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Polymerase chain reaction of nanoparticle-bound primers

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Abstract

Using one or two primers respectively bound to the surface of Au nanoparticles (AuNPs) or magnetic nanoparticles (MNPs), polymerase chain reaction (PCR) based on nanoparticles was systemically studied, agarose gel electrophoresis and atomic force microscopy (AFM) were respectively used to detect and observe the PCR product. The results obtained indicated that with either one or two primers respectively bound to the nanoparticle surface, PCR can proceed successfully under optimized condition and is subject to certain rules, consequently a symmetric PCR technique and an asymmetric PCR technique based on nanoparticles have been developed. A kind of nanostructured aggregates can be constructed by a symmetric PCR using two nanoparticle-bound primers.

Keywords: AuNPs; MNPs; AFM; Symmetric PCR; Asymmetric PCR; Nanostructured aggregates

1. Introduction

PCR is an amplification technique for target nucleic acid sequence in vitro, and is one of the most important experimental methods in molecular biology [1]. Recently, some groups have studied several kinds of solid PCR: Lockley group [2], Adessi and co-workers [3], Koch group [4], and Turner et al. [5] have studied PCR based on nylon, glass, microtiter well, and microwell, respectively. What about PCR based on nanoparticles? If PCR based on nanoparticles can proceed successfully, it will have broad application in the detection and isolation of PCR product as well as material science. With nanotechnology rapidly advancing in life science, people have been studying and solving vital issues in life science using nanotechnology [6–8], studying PCR based on nanoparticles and integrating PCR technique with nanotechnology will evoke great interests. In this paper, we systemically studied PCR based on nanoparticles, agarose gel electrophoresis and AFM were respectively used to detect and observe the PCR product. The results obtained indicate that with one or two primers respectively bound to the surface of AuNPs or MNPs, PCR can proceed successfully under optimized condition and is subject to certain rules, a symmetric PCR technique and an asymmetric PCR technique based on nanoparticles have been developed. More interestingly, a kind of nanostructured aggregates can be constructed by a symmetric PCR using two nanoparticle-bound primers.

2. Materials and methods

2.1. Materials

Approximately 13 nm diameter AuNPs were prepared by the citrate reduction of $HAuCl_4$ [9]. About 50 nm diameter MNPs were prepared by adopting reverse microemulsion method [10]. Mercaptoethanol (MCE) and Agarose II were respectively purchased from Sigma and Bio Basic Inc. (EMO, Canada). Primers, Taq DNA polymerase, and dNTPs used in this work were purchased from Shanghai Sangon Biologic Engineering Technology and Service Co. Ltd (Shanghai, P.R. China). Primers with sequence used in experiments as follow: Forward primer 1 (F1): 5′-GGGA-TAACGCAGGAAAGA-3′, reverse primer 1 (R1): 5′-AGGGTCGGAACAGGAGA-3′. Forward primer 2 (F2):

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5′-TATTGGGCGCTCTTCCGCTTCCTC-3′, reverse primer 2 (R2): 5′-TCTTTATAGTCCTGTCGGGTTTCG-3′. Forward primer 3 (F3): 5′-CGCTCTTCCGCTTCCTC-5′, reverse primer 3 (R3): 5′-AGCACCGCCTACATACCTC-5′. Plasmid pbluescript SK (pSK) was extracted from *Escherichia coli* and served as template. Other reagents available were analytical grade.

