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Transformation of *Escherichia coli* and protein expression using lipoplex mimicry

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ABSTRACT

We investigated a "one-step" method for transformation of and protein expression in *Escherichia coli* (*E. coli*) using a complex of *n*-stearylamine, a cationic lipid, and plasmid DNA, which mimics lipoplex-based approaches. When *E. coli* cells were treated with the cationic lipid-plasmid complex, the transformation efficiencies were in the range of approximately $2-3 \times 10^6$ colony-forming units. Further increase in the efficiency was obtained by co-treatment with calcium chloride (or rubidium chloride) and the complexes. Moreover, after DNA transfer, *E. coli* cells successfully expressed plasmid-encoded proteins such as cytochrome P450s and glutathione-*S*-transferase without overnight incubation of the cells to form colonies, an indispensable step in other bacterial transformation methods. In this study, we provide a simple method for *E. coli* transformation, which does not require the preparation of competent cells. The present method also shortens the overall procedures for transformation and gene expression in *E. coli* by omitting the colony-forming step.

1. Introduction

Since Mandel and Higa [1] demonstrated that calcium chloride ($CaCl_2$) treatment renders *Escherichia coli* (*E. coli*) susceptible to uptake of exogenous DNA, several chemical methods have been developed to increase transformation efficiency. Among them, Hanahan [2] provided a method that produces a transformation yield of $>10^8$ colony-forming units (CFU), which is one of the most widely used procedures. In addition, a method for the preparation of "ultra-competent" *E. coli* cells has also been proposed [3]. Although these methods are effective in transferring DNA to *E. coli* cells, and thereby have been conventionally used, they involve rather cumbersome steps and require a relatively long time to complete. To overcome these problems, a so called "one-step" method using polyethyleneglycol/dimethyl sulfoxide broth has been suggested, which is rapid, convenient, and effective in the preparation and transformation of competent *E. coli* cells [4].

Cationic lipids have been widely used to deliver nucleic acids into targeted cells as a form of lipoplex, a liposome/DNA complex, due to the charge interaction between the cationic lipids and polyanionic nucleic acids [5]. Among them, nanoparticles containing 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) or N(1-[2,3-dioleyloxy]propyl)-N,N,N-trimethylammonium chloride (DOTMA) have been well characterized in gene delivery systems. Another cationic lipid, *n*-stearylamine, has been also adopted for delivery of linear DNA or circular plasmid DNA [6]. However, these cationic lipid-based systems are generally used for transfection process introducing nucleic acids into eukaryotic cells by non-viral methods and their applications to prokaryotic cells are strictly limited.

In this study, we developed a new "one-step" method that mimics lipoplex-based DNA transfection of eukaryotic cells. By using this method, plasmid DNAs were transferred to *E. coli* and the plasmid-encoded genes such as cytochrome P450 (CYP) and glutathione-*S*-transferase (GST) were successfully expressed without any preparation of competent cells or time-consuming steps such as overnight incubation to form colonies.

2. Materials and methods

2.1. Materials

n-Stearylamine (synonym: octadecylamine), $NADP^+$, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, 7-

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ethoxyresorufin, testosterone, δ-aminolevulinic acid (δ-ALA), and 6β-hydroxytestosterone were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). pGEX-2T plasmid encoding GST protein and glutathione-Sepharose 4B were purchased from GE Healthcare Biosciences (Piscataway, NJ, USA). Recombinant rat NADPH-P450 reductase was expressed in *E. coli*, and purified as described previously [7]. The cDNA for human cytochrome P450 3A4 (CYP3A4) with *N*-terminal truncation [8] or for human cytochrome P450 1A2 (CYP1A2) with *N*-terminal truncation [9] was subcloned into an expression vector, pCW, to generate pCW3A4 and pCW1A2 plasmid, respectively.

2.2. Preparation of lipoplexes

A stock solution of *n*-stearylamine was prepared by dissolving 35 mg of the lipid in 1 mL $CHCl_3$. Each indicated amount of *n*-stearylamine solution was transferred to round-bottom glass tubes. After evaporation of the solvent by a stream of nitrogen gas, the dry lipids were mixed with buffer solution (25 mM Tris-HCl, pH 7.4, and 100 mM NaCl) containing 25 ng of pGEX-2T or the plasmids encoding CYPs (pCW3A4 and pCW1A2). Throughout the studies, a fixed amount of plasmid was used. The mixture was then emulsified by vortex mixing and subsequent brief sonication in a VWR Q700 probe sonicator (with a continuous ultrasound for 30 s and output power of 120 W) at room temperature. The particle sizes and zeta potentials of lipoplexes were measured using a Zetasizer Nano ZS (Malvern Instruments Inc., Westborough, MA, USA). The resultant lipoplexes had zeta potentials of 25–50 mV, depending on the concentration of *n*-stearylamine. The particle sizes of lipoplexes were determined to be approximately in the range of 30–45 nm.

