Ethanol-induced Enhancement of the Transformation of *Escherichia coli* by Plasmid DNA

Suchitra Sarkar, Sujan Chaudhuri and Tarakdas Basu*

Department of Biochemistry and Biophysics,
University of Kalyani, Kalyani 741235, India

The transformation efficiency (TR) of CaCl₂-treated competent *E. coli* cells by plasmid DNA decreased gradually by the presence of increasing ethanol concentration (up to 10% v/v) in the cell population during transformation. This was due to ethanol-induced leaching of lipopolysaccharide (LPS) from the competent cell surface. On the other hand, when the ethanol was removed after treatment of the competent cells for 30 min and then the cells were allowed to transform, the (TR) enhanced compared to that of control cells; the enhancement was found to be maximum (> 100%) by the treatment of the cells with 5% (v/v) ethanol.

Keywords: ethanol, *E. coli*, calcium chloride, plasmid DNA, LPS, transformation efficiency

The fast increasing use of gene technology has necessitated re-evaluation and development of its basic methods, such as the cell transformation by DNA through introduction of foreign DNA. *E. coli* is normally very poor at taking up DNA from an external source. Bacterial cells, which can take up foreign DNA, are described as 'competent'. This occurs when the cells are suspended in ice-cold calcium chloride (Mandