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Size-dependent PCR Inhibitory Effect Induced by Gold Nanoparticles

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***Abstract*—In this work, the effect of gold nanoparticles (AuNPs) with diameters of around 5, 10 and 20 nm on PCR efficiency is evaluated respectively using a real-time PCR machine. Gold nanoparticles show no obvious effect on PCR at low particle concentration. When the concentration is increased, PCR inhibition is observed. At the same particle concentration, gold nanoparticles of different sizes show different inhibitory effects on PCR. It is found that Taq polymerase can interact with AuNPs. The interaction is probably due to the binding of polymerase to AuNPs therefore lowering the concentration of free polymerase. It is also found that bovine serum albumin can interact with gold nanoparticles. It is believed that BSA blocks the surface of AuNPs from forming biding sites for polymerase. It can be used as an additive to reverse the inhibitory effect caused by gold nanoparticles.**

I. Introduction

Polymerase Chain Reaction (PCR) is a commonly used technology that enables researchers to produce millions of copies of a specific region of DNA in vitro. It was developed in 1984 and has been widely used in gene amplification, DNA cloning, functional analysis of DNA, diagnosis of diseases and so on [1-3]. PCR is a complicated chemical process that includes a number of necessary components. Due to their small sizes, nanoparticles possess outstanding properties that are not seen in their bulk forms. This results in many potential applications in biotechnology [4, 5]. The evaluation of the effect of nanoparticles on PCR is a relatively new research area and attracts more attention recently. In 2005, Li *et al.* [6] reported that gold nanoparticles (AuNPs) can be added in PCR reaction solutions to avoid nonspecific amplification products at lower annealing temperatures. According to their explanation, AuNPs' effects that are similar to single-stranded DNA binding protein should account for the improvement. As for how AuNPs affect PCR efficiency, some researchers found that Au nanoparticles can improve the efficiency of PCR [7, 9] but some researchers reported contradictory observations [9, 10]. In 2005, Li *et al.* [7] found that AuNPs can improve the efficiency of PCR at an appropriate concentration when using a fast-speed cycling PCR machine. The authors suspected that the excellent heat conductivity of the aqueous solution due to suspended AuNPs plays the most important role in increasing the efficiency of the PCR by dramatically shortening the time required [8]. In 2008, Yang *et al.* [9] evaluated AuNPs in PCR with SYBR Green I. They suggested that a complex interaction exists between AuNPs and native Taq DNA polymerase, which can either improve or reduce PCR efficiency at different concentrations of AuNPs. Vu *et al*. [10] demonstrated that AuNPs have such an effect that they tend to favor smaller products than larger ones. PCR inhibition was also reported when AuNPs were added. The researchers suspect that the effect is attributed to the ability of AuNPs to adsorb DNA polymerase.

Although AuNPs are widely used and tested in nanoparticle PCR experiments, no research work has been conducted to understand how sizes of AuNPs could affect PCR. This paper focuses on evaluating the effect of AuNPs of different sizes on PCR efficiency. The study demonstrates that AuNPs have inhibitory effect on PCR efficiency at particle concentrations above a certain value and AuNPs of larger sizes can cause complete PCR inhibition at a lower particle concentration than those of smallerer sizes. The effect should be attributed to the interaction of DNA polymerase and AuNPs. The inhibitory effect could be reduced by adding an appropriate amount of BSA or DNA polymerase to the PCR reaction solution.

II. Materials and Methods

A. Gold Nanoparticles (AuNPs)

Aqueous solutions of well dispersed AuNPs of three different sizes are prepared by Sigma. The sizes are 5, 10 and 20 nm in diameter. AuNPs are synthesized from an aqueous solution of ~0.01% $HAuCl_4$ contain <0.01% tannic acid, <0.04% sodium citrate as stabilizer and 0.02% sodium azide as preservative. High resolution TEM images are shown in Fig. 1.