2.3. Transformation of E. coli

Transformation of *E. coli* BL21 (for pGEX-2T), BL21 (DE3) (for pET-24a), or DH5αF′IQ (for pCW3A4 and pCW1A2) cells was performed by mixing the prepared lipoplexes and 1 mL of cells (optical density at 600 nm [OD_{600}] = 0.4–0.5) in Luria-Bertani (LB) media (1% (w/v) NaCl, 0.5% (w/v) yeast extract, and 1% (w/v) Tryptone) in the presence or absence of $CaCl_2$ or rubidium chloride ($RbCl_2$) for each indicated time at 37 °C with gentle shaking. The mixtures were plated on agar plates (1.5% (w/v) agar in LB media) containing ampicillin (50 μg/mL), and incubated at 37 °C for 17 h. Transformation efficiencies were expressed as the number of transformants per microgram of DNA (colony-forming unit, CFU).

2.4. Expression of CYPs and preparation of membrane fractions

After incubation of the mixture (lipoplexes and *E. coli* cells) for 1 h at 37 °C with gentle shaking, 50 μL of the sample was transferred to 5 mL of Terrific Broth (TB) containing 0.2% (w/v) bactopeptone, 1.0 mM thiamine, ampicillin (50 μg/mL), and trace elements [10], and incubated at 37 °C with vigorous shaking. When the OD_{600} of the culture reached approximately 0.4–0.5, gene expression was induced by the addition of 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 1.5 mM δ-ALA. Every 3 h, a portion of the culture was sampled and the expression level of CYPs was quantitated by Fe^{2+}–CO versus Fe^{2+} difference spectra with whole cells as described [11] in 100 mM Tris-Cl (pH 7.4) containing 20% (v/v) glycerol, 10 mM CHAPS, and 1 mM Na-EDTA using a Shimadzu UV-1650 PC spectrophotometer. Membrane fractions of *E. coli* cells were prepared as described previously [8].

2.5. Enzymatic assay of CYPs

The catalytic activities of enzymes were measured with membrane fractions. Testosterone 6β-hydroxylation and ethoxyresorufin *O*-deethylation activities were determined for CYP3A4 [12] and CYP1A2 [13], respectively. The activity assay was performed in 100 mM potassium phosphate buffer (pH 7.4) with a reaction volume of 500 μL. The membrane fraction (0.4 μM) and NADPH-cytochrome P450 reductase (2 μM) were mixed in the presence of testosterone or 7-ethoxyresorufin as a substrate for CYP3A4 or CYP1A2, respectively. The reaction was started by adding an NADPH-generating system (0.5 mM $NADP^+$, 5 mM glucose-6-phosphate, 0.5 unit glucose-6-phosphate dehydrogenase/mL), and after incubating the sample at 30 °C for 10 min. The reaction of CYP3A4 was stopped by adding 50 μL of 1.0 N HCl containing 2.0 M NaCl and the resulting product was extracted and analyzed by HPLC with UV detection at 240 nm. The reaction of CYP1A2 was terminated by adding 500 μL of cold methanol and the formation of resorufin was assayed by measuring the fluorescence intensity at 590 nm (535 nm of excitation wavelength).

2.6. Expression and purification of glutathione-S-transferase

GST protein was expressed in *E. coli* BL21 strain using the similar methods to those for CYPs with pGEX-2T plasmid: after mixing *E. coli* cells and lipoplexes for 1 h at 37 °C, 500 μL of the sample was transferred to 50 mL of TB/ampicillin media and the mixture was incubated until the OD_{600} of the culture reached approximately 0.5. The protein expression was then induced by the addition of 1 mM IPTG and further incubated for 4 h. After harvest of cells by centrifugation (5000 × *g*, 10 min, and 4 °C), expressed GST was purified using glutathione-Sepharose chromatography by following the instructor's manual.

2.7. Statistical analysis

Statistical data are expressed as the means ± standard deviations (S.D.) of five independent experiments. Two-tailed Student's *t*-tests were used to analyze differences between groups. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Lipoplex-induced transformation of E. coli

First, we tested a feasibility of *n*-stearylamine/pGEX-2T plasmid complexes (lipoplexes) as a tool for the transformation of *E. coli* by measuring the transformation efficiencies over time. As shown in Fig. 1A, the CFU values increased with incubation time and reached a plateau at around 50–60 min when 15 mM (*n*-stearylamine concentration) lipoplexes were simply mixed with growing cells for each indicated time and then colonies were obtained on an agar plate. At this time, the maximal CFU value was approximately 2.5×10^6. In contrast, further incubation over 60 min reduced the CFU value, although the reason for this reduction is unclear. On the basis of this result, we used 60 min as the time required for effective transformation of *E. coli* throughout the studies. As a control experiment, however, no colony formation was observed regardless of the incubation time when *n*-stearylamine was used in the absence of plasmid DNA (data not shown). Also, when the experiment was repeated with pET-24a (+) plasmid containing kanamycin-resistance gene, the transformation efficiency and the incubation-time dependency were similar to those using pGEX-2T vector (data not shown). Therefore, these results suggest that the present method is independent on the type of antibiotic-resistant