2.2. Methods

AuNP-bound primers were prepared using 5′-end-SH-$(CH_2)_6$-modified primers and AuNPs [9]. MNP-bound primers were prepared using 5′-end-HO-$(CH_2)_6$-S-S-$(CH_2)_6$-O-(PO_3)-modified primers and thiol-functionalized MNPs [10]. The PCR reaction was performed in 30 μl mixture contained 1× Reaction Buffer (50 M KCl, 10 mM Tris–HCl pH 9.0 at 25 °C, 0.1% TritonX-100, 1.5 mM $MgCl_2$), 2 mM each of four dNTPs, 1 μl of pSK (amount to 5 ng), 0.5 U of Taq DNA polymerase, about 20 pmol each of primer. The PCR reaction was performed in MJR PTC-100 PCR system (Whatman Biometra). The amplifying procedure for R1/F1: (1) 2 min at 94 °C; (2) 20 or 45 cycles of 20 s at 94 °C, 20 s at 46 °C, 50 °C, 54 °C 56 °C, 58 °C or 60 °C, 20 s at 72 °C; (3) 3 min at 72 °C. Procedure for R2/F2: (1) 3 min at 94 °C; (2) 20 or 45 cycles of 50 s at 94 °C, 50 s at 52 °C, 56 °C, 60 °C, or 65 °C, 30 s at 72 °C; (3) 4 min at 72 °C. Procedure for R3/F3: (1) 3 min at 94 °C; (2) 35 cycles of 50 s at 94°C, 50 s at 65 °C, 30 s at 72 °C; (3) 4 min at 72 °C. After the reaction, MCE was added to each sample to remove the PCR product from the nanoparticles surface, and the final volume ratio was brought to 3%. They were allowed to react for 8 h at 37 °C, and then samples were centrifuged (or deposited in normal external magnetic field), and 10 μl of the solution was removed from each sample to save for analysis using agarose gel. When the PCR product was observed using AFM, it was directly loaded on the mica after PCR.

3. Results and discussion

5′-Thoil modified primers were covalently attached to the AuNP surface via Au–S bonds [9]. Firstly, F1 was attached to the AuNP surface (AuNP-F1) and R1 was freely diffusing. Its amplified fragment was 217 bp and symmetric PCR was performed using 20 cycles in different annealing temperatures. The electrophoretic results were shown in Fig. 1a. Almost the same results were attained when this experiment was done using AuNP-R1 and F1. This fact implies that when only one primer is attached to the AuNP surface, symmetric PCR can go on successfully and is hardly affected by the variation of annealing-temperature. To confirm the result, primers F2/R2, whose amplified fragment is 277 bp, was used. The same rule described above was attained when symmetric PCR was performed using either AuNP-R2 and F2 or AuNP-F2 and R2.

Fig. 1b shows agarose gel analysis of symmetric PCR product elongated by AuNP-R1 and AuNP-F1 under the same condition as Fig. 1a. The result indicates that symmetric PCR with two AuNP-bound primers can also proceed successfully and the variation of annealing temperature hardly affects it. When symmetric PCR was performed using AuNP-R2 and AuNP-F2, the same rule was achieved.

AFM was adopted to observe the PCR product. The tapping-mode was selected and the measurement was performed on a Nanoscope IIIa Multimode scanning probe microscope from Digital Instruments (Veeco Metrology Group, Santa Barbara, CA). The tips were manufactured by nanosensors (Wetzlar-Blankenfeld, Germany). Fig. 2 shows the AFM image of symmetric PCR product elongated by AuNP-R2 and AuNP-F2 when a symmetric PCR was performed using 45 cycles and the annealing temperature was 65 °C. From the image, it is clear that AuNPs can possibly be interconnected by symmetric PCR product and form nanostructured aggregates. The formation process of nanostructured aggregates is a kind of AuNP self-assembly during symmetric PCR procedure via symmetric PCR product. Symmetric PCR product, that is, dsDNA, has the functions of reversible denaturalization and renaturation, therefore this kind of nanostructured aggregates might disperse and congregate reversibly under certain condition.

Symmetric PCR based on MNPs was studied by selecting R1/F1 R2/F2 as primers. 5′-disulfide modified

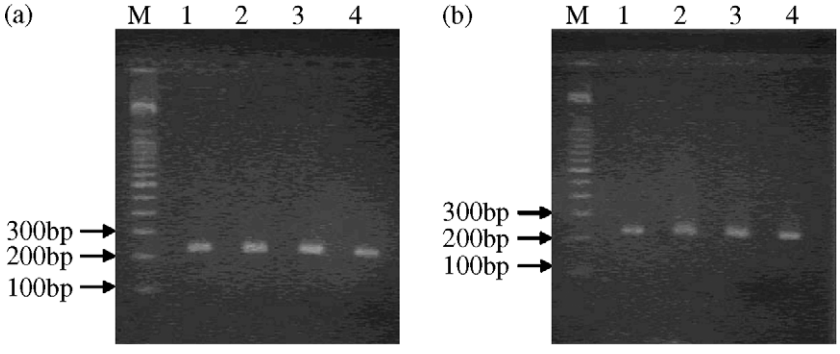

Fig. 1. 3% agarose gel analysis of symmetric PCR product: (a) elongated using AuNP-F1 and R1, (b) elongated using AuNP-R1 and AuNP-F1. M: 100 bp molecular size marker. Lanes 1–4: the annealing temperatures were 46 °C, 50 °C, 54 °C, 58 °C, respectively.

