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DNA template-synthesized silver nanoparticles: A new platform for high-performance fluorescent biosensing of biothiols

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To develop the high-performance fluorescent bio-sensors, the metal nanoparticles were employed as nanoquenchers and attracted reasonable attention in the design of fluorescent biosensors. In this work, silver nanoparticles (AgNPs) were obtained via reduction of Ag^+ on FAM-labeled DNA template. For the tight binding between AgNPs and DNA, the template-synthesized AgNPs turned out high quenching efficiency and could be applied as super nanoquenchers to establish the biosensing platform for fluorescent detection. As an example, the template-synthesized DNA-AgNPs conjugates were employed in sensing thiols. By forming S–Ag bonds, thiols interact intensely with AgNPs and replace the FAM-labeled DNA off from the surface of AgNPs, resulting in a fluorescence enhancement. Besides the advantages of lower background and higher signal-to-background ratio (S/B), the conjugates present better stability, making them applicable in complicated biological fluids. To further evidence the feasibility of sensing thiols in real samples, the thiols in human urine were detected. The total amount of free thiols found in human urine was ranging from 229 μM to 302 μM with the proposed sensor. To conclude the reliability, low content of Cys was added and the recovery was 98%–103%.

silver nanoparticles, DNA template-synthesized, thiols, fluorescent sensing

1 Introduction

Metal nanoparticles, which are of extremely small size, display unique optical and electrochemical activities and have been currently interest in the field of biochemistry and nanotechnology [1–5]. In recent years, to develop sensitive, rapid and cost-effective biosensors, metal nanoparticles were employed as super nanoquenchers instead of traditional organic dyes in design of fluorescent sensors [6–13]. For the much higher quenching efficiency, metal nanoparticle-based fluorescent sensors were successfully developed with advantages of lower background and better sensitivity. Generally, the fluorophores are combined with metal nanoparticles in two ways. As shown in Scheme 1(a), by strong interaction between thiol groups and metal atoms, thiol contained fluorophores are covalently immobilized on the surface of nanoparticles [6, 7]. Though it provides a tight link, the fluorophores could not be effectively removed away from the nanoparticle surface, limiting the restoration of fluorescence. An alternative way is to non-cross link them by weak interactions as electrostatic force and π-π stacking interaction (Scheme 1(b)). Sensors for label-free fluorescent detections are developed under this strategy with advantages of simplicity and fast responding [8–13]. However, the relatively weak interactions make the non-cross linked combinations unstable in complex conditions, which increase the background and limit their applications in real samples. To further improve the performance of these sensors, new methods for combination of fluoro-phores and nanoparti-

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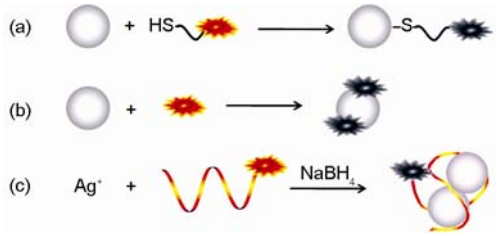

Scheme 1 Three different ways to conjugate metal nanoparticles with fluorophores.

cles, with both advantages of better stability and effectively fluorescence restoration, are required.

Silver is one of the most commonly used materials for making up nanoparticles [14, 15]. When silver nanoparticles (AgNPs) are favorably applied in the fields of surface enhanced raman scattering, colorimetric assay as well as electrochemical assay, few attention is paid on the quenching capability of AgNPs. Moreover, different from other metals, AgNPs could be template-synthesized on macromolecules [16, 17]. For example, templated by DNA, which is rich of amino groups and heterocyclic nitrogen atoms and has high affinity for metal ions, Ag^+ can be enriched and reduced to form AgNPs with various sizes and shapes, such as fluorescent silver clusters [18, 19], nano-wires [20] or nano-rings [21]. Due to the tighter binding between template-synthesized AgNPs and the DNA, AgNPs turn out much closer to the fluorophores and result in a higher quenching efficiency (Scheme 1(c)). Moreover, the interaction between template-synthesized AgNPs and DNA is also much weaker than that of covalent link. Therefore, the fluorophores could be removed away from the nanoparticle surface effectively, making the fluorescence highly restored. Providing a new strategy for combining fluorophores and metal nanoparticles, template synthesis of AgNPs turns out to be promising in design of high performance fluorescent biosensors.