B. DNA template and PCR Reagents

Salmonella enterica ATCC 13311 is used to prepare the DNA template. The target gene is *invA* gene for *Salmonella* species (GenBank accession number: M90846 [11]). The primers and probe used in the experiments are: forward primer, 5'-TCGTCATTCCATTACCTACC-3'; reverse primer, 5'-AAACGTTGAAAAACTGAGGA-3'; TaqMan probe, FAM-CTGGTTGATTTCCTGATCGCA-BHQ1 [12]. iTaq polymerase, 10X PCR buffer and magnesium chloride are obtained from Biorad. Primers, TaqMan probe, dNTP mix (dATP, dCTP, dGTP and dTTP) and bovine serum albumin

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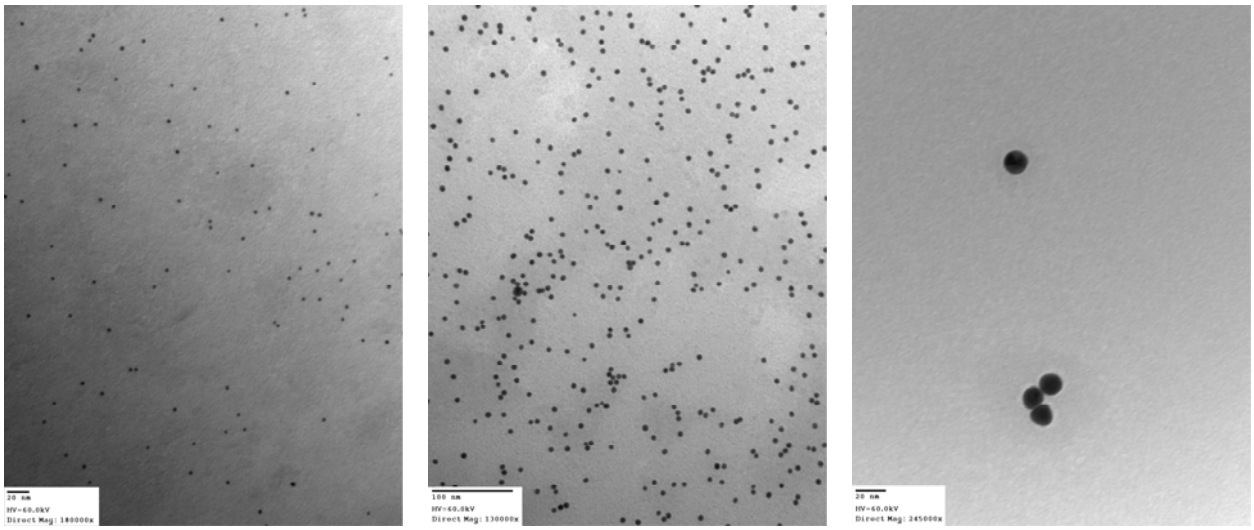


(a) (b) (c)

Fig. 1. TEM images of the gold nanoparticles used in the experiments

(BSA) are obtained from Sigma. PCR is performed in a 25 µL reaction volume containing 300 nM forward primer, 300 nM reverse primer, 100 nM Taqman probe, 200 µM dNTPs, 2.5 µL of 10X PCR buffer, 0.625 U of iTaq polymerase, 3.5 mM magnesium chloride, 10 µg of BSA , 5 µL of DNA template and an appropriate amount of AuNPs. DNA template concentration is adjusted from 30 ng/mL to 30 pg/mL as a standard series with each step differing by 10 fold.

C. Real-time PCR System and PCR Program

iCycler iQ real-time PCR machine from Bio-Rad is used in the experiments. Fluorescent signal is detected at the end of each cycle. Excitation light is set at 490 nm and emission light is detected at 530 nm. The PCR program for Salmonella DNA is set as follows: 3 minutes at 95°C for pre-denaturation, 50 cycles of amplification at 95°C for 15 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and 10 minutes at 72°C for final extension.

