Nanobiotechnology

DOI: 10.1002/ange.200500403

Nanoparticle PCR: Nanogold-Assisted PCR with Enhanced Specificity**

Haikuo Li, Jiehuan Huang, Junhong Lv, Hongjie An, Xiaodong Zhang, Zhizhou Zhang, Chunhai Fan,* and Jun Hu**

The polymerase chain reaction (PCR) revolutionized molecular genetics and has become one of the most popular techniques in modern biological and medical sciences.[1] Owing to the exponential amplification ability of PCR, one could start from even a single copy of target DNA to produce large amounts of DNA copies for sequencing, molecular diagnosis, or genetic analysis.[2–4] This remarkable amplification ability is critical in many circumstances, such as early-stage diagnosis of HIV or cancers. Given the rapidly increasing interest in optoelectronic DNA biosensors, the extremely high detection sensitivity of PCR has not been surpassed until now.[5–9] However, the specificity of PCR does not match its unparalleled sensitivity.[10] It is well known that even with sophisticated optimization, PCR specificity is not always satisfactory (e.g. in multiple-round PCR or multiplex PCR).[10] Herein, we report a novel PCR method that employs inexpensive gold nanoparticles to effectively avoid nonspecific PCR reactions.

Gold nanoparticles have found broad and important applications in biology; for example, a variety of ultrasensitive biosensor strategies have been reported which take

[*] H. Li, J. Huang, H. An, Dr. X. Zhang, Prof. Dr. Z. Zhang, Prof. Dr. C. Fan, Prof. Dr. J. Hu
Bio-X DNA Computer Consortium
Shanghai Jiao Tong University
Shanghai 200030 (China)
Fax: (+86) 21-6407-7361
E-mail: zhangzz@sjtu.edu.cn
fchh@sinap.ac.cn
jhu@sjtu.edu.cn

H. Li, J. Huang, H. An, Dr. X. Zhang, Prof. Dr. Z. Zhang, Prof. Dr. J. Hu
Bio-X Life Science Research Center
Shanghai Jiao Tong University
Shanghai 200030 (China)
Fax: (+86) 21-6407-7361

Dr. J. Lv, Prof. Dr. C. Fan, Prof. Dr. J. Hu
Shanghai Institute of Applied Physics
Chinese Academy of Sciences
Shanghai 201800 (China)
Fax: (+86) 21-5955-6902

[**] This work was supported by the Chinese Academy of Sciences, the National Natural Science Foundation (10335070 and 20404016), the Ministry of Science and Technology of China (2002CCA00600, 2003A310A02, and 2003AA226011), the Shanghai Municipal Commission for Science and Technology (0452M068, 03DZ14025, and 02JC14029), and the Ministry of Personnel. We also thank Prof. Kevin Plaxco (UCSB) for helpful comments.

advantage of gold nanoparticles.[8,9,11–14] Gold nanoparticles are nontoxic, biocompatible nanomaterials that can be either obtained from commercial sources or conveniently produced in laboratories,[15] and owing to the availability of versatile chemistry for functionalization at their surface as well as their unique optoelectronic properties[9,11] gold nanoparticles provide a particularly useful platform for attachment of biomolecules.[15] However, to the best of our knowledge, the use of gold nanoparticles to improve the performance of PCR has not been reported.

Herein, we employed an error-prone two-round PCR as our model system. Amplification of very low quantities of copies of target genes usually increases the possibility of amplifying nonspecific sequences.[16] In the first round, we amplified a 283-bp sequence using a λ-DNA template with 35 PCR cycles; in the second round PCR, this 283-bp DNA was employed as the template for the other 35-cycle PCR amplification sequence. Nonspecific PCR products tend to accumulate after two rounds of PCR, as manifest by a broad molecular size distribution of amplified products in agarose gel electrophoresis (Figure 1). Strikingly, in the presence of

