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Graphical Abstract

Label-free DNA detection based on oligonucleotide-stabilized silver nanoclusters and exonuclease III-catalyzed target recycling amplification

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A label-free DNA biosensor with high sensitivity and selectivity is constructed by DNA-Ag NCs and Exo III-catalyzed target recycling amplification.
Label-free DNA detection based on oligonucleotide-stabilized silver nanoclusters and exonuclease III-catalyzed target recycling amplification

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As an emerging class of metal nanoclusters, oligonucleotide-stabilized silver nanoclusters (DNA-Ag NCs) show a number of applications in biosensing and bionanotechnology. Herein, we develop a label-free DNA sensor based on DNA-Ag NCs and exonuclease III (Exo III)-catalyzed target recycling amplification. The fluorescence of single-strand DNA-stabilized Ag NCs can be enhanced through hybridization with the guanine-rich DNA. With the addition of target DNA, the fluorescence intensity decreases comparable with that of DNA duplex-stabilized Ag NCs, which is attributed to the competitive hybridization reaction. With the help of Exo III, the fluorescence intensity decreases more obviously. The calibration range for target DNA is 0.3 to 30 nM, and the detection limit is 0.2 nM. The sensor offers 100-fold improvement in detection sensitivity compared with that obtained without Exo III. The proposed strategy also shows excellent selectivity, which can differentiate between perfectly matched and mismatched target DNA. Therefore, the strategy presents a promising platform for DNA detection with high sensitivity and selectivity.

Introduction

Noble metal nanoclusters, comprising of several to hundreds of atoms, have gained much attention owing to their unique fluorescence properties and potential applications in biosensor and bionanotechnology. Among these noble metal clusters, silver nanoclusters (Ag NCs) possess good solubility, excellent photophysical properties and low toxicity, which makes them become promising substitutes for organic dyes and quantum dots for the aforementioned applications. More strikingly, oligonucleotide-stabilized silver nanoclusters (DNA-Ag NCs) exhibit outstanding features, such as their facile synthesis, tunable fluorescence emission and high photostability. The fluorescence emission spectra of DNA-Ag NCs are highly DNA-sequence-dependent, which can be tuned throughout visible and near IR region just by changing the sequence and length of oligonucleotides. Besides, the fluorescence intensity of DNA-Ag NCs can be enhanced by 500-fold when they are in close proximity to guanine-rich (G-rich) DNA sequences. Moreover, DNA-Ag NCs have been successfully applied to cell imaging and enzyme-catalyzed signal amplification. As shown in Scheme 1A, N-DNA-Ag NCs have an Ag NCs-nucleation sequence. The single-strand DNA-stabilized Ag NCs are synthesized with N-DNA and exhibit almost no fluorescence. Upon addition of G-rich DNA (G-DNA) to the solution of N-DNA-stabilized Ag NCs (N-DNA-Ag NCs), the G-rich overhang gets close proximity to the prepared N-DNA-Ag NCs through hybridization, and the fluorescence intensity increases dramatically. When a low concentration of target DNA (T-DNA) is added to the mixture of N-DNA-Ag NCs and G-DNA, more stable T/G-DNA duplex can be formed. And the fluorescence intensity decreases due to the decrease in the amount of N/G-DNA duplex-stabilized Ag NCs (N/G-DNA-Ag NCs) and the formation of more N/DNA-Ag NCs.

Herein, we report a sensitive, selective, and label-free strategy for DNA detection based on DNA-Ag NCs and Exo III-catalyzed target recycling amplification. As shown in Scheme 1A, N-DNA has an Ag NCs-nucleation sequence. The single-strand DNA-stabilized Ag NCs are synthesized with N-DNA and exhibit almost no fluorescence. Upon addition of G-rich DNA (G-DNA) to the solution of N-DNA-stabilized Ag NCs (N-DNA-Ag NCs), the G-rich overhang gets close proximity to the prepared N-DNA-Ag NCs through hybridization, and the fluorescence intensity increases dramatically. When a low concentration of target DNA (T-DNA) is added to the mixture of N-DNA-Ag NCs and G-DNA, more stable T/G-DNA duplex can be formed. And the fluorescence intensity decreases due to the decrease in the amount of N/G-DNA duplex-stabilized Ag NCs (N/G-DNA-Ag NCs) and the formation of more N/DNA-Ag NCs.

The sensitive DNA detection is of vital importance for mutation identification, gene therapy and monitoring of hybridization reaction. Recently, the strategies based on enzyme-catalyzed signal amplification have been performed via polymerase, nicking endonuclease and exonuclease for sensitive DNA detection. Among them, exonuclease III (Exo III) is a kind of exo-III that can selectively catalyze the stepwise removal of mononucleotides from blunt or recessed 3′ termini of double-stranded DNA. Compared with nicking endonuclease, Exo III does not require specific recognition sequences, which makes it become an ideal candidate for constructing a universal signal amplification platform. The method based on Exo III-catalyzed signal amplification has been carried out to obtain sensitive DNA detection in combination with graphene oxide or gold nanoparticles. Recently, our group has developed a biosensor for colorimetric DNA detection based on gold nanoparticles and Exo III. The sensor offers the detection sensitivity as low as 2 nM and good selectivity with naked eyes.

