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Effects of nanoparticles on fluorescence enhancement of the complexes of dsDNA and SYBR Green I

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Abstract Fluorescence enhancement in a DNA-dye system is favorable for sensitive and accurate DNA detection/sensing technologies. In this paper, we report that the fluorescence of the double-stranded DNA (dsDNA) and SYBR Green I (SG) system (dsDNA-SG) can be effectively enhanced by negatively charged magnetic iron oxide (Fe_2O_3@DMSA) and gold nanoparticles in suitable concentrations, but positively charged nanoparticles quench the fluorescence. Effects of the Fe_2O_3@DMSA on the fluorescence intensities are investigated with dsDNA-SG of different lengths or complexities. The results show that nanoparticles perform similarly in enhancing fluorescence intensity for several kinds of dsDNA. However, the dsDNA concentration determines the fluorescence amplitude. It shows that fluorescence intensity of lower concentration dsDNA is enhanced remarkably in DNA-SG. The finding may be useful in sensitive biomolecular detection.

Key words Surface charges, Fluorescence enhancement, Nanoparticles, Double-stranded DNA, SYBR Green I

1 Introduction

Sensitive and accurate DNA detection is crucial in the modern biology and medicine[1,2]. It has been the base of genetic analysis[3–8] and diagnosis[9,10], and antiviral research[11,12]. DNA assays have been developed using radioactive labels[13,14], electro-chemical tags[8,15], and nanostructure-based labels[8,16, 17]. Fluorescent method has become the dominant DNA detection/sensing technology in the biology and medicine[18] because it is sensitive, accurate, and easy to operate[19].

SYBR Green I (SG), an asymmetrical cyanine dye[2,20,21], is widely used as a nucleic acid stain because of its outstanding optical properties, such as long wavelength absorption, high molar extinction coefficient, and high quantum yield[22–24]. The SG preferentially binds to double-stranded DNA (dsDNA) instead of single-stranded DNA (ssDNA) and RNA[25–27], and emits strong fluorescence when it is excited by blue light[27]. The method exhibits minimal background fluorescence, leading to a high signal-to-background ratio[28].

Nanoparticles have received great attention in biology and medicine due to their unique electric[29], optical[30], and catalytic[31–33] properties. Particularly, magnetic iron oxide and gold nanoparticles can serve as large surface area platforms for functional groups[34] that interact with many biomolecules[9,28,35,36], hence their detection. In many nanoparticle-based bio-assay systems[16,37], fluorescence is normally the signal that reports the presence of target biomolecules. Then, it is important to investigate effect of the nanoparticles with different surface properties on fluorescence.

In this work, nanoparticles, dsDNA and SG were used for studying fluorescence enhancement effect. We found that the fluorescence of dsDNA-SG could be effectively enhanced by negatively charged magnetic iron oxide (Fe_2O_3@DMSA) and gold nanoparticles (AuNPs) at their suitable concentrations. Also, the fluorescence enhancement was adapted to kinds of length or complexity dsDNA. This suggests a new method for effective biomolecule detection.

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2 Materials and Methods

SYBR Green I (S9430) and AuNPs (5 nm, G1402) were purchased from Sigma Co. *Taq* DNA polymerase (EX-*Taq*) and its HS version were purchased from TaKaRa Bio. Inc. $HAuCl_4$ (AR) was purchased from Sinopharm Chemical Reagent Co. Ltd. DNA and sodium citrate trihydrate (99.0%) were purchased from Shanghai Sangon Biological Engineering and Technology and Service Co. Ltd (SSBETS). Stable aqueous sols of Fe_2O_3 modified with 3-dimercaptosuccinic acid (DMSA), L-glutamic acid (Glu), 3-amino-propyltriethoxysilane (APTS), and polylactide (pLL) (from Southeast University of China) were synthesized as previously reported[38].