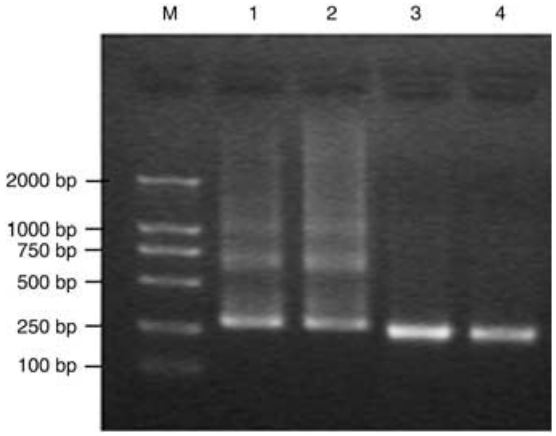

Figure 1. The effect of gold nanoparticles on the specificity of PCR. PCR was performed by employing a 283-bp target sequence from a λ-DNA template, and PCR products were analyzed by agarose gel electrophoresis (1.5%). Lane M is for markers; lanes 1 and 2 show the results of PCR performed in the absence of gold nanoparticles; lanes 3 and 4 show the results of PCR performed with gold nanoparticles (0.4 nM of 10-nm gold nanoparticles). bp = base pair.

0.4-nM gold nanoparticles (10 nm, Sigma) we only observed a single predominant band corresponding to the 283-bp target, and the yield of this target was also much higher in this case (Figure 1). Control experiments show that the high specificity of gold-nanoparticle-assisted PCR (nanoparticle PCR) arises solely because of the effect of gold nanoparticles. We performed nanoparticle PCR by using freshly prepared gold nanoparticles that were free of additives such as tannic acid and sodium azide[17] and observed similar electrophoresis patterns (data not shown). In contrast, we did not observe any improvement by using solely the medium in which the gold nanoparticles were provided (the gold nanoparticles were removed by centrifugation).[18] The concentration of gold nanoparticles is critical for obtaining optimal PCR results. We observed that nonspecific "tailing" bands gradually diminished by increasing the concentration of gold nanoparticles in the range of 0.2–0.8 nM. However, the presence of excess gold nanoparticles (1.0 nM) effectively inhibited the PCR reaction, as manifest by the significantly lowered yield of PCR (Figure 2). This inhibition effect is discussed later.

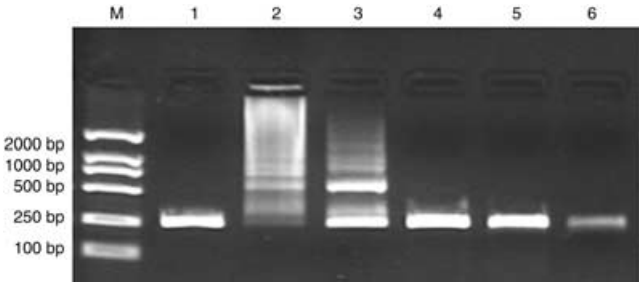

Figure 2. The effect of the concentration of gold nanoparticles on the specificity of PCR: lane M: (markers) 0; lane 1: 0.6 nM; lane 2: 0; lane 3: 0.2 nM; lane 4: 0.4 nM; lane 5: 0.8 nM; lane 6: 1.0 nM.

In conventional PCR, it is essential to find the optimal annealing temperature by the so-called "touchdown" PCR[19] to obtain optimal yields (which requires a lower annealing temperature) and specificity (which requires a higher annealing temperature) of the target. Our nanoparticle PCR strategy allows reliable PCR amplification even at lowered annealing temperatures, obviating time-consuming "touchdown" PCR. As demonstrated in Figure 3, in the presence of 0.4-nM gold nanoparticles we could obtain a single product band at a nonoptimal annealing temperature of 40 °C. We even observed a product band with minor tailing bands at an annealing temperature as low as 25 °C, in sharp contrast to normal PCR in the absence of gold nanoparticles which produces negligible amplified target DNA at this temperature. This specificity could be further improved by using 0.6-nM gold nanoparticles, which produced a single product band at the annealing temperature of 25 °C (Figure 3). Therefore, it is possible to 1) employ gold nanoparticles to perform PCR without the need for sophisticated optimization of annealing temperature and/or 2) to increase PCR yields at lowered annealing temperatures without compromising PCR specificity.

