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Colorimetric Sensing of Adenosine Based on Aptamer Binding Inducing Gold Nanoparticle Aggregation

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A simple and rapid colorimetric approach for the determination of adenosine has been developed via target inducing aptamer structure switching, thus leading to Au colloidal solution aggregation. In the absence of the analytes, the aptamer/gold nanoparticle (Au NP) solution remained well dispersed under a given high ionic strength condition in that the random-coil aptamer was readily wrapped on the surface of the Au NPs, which resulted in the enhancement of the repulsive force between the nanoparticles due to the high negative charge density of DNA molecules. While in the presence of adenosine, target-aptamer complexes were formed and the conformation of the aptamer was changed to a folded structure which disfavored its adsorption on the Au NP surface, thus leading to the reduction of the negative charge density on each Au NP and then the reduced degree of electrostatic repulsion between Au nanoparticles. As a result, the aggregation of the Au colloidal solution occurred. The changes of the absorption spectrum could be easily monitored by a UV-Vis spectrophotometer. A linear correlation exists between the ratio of the absorbance of the system at 522 to 700 nm ($A_{522\ nm}/A_{700\ nm}$) and the concentration of adenosine between 100 nmol•L^{-1} and 10 μmol•L^{-1}, with a detection limit of 51.5 nmol•L^{-1}.

Keywords colorimetric sensing, modification-free Au NP aggregation, adenosine, aptamer, conformational change

Introduction

Gold nanoparticles (Au NP), based on their unique size-dependent surface plasmon resonance absorption, have been successfully used as a colorimetric probe for the detection of various substances.[1-3] Generally, well-dispersed Au nanoparticles appear red whereas aggregated Au nanoparticles change to blue.[4] Au NP-based assays possess advantages as color changes of Au NP indicating molecular recognition events can be directly observed by the naked eyes without the requirement for sophisticated instruments. The extremely high extinction coefficient (10^8—10^{10} L•mol^{-1}•cm^{-1}) of Au NP provides good sensitivity. Therefore, Au NP-based assays have been used in biological and chemical fields.[5,6]

Aptamers generated by an *in vitro* selection process termed systematic evolution of ligands by exponential enrichment (SELEX)[7] are functional DNA or RNA structures that can bind with high affinity and specificity to various targets, such as small inorganic or organic substances,[8,9] proteins,[10] and even cells.[11] Due to their salient advantages, including simple synthesis and storage, easy labeling, good stability and wide applicability, a variety of aptamer-based analytical methods have been developed for molecular recognition and detection, including electrochemistry,[12,13] fluorescence,[14,15] surface plasmon resonance,[16] atomic force microscopy[17] and quartz crystal microbalance.[18] The aptamer-based colorimetric methods with Au NP as sensing elements have also been developed.[19,20] Unfortunately, most of these methods need modification and separation, which require high cost and long analytical time. Therefore, developing colorimetric methods using modification-free Au NP to simplify the detection process would be of considerable interest. As we know, upon binding with their targets, aptamers usually undergo significant structural variations.[21] Moreover, according to the literature,[22,23] unfolded single-stranded DNA (ssDNA) could stabilize the Au NP in the presence of salt of a given high concentration, while the duplex structure or folded structure would destroy this protecting function and make the Au NP aggregate. As a result, by exploiting these properties, colorimetric sensing using unmodified Au NP based on the discriminating effects of different DNA structures on the nanoparticles has been developed for the detection of nucleic acids,[22] metal

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ions[23] and enzymatic activities.[24]

In this paper, by employing aptamer and Au NP in the presence of a moderate amount of salt, a simple and rapid colorimetric assay was proposed for the detection of adenosine based on adenosine-aptamer complexes inducing the aggregation of Au NP. A 27-nt (27 bases with the sequence of 5'-ACCTG GGGGA GTATT GCGGA GGAAG GT-3') DNA aptamer against adenosine selected by Huizenga and Szostak[25] was used in the experiment. Random-coil aptamer was adsorbed onto Au NP surface through coordination between Au and nitrogen atoms. Owing to the high negative charge density of DNA on each Au NP surface, the colloidal solution kept dispersed under a given high ionic strength condition. With the existence of adenosine, the target-aptamer complexes were formed and the conformation of the aptamer was changed to a folded G-quadruplex/duplex structure, which disfavored the adsorption on the Au NP surface. As a result of the decreased zeta potential on each Au NP and the subsequent reduced degree of electrostatic repulsion between Au nanoparticles, aggregation of the Au NP occurred. Therefore, the color of the solution changed from red to blue, thus forming the basis of adenosine detection.

Experimental

Reagent

DNA aptamer for adenosine and control DNA were obtained from Takara Biotechnology Co. Ltd., which have the sequence of ACCTG GGGGA GTATT GCGGA GGAAG GT and AGCAA CCTCA AACAG ACACC ATGG, respectively. Adenosine, cytidine, uridine, and guanosine were obtained from Generay Biotech Co. Ltd. Chloroauric acid, trisodium citrate and other chemicals were of commercially available analytical reagent grade. Phosphate buffer solution (PBS, pH 7.4) was prepared by using 10 mmol•L^{-1} Na_2HPO_4 and 10 mmol•L^{-1} KH_2PO_4. Ultrapure water (resistivity >18 MΩ•cm^{-1}) was used throughout the experiments.

Apparatus

All UV-Vis absorption spectra were made using a Shimadzu UV-2450 spectrophotometer with a 1-mm path length quartz cell. The experimental temperature was controlled with a DKB-501A thermostat made by Shanghai Jinghong Experimental Equipments Co. Ltd. Transmission electron microscopy (TEM) photograph was taken with a JEM-100CXⅡ transmission electron microscope.

Preparation of Au NP

All glassware needed to prepare Au colloid was cleaned using freshly prepared aqua regia (3 parts of HCl plus 1 part of HNO_3) and rinsed thoroughly with ultrapure water. Au nanoparticles were prepared using the citrate reduction method described elsewhere.[26] Briefly, to 100 mL of ultrapure water, 1 mL of 1% (*w*/*V*, g/mL) $HAuCl_4$•$4H_2O$ solution was added and heated to a rolling boil with continuous stirring. Then 3.0 mL of 1% (*w*/*V*) sodium citrate solution was quickly added. After about 40 min of heatment, the heater was removed and the mixture stirred continuously until it cooled. Gold colloidal solution thus prepared was stored in a dark glass bottle at 4 ℃. The gold nanoparticles were characterized with a JEM-100CXⅡ transmission electron microscope, and the size was determined to be *ca*. 13 nm (Figure 1).

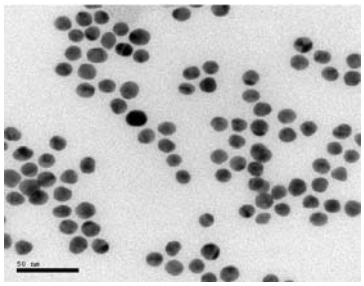
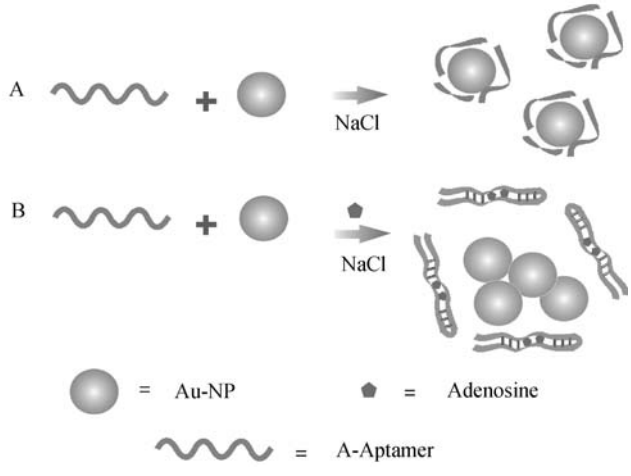


Figure 1 Transmission electron microscopy image of Au NP stabilized by citrate.

Analytical procedure

A schematic representation of the analytical process of this colorimetric assay is shown in Scheme 1. Briefly, 100 μL of 300 nmol•L^{-1} aptamer against adenosine diluted with PBS was firstly mixed with 100 μL of gold colloidal solution for 10 min at room temperature. Subsequently, 100 μL of adenosine at an appropriate concentration was added to the solution and incubated at 37 ℃ for 30 min. Colorimetric detection was performed immediately after the addition of 50 μL of 700 mmol•L^{-1} NaCl. The UV-Vis absorption spectra were recorded with a UV-2450 spectrophotometer between 300—800 nm.