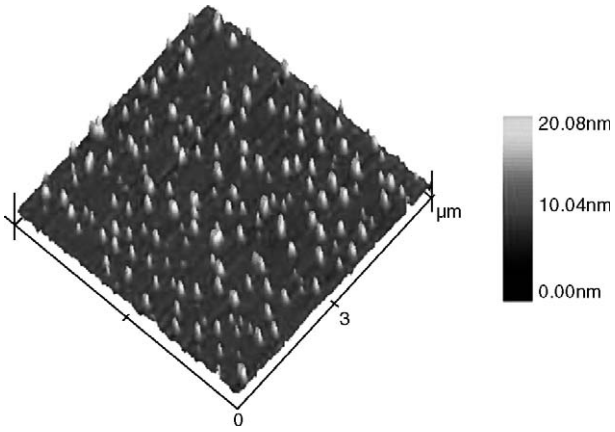

Fig. 2. Large-scale (6 μm) AFM image of symmetric PCR product elongated by AuNP-R2 and AuNP-F2.

primers were covalently immobilized onto the MNP surface via S–S bonds [10]. Fig. 3a shows the agarose gel analysis of symmetric PCR product elongated by MNP-F1 and R1. Fig. 3b shows agarose gel analysis of symmetric PCR product elongated by MNP-R1 and MNP-F1. In Fig. 3a, the DNA bands are uniform and the specificity is high in different annealing-temperature, but in Fig. 3b with the increasing of annealing-temperature, the bands become narrower and finally disappeared. The experiment result indicates that with only one MNP-bound primer, symmetric PCR can proceed successfully, and the annealing temperature variation hardly affects it; with two MNP-bound primers, symmetric PCR just can proceed at appropriate annealing temperature, and the annealing temperature variation greatly affects it. The same rule was attained when symmetric PCR was performed using one or two primers of R2/F2 immobilized on MNPs.

Fig. 4 shows the AFM image of PCR product elongated by MNP-R1 and MNP-F1 when a symmetric PCR was performed using 20 cycles and the annealing temperature was 56 °C. According to the image, MNPs can also be interconnected by symmetric PCR product and form nanostructured aggregates. This fact further confirmed that

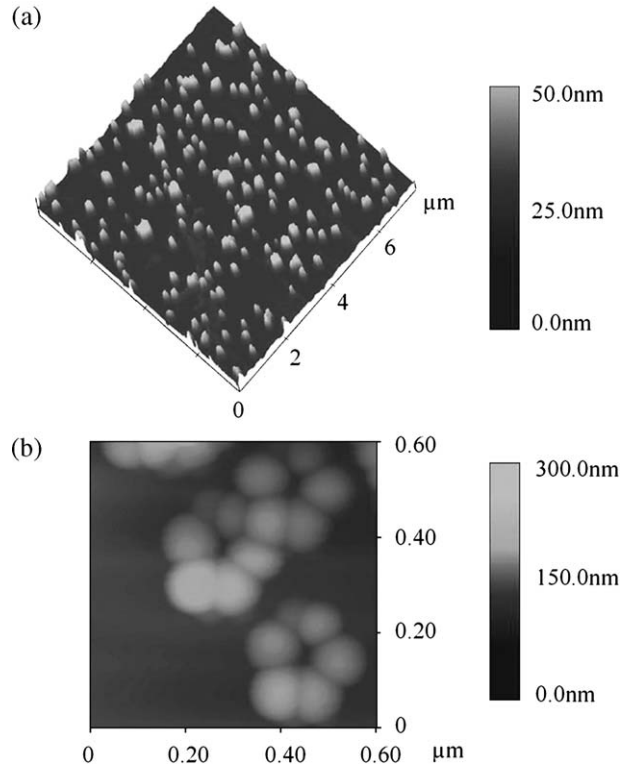

Fig. 4. AFM image of symmetric PCR product elongated by MNP-R1 and MNP-F1: (a) large-scale (8 μm) AFM image, (b) small-scale (0.60 μm) AFM image.

nanostructured aggregates could be prepared by a symmetric PCR with two nanoparticle-bound primers. It can be seen in the image that the size of magnetic nanostructured aggregates is much larger than Au ones. This might be due to that the diameter of MNPs (50 nm) is much bigger than that of AuNPs (13 nm).