Using template-synthesized DNA-AgNPs conjugates, a fluorescent thiol sensor is thus proposed as an example. FAM-labeled oligonucleotides are used as DNA template for the formation of DNA-AgNPs conjugates. Using diluted $NaBH_4$ as reductive reagent, AgNPs with diameters of 3–5 nm are synthesized and work as super nanoquenchers. In the presence of biothiols, such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), the strong interaction between thiol group and silver makes DNA released from the AgNPs surface. Thus the fluorescence is restored. As the conjugates have better stability in complex conditions than that of non-covalently linked DNA/AgNPs complex, the proposed sensor could be applied in real samples.

2 Experimental

2.1 Chemicals and apparatus

Oligonucleotides with random sequence (P1: 5′-FAM-TCT AAA TCA CTA TGG TCG C-3′ and P2: 5′-TCT AAA TCA CTA TGG TCG C-3′) were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). $AgNO_3$ (99.9999%) and $NaBH_4$ were purchased from Sigma-Aldrich. All other chemicals were of analytical reagent grade and used as received. Ultrapure water with an electric resistance of 18 MΩ, supplied by a Milli-Q water purification system (Millipore, Billerica, MA), was used in preparing the solutions.

UV-vis absorption spectra were performed on a Hitachi U-4100 spectrophotometer (Kyoto, Japan). The fluorescence was measured on a Hitachi F-7000 fluorescence spectrofluorometer (Kyoto, Japan). The fluorescence polarization assay was performed on a PTI QM4 Fluorescence System (Photo Technology International, Birmingham, NJ) with accessories of a motorized polarizer and the results were analyzed with FeliXTM software (version 1.2) supplied by the manufacturer. Transmission electron microscope (TEM) images were recorded on a Tecnai F20 (FEI Co.) using an accelerating voltage of 200 kV. Samples were prepared by placing a 5 μL drop of DNA template-synthesized AgNPs on a 3 mm copper TEM grid and allowing the droplet to evaporate to dryness.

2.2 Preparation of P1-AgNPs conjugates

50 nM P1 in 10 mM PBS (pH 7.4) was previously cooled in ice water. Following the addition of 10 μM Ag^+, 10 μM freshly prepared $NaBH_4$ was added into the mixture. After 30 min incubation in ice-water for complete reaction, the P1-AgNPs conjugates were obtained and stored under 4 °C before use.

2.3 Detection of Cys

Cys was freshly prepared and diluted to different concentrations. 2 mL of DNA-AgNPs conjugates (prepared with 50 nM P1, 10 μM Ag^+ and 10 μM $NaBH_4$) was firstly brought into a cuvette and then a continuous titration of Cys was performed. The total volume added was less than 5% and the volume influence could be neglected.

2.4 Detection of urinary thiols

Human urine was kindly provided by healthy volunteers and stored at 4 °C before detection. Typically, 500 μL DNA-AgNPs conjugates were brought into a cuvette and then mixed with 5 μL urine samples. After 5 min incubation, the fluorescence was detected. The recovery was obtained via adding 100 μM Cys to urine samples.

3 Results and discussion

3.1 Synthesis of DNA-AgNPs conjugates

Through fluorescence resonance energy transfer (FRET)

process or photo-induced electron transfer (PET) process, most fluorescent dyes could be quenched by metal nanoparticles [6–13]. When gold nanoparticles (GNPs) were employed as metal nanoquenchers to establish fluorescent platforms for biosensing and received great success during last decades, however, little attention was paid to such applications of AgNPs. The most possible reasons could be concluded as follows: Firstly, the synthesis of water soluble AgNPs, with uniform size and shapes, is much more difficult than that of GNPs. Secondary, due to the relatively weak adsorption capability of AgNPs, non-covalently linked AgNPs/fluorophore combinations turn out unstable in complex environments and emit high background fluorescence. Finally, AgNPs tend to aggregate in aqueous solution and lose their quenching capability. To establish a high performance fluorescent platform of sensing, water soluble AgNPs, as well as a tight combination with fluorophores, are required. Fortunately, it was well demonstrated AgNPs can be template-synthesized via a reduction of Ag^+ on the template of oligonucleotides with different size and shapes, depending on the ratio of Ag^+ to DNA. When the ratio is low, small-sized silver nanoparticles or silver clusters are formed on DNA template. However, the higher ratio of Ag^+ to DNA will result in the formation of large-sized AgNPs, which were sometimes mentioned as nanowires or nanorings. To reveal the size and shapes of AgNPs synthesized under different ratios, transmission electron microscopy was undertaken. As shown in Figure 1, when the ratio is about 10, the AgNPs synthesized turn out sphere shapes and a diameter distribution of 3–5 nm. For the effective wrapping of DNA, the AgNPs were well dispersed in aqueous solution and no significant aggregation was found. When the ratio was increased to about 100, the diameter of AgNPs grew to about 6–8 nm. Though a tiny aggregation was found, most of the AgNPs were mono-dispersed. Further increasing the Ag^+ concentration results in a dramatic aggregation of AgNPs, indicating the lower capability of DNA in stabilizing large-sized AgNPs.

