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High Transformation Efficiency of *Escherichia coli* with Plasmids by Adding Amino Modified Silica-nanoparticles

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Abstract: The calcium chloride method is an efficient and convenient technique to prepare *Escherichia coli* competent cells. The transformation efficiency of the competent cells when prepared in the laboratory is generally below 10^6 cfu μg^{-1} (colony forming units μg^{-1}) DNA and thus its application is limited in some extent. Here we report the utilization of a-SiNPs (amino modified Silica-Nanoparticles) as a help material to improve the transformation efficiency of *E. coli* competent cells that are prepared with calcium chloride method. Addition of a-SiNPs increases the transformation efficiency of *E. coli* to 10^7-10^8 from below 10^6 cfu μg^{-1} DNA in the laboratory.

Key words: Silica-Nanoparticles, *Escherichia coli*, transformation efficiency, AEAPS

INTRODUCTION

The calcium chloride method is the first reported method to prepare *E. coli* competent cells used in chemical transformation, whose transformation efficiency is $<10^7$ cfu μg^{-1} DNA (Cohen *et al*., 1972). Then Hanahan's and Inoue's methods were developed to prepare competent cells in order to improve the efficiency of chemical transformation to 10^6-10^8 cfu μg^{-1} (Hanahan, 1983; Inoue *et al*., 1990) which however require rather strict and complicated processes compared with calcium chloride method. Therefore, most researchers choose electroporation when higher transformation efficiency ($>10^6$ cfu μg^{-1}) is required, even though electroporation method requires special apparatus and additional treatment to DNA samples, perhaps taking some DNA samples away (Chassy and Flickinger, 1987; Dower *et al*., 1988).

In recent years, nanoparticles have been gradually introduced to biological researches and made some noticeable technical progresses (Liu *et al*., 2004; Anonymous, 1994). So far, the studies and applications on nanoparticles are mostly focused on cell transfection. As a nonviral transfection system *in vitro*, surface modified silica-nanoparticles bring high efficiency of cell transfection and therefore are regarded as potential drug deliverers *in vivo* (Kneuer *et al*., 2000a, b; He *et al*., 2003; Santra *et al*., 2001; Sameti *et al*., 2003). a-SiNPs are nanoparticles made from TEOS (tetraethoxysilane) and AEAPS (N-(β-aminoethyl)-γ- aminopropyltriethoxysilane) (Fig. 1). The particle surface is covered with positive electrical amidogen, which makes the particles easier to bind negatively charged DNA and cell surface membrane in a similar way (Santra *et al*., 2001). In this study, we used a-SiNPs as a surface modified nanoparticles to bind and transport DNA onto *E. coli* membrane and obtained a high-transformation efficiency up to 10^8 cfu μg^{-1}.

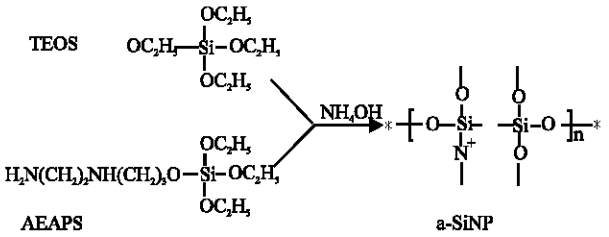

Fig. 1: Synthetic scheme of a-SiNP

MATERIALS AND METHODS

Reagents: All chemicals and biologicals were commercially available and used as received. 2-(2-Aminoethylamino-)-5-nitropyridine (AEAPS) was obtained from Sigma company. Cyclohexane, tritonx-100,

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n-hexanol, ethyl silicate (all chemicals were analytical grade), DNase and all components of *E. coli* growth medium were obtained from Shanghai Sangon Biological Engineering Technology and Services.

a-SiNPs preparation: It is simple and convenient to prepare a-SiNP[8] and the diameter of nanoparticles can be adjusted by the volume of each material. Calculate the concentration of a-SiNPs and reserve them at 4°C.

Transformation procedure: *E. coli* competent cells are prepared as standard manual. Before transformation, mix the autoclaved sterile a-SiNP and DNA (pBluescript SK+ plasmid DNA was applied in present study) in the mass proportion of 20:1 (Notice: The total volume should be under 10 μL for every 200 μL competent cells) and place the mixture at room temperature for 10 min. Afterwards, perform the chemical transformation according to standard protocol (He *et al*., 2003). Simple described as the following: add the DNA and a-SiNP complexes to competent cells in ice-water; incubate on ice for 30 min; heat shock for 90 sec and chill on ice for 1 min; add 1 mL SOC medium and incubate at 37°C with soft shake for 1 h; dilute and plate.

RESULTS AND DISCUSSION

To analyse the Zeta potential of a-SiNPs in *E. coli* transformation, a-SiNPs (~50 nm) are directly synthesized by using the synchronous hydrolysis of TEOS and AEAPS in water-in-oil microemulsion. Because of the protonation of the amino group on the a-SiNPs, the separated a-SiNPs possess a positive charge of nearly +30 mV at neutral pH (He *et al*., 2003). Zeta potential analysis shows that the potential of DNA-a-SiNPs complexes decreases as more DNAs were added to the complex (Fig. 2A). Furthermore, DNA binding ability of a-SiNPs is saturated when the weight ratio of a-SiNPs to DNA is nearly 10:1. Figure 2B shows the agarose gel electrophoresis of a-SiNP/DNA complexes according to the weight ratio. The movement of DNA was retarded and remained at the top of the gel when a-SiNPs/DNA weight ratio is greater than 10:1, indicating that DNAs form complexes with a-SiNPs.