Scheme 1 Schematic description of colorimetric sensing of adenosine based on the aggregation of the modification-free Au NP induced by an aptamer-target complex (A in red color and B in blue color)



Results and discussion

Mechanism for the colorimetric method

Due to the specific optical absorption peak around 520 nm caused by surface plasmon resonance, the colloidal solution of Au NP with diameter of 13 nm exhibits a red color.[27] The color of gold colloid would change according to the aggregation degree of Au NP in solution. Generally, the as-prepared Au nanoparticles of *ca*. 13 nm diameter were well dispersed against aggregation by a negatively charged coating of citrate ions. However, when 0.1 mol•L^{-1} NaCl was added, which screened the electrostatic repulsion between the ion-coated Au NP, the aggregation would be readily induced, resulting in a broad absorption spectrum (Figure 2, curve a) and blue color (inset in Figure 2: right tube). This phenomenon could be simply monitored by a UV-Vis spectrophotometer or observed by naked eyes.

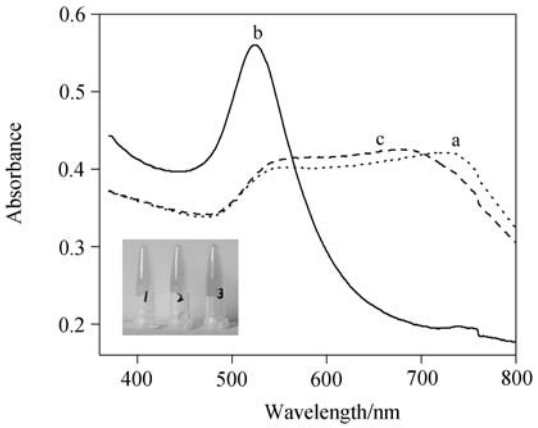


Figure 2 UV-Vis spectra of Au NP solutions after addition of (a) NaCl, (b) aptamer+NaCl and (c) aptamer+adenosine+NaCl. Inset: Photograph of Au NP solutions after addition of NaCl (right tube: in blue color), aptamer+NaCl (left tube: in red color), aptamer+adenosine+NaCl (middle tube: in blue color).

Interestingly, if certain amounts of adenosine aptamer, a short single-stranded DNA of 27-nt, were added to the gold colloidal solution, the aggregation would not appear in the presence of salt of relatively high concentration. This might be explained that DNA bases possess high affinity with gold via coordination between Au and nitrogen atoms, which favored the adsorption of the aptamer on the Au NP surface and presumably led to a redistribution of charge that made the surface appear more negatively charged, thus retained the original absorption spectrum (Figure 2, curve b) and color (inset in Figure 2: left tube) of the gold colloidal solution. Once the target adenosine was added to the system, it would interact with the aptamer to form a G-quadruplex/duplex structured complex, which impeded the exposure of DNA bases to Au NP. In addition, contrast to the soft and random coil-like structure of the unfolded aptamer, the conformation of G-quadruplex/duplex is rigid and difficult to wrap on Au NP. Therefore, the formation of G-quadruplex/duplex would decrease the affinity with Au NP and consequently decrease the ability to prevent the gold colloid from salt-induced aggregation. Under such high ionic strength conditions, the system with adenosine existence produced a color change from red to blue (inset in Figure 2: middle tube) and meanwhile the absorption spectrum became broader (Figure 2, curve c), just like unmodified Au NP (Figure 2, curve a). From curve b in Figure 2, it was observed that only one absorption peak located at 522 nm for the Au NP/aptamer system. Nevertheless, in the presence of adenosine, one can see that the 522 nm peak shifted to *ca*. 540 nm and decreased, at the same time, a new wide absorption band (590—750 nm) appeared (Figure 2, curve c). The ratio of $A_{522\ nm}/A_{700\ nm}$ was taken to evaluate the degree of colloid aggregation, which constructed the basis for a colorimetric detection of adenosine.

