Available online at www.sciencedirect.com
SCIENCE DIRECT
Enzyme and Microbial Technology 39 (2006) 366–370
ENZYME AND MICROBIAL TECHNOLOGY
www.elsevier.com/locate/emt

Transformation of *Escherichia coli* mediated by magnetic nanoparticles in pulsed magnetic field

Ji-Yao Chen, Ya-Ling Liao, Tzu-Hsien Wang, Wen-Chien Lee *

Department of Chemical Engineering, National Chung Cheng University, Chiayi 621, Taiwan

Received 30 August 2005; received in revised form 25 October 2005; accepted 10 November 2005

Abstract

A magneto-transformation method was developed for transferring plasmid DNA into *Escherichia coli*. Superparamagnetic nanoparticles (Fe_3O_4) having the saturation magnetization up to 68 emu/g were prepared by the method of co-precipitation at alkaline and thermal conditions, coated with polyethyleneimine (PEI), and then complexed with negatively charged DNA molecules. Plasmid DNA (pGEX-1λT) attached to the PEI-coated magnetic nanoparticles could be transformed into *E. coli* DH1 by the application of a high magnetic field in pulses. Experimental results suggested that magnetic nanoparticles could well assist DNA delivery into bacterial cells. Factors that could significantly influence the transformation efficiency included the multiple pulsing in magnetic field and concentrations of DNA and magnetic nanoparticles. The highest transformation efficiency could be achieved by pulsing for three times in the 2.15 T magnetic field. The survival declined drastically at the additional pulses. Magneto-transformation of cells with 25 μg DNA and 16 μg magnetic nanoparticles resulted in transformants number in the range from 1600 to 2480, corresponding to efficiency from 64 to 99 cfu/μg DNA. Although the yield of transformants increased with input DNA, the transformation efficiency decreased with DNA dosage. Superparamagnetic nanoparticles played a crucial role in the bacterial transformation in strong magnetic field. Increasing the amount of input magnetic nanoparticles decreased both the survival and transformation efficiency. The higher dosage of nanoparticles led to more dead cells.

Keywords: Magneto-transformation; Superparamagnetic nanoparticle; Pulsed magnetic field; Gene transfer; *Escherichia coli*

1. Introduction

The introduction of plasmid DNA into a host like *Escherichia coli* is an essential step in the construction of recombinant strain for the overexpression of exogenous gene. Several protocols have been established to transfer foreign DNA into prokaryotic cells based on the calcium chloride method and electroporation [1]. Electroporation, which is also known as electrotransformation, was developed originally to introduce DNA into mammalian cells [2], but it has lately been used to transform *E. coli* and other bacteria [3–7]. It involves a brief high-voltage electric discharge that renders cells permeable to DNA, so that the plasmid DNA can simultaneously diffuse into the host cells. Higher transformation efficiencies can be achieved by using high voltages or longer pulses. The present paper shows that in the strong, pulsed magnetic field, foreign DNA was able to penetrate into *E. coli* mediated by the magnetic nanoparticles. This transformation process can be termed as the magneto-transformation.

Conjugations of nanoparticles and DNA have widely been used in drug delivery, gene therapy and gene transfection. For the transferring DNA into eukaryotic cells, the use of nanoparticles, either polymeric or inorganic, has been found to enhance the transfection yield [8]. Nanoparticles in this instance act as a condensation agent to enrich DNA on the cell surface. Magnetic nanoparticles were recently prepared for the coupling of avidin, which could attach a single-stranded DNA through the avidin–biotin interaction and hybridize with a DNA target [9]. As described in this paper, magnetic nanoparticles, which were modified on the surface by coating with a polycationic polymer, were able to form complexes with plasmid DNA and be carriers for DNA delivery to bacteria.

2. Materials and methods

2.1. Preparation of magnetic nanoparticles

Superparamagnetic particles Fe_3O_4 were prepared by co-precipitating di and trivalent Fe ions (in the molar ratio of 1:2) by alkaline solution and treating under hydrothermal conditions. Briefly, to an aqueous solution mixture of $FeCl_2{\cdot}4H_2O$ (0.1 M) and $FeCl_3{\cdot}6H_2O$ (0.2 M), the solution of NaOH (1 M) was dropwisely

* Corresponding author. Fax: +886 5 2721206.
E-mail address: chmwcl@ccu.edu.tw (W.-C. Lee).

0141-0229/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2005.11.035

added under continuous stirring at 300 rpm. The precipitate was heated at 40, 60 or 80 °C for 1 h. The pH was maintained at a constant value during the reaction process, described by the following reaction scheme:

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightleftharpoons Fe_3O_4 + 4H_2O$$

2.2. Characterization of magnetic nanoparticles

The size of prepared iron oxide particles was examined by transmission electron microscopy (TEM) and photon correlation spectroscopy (PCS). The magnetization curves of the nanoparticles samples were measured by a superconducting quantum interference device (SQUID). X-ray diffraction (XRD) was used to investigate the crystal structure of the samples.

