimetric method to detect K^+ based on unmodified AuNPs as probes and the G-quadruplex aptamer as the recognition element. Anti-K^+ aptamer with a random coil structure can be easily absorbed onto the surface of AuNPs, this added negative charges to the AuNPs, increased their repulsion and stabilized the AuNPs. When added NaCl solution with high concentration and K^+ with various concentrations into the solution, the conformation of anti-K^+ aptamer changed from a random coil structure to a G-quadruplex one, and therefore lost the ability of protecting AuNPs from aggregation. The change could be detected by monitoring the color change of the AuNPs even with the naked eyes. The detection limit of 0.42 nM and the linear range from 1 μM to 1 mM were obtained. Of note, compared to our previous work,[18] this new protocol has several advantages. First, the design of the sensor is more simple and time-saving than previous ones, the aptamer doesnot require modification of –SH; Second, this sensor offers a lower detection limit (0.42 nM) than before (5 nM); Last, during the experiment, with the increase of K^+ concentration, the change of color is more obvious than before.

Experimental

Materials and apparatus

The aptamer of K^+ used in this work was synthesized by Sangon Antibody R & D Center (Shanghai, China). The sequence of oligonucleotide employed is: 5'-TTTGGTTGGTGTGGTTGGTTT-3'.[13,19] Sodium tetrachloroaurate (III) ($HAuCl_4$) and sodium citrate were purchased from Sigma-Aldrich (USA). All other reagents are of analytical reagent grade. All solutions were prepared with doubly distilled water (pH=7.0).

UV-Vis absorption spectra were recorded on an UV-2550 Spectrophotometer (Shimadzu Corporation). TEM observations were carried out with a JEOL JEM2010 microscope at 200 kV.

Preparation of AuNPs

Citrate-stabilized AuNPs were prepared by thermal reduction of $HAuCl_4$ with sodium citrate.[20] Spherical AuNPs of about 15 nm were observed by TEM (Fig. S1A). UV-visible

spectrophotometry (UVs) absorption peak appeared at about 528 nm, consistent with the absorption of the AuNPs of this size (Fig. S1B).

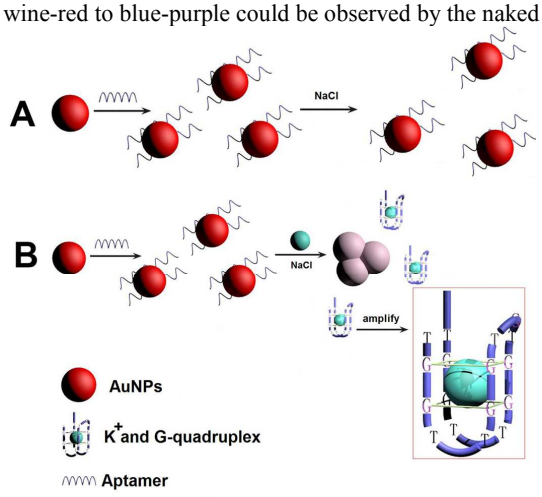
General procedure of colorimetric sensing of K^+

First, 1.2 μL K^+-aptamer binding aptamer solution (100 μM) was added into a 1.5 mL plastic vial containing 1200 μL AuNP solution (10 nM). After incubation for 10 min, then 10 μL of NaCl (4.8 M) solution was added. After incubation for 5 min, a series of different concentrations of K^+ ranging from 10 nM to 1 mM in the vial were made. Reacting for 5 min, the resulting solution was transferred to a 1cm path length quartz cuvette for spectral recording. The UV-vis absorption spectra were measured over the wavelength range from 400 nm to 700 nm. All assays were performed at room temperature.