III. Results and Discussion

In the first experiment, different particle concentrations of AuNPs of each particle size are tested. In each PCR run, there are a set of reference tubes containing no AuNPs and a set of experimental tubes each containing a certain amount of AuNPs of a specific size. It is observed that at low particle concentrations, for example 5 nm AuNPs at 3.5 nM, 10 nm AuNPs at 0.47 nM and 20 nm AuNPs at 0.04 nM, there is no obvious change to the amplification curves. As the particle concentration is increased, PCR inhibitory effect is observed for AuNPs of all sizes. The effect of AuNPs of all three sizes at the same particle concentration is then evaluated. 5, 10 and 20 nm AuNPs at the same particle concentration of 1.9 nM in the final reaction volume are tested. Particle concentration of 1.9 nM for the 5 and 10 nm AuNPs equals 0.7 and 5 µL of the original solutions respectively. However, the original particle concentration of the 20 nm AuNPs is too small to be used in the experiments. Therefore, more concentrated solution is prepared by first centrifuging the stock solution and then removing a certain amount of the supernatant according to the desired particle concentration. 8X concentrated solution is prepared and 9.8 µL is needed to achieve 1.9 nM in the final reaction volume. It is observed that AuNPs of different sizes show different inhibitory effect on PCR. At 1.9 nM, 5 nm AuNPs almost have no effect on PCR while 10 nm AuNPs cause partial PCR inhibition as shown in Fig. 2. At this particle concentration, 20 nm AuNPs lead to complete PCR inhibition with no amplified DNA fragments. Similar

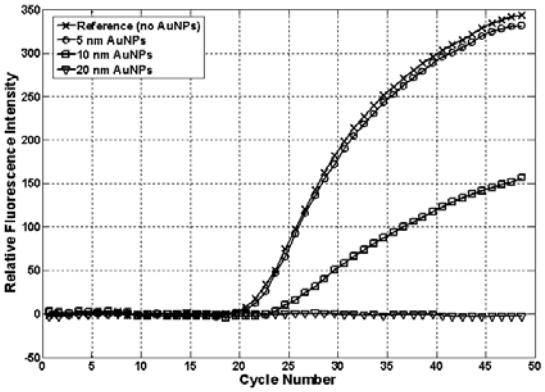
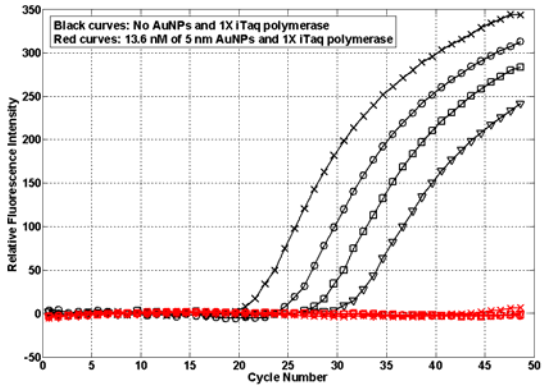


Fig. 2. Amplification curves of the PCR with 5, 10 and 20 nm AuNPs at the same particle concentration of 1.9 nM. AuNPs of smaller size have less inhibitory effect on PCR than those of larger size. Starting DNA concentration is 30 ng/mL. ×− Reference curve with no AuNPs, ○− Amplification curve with 5 nm AuNPs at 1.9 nM, □− Amplification curve with 10 nm AuNPs at 1.9 nM, ▽− Amplification curve with 20 nm AuNPs at 1.9 nM.

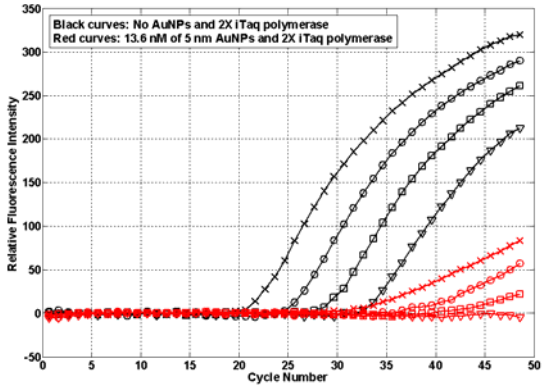
behavior is observed for all DNA template concentrations from 3 ng/mL to 30 pg/mL. Fig. 2 suggests that a higher particle concentration of smaller AuNPs is required to cause complete PCR inhibition than AuNPs of larger sizes.