A linear dsDNA of 886 bp was used, and amplified by circular plasmid pBR-322, which was used as the template by a normal PCR protocol. The upstream primer (P1: 5′-TGA CGA CCA TCA GGG ACA-3′) and downstream primer (P2: 5′-TAA AGC GGG CCA TGT TAA-3′) were synthesized by SSBETS Co. Ltd. The PCR products were purified by Takara DNA Fragment purification kit Ver.2.0, and diluted to appropriate concentration with pure water. The 13-nm gold nanoparticles were synthesized as previously reported [39,40].

The dsDNA of different concentrations was mixed with SYBR Green I (final concentration of 0.2×) in Mill-Q water to a final volume of 1 mL. DNA samples were kept in dark at room temperature for 5–30 min, and transfer into quartz cuvettes, disposable acrylic fluorescence cuvettes or microplate wells. The 497-nm excitation and 530-nm emission spectra for the SYBR Green I-bound dsDNA were measured by a F-4500 fluorescence spectro-photometer (Hitachi).

3 Results and Discussion

3.1 Fluorescence enhanced by Fe_2O_3@DMSA

Fluorescence emission of the dsDNA-SG was evaluated in absence and presence of the Fe_2O_3@ DMSA nanoparticles. Thereinto, F_0 and F represent the fluorescence intensity without and with the nanoparticles, respectively. Fluorescence amplitude is characterized as F/F_0. As shown in Fig.1, the fluorescence enhancement of the dsDNA-SG varies with the Fe_2O_3@DMSA nanoparticle concentration, enhanced at the concentrations of below 20 ng/μL, with a maximum F/F_0 ratio at 2.5 ng/μL, where it began to decrease gradually. With over 20 ng/μL of the nanoparticles, the fluorescence was suppressed.

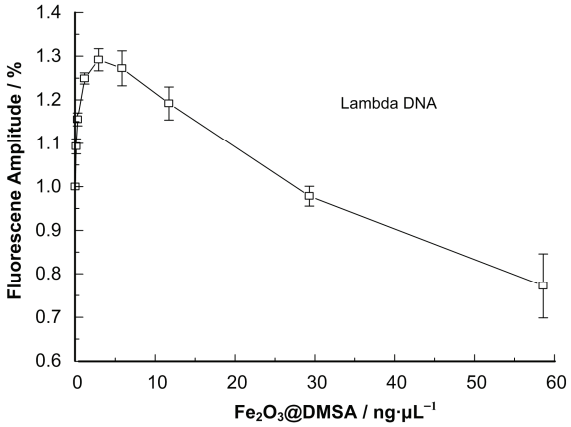

Fig.1 Fluorescence intensity of Lambda DNA and SYBR Green I system plotted against the concentration of the Fe_2O_3@DMSA particles. The concentration of dsDNA was 0.3 ng/μL. The *Y*-axis represents the ratio of the measured fluorescence intensities with (F) and without (F_0) addition of nanoparticles in the systems.

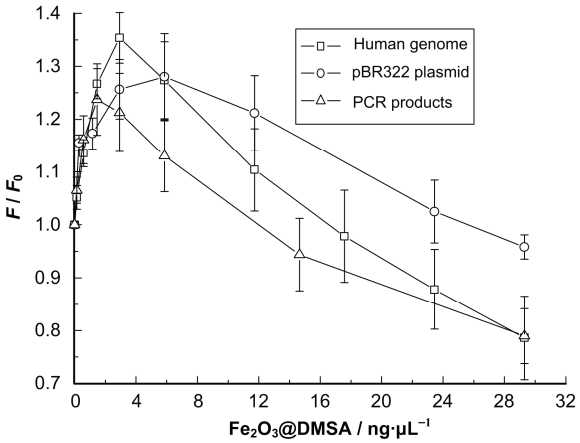

Fig.2 The fluorescence intensity of dsDNA-SYBR Green I systems plotted against the concentration of Fe_2O_3@ DMSA nanoparticles. The concentrations of DNA were 0.1 ng/μL (human genome) and 0.3 ng/μL (pBR322 plasmid, 886bp PCR product), respectively.