Results and discussion

Principle of the colorimetric detection

In this work, the idea of using DNA probes and unmodified AuNPs to detect K^+ is based on the discovery that the citrate anions-protected AuNPs undergo immediate aggregation in the presence of NaCl and K^+-G-quadruplex (Scheme 1). The AuNPs in solution were stabilized by adsorbed citrate anions as their repulsion to prevent AuNPs from aggregating. When the single-stranded (ss) flexible DNA with a random coil structure was absorbed onto the surface of AuNPs through non-covalent interactions between the exposed nitrogen bases and the AuNPs, this added negative charges to the AuNPs, increased their repulsion and stabilized the AuNPs. When NaCl solution with high concentration was added into the solution, the stabled ssDNA-AuNPs protected the AuNPs from NaCl-induced aggregation in the absence of K^+ (Scheme 1A). However, upon the addition of K^+, the conformation of DNA in solution changed from a random coil structure to a secondary structure, a compact rigid G-quadruplex one (Fig. S2). The rigid G-quadruplex structure prevented the exposure of the nitrogen bases to the AuNPs, and thus lost the ability of protecting the AuNPs from NaCl-induced aggregation (Scheme 1B). The visible color change from wine-red to blue-purple could be observed by the naked eye.



Scheme 1

Scheme 1 Schematic illustration of the colorimetric detection of K^+ using anti- K^+ aptamer and unmodified AuNPs.

Characterization of aptasensor

In order to clarify the colorimetric properties of the resulting aptasensor, a series of UV-vis spectra of the AuNP solution under different conditions were measured for monitoring the process of the fabrication of the aptasensor for each step. Fig. 1A shows UV-vis spectra of the AuNP solution under different experimental conditions. As we expected, in the presence of ssDNA, compared with that of AuNPs, there was only a similar characteristic surface plasmon resonance (SPR) absorption band of ssDNA-AuNPs at about 530 nm, the absorbance peak value was larger (Fig. 1A). Correspondingly, the color of the AuNP solution still remained red (Fig. 1B). We presumed that ssDNA could be adsorbed onto the surface of AuNPs, thus added negative charges to the AuNPs and increased their repulsion. After the addition of NaCl, compared with that of DNA-AuNPs, it was observed that a SPR absorption band was still at about 530 nm, the absorbance peak value was smaller (Fig. 1A). Correspondingly, the color of the AuNP solution changed from red to dark red (Fig. 1B). We supposed that although there was a little aggregation among AuNPs induced by NaCl, in a large part, the repulsion among the ssDNA-AuNPs enhanced the stability of AuNPs against the NaCl-induced aggregation. However, upon the addition of K^+(10 nM), it was observed that a SPR absorption band of AuNPs red shifted with a shoulder band appearing at about 580 nm (Fig. 1A) and the color of AuNP solution changed from dark red to violet (Fig. 1B). In addition, the absorption at 530 nm decreased with the increase of the concentration of K^+ from 19 nM to 1 mM, the absorption peak at 580 nm was more obvious (inset of Fig. 1A), indicating the extent to the aggregation of AuNPs was much stronger. Correspondingly, the color of AuNP solution (19 nM-0.1 mM) changed from violet to blue. When K^+ concentration increased to 1 mM, the color of AuNP solution changed from blue to pale blue, which indicated that AuNPs were almost completely aggregated (Fig. 1B). Furthermore, Fig. 1C and D showed the morphology change of AuNPs through TEM. As shown in Fig.1C, the AuNPs were still dispersed in the presence of DNA-AuNP-NaCl. Fig.1D showed that the AuNPs were in an aggregated state in the presence of DNA-K^+-AuNP-NaCl. All these results were in good agreement with our assumption, indicating that K^+ induced the conformation change of aptamer from a random coil structure to a G-quadruplex one.