The problems associated with specificity of PCR possibly arise owing to performance of the procedure in vitro. During DNA replication in vivo, DNA polymerase is assisted by a variety of proteins/enzymes, which ensure the specificity of DNA replication.[20] For example, single-stranded DNA-binding protein SSB selectively binds to single-stranded DNA (ssDNA) and not to double-stranded DNA (dsDNA). This selectivity largely minimizes mispairing between primers and templates during DNA replications.[20] Indeed, mimicking Nature's strategy, Nielson et al., among others, successfully employed a thermostable SSB to increase PCR specificity and fidelity.[21–23] Note that the use of thermostable SSB from the *T. thermophilus* strain is critical to withstand the temperature cycles (up to 94 °C) involved in PCR.[23] Here, similarly enhanced PCR specificity is observed for our nanoparticle

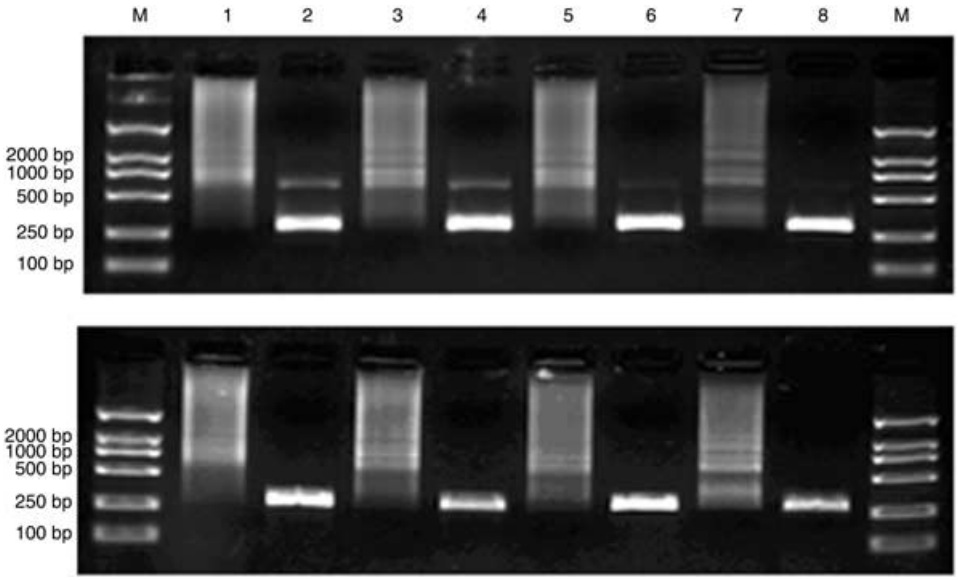

Figure 3. The effect of the annealing temperature on the specificity of PCR: lanes 1, 2: 25 °C; lanes 3, 4: 30 °C; lanes 5, 6: 35 °C; lanes 7, 8: 40 °C; lane M is for markers; lanes 1, 3, 5, and 7 show the results of PCR performed in the absence of gold nanoparticles; lanes 2, 4, 6, and 8 show the results of nanoparticle PCR (the concentration of gold nanoparticles is 0.4 and 0.6 nM in the upper and lower panels, respectively).

PCR system. We reason that this enhancement arises also as a result of a similar mechanism to SSB-assisted PCRs. Indeed, both gold nanoparticles and SSB significantly enhance the specificity of this error-prone two-round PCR (see the Supporting Information). However, in this case, the enhancing effect of gold nanoparticles exceeds that of the commercially available SSB.

