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Regulation of PCR efficiency with magnetic nanoparticles in a rotating magnetic field

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ABSTRACT

The polymerase chain reaction (PCR) method is widely used for the reproduction and amplification of specific DNA segments in vitro, and a novel PCR method using nanomaterials such as gold nanoparticles has recently been reported. This paper reports on the regulation of PCR efficiency with superparamagnetic nanoparticles in a rotating magnetic field. The level of efficiency was successfully regulated in a rotating magnetic field by the authors, and decreased with increasing frequency of the field. The results obtained show that simply controlling the structure and dynamics of magnetic nanoparticle clusters in a rotating magnetic field can regulate PCR efficiency.

1. Introduction

The polymerase chain reaction (PCR) is a technique that allows reproduction and amplification of specific DNA segments using DNA polymerase in vitro [1]. Currently, the method is widely used in various fields. In some cases, non-specific DNA fragments are often produced using primer dimers, GC-rich regions of template DNA and low annealing temperatures [2,3]. Non-specific DNA fragment production is generally suppressed through optimized primer design, buffer conditions including magnesium concentration, and denaturing/annealing temperatures [4,5]. It is furthermore well known that the specificity and efficiency of PCR can also be improved using a variety of additives and agents such as formamide, glycerol, single-stranded DNA-binding protein (SSB) and tetramethylammonium chloride (TAMAC) [6–9]. Recently, it has been reported that nanomaterials such as gold nanoparticles can also be used to improve PCR efficiency. Li et al. [10] reported that such nanoparticles inhibited nonspecific products in PCR at low annealing temperatures. Li et al. [11] also reported that PCR efficiency can be dramatically increased using gold nanoparticles in PCR reagents. According to these reports, the outstanding heat transfer properties of gold nanoparticles enhance PCR efficiency and shorten reaction times. However, there have been a number of contradictory reports stating that gold nanoparticles have remarkably negative effects on PCR efficiency [12–14]. These reports suggested that such nanoparticles could inhibit PCR efficiency at high levels of concentration because of the interaction between Taq polymerase and gold nanoparticle surfaces. In our previous study, we have already reported the effects of superparamagnetic nanoparticles on PCR efficiency without an external magnetic field, and clarified the mechanism behind the effects of superparamagnetic particle on PCR efficiency by estimating the structures of such clusters in PCR [15]. We concluded that superparamagnetic nanoparticles tend to inhibit PCR efficiency depending on the structure and sizes of magnetic nanoparticle clusters. We clarified that some amount of Taq polymerase in a PCR solution was captured in the spaces among magnetic nanoparticle clusters formed thermal aggregation, and that it was captured more efficiently by the motion effect brought about in them by heat treatment in the PCR thermal cycles. Consequently, the amount of Taq polymerase that should be used in PCR amplification is reduced in the PCR solution, and as a result, PCR efficiency was reduced. We also suggested the possibility of regulating PCR efficiency using their outstanding specific magnetic properties in external magnetic fields. Magnetic particles are classified into two types: ferromagnetic and paramagnetic/superparamagnetic. Ferromagnetic particles have a permanent magnetic dipole moment [16,17], while such a moment is induced only by an external magnetic field in paramagnetic particles [18,19]. In the dispersion systems of both types of magnetic particle, chain clusters are formed by particles via dipole–dipole interaction in an external DC magnetic field [16–19]. When a rotating magnetic field is applied, these chain clusters rotate with the field's rotation [20–24]. Kang et al. [25] simulated the chaotic mixing induced by a magnetic chain in a rotating magnetic field. Mizuki et al. [26] previously focused on

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the magnetic properties of paramagnetic particles, and reported that the enzyme activities of α-amylase immobilized on superparamagnetic particles are regulated in a rotating magnetic field.

In this Letter, we report on the regulation of PCR efficiency with superparamagnetic nanoparticles in a rotating magnetic field. We applied an external rotating magnetic field to a PCR solution containing such particles during the PCR process and changed the structure and dynamics of nanoparticle clusters by controlling the frequency of the rotating magnetic field in vitro. We investigated the dependence of PCR efficiency on the frequency of the magnetic field. The results indicate that PCR efficiency can be easily regulated simply by controlling the external magnetic field, while the regulation of conventional PCR efficiency requires the addition of expensive reagents and precise control of reaction temperatures. Moreover, the results show the potential of magnetic nanoparticles to perform the role of a nano-size magnetic stirrer in vitro.

2. Experiment details

The magnetic nanoparticle medium used in this study was a water-based ferrofluid containing superparamagnetic nanoparticles (EMG607, Ferrotec Japan) as shown in Figure 1a. This was developed for biomedical applications such as utilization as a magnetic resonance imaging contrast medium and drug delivery systems. The EMG607 superparamagnetic nanoparticles had an average diameter of 10 nm as shown in Figure 1b, and were composed of mixtures of magnetite (Fe_3O_4) and maghemite (Fe_2O_3) iron oxides. Their surfaces were modified with an anionic surfactant to improve dispersion, and the magnetic particle fluids were centrifuged to remove excess surfactant molecules. Additionally, these fluids were diluted to a specific concentration with distilled water, and the magnetic nanoparticles were then dispersed using ultrasonication.