In the absence of sodium chloride, when only adenosine was added to the gold colloidal solution, the aggregation appeared as well (left tube in inset and curve a in Figure 3) due to the possible displacement of weakly bound citrate ions via strong metal-ligand interactions between adenosine and gold. However, under such a condition, adenosine could not cause the aggregation of the aptamer-protected gold colloid (right tube in inset and curve b in Figure 3). This control experiment confirms that it is the adenosine-binding-induced aptamer structural switching, not adenosine itself, that destabilizes gold colloid under a moderate amount of salt.

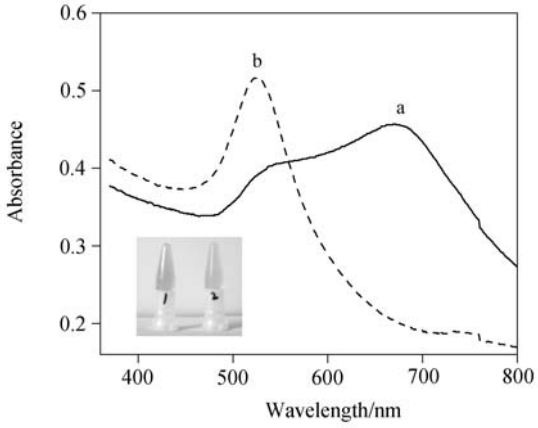


Figure 3 UV-Vis spectra of Au NP solutions in the absence of sodium chloride after addition of (a) adenosine, (b) aptamer + adenosine. Inset: photograph of Au NP solutions without sodium chloride after addition of adenosine (left tube: in blue color), aptamer and adenosine (right tube: in red color).

Analytical performance

To test the specific recognition of adenosine by its aptamer, control experiment was conducted with another random ssDNA sequence, which would keep the soft and uncoiled structure even in the presence of adenosine, thus still wrapped on the Au NP and did not

cause obvious color changes to the colloidal solution upon the addition of NaCl of a given high concentration (Figure 4). We also examined the effects of other three adenosine analogues, cytidine, guanosine and uridine, on the stability of the Au NP/aptamer/NaCl system. After the addition of 100 μmol•L^{-1} of each analogue instead of adenosine, the colloidal solution retained its original red-color (Figures 4a, 4c—4e), meanwhile, the response values of $A_{522\ nm}/A_{700\ nm}$ were as high as the blank value (Figure 4B, black bars), indicating that the adenosine aptamer was still adsorbed on the Au NP surface and protected the Au NP from aggregation. The influence of these analogues on the detection of adenosine was further investigated. From the grey bars in Figure 4B, one can observe that response value of $A_{522\ nm}/A_{700\ nm}$ with adenosine is almost unaffected in a background of its analogues at 10-fold excess concentration of the analyte. These results demonstrated that high specificity was guaranteed for adenosine assay with the proposed method.

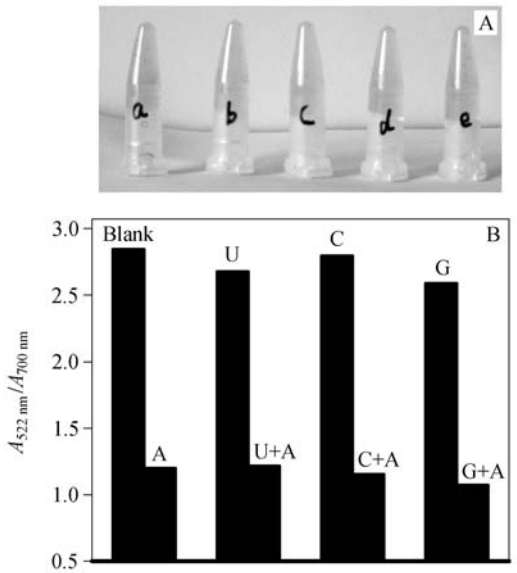


Figure 4 Selectivity of the modification-free Au NP based colorimetric method for adenosine determination. (A) Photograph of Au NP solutions after addition of (a) aptamer+adenosine+NaCl, (b) control DNA + adenosine + NaCl, (c) aptamer + cytidine+NaCl, (d) aptamer+guanosine+NaCl, (e) aptamer+uridine+NaCl (a in blue color and b—e in red color). (B) Effects of different substances on the absorbance ratio of $A_{522\ nm}/A_{700\ nm}$ of Au NP/aptamer/NaCl (blank) system. The concentration of other three analogues was 100 μmol•L^{-1}, and 10 μmol•L^{-1} for adenosine (A, C, U and G refer to adenosine, cytidine, uridine, and guanosine, respectively).