3.2 Quenching efficiency

Using FAM-labeled oligonucleotides (P1) as DNA template, the AgNPs were template-synthesized and turned out strong quenching efficiency to the fluorophores labeled on DNA.

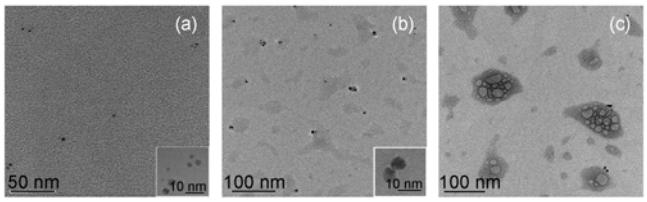

Figure 1 TEM images of DNA-AgNPs conjugates template-synthesized under different ratios of Ag^+ to DNA. 1 μM 19-mer ss-DNA (P2) was used as DNA template and (a) 10 μM, (b) 100 μM, (c) 1 mM Ag^+ and $NaBH_4$ were used for the synthesis of AgNPs.

As indicated in Figure 2(a), the fluorescence of P1 was quenched dramatically when the total amount of Ag^+ used in preparation increased. To evidence the fluorescence quenching was mainly caused by the formation of AgNPs on DNA template, fluorescence polarization was simultaneously detected during the synthesis of P1-AgNPs conjugates. The fluorescence polarization of a fluorophore reflects the molecule's ability to rotate in its microenvironments, which include viscosity of the solution, and the size and mass of the molecule to which the fluorophore is attached [22]. Therefore, fluorescence polarization can be used to judge whether the fluorescent oligonucleotide is free in aqueous solution or immobilized on AgNPs surface. For oligonucleotides in PBS, the polarization was about 0.020 indicating the relatively small mass. When the Ag^+ concentration increased to 10 μM, the polarization gradually increased to about 0.409, which indicating the increase of mass and the formation of AgNPs on DNA.

The quenching efficiency of DNA template-synthesized AgNPs turned out much higher than that of non-template-synthesized AgNPs. To make them comparable, the mass concentration was used for both of template or non-template synthesized AgNPs. As shown in Figure 3, though the non-template-synthesized AgNPs can partly quench the fluorescence, the template-synthesized AgNPs turn out much higher quenching efficiency. When the mass concentration of AgNPs was about 1.27×10^{-3} g/L (about 10 μM), the

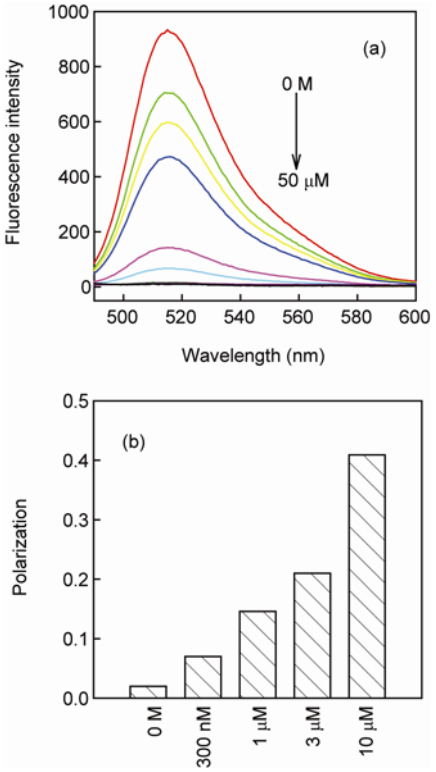

Figure 2 (a) Fluorescence emission spectra for P1-AgNPs conjugates prepared with different concentration of Ag^+ and 10 μM $NaBH_4$; (b) fluorescence polarization changes for P1-AgNPs conjugates prepared with different concentration of Ag^+.