It is suspected that PCR inhibition is caused by the interaction between some chemical(s) in the PCR solution and the added AuNPs. Experiments are designed and performed to evaluate which chemical(s) interact with AuNPs. The experiments are performed as follows: either increasing or reducing the concentration of a chemical component one at a time to see whether the amplification curves show an obvious change. It is observed that changing the concentration of iTaq polymerase and BSA affects the amplification curves.

Complete PCR inhibition is observed when 5 nm AuNPs at 13.6 nM are added to the PCR reaction solution containing 0.625 U of iTaq polymerase (1X) per reaction as shown in Fig. 3(a). When the amount of iTaq polymerase is increased to 1.25 U (2X) per reaction, DNA amplification is observed although the product yield is suppressed and less DNA fragments are amplified as shown in Fig. 3(b). Similar behavior is observed for 10 and 20 nm AuNPs. Fig. 3 clearly



(a)

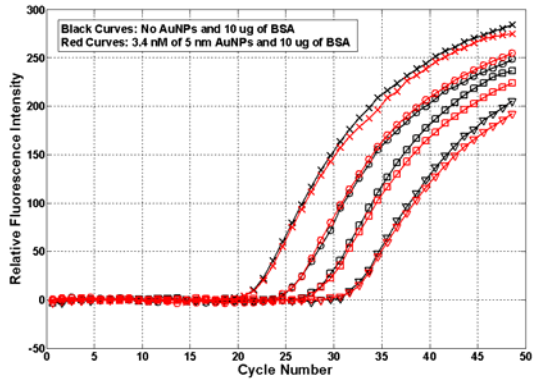


(b)

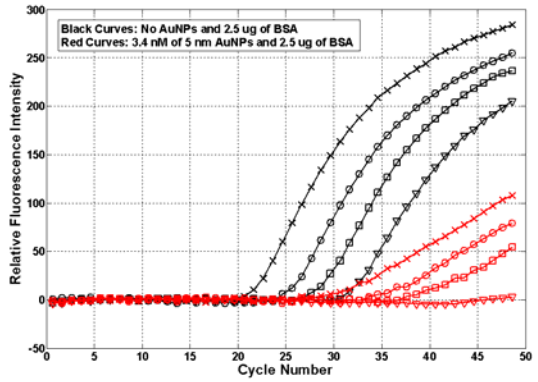
Fig. 3. Amplification curves of the PCR with AuNPs and various amounts of iTaq polymerase. DNA template concentration is ×− 30 ng/mL, ○− 3 ng/mL, □− 300 pg/mL, ▽− 30 pg/mL. Black curves are reference curves and red curves are experimental curves. (a) 0.625 Uof iTaq polymerase (1X) per reaction, (b) 1.25 U of iTaq polymerase (2X) per reaction.