Herein, we report a sensitive, selective, and label-free strategy for DNA detection based on DNA-Ag NCs and Exo III-catalyzed target recycling amplification. As shown in Scheme 1A, N-DNA has an Ag NCs-nucleation sequence. The single-strand DNA-stabilized Ag NCs are synthesized with N-DNA and exhibit almost no fluorescence. Upon addition of G-rich DNA (G-DNA) to the solution of N-DNA-stabilized Ag NCs (N-DNA-Ag NCs), the G-rich overhang gets close proximity to the prepared N-DNA-Ag NCs through hybridization, and the fluorescence intensity increases dramatically. When a low concentration of target DNA (T-DNA) is added to the mixture of N-DNA-Ag NCs and G-DNA, more stable T/G-DNA duplex can be formed. And the fluorescence intensity decreases due to the decrease in the amount of N/G-DNA duplex-stabilized Ag NCs (N/G-DNA-Ag NCs) and the formation of more N/DNA-Ag NCs.
Experimental

Reagents

All oligonucleotides were purchased from Sangon Biotechnology Co., Ltd (Shanghai, China). All sequences of the oligonucleotides used in the assay were listed in Table S1 (ESI†). The oligonucleotides were stored in phosphate buffer solution (20 mM phosphate, 1 mM magnesium acetate, pH 7.4). Exonuclease III (Exo III) was obtained from TAKARA biotechnology Co., Ltd. (Dalian, China) and used without additional purification. Silver nitrate (AgNO₃) and sodium borohydride (NaBH₄) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Nanjing, China). Other chemicals were of analytical grade. All aqueous solutions were prepared with double distilled water.

Apparatus

UV-vis absorption spectra were recorded with a UV-visible spectrometer (Shimadzu UV-2450, Japan), and fluorescence measurements were performed on FluoroMax-4 spectrophotometer (Horiba, Japan). The morphologies of Ag NCs were observed with transmission electron microscopy (TEM, JEM-2010, Japan). CD spectra of DNA were collected by a Chirascan Apparatus (Dalian, China) and used without additional purification. Silver nitrate (AgNO₃) and sodium borohydride (NaBH₄) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Nanjing, China). Other chemicals were of analytical grade. All aqueous solutions were prepared with double distilled water.

Synthesis of N-DNA-Ag NCs

N-DNA-Ag NCs were synthesized according to the previous literature with minor modification. Briefly, N-DNA (4 μM) and 24 μM AgNO₃ were sequentially mixed with 20 mM phosphate buffer (1.0 mM Mg²⁺, pH 7.4). After cooling on the ice for 15 min, 24 μM NaBH₄ aqueous solution (freshly prepared) was added quickly, followed by vigorous shaking for 1 min. The reduction time of the mixture in the dark at 4 °C was at least 3 h (ESI, Fig. S1†). The prepared N-DNA-Ag NCs solution was stored in the dark at 4 °C prior to use.

DNA detection

In a typical hybridization assay, 0.5 μM G-DNA, 15 U Exo III and various concentrations of target DNA were incubated at 37 °C for 2 h. The mixture was heated at 80 °C for 20 min to deactivate Exo III and then slowly cooled to room temperature. Afterwards, 0.5 μM N-DNA-Ag NCs solution was added to the above solution. The mixture was incubated at 37 °C for 1 h (ESI, Fig. S2†). The concentration of target DNA was detected by measuring the fluorescence decrease compared with the fluorescence intensity of N/G-DNA-Ag NCs.

Results and discussion

Characterization of DNA-Ag NCs

The formation of DNA-Ag NCs could be characterized by the transmission electron microscope (TEM). As shown in Fig. 1A, DNA-Ag NCs were monodispersed with an average size of approximately 2 nm. Fig. 1B showed that N/G-DNA-Ag NCs exhibited strong fluorescence emission at 623 nm with excitation at 564 nm. The characteristic absorption peak was observed in the UV-vis spectra, which had a maximum wavelength that was identical to that of the fluorescence excitation peak (ESI, Fig. S5†, curve b). The N/G-DNA-Ag NCs displayed pale pink color under room light and bright red color under UV lamp irradiations (Inset in Fig. 1B). The room temperature quantum yield of the N/G-DNA-Ag NCs was calculated to be about 15% against the reference of Rhodamine B in water (ESI, Fig. S3†), which was comparable with the reported results. Furthermore, N-DNA-Ag NCs and N/G-DNA-Ag NCs were stable up to one week in the dark at 4 °C (ESI, Fig. S4†).