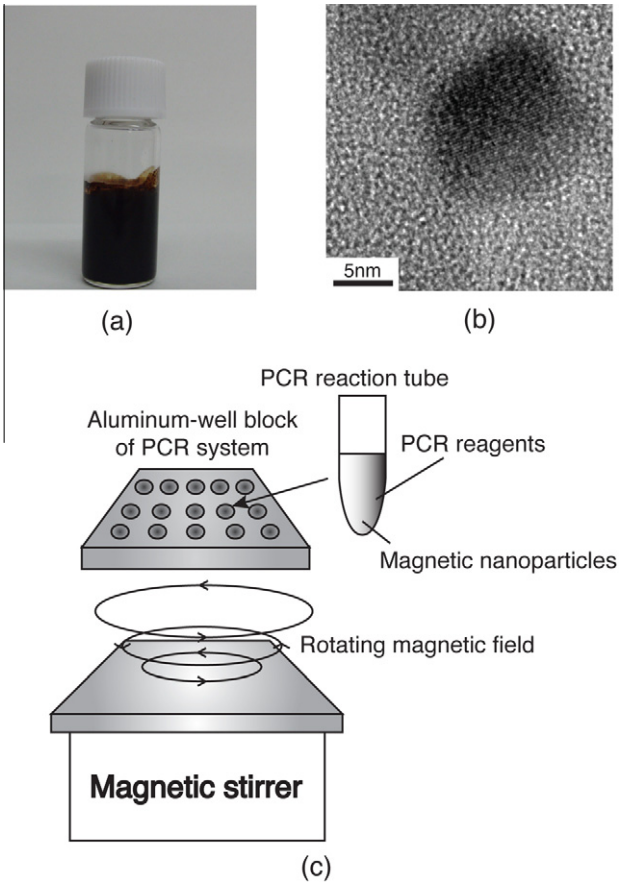

Figure 1. (a) Photograph of water-based ferrofluid containing superparamagnetic nanoparticles (EMG607); (b) TEM image of individual magnetic nanoparticles; (c) schematic diagram of PCR amplification using a rotating magnetic field.

Experimental PCR amplification using a rotating magnetic field is outlined here. The strain used in this study was Escherichia coli DH5a cultivated in LB medium. Cells were harvested by centrifugation and suspended in TEN buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA, 100 mM NaCl), and 0.3 g of glass beads were added. The cells were broken up by being shaken for 10 min on a vortex mixer at maximum speed, and nucleic acid was extracted through phenol/chloroform treatment and ethanol precipitation. An approximately 1500-bp segment of the 16S rRNA gene was amplified by PCR with the forward and reverse primers 5′-AGA GTT TGA TCC TGG CTC AG-3′ (positions 8–27 according to *Escherichia coli* numbering) and 5′-GGC TAC CTT GTT ACG ACT T-3′ (positions 1510–1492) using an Ex Taq polymerase kit (TaKaRa). PCR thermal cycling was carried out in 0.2-ml reaction tubes in a GeneAmp PCR System 9700 (Applied Biosystems). Amplification was performed in the following 50-μl mixture: 5.0 μl of 10 × Ex Taq PCR buffer, 4.0 μl of dNTP mixture (dATP, dTTP, dGTP and dCTP; 2.5 mM each), 1.0 μl of forward primer, 1.0 μl of reverse primer (10 mM each), 27.3 μl of distilled water, 0.4 μl of Ex Taq polymerase (1 U/μl), 1.0 μl of template DNA (100 ng of DNA) and 10 μl of magnetic nanoparticles (EMG607: 1.0 μg/μl). The thermal profile for amplification began with an initial denaturation step (2 min., 96 °C) followed by 25 cycles of denaturation (20 s., 96 °C), annealing (20 s., 56 °C) and extension (90 s., 72 °C), then a final terminal extension step (2 min., 72 °C). An external rotating magnetic field was applied to the PCR reagents in vitro throughout the PCR thermal cycles. A rotating magnetic field was produced using a conventional magnetic stirrer (RCX-1000D, EYELA) as shown in Figure 1c. The magnetic stirrer embedded a high precision speed control system, and the rotating speed provided us the frequencies around the centre of PCR liquid. The frequency of the rotating magnetic field was increased stepwise from 0 to 20 Hz. In addition, the magnetic field intensity applied to the center of the sample was measured using a precision gaussmeter. The intensity was maintained at a constant 4.5 mT throughout the whole experiment. Aliquots of 5.0 μl from PCR were analyzed using agarose gel (1.2%) electrophoresis, and the product yield was confirmed using the InGenius system (Syngene).

3. Results and discussion

We first examined the effect of the rotating magnetic field on PCR efficiency with magnetic nanoparticles, then performed PCR amplification while changing the frequency of the magnetic field. Figure 2 shows amplified DNA bands observed using agarose gel electrophoresis with different magnetic field frequencies. The control sample was amplified without an external magnetic field. We observed that the fluorescence intensity of DNA bands decreased with increasing frequencies of the rotating magnetic field, and PCR amplification consequently tended to be inhibited with higher frequencies. Figure 3 shows the dependence of the PCR product yield with EMG607 magnetic nanoparticles on the frequency of the rotating magnetic field in quantitative terms. It clearly shows that the yields were linearly regulated with increasing frequencies. In the DC magnetic field applied, the yields were significantly lower than that of the control sample. When the frequency was increased stepwise from 1.0 to 20 Hz, the yields also decreased depending on the frequency, which was finally inhibited at 20 Hz. These results indicate that PCR efficiency can be controlled by an external magnetic field, and consequently that the regulator