2.3. Transformation in pulsed magnetic field

The plasmid pGEX-1λT obtained from Amershan Pharmacia Biotech was used for transformation. The host cell *E. coli* DH1 was cultured in LB medium at 37 °C until the OD_{600} reached 0.5–0.7. For the transformation, 40 μL of cell suspension was mixed with 10 or 50 μL of the solution consisting of DNA, PEI-coated magnetic nanoparticles, and HEPES buffer in a 1.5-mL microtube. Host cell, DNA, and magnetic nanoparticle alone were respectively employed for control experiments. After repeated exposure in the pulsed magnetic field generated by a commercial magnetizer (Model UC-2420M, Jin Fair, Taiwan) with a magnetic field strength of 2.15 T or other specified value, the cells were mixed immediately with 1 mL SOC medium for static culture at 37 °C for 1 h. The SOC medium consists of 2% bacto tryptone, 0.5% bacto yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2 \cdot 6H_2O$, 10 m $MgSO_4 \cdot 6H_2O$, and 20 mM glucose. The culture was then diluted into a series of fractions with different dilution ratios. About 100 μL of each diluted culture were plated on LB agar containing 100 μg/mL ampicillin, as well as on ampicillin-free LB agar. Plates were statically cultured at 37 °C for 24 h. The transformation efficiency and survival were calculated based on the numbers of CFU generated from the cultured plates with and without ampicillin. The transformation efficiency was defined as CFU per μg of DNA used. The survival defined here was the ratio of total CFU with magneto-transformation to that without magneto-transformation.

3. Results and discussion

3.1. Preparation of superparamagnetic nanoparticle

Superparamagnetic nanoparticles were prepared by the method of alkaline precipitation at various combinations of pH and temperature. The particle diameter of prepared magnetic nanoparticles was determined by photon correlation spectroscopy measurement to be in the range of 10–100 nm. Fig. 1 show the X-ray diffraction spectra and magnetization curve for the nanoparticles prepared at pH 10 and 80 °C. The XRD data (Fig. 1a) suggests that the nanoparticle is magnetite (Fe_3O_4) crystal with inverse cubic spinel structure having six diffraction peaks: {2 2 0}, {3 1 1}, {4 0 0}, {4 2 2}, {5 1 1} and {4 4 0}. These nanoparticles were thus proved to be superparamagnetic and the saturated magnetization was measured by SQUID at 25 °C to be 65 emu/g.

The magnetization of the nanoparticles was influenced by the preparation conditions. A higher temperature used for the co-precipitation process could result in nanoparticles with a higher value of saturation magnetization. When 60 °C was employed, for example, the saturation magnetization of magnetic nanoparticles was determined to be in the range from 52 to 62 emu/g. While the saturation magnetization of magnetic nanoparticles

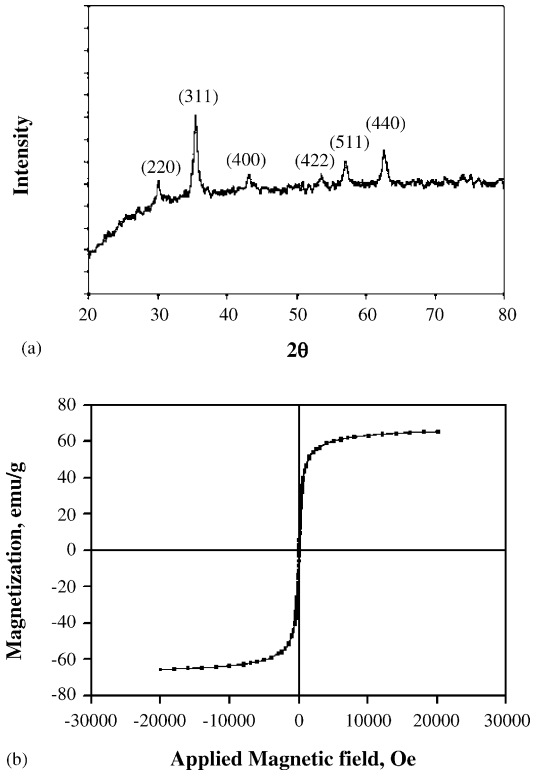

Fig. 1. XRD (a) and magnetization curve (b) of magnetic nanoparticles prepared at pH 10 and 80 °C.

prepared at 80 °C could reach 53–68 emu/g. The pH value was of little influence in the range from 10 to 12. However, the highest pH value (pH 12) could result in the highest saturation magnetization. When the co-precipitation took place at lower temperature such as 40 °C, the saturation magnetization of the resultant nanoparticles became small, suggesting that thermal conditions was essential for the formation of magnetite crystals.

The superparamagnetic property is crucial for the application in magneto-transformation. Superparamagnetic nanoparticles could move instantly in the magnetic field as soon as they were magnetized. After the removal of magnetic field, these nanoparticles became non-magnetic, as shown in Fig. 1b.

The surface coating of magnetic nanoparticles was achieved using 0.5 g/L polyethyleneimine (PEI). For the coating, the molar ratio of PEI to Fe_3O_4 was kept at low level (about 0.13%). Amino groups on the resultant particles were used for complexing with negatively charged DNA molecules. The zeta potential of nanoparticles was increased by the coating of positively charged PEI. The average particles size of PEI-coated particles was 65.2 nm.

3.2. Transformation mediated by magnetic particles in pulsed magnetic field

Fig. 2 schematically shows the hypothetical mechanism of magneto-transformation. The DNA construct preparing by complexing plasmid DNA with PEI-coated superparamagnetic nanoparticles was mixed with the bacterial cell suspension and placed in the fixture of the magnetizer. The magnetizer consist-