Verification of the assay principle

The principle of the assay was shown in Scheme 1. In our assay, N-DNA involved an Ag NCs-nucleation sequence (pink color), a hybridization sequence and an adenine-rich sequence (black color). G-DNA involved a sequence that was complementary to N-DNA (green color) and a G-rich sequence (orange color).
Methods

Accepted Analytical Methods

The circular dichroism (CD) spectra were also used to verify the principle of the designed strategy (Fig. 3). CD spectra showed significant enhancement of peak strategy and a shift of peak position from 251 nm to 248 nm before (curve a) and after (curve b) hybridization of N-DNA-Ag NCs with G-DNA. With the addition Exo III to the mixture of N-DNA-Ag NCs and G-DNA, the ellipticity at 248 nm had almost no change compared with N/G-DNA-Ag NCs (curve c). In the presence of a low concentration of T-DNA, the peak intensity at 248 nm became a little stronger (curve d). The reason was that the formation of T/G-DNA duplex led to the decrease in the amount of N/G-DNA-Ag NCs. Therefore, the ellipticity of the former was stronger than that of the latter. This was in accordance with the reported results that the ellipticity of the DNA-Ag NCs was weaker than that of free DNA due to the formation of folded DNA structure.11,47

In the presence of Exo III and T-DNA simultaneously, an obvious decrease of peak intensity and a shift of peak position from 248 nm to 249 nm were observed (curve e), which was close to the spectra of N-DNA-Ag NCs. This proved that a number of N-DNA-Ag NCs existed in the solution, which was attributed to the fact that a number of G-DNA was digested by Exo III. Besides, Exo III amplification could be further verified by the corresponding UV-vis absorption spectra, which were recorded in Fig. S5 (ESI†). All of the above results demonstrated the successful Exo III-catalyzed target recycling amplification.

Optimization of assay conditions

The ratio of G-DNA to N-DNA-Ag NCs (G/N ratio) would affect the sensitivity for DNA detection. As shown in Fig. 4, the fluorescence intensity increased with the increasing G/N ratio and reached a maximum value at the G/N ratio of 1.5 (curve a). However, the increase of G/N ratio led to the rapid decrease of signal to background (S/B) ratio ((F₀-F)/F₀) from 0.94 to 0.05 (curve b). F₀ corresponded to the fluorescence intensity of N-G-DNA-Ag NCs, and (F₀-F) corresponded to the fluorescence decrease of the solution comparable with N/G-DNA-Ag NCs.

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The S/B ratio reduced to as low as 0.43 at the G/N ratio of 1.5. Considering the detection sensitivity, the ratio of 1 (S/B = 0.71) was selected as the optimal G/N ratio.

The enzymatic reaction condition was also vital to the assay. Fig. 5A showed that the fluorescence decrease increased significantly with increasing amount of Exo III and was nearly saturated at 15 U. The amount was close to the reported result, in which the sensor exhibited high detection sensitivity and good double-stranded selectivity by using 16 U Exo III. The Exo III-catalyzed target recycling reaction was rapid, and the fluorescence decrease reached a platform after 30 min in the presence of 15 U Exo III (Fig. 5B). In order to obtain complete cleavage effect of Exo III, 2 hours were chosen as incubation time in the whole assay.

Sensitivity and selectivity of the sensor

Under the optimal assay condition, the fluorescence intensity gradually decreased with increasing concentration of T-DNA either in the absence or presence of Exo III. In the absence of Exo III, the fluorescence decrease was linear with the concentration of T-DNA in the range of 30-200 nM with a correlation coefficient of 0.997, and the detection limit was 20 nM in terms of 3 times deviation of blank sample. In the presence of Exo III, the fluorescence decrease was linear with the concentration of T-DNA in the range of 0.3-30 nM with a correlation coefficient of 0.999 (Fig. 6). The detection limit was reduced to 0.2 nM, which was much lower than that of other biosensors based on DNA-Ag NCs (12.5 nM-200 nM). Moreover, the sensor assisted with Exo III had about 100-fold improvement in the sensitivity with respect to that without Exo III.

Conclusions

In this work, a label-free DNA biosensor based on DNA-Ag NCs and Exo III-catalyzed target recycling amplification has been constructed. With the help of Exo III, the detection limit is as low as 0.2 nM, and the sensor has 100-fold improvement in sensitivity with respect to that without Exo III. Using DNA-Ag NCs as fluorescent probes and G-rich DNA as signal enhancers, the strategy needs no separation procedures, complex labeling processes and sophisticated instruments. Besides, the strategy shows high selectivity toward target DNA and provides a
plausible technique for discriminating perfectly matched target DNA from mismatched target DNA. Thus, this innovative strategy presents a promising platform for sensitive and selective detection of DNA.

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Notes and references

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