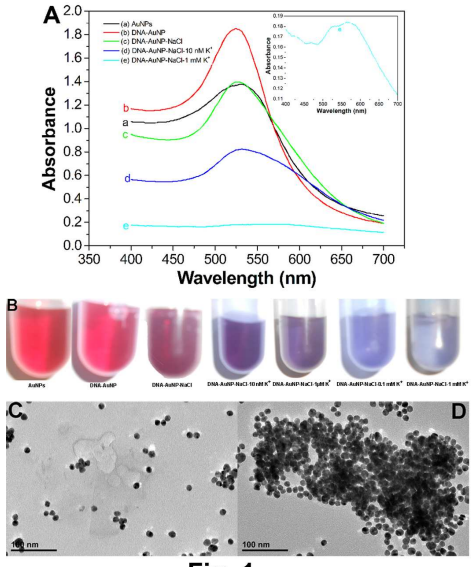


Fig. 1

Fig.1 (A) UV-vis absorption spectra of AuNPs under different

experimental conditions. Experimental condition are 40 mM NaCl, 0.1μM aptamer, 10 nM AuNPs, T=25 °C. (B) The photographs corresponding to (A). (C) TEM images of 10 nM AuNPs-0.1 μM aptamer-40 mM NaCl. (D) TEM images of 10 nM AuNPs-0.1 μM aptamer-1 mM K+-40 mM NaCl.

Optimization of experimental conditions

To achieve better assay results, a suitable concentration of NaCl, the concentration of aptamer, and the binding time of aptamer and K^+ can make sensing effect of K^+ detection more effective. First, the effect of the concentration of NaCl in the concentration range of 0–80 mM was studied. When the concentration of NaCl was 40mM, the absorbance peak intensity at 530 nm reached the maximum. Thus, 40 mM NaCl was chosen for this work on basis of higher sensitivity. In addition, the effect of the concentration of aptamer was studied in the concentration range of 0-1μM, the absorbance peak intensity at 530 nm reached the maximum value when the concentration of aptamer was 0.1 μM. Thus, aptamer of 0.1 μM was chosen for this experiment. Finally, the effect of binding time of aptamer-K^+ over the range of 0-20 min was investigated, the absorbance intensity reached stability at 10 min . Thus, 10 min was chosen for the following experiment

Colorimetric sensing of K^+

According to the above-mentioned general procedures, different concentrations of K^+ in the range of 0-1 mM were measured. As can be shown in Fig. 2. Fig. 2A depicted the UV-vis absorbance spectra of AuNPs in the presence of 40 mM NaCl solution under different concentrations of K^+ (0-1 mM). Increase of concentrations of K^+ led to increase in absorbance peak at 530 nm up to 0.5 mM, as shown in Fig. 2B. The ΔA_{530} was proportional to the log value of K^+ concentration over the range of 1 μM-1 mM (inset of Fig. 2), the linear regression equation was ΔA_{530} = 1.318 + 0.033lgC (C: M, R = 0.997), with a detection limit of 0.42 nM (3σ/slope), which compared well with those obtained from optical assay for K^+ detection, as shown in Table 1.

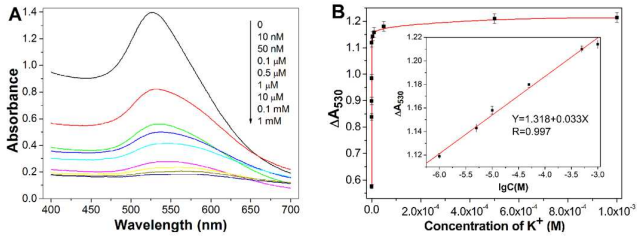


Fig. 2

Fig. 2 (A) The UV-vis absorbance spectra of AuNPs in the presence of 40 mM NaCl under different concentrations of K^+ (0-1 mM). (B) the peak absorbance change at 530 nm as a function of K^+ concentration. Inset: the peak absorbance change of 530 nm is linear with logarithm of K^+ concentration over the range from 1 μM to 1 mM.

Table 1 Comparison between the proposed colorimetric method and other optical techniques for K^+ detection.