Analogous to SSB, gold nanoparticles bind to ssDNA much more strongly than to dsDNA.[8] While not yet fully understood, it is generally recognized that the selectivity of gold nanoparticles for binding to ssDNA arises for various reasons. First, for electrostatic reasons the binding of anionic DNA strands to negatively charged surfaces of citrate-stabilized gold nanoparticles is not favored. Note that dsDNA has a higher surface charge density and thus experiences more repulsion than ssDNA.[6] Second, the four bases—adenine, guanine, thymine, and cytosine (A, G, T, and C)—contain nitrogen atoms that display high affinities to gold.[24,25] Formation of a DNA duplex prevents the exposure of the bases to gold surfaces and thus reduces interactions between the two.[7] Third, the rigidity of dsDNA does not favor the wrapping of dsDNA around gold surfaces, while ssDNA is a "soft", flexible polymer that has a much higher freedom to wrap around gold nanoparticles. (Note that the persistence lengths of ssDNA and dsDNA are approximately 1 and 50 nm, respectively.[26]) However, an excess of gold nanoparticles can significantly reduce PCR yields which suggests that even correctly matched DNA may be adsorbed during PCR amplification (Figure 2). This effect is not unexpected as dsDNA may still bind to gold nanoparticles, although with a much lower affinity relative to ssDNA–gold binding.[27]

An approximate concentration of 10 μM of 1,6-dithiothreitol (DTT) is present in PCR solutions and is required for storage of Taq polymerase, the enzyme that is used to copy DNA. This thiol could self-assemble on gold nanoparticles through Au–S bonds and possibly cross-link them. As DTT-free Taq is not active, we cannot exclude the effect of DTT at present; however, we suggest that its effect is relatively small for the following reasons. First, the concentration of DTT is fairly low. Usually millimolar or higher concentrations of thiols are required for the preparation of densely packed monolayers on gold surfaces.[28] Second, Au–S bonds are labile at elevated temperatures.[29,30] PCR reactions were carried out at a high temperature at which Au–DTT complexes are unstable. Third, the PCR reaction was largely inhibited in the presence of 1.0 nM of gold nanoparticles (Figure 2). If the presence of 10 μM of DTT is enough to cross-link all gold nanoparticles, this inhibition effect could not have occurred. It was mentioned earlier that PCR specificity was enhanced by increasing the concentration of gold nanoparticles in the range of 0.2–0.8 nM. This observation suggests that DTT may cross-link some gold nanoparticles, while the excess gold nanoparticles ensure the enhancing effect. However, the mechanism of nanogold-assisted PCR remains to be explored.

In conclusion, we have developed a highly selective nanoparticle PCR strategy by employing gold nanoparticles. We have demonstrated that in the presence of appropriate concentrations of gold nanoparticles, PCR amplification can be optimized with respect to both yields and specificity. Thus the use of these highly stable, commercially available, and inexpensive inorganic nanomaterials open new opportunities for improving PCR, which is the most important standard method in molecular biology. Besides the two-round PCR employed here, gold nanoparticles may also be applied to many other PCR reactions that require either high specificity or high yields, such as single-molecular, multiplex, and long-distance PCRs. Further investigation into the effect of gold nanoparticles on these PCR reactions is required.

Experimental Section

λ-DNA templates and Ex Taq DNA polymerase were obtained from TaKaRa Bio. Inc. Colloidal gold nanoparticles (10 nm, 0.01% $HAuCl_4$) were either purchased from Sigma or freshly produced in our laboratory following the reported procedure.[31] (Gold nanoparticles from Sigma contain $<0.01\%$ tannic acid, 0.04% trisodium citrate, 0.26 mM potassium carbonate, and 0.02% sodium azide.) For PCR reactions, final concentrations of primers = 0.1 μM, template = 20 $\mu g L^{-1}$, and deoxynucleotide triphosphates (dNTPs) = 0.5 mM; reaction conditions: 2 min at 94°C for denaturation, followed by 35 cycles of a–c: a) 45 s at 94°C (denaturation), b) 1 min at 50°C (annealing), and c) 1 min at 72°C (elongation). Then after an additional extension step of 72°C for 5 min, PCR tubes were mainatined at 4°C. PCR products were analyzed by agarose gel electrophoresis (1.5%). Aliquots of the solution of gold nanoparticles were added to the PCR reaction system to an appropriate concentration.

Received: February 3, 2005
Revised: April 4, 2005
Published online: June 8, 2005

Keywords: DNA replication · gold · nanotechnology · polymerase chain reaction

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