To quantitatively detect adenosine with this aptamer structure-induced Au NP aggregation phenomenon, different amounts of adenosine were incubated with the Au NP/aptamer solution for 30 min, then the UV-Vis spectra were recorded as soon as the addition of NaCl solution. As shown in Figure 5A, with the increase of the adenosine concentration, the absorption peak at 522 nm gradually decreases whereas the broad absorption band between 590 and 750 nm gradually increases. The absorbance ratio of $A_{522\ nm}/A_{700\ nm}$ was employed to quantitatively measure the adenosine concentration. Figure 5B shows that the absorbance ratio exhibits a dynamic correlation with the concentration of adenosine. A linear correlation of adenosine was obtained in the range from 100 nmol•L^{-1} to 10 μmol•L^{-1} (shown in the inset of Figure 5B). The regression equation was $A_{522\ nm}/A_{700\ nm} = 1.858 - 0.714\lg[c_{adenosine}/(\mu mol \cdot L^{-1})]$ with a correlation coefficient of 0.9946. The detection limit was estimated to be 51.5 nmol•L^{-1} in terms of the rule of three times standard deviation over the blank.

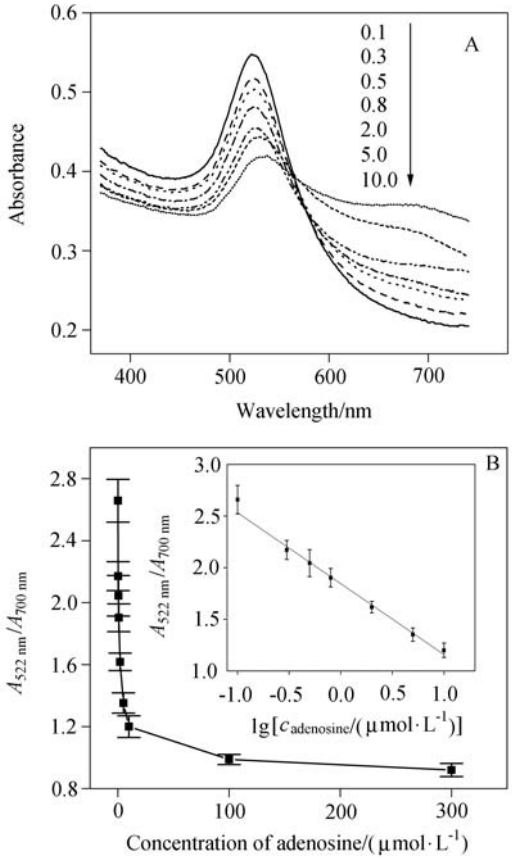


Figure 5 (A) UV-Vis spectra of the colorimetric sensing for adenosine with varying concentration (μmol•L^{-1}); (B) Plot of ratio of $A_{522\ nm}/A_{700\ nm}$ versus various concentrations of adenosine. Inset: calibration curve of ratio of $A_{522\ nm}/A_{700\ nm}$ versus logarithmic concentration of adenosine.

Conclusion

The present work has developed a simple, inexpensive, rapid and specific aptamer-based method for adenosine assay using an unmodified Au NP probe, which possesses the following advantages: firstly, Au NP can be easily and cost-effectively prepared and hold excellent colorimetric detection nature. Next, owing to the electrostatic interaction force between the nanoparticles, this non-crosslinking aggregation process is considerably rapid. In addition, some complicated and

time-consuming steps such as modification and separation are avoided. Finally, because the entire process is conducted in homogeneous solutions, it is readily suitable for highthroughput assays. This approach has demonstrated its applicability to the determination of metal ions, DNA, proteins and small molecules. It also shows great potential applications to other target molecules (for example, peptides), which could affect the electrophoretic properties of Au NP in the future.

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