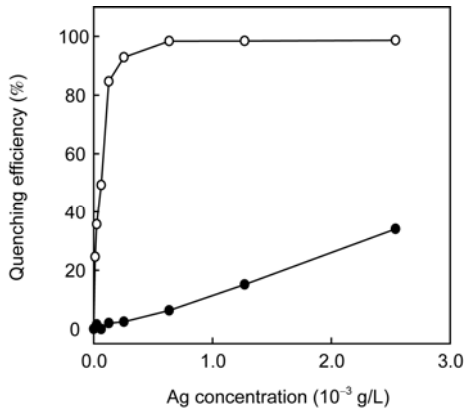

Figure 3 The fluorescence intensity ratio F_0/F (where F_0 and F are the fluorescence intensities of P1 and AgNPs complexed P1) for non-template-synthesized AgNPs (●) and template-synthesized AgNTs (○) plotted against the AgNPs mass concentration.

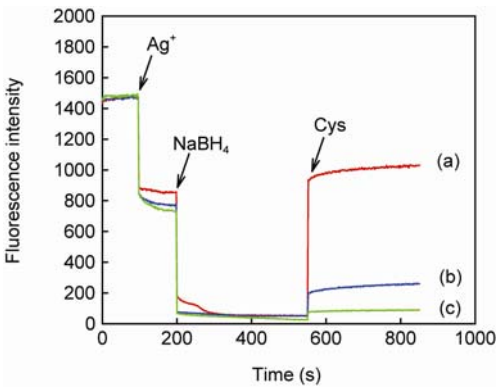

Figure 4 Real-time fluorescence records of P1 (50 nM) upon additions of 10 μM (a), 100 μM (b) and 1 mM (c) Ag^+ and $NaBH_4$ and subsequent 1 mM Cys. The transitions between each regime are marked with an arrow. Fluorescence emission was recorded at 518 nm with an excitation wavelength of 480 nm.

quenching efficiency for template-synthesized AgNPs researched as high as 98.4%. But for non-template-synthesized AgNPs, a same concentration of AgNPs only results a fluorescence quenching of 15.1%. The extremely high quenching efficiency for template-synthesized AgNPs was mainly caused by the tight binding between AgNPs and oligonucleotides. For the complete wrapping and contacting of AgNPs and DNA, the template-synthesized AgNPs turn out much closer to the fluorophore and higher quenching efficiency.

3.3 Binding affinity between template-synthesized AgNPs and DNA

Thiol replacement was used to further investigate the binding affinity between AgNPs and DNA. For the strong interaction between thiol group and silver atoms, non-covalently immobilized fluorophores could be replaced quickly by thiols, giving out a dramatic fluorescence enhancement. But for covalently linked fluorescent molecules, the tight binding between AgNPs and the fluorophores makes the replacement much more difficult. Rather than covalent or non-covalent link, the template synthesis of DNA-AgNPs conjugates provides a third way to combine AgNPs together with the fluorophores. As shown in Figure 4, template-synthesized AgNPs turn out size-dependent binding affinity to DNA. When using low concentration of Ag^+, the AgNPs formed were of small size and bond with the oligoculeotides weakly, which could be simply replaced by adding 1 mM Cys (line a) and resulted in a quick and dramatic fluorescence increase. However, large-sized AgNPs synthesized with higher Ag^+ concentration turned out higher binding affinity towards DNA and the addition of 1 mM Cys could hardly release the oligonucleotides from AgNPs surface. Therefore, less fluorescence enhancement was observed (line b and c). The different binding affinities could be explained by the different binding modes. When small-sized AgNPs, which could be effectively wrapped by oligonucleotides, turned out conjugates in mode of AgNPs-in-DNA, in which soft-structured DNA was outside and easy to be replaced by thiols, large-sized AgNPs, synthesized with high concentration of Ag^+, turned out conjugates in mode of DNA-in-AgNPs. For the large-sized AgNPs could be hardly stabilized by the oligonucleotides, an aggregation took place, during which the DNA was embedded inside the aggregations. For the rigid structure of AgNPs, oligonucleotides were tightly trapped and unable to release. When small-sized AgNPs combined with oligonucleotides with weak binding affinity, large-sized AgNPs combined with them more tightly. Therefore, by controlling the reduction process of synthesis, DNA-AgNPs conjugates with a tunable binding strength, as well as a tunable stability, could be obtained, which turn out more convenient for different applications.