To test the effect of a-SiNPs on chemical transformation, we performed standard chemical transformations with a-SiNPs/DNA complexes mixed according to various weight ratios (Cohen, 2001). *E. coli* competent cells were prepared with calcium chloride method. Figure 3 shows that when the plasmid DNA was transformed alone, the transformation efficiency of the competent cells is about 10^5 cfu μg^{-1}. Where the weight ratio of a-SiNP/DNA is ≤10:1, the transformation efficiency shows a modest increase or even some decrease with the addition of a-SiNPs. In these cases,

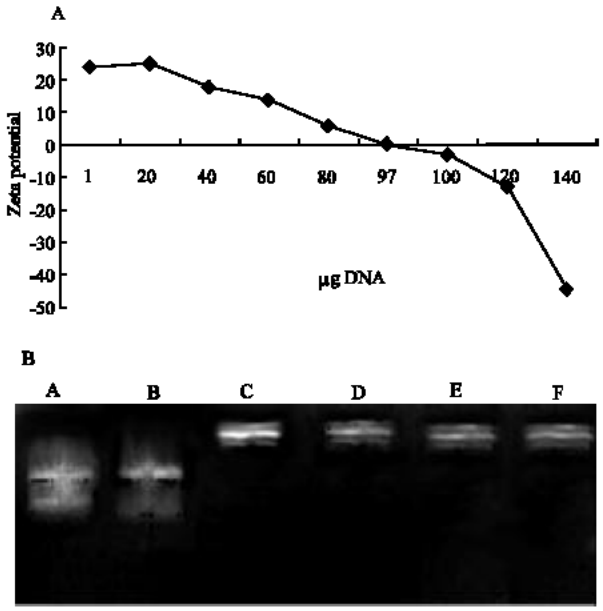

Fig. 2: a-SiNPs can bind with DNA (pGL3 control plasmid DNA) to form complexes. (A) The zeta potential of DNA-a-SiNPs complexes decreases with increasing addition of DNA. We suspended the 1 mg a-SiNPs and various amount of DNA in ultrapure water and titrated the DNA-a-SiNPs complexes at neutral pH. When 97 μg DNA was added to the solution with a weight ratio of a-SiNPs/DNA nearly 10:1, the potential of DNA-a-SiNPs complexes is 0 mV. As the weight ratio decreased, the solution begins to possess negative charge. (B) Agarose gel electrophoresis of DNA-a-SiNPs complex according to weight ratios (1 μg DNA in each lane), Lane A (1:0); lane B (1:1); lane C (1:10); lane D (1:15); lane E (1:20); lane F (1:100). The complexes of DNA-a-SiNPs were prepared by mixing plasmid DNA in phosphate-buffered saline (pH 7.4) with various amounts of a-SiNPs suspended in ultra pure water, then incubated at room temperature for 10 min and followed by 1% agarose gel electrophoresis

a-SiNPs lose their positive charge due to binding with DNA molecules and the negatively charged a-SiNP/DNA complexes could not be absorbed efficiently by the negative charged cell surface membrane. When the a-SINP/DNA ratio is >10:1 (0.1 in the Fig. 3), the transformation efficiency increases accordingly. Transformation efficiency reaches a plateau at a- SiNP/DNAratio 20:1 (0.2 in the Fig. 3). However, continuous increase of a-SiNPs did not improve the transformation efficiency further after the ratio reaches 20:1 (SiNP/DNA). Therefore the a-SiNP and DNA weight ratio of 20:1 (0.2 in the Fig. 3) is the most efficient condition for *E. coli* transformation. Furthermore, we did

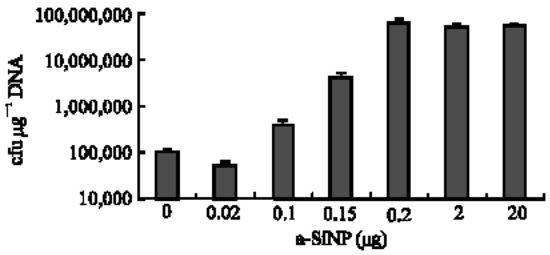

Fig. 3: Transformation efficiency of competent cells (DH5α) with DNA/a-SiNPs complexes prepared according to various weight ratios of DAN and a-SiNPs. Plasmid DNA pBluescript SK+ was prepared by plasmid purified kit protocol (Qigen, Co.) and 10 ng was used in each transformation. Transformations were performed according to standard protocol (13). The error bares represent standard deviation as mean ±SD (n = 3)

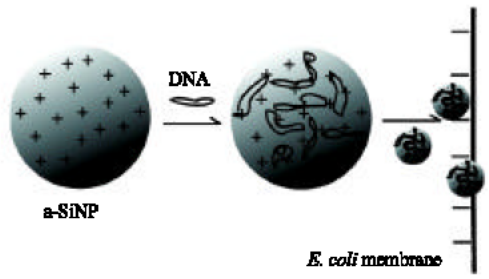

Fig. 4: a-SiNPs taking positive charge can carry DNA to cell membrane

not observe any negative effect of a SiNP to *E. coli* cell growth after transformation even when 20 μg a-SiNPs were added to 200 μL competent cells as shown in the supporting materials.

CONCLUSIONS

The present study shows that the a-SiNPs can act as a help material to greatly improve the transformation efficiency of chemical transformation 3 of *E. coli* cells (Fig. 4). The use of silica-nanoparticles will widen the application of calcium chloride method for competent cell preparation as well as chemical transformation of *E. coli* cells.

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