From the results of symmetric PCR based on AuNPs and MNPs, it can be deduced that the kind of nanoparticles might has no effect on symmetric PCR amplification based on them, but the diameter has some effect. It might be because of that the larger the diameter of nanopartilces is, the greater the effect of the steric resistance caused by the

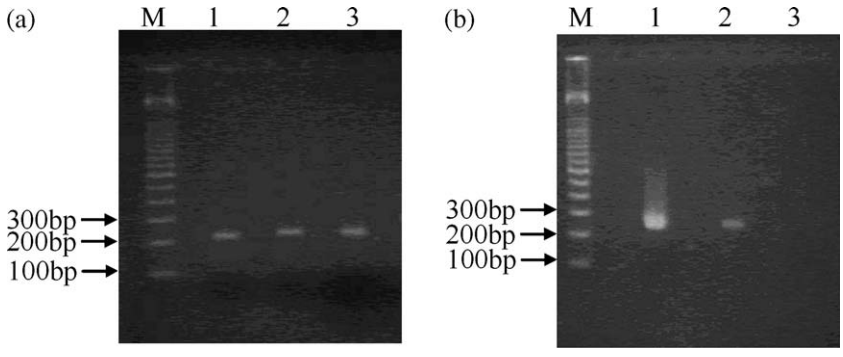

Fig. 3. 3% agarose gel analysis of symmetric PCR product: (a) elongated by MNP-F1 and R1, (b) elongated by MNP-R1 and MNP-F1. M: 100 bp molecular size marker. Lanes 1–3: the annealing temperatures were 50 °C, 56 °C, 60 °C, respectively.

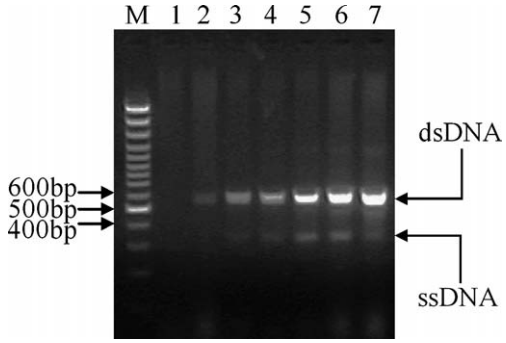

Fig. 5. 2% agarose gel analysis of asymmetric PCR product elongated using MNP-F3 and R3. M: 100 bp molecular size marker. The annealing temperature was 65 °C.

roughness of nanoparticle surface to the hybridization and extension of primers will be.

Target ssDNA along with dsDNA can be generated by an asymmetric PCR when one primer is used at higher concentration than the other [11]. If asymmetric PCR is performed using one MNP-bound primer and at higher concentration, plenty of MNP-bound ssDNA will be attained, and the isolation of asymmetric PCR product will become very convenient because of the superparamagnetism of MNPs. F3 was immobilized onto MNPs (MNP-F3) and R3 was freely diffusing used as limiting primer. PCR was performed using 35 cycles and the annealing temperature was 65 °C. The result is shown in Fig. 5. In seven reactions, the amount of MNP-F3 was same and about 25 pmol, but the amount of R3 was 0.1, 0.25, 0.4, 0.5, 1, 2.5 and 5 pmol, respectively. In the image, there are all two bands in lanes 2~7, the upper bands are the 582 bp target product, and the lower bands verified by sequencing are corresponding to the expected ssDNA. Due to ethidium bromide stained ssDNA being inherently less efficient in contrast to dsDNA, the ssDNA band intensities are not such high as the dsDNA ones though the amount of ssDNA is much greater than that of dsDNA [12]. The same result was attained when this experiment was performed using MNP-R3 and F3. This demonstrates that with whichever MNP-bound primer, asymmetric PCR can proceed, but only when the two primers are at optimum concentration ratio, the amount of ssDNA is at most. The rule was verified when asymmetric PCR was performed using whichever MNP-bound primer of R2/F2.

In conclusion, with one or two primers respectively bound to the surface of AuNPs or MNPs, PCR can proceed successfully under optimized condition and is subject to certain rules, a symmetric PCR technique and an asymmetric PCR technique based on nanoparticles have been developed. A kind of nanostructured aggregates can be constructed by a symmetric PCR using two nanoparticle-bound primers.

Acknowledgments

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