3.4 Detection of thiols

To evidence the potential application of template-synthesized DNA-AgNPs conjugates as a high-performance fluorescent platform for biosensing, a thiol sensor was presented. As shown in Figure 5(A), the fluorescence of P1-AgNPs conjugates turned out to be low fluorescence. The presence of Cys displaced oligonucleotides from AgNPs, resulting in a release of fluorescent oligonucleotides and a dramatic fluorescence enhancement. For the high quenching efficiency of template-synthesized AgNPs, the sensor turned out extremely low background signal and the ratio of F to F_0, where F_0 and F are the fluorescence intensities of DNA conjugates before and after interacting with Cys, reached about 50 in presence of 5 μM Cys. Moreover, using DNA-AgNPs conjugates synthesized with different concentration of Ag^+, the sensitivity and the detection range could be quite varied (Figure 5(B)). When the Ag^+ concentration was as

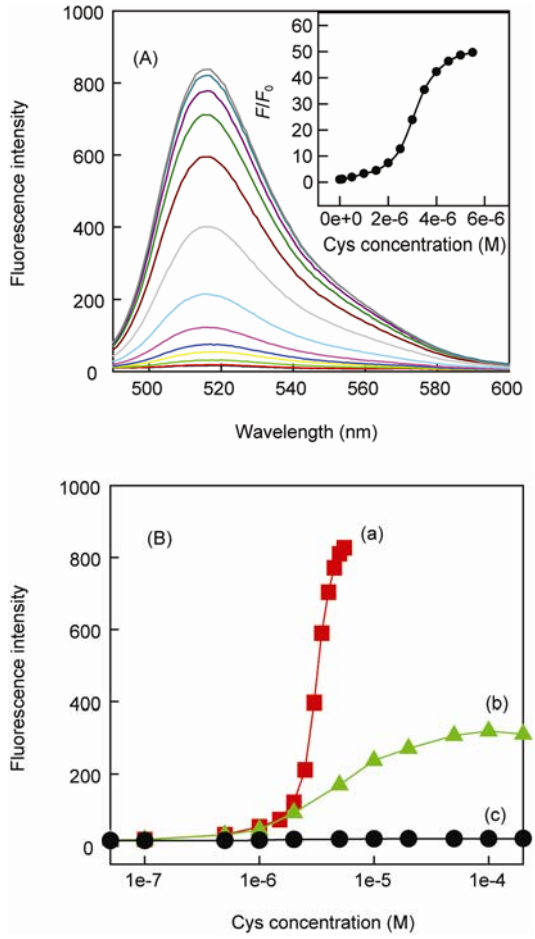

Figure 5 (A) The fluorescence emission spectra for P1-AgNPs conjugates in responding to different concentration of Cys. Inset: Cys concentration-dependent change in F_0/F; (B) the fluorescence intensity as a function of Cys concentration. P1-AgNPs conjugates were synthesized via reduction of 10 μM (a), 100 μM (b), and 1 mM Ag^+ and $NaBH_4$, 50 nM P1 was used as template.

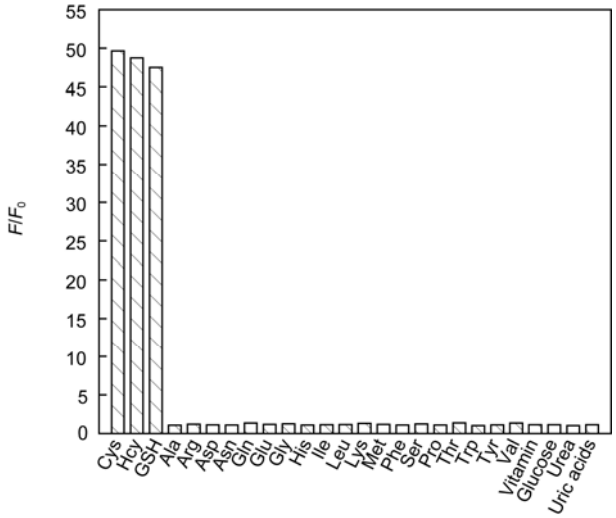

Figure 6 F/F_0 of the sensing system in responding to 5 μM of different amino acids, GSH, Hcy, vitamin B, glucose, urea and uric acids.

low as 10 μM, the DNA-AgNPs conjugates presented better sensitivity and a relatively narrow detection range. Though the sensitivity could be further improved by using lower concentration of Ag^+, the dramatic increase of background decreased the ratio of signal to background. Using higher concentration of Ag^+, a much wider detection range could be obtained, however, when the Ag^+ concentration increased to even higher, about 1 mM, for the DNA became embedded in aggregations, no significant signal enhancement was observed in presence of Cys. The selectivity was also investigated by adding 5 μM of each chemical into the solution of DNA-AgNPs conjugates. For different thiols, such as Cys, Hcy and GSH, a similar fluorescence enhancement was detected, however, for other biomolecules, such as amino acids, vitamins, glucose, urea and uric acids, no obvious fluorescence enhancement was found (Figure 6), indicating the detection was thiol selective.