The effect of fluorescence enhancement was checked, too, on different dsDNA fragments. The pBR322 plasmid, a 4.3-kbp linear dsDNA, was digested by EcoR I (restriction endonuclease) from pBR322 vector. PCR products refer to 886-bp linear dsDNA was amplified using PCR from circular pBR322 plasmid. As the controls of longer Lamda dsDNA (48-kbp), 886-bp and 4.3-kbp dsDNA were used to study the effect of DNA length on the fluorescence enhancement. In addition, human

genome, as a representation of complex dsDNA mixtures, was used to perform similar experiments. As shown in Fig.2, the length and complexity of dsDNA have little effect on fluorescence enhancement in the test system with Fe_2O_3@DMSA nanoparticles, of which concentrations of 2.0, 5.0 and, 2.5 ng/μL are suitable to reach the maximum F/F_0 ratio for the PCR products, pBR322 plasimid and human genome, respectively. Further increase of the nanoparticle concentration, the fluorescence amplitude reduced gradually. According to Figs.1 and 2, for the same amount of dsDNA (0.3 ng/μL, 886-bp, 4.3 kbp and 48 kbp), the effective nanoparticle concentrations (F/F_0<1) are 13.0, 24, and 27 ng/μL.

Unlike the Fe_2O_3@DMSA nanoparticles in low concentrations, the dsDNA concentration affected F/F_0 remarkably. Fig. 3 shows the F/F_0 of the 0.2 ng/μL and 0.4 ng/μL dsDNA-SYBR Green I systems. The F/F_0 ratio of the 0.2 ng/μL system is much higher than that of the 0.4 ng/μL system. This was proved by the results obtained with a series of diluted dsDNA (data not shown).

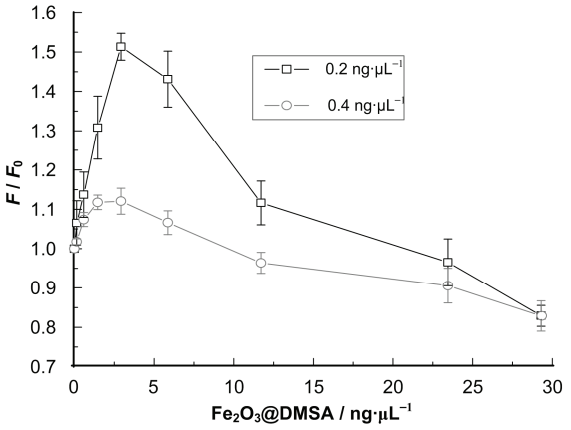

Fig.3 Effect of DNA concentration in fluorescence enhancement using Fe_2O_3@DMSA.

3.2 Effect of surface charge of Fe_2O_3 nanoparticles

The Fe_2O_3@DMSA nanoparticles are negatively charged, and it is interesting to see whether the surface charge affects the fluorescence enhancement. Three kinds of positively charged Fe_2O_3 nanoparticles, i.e. Fe_2O_3@APTS, Fe_2O_3@Glu and Fe_2O_3@pLL were synthesized using published methods[38]. They were introduced into dsDNA and SYBR Green I systems, but no fluorescence enhancement was observed in any system with the positively charged Fe_2O_3 nanoparticles. In contrast, the fluorescence was suppressed. The results indicated that fluorescence enhancement of dsDNA and SYBR Green I system by the Fe_2O_3 nanoparticles heavily depends on the surface charge of nanoparticles.