Method	Technique	LOD	References
Optical	Colorimetric	0.42 nM	This work
Optical	Colorimetric	5 nM	[18]
Optical	Fluorescence	10.9 μM	[19]
Optical	Fluorescence	0.4 mM	[21]
Optical	Fluorescence	0.005 mM	[22]
Optical	Fluorescence	2.5 μM	[23]

Selectivity for detecting K^+

To test the selectivity of the colorimetric sensor for K^+ (1 mM) analysis, other ions including Ca^{2+}, Mg^{2+}, Na^+, Hg^{2+}, and NH_4^+ (each at 1 mM) as the potential interference ions were tested under the same conditions. As shown in Fig. 3A, upon the addition of K^+, there was an obvious change in UV-vis adsorption spectra compared with the background, whereas no or just a little spectral change in the absence (blank) or presence of other interference ions occurred. The data obtained from Fig. 3A showed that the absorbance peak value in the presence of K^+ was dramatically smaller than those of blank and the interference ions (Fig. 3B). These results clearly indicated that our method could be used to detect K^+ with high selectivity.

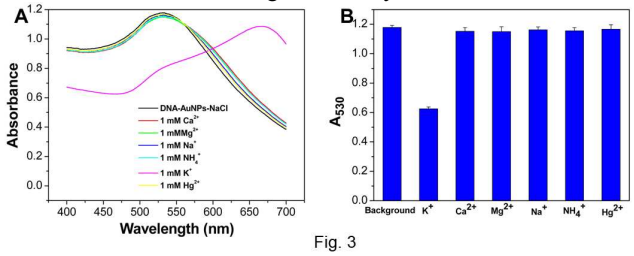


Fig. 3

Fig.3 Specificity assays. (A) The UV-vis absorption spectra of DNA-AuNP-NaCl solution in the presence of different ions including K^+, Ca^{2+}, Mg^{2+}, Na^+, Hg^{2+}, and NH_4^+ (each at 1 mM) under the same conditions. (B) The absorbance peak values of K^+ and other interference ions (Ca^{2+}, Mg^{2+}, Na^+, Hg^{2+}, and NH_4^+).

Analytical applications

In order to evaluate the application of this assay for detection of K^+ in biological system, this assay was used to detect K^+ concentration in human urine samples. As we know, the level of K^+ in urine is indicative of certain kidney diseases and the normal concentration of K^+ in the urine is in the range of 25-125 mM.[19] However, in real sample analysis, the complexity of urine samples may lead to the loss of selective interaction between the K^+ and the DNA-AuNPs, which would lead to no signal suppression at 530 nm. Therefore, we filtered the urine samples from four healthy volunteers through 0.2 mm membrane, and diluted the samples 100-fold using deionized water. On the basis of an *F*-test at a 95% confidence level, the K^+ concentrations in four urine samples determined by the colorimetric sensor agree very well with the values obtained from atomic absorption spectroscopy (AAS). The analytical results were shown in Table 2, and the relative standard deviation (R.S.D) was in the range of 0.85-3.58%. In addition, the recovery studies were carried out on 100-fold diluted urine sample to which known amounts of K^+ were added, and the spiked recoveries were changed from 90 to 96% (Table 2). The experimental results demonstrated that this method might be used to detect K^+ in biological systems.

Table 2 Analytical results for K^+ in human urine samples using the proposed method and atomic absorption spectroscopy (AAS).

Sample	Colorimetric (mM)	AAS (mM)	Added K+ (mM)	Founded K+ (mM)	Recovery (%)	R.S.D (%)
S1	0.38	0.37	0.10	0.093	93	1.15
S2	0.40	0.42	0.10	0.090	90	1.74
S3	0.35	0.38	0.10	0.096	96	3.35
S4	0.52	0.50	0.10	0.091	91	3.24

Conclusions

To sum up, a simple system for colorimetric detection of K^+ at room temperature based on unmodified AuNPs as probes and the G-quadruplex aptamer as the recognition element was developed. In the presence of K^+, the aptamer underwent a conformation change from a random coil structure to a compact rigid G-quadruplex one. The structural change and the addition of NaCl made aptamer lose the ability of protect AuNPs from NaCl-induced aggregation. The assay had a wide linear range of 1 μM-1 mM and a detection limit of 0.42 nM. It was cost-effective and ease of operation in the comparison with those of literature. Moreover, in the presence of other interference ions, this method was able to detect K^+ with high selectivity. What was more important, satisfactory results were obtained when it was applied in the detection of K^+ in human urine samples.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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