suggests that there is an interaction between AuNPs and iTaq polymerase. Since DNA polymerase is an indispensible component to make PCR work, the interaction between AuNPs and iTaq polymerase should account for the PCR inhibition caused by AuNPs. We performed two experiments to explore the probable mechanism of the interaction. In the first experiment, a mixture containing 20 nm AuNPs, iTaq polymerase and water and is prepared and well mixed. The concentration of iTaq polymerase is chosen to be 0.625 U per reaction while the amount of AuNPs is chosen such that it causes partial PCR inhibition when added to the PCR reaction solution. The mixture is centrifuged at 12,000 rpm for 6 minutes and the supernatant is carefully transferred to a new tube. Other PCR chemicals are added to the new tube at their optimal concentrations. The resulting PCR mix is aliquoted into PCR tubes along with DNA template for a PCR run. Partial PCR inhibition is observed after 50 PCR cycles. The observation suggests that AuNPs can interact with iTaq polymerase to inhibit PCR. The second experiment follows the procedure in the first experiment. The only difference is the amount of AuNPs which is chosen such that it causes complete PCR inhibition when added to the PCR reaction solution. No DNA amplification is observed after 50 cycles. Based on the observations from both experiments, we propose a probable mechanism which is AuNPs and iTaq polymerase bind to each other when they are mixed together. This could explain the observations for both experiments. In the first experiment, when limited amount of AuNPs is present, there is still free iTaq polymerase in the supernatant although the concentration is low and that is why partial PCR inhibition is observed. In the second experiment, when excessive amount of AuNPs is used, all iTaq polymerase is bound to AuNPs leaving no free polymerase in the supernatant to participate in PCR and that is why complete PCR inhibition is observed.

It is also observed that the amplification curves could be changed dramatically by changing the amount of BSA in the reaction solution. When 5 nm AuNPs at 3.4 nM are added to the PCR solution containing 10 µg of BSA, no PCR inhibition is observed for all standard DNA series as shown in Fig. 4(a). However, when the amount of BSA is reduced to 2.5 µg, PCR inhibition is observed as shown in Fig. 4(b). This suggests that BSA can also interact with AuNPs. PCR inhibition caused by AuNPs can be reversed by adding more BSA to the reaction solution. An experiment is performed to confirm the interaction. BSA, 20 nm AuNPs and water are well mixed and incubated at room temperature for 5 minutes. The amounts of BSA and 20 nm AuNPs are chosen such that when they are added to the PCR reaction solution. There is no obvious PCR inhibition. A centrifugation step is followed at 12,000 rpm for 6 minutes and the supernatant is removed. Water is then added to the original tube to replace the supernatant removed. The new mixture is well dispersed followed by the centrifugation, supernatant removal and water replenish steps again. After repeating the same process for 5 times, the amount of BSA is negligible in the solution. The final



(a)



(b)

Fig. 4. Amplification curves of the PCR with 5 nm AuNPs and various amounts of BSA. DNA template concentration is ×—30 ng/mL, ○—3 ng/mL, □—300 pg/mL, ▽—30 pg/mL. Black curves are reference curves and red curves are experimental curves. (a) 10 µg of BSA per reaction and (b) 2.5 µg of BSA per reaction.

concentration of AuNPs in the solution is controlled by removing a desired volume of the supernatant. AuNPs after BSA treatment are then transferred to the master mix for a 50-cycle PCR run. The concentration of the AuNPs in the PCR reaction solution is chosen such that it causes complete PCR inhibition when the same concentration of the AuNPs before BSA treatment is used. It is observed that there is only partial PCR inhibition. The observation suggests that AuNPs after BSA treatment have less inhibitory effect on PCR than they are treated before. As we know, BSA is a commonly used material to block PCR inhibitors. The way BSA works in the experiments is probably the same mechanism as it blocks the surface of AuNPs from forming biding sites for polymerase.

IV. Conclusions

We have demonstrated that AuNPs can interact with iTaq polymerase to inhibit PCR. AuNPs have no obvious effect on PCR efficiency at low particle concentration while they inhibit PCR at concentrations above a threshold value. The size of AuNPs has an impact on the PCR inhibitory effect. At the same particle concentration, AuNPs of smaller sizes have less inhibitory effect on PCR than those of larger sizes. More AuNPs of smaller sizes are needed to result in complete PCR inhibition than those of larger sizes. The inhibitory effect caused by AuNPs is probably due to the binding of iTaq polymerase and AuNPs. BSA can also interact with AuNPs by blocking the surface of AuNPs from forming binding sites for polymerase. The PCR inhibitory effect caused by AuNPs can be alleviated by adding more iTaq polymerase or BSA to the PCR reaction solution.

Acknowledgments

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