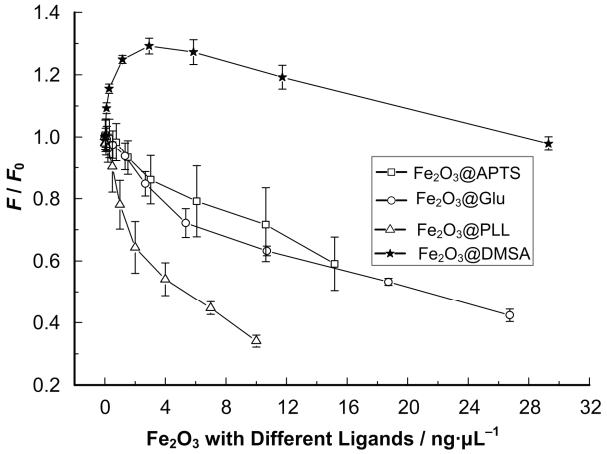

Fig.4 The fluorescence intensity of dsDNA-SYBR Green I systems plotted against the concentration of Fe_2O_3@ DMSA nano- particles with different ligands.

3.3 Effect of negatively charged Au nanoparticles

Negatively charged Fe_2O_3 nanoparticles can enhance the fluorescence intensity of dsDNA and SYBR Green I systems. Therefore, it is necessary to detect other negatively charged nanoparticle. Au nanoparticles, a suitable candidate in biomolecular detection, were introduced into DNA-dye system. Reportedly, Au would effectively quench the fluorescence[41–43].

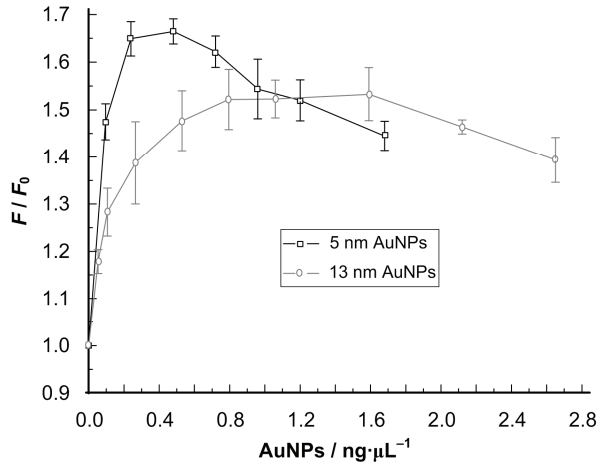

Fig. 5 Fluorescence enhancement using AuNPs with different sizes in dsDNA and SYBR Green I system.

However, we found similar phenomenon to the

negatively charged Fe_2O_3 nanoparticles. Fig.5 shows that Au nanoparticles of Φ5 nm and Φ13 nm enhanced the fluorescence intensity of dsDNA and SYBR Green I systems, and the system of Φ5 nm nanoparticles has higher fluorescence enhancement than the system of Φ13 nm Au nanoparticles, because of the larger specific surface area of smaller nanoparticles.

We think that the nanoparticle effect on the fluorescence intensity can be ascribed to the specific interactions among the nanoparticles, SG and dsDNA. Negatively charged surface of nanomaterials is inclined to absorb the free SG with positively charges, and this reduces self-quenching of the free dye[23,27], Then, it leads to fluorescence enhancement indirectly. On the other hand, due to electrostatic interactions, positively charged nanoparticles also bind to dsDNA, which compete with SG and reduce its binding with dsDNA, resulting in the reduction of fluorescence emission in this system. Lower concentration of nanoparticles may keep new balance of SG, and dsDNA at higher fluorescent intensity.

4 Conclusions

We investigated the effects of several nanoparticles on the fluorescence intensity of the dsDNA-SG, such as Fe_2O_3@DMSA, Fe_2O_3@APTS, Fe_2O_3@Glu, Fe_2O_3@ pLL and AuNPs. The negatively charged nanoparticles can induce a remarkable fluorescence enhancement at their suited concentrations but not the opposite charged one. In addition, we demonstrate that the properties of dsDNA, including length and complexity have little effect on fluorescence enhancement. However, the dsDNA concentration will determine the fluorescence amplitude. The result shows fluorescence intensity of lower concentration dsDNA is likely to be enhanced in our experiment system. We believe that our finding may be applied in DNA detection-based on fluorescent dye.

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