Numerous of thiol sensors were reported during last decades. Besides synthetic organic probes [23–26], metal nanoparticles, such GNPs and gold nanorods, were involved in sensing thiols, due to their easy synthesis and biocompatibility [27–29]. The presence of thiols reacted and immobilized on the surface of nanoparticles and induced them to aggregate, turning out a colour change. To further improve the sensitivity, metal nanoparticles were non-covalently linked with fluorophores to fabricate fluorescent thiol sensors [30]. Through a thiol-induced displacement, fluorophores were released and resulted in a fluorescence enhancement. These sensors provide selective and sensitive detections of thiols, however, for the non-covalently linked metal nanoparticles/fluorophore complex are unstable in complex conditions, most of them can not to be applied in real biological fluids. The template-synthesized DNA-AgNPs conjugates were of higher stability than those of non-covalently linked mixtures. By examining the substrate-induced fluorescence enhancement, it was demonstrated metal ions in the 100 mM range do not affect the fluorescence intensity of P1-AgNPs, suggesting that these metal ions do not destroy the nanostructure of AgNPs. Inorganic and organic anions, as well as amino acids, such as L-glycine (Gly), leucine (Leu), histidine (His), glutamic acid (Glu), aspartic acid (Asp), and methionine(Met) in the 10 mM range do not induce any fluorescence enhancement of P1-AgNPs. Furthermore, the detection of Cys in co-existence of the above chemicals turned out no obvious difference from that of standard Cys samples, indicating the strong anti-interference ability of the proposed sensor (Figure 7). These results clearly give an evidence for the potential capability of sensing Cys in complex conditions using DNA-AgNPs conjugates.

To demonstrate the availability of sensing thiols in biological fluids, human urine, without any pretreatment, was used as real sample and the total thiol concentration was measured. As listed in Table 1, the total thiol concentration

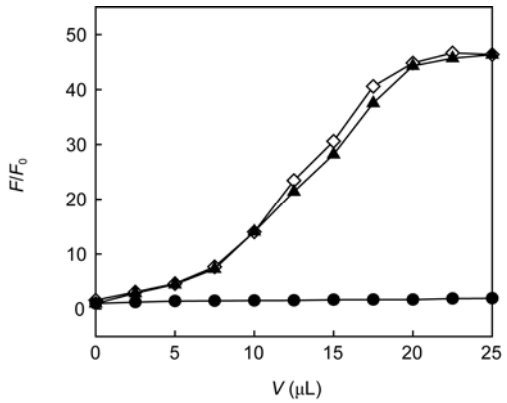

Figure 7 Fluorescent titration using 100 μM Cys solution (-◇-), the mixture containing 100 mM NaCl, 1 mM of vitamins, glucose, urea and uric acid, and 100 μM of amino acids, including Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val (-●-), and the above mixture with additional 100 μM Cys (-▲-).

Table 1 Assay results of human urine samples and the recovery[a)]

Samples	Total free thiols		
	added (μM)	found (μM)	recovery (%)
Urine 1	0	302±12	-
	100	403±5.3	101
Urine 2	0	257± 3.4	-
	100	360±4.5	103
Urine 3	0	229 ± 7.2	-
	100	327 ± 1.7	98

a) Mean value of three measurements.

determined in urine samples was varied from 2.29×10^{-4} M to 3.02×10^{-4} M, which was agreement with those obtained by other methods [31–33]. In order to conclude on the reliability of the proposed method, urine samples with low additional content of Cys were also analyzed and the recovery was about 98%–103%.

4 Conclusions

Using FAM-labeled DNA as template, AgNPs were template-synthesized and applied as super nanoquenchers to establish fluorescence platform for biosensing. Different from covalent link or non-covalent link, template-synthesized AgNPs have a tunable binding affinity to the oligonucleotides and turn out advantages as higher quenching efficiency, better stability as well as substantial signal enhancement in fluorescent detection. A thiol sensor was proposed using template-synthesized DNA-AgNPs conjugates and the detection was proved sensitive and thiol selective. Comparing with other nanoparticle-based thiol sensors, the conjugates have better performance in complex conditions, even in co-existence of high concentration of salts and biological molecules. To further evidence its applications in biological fluids, urinary thiols were detected without sample pretreatment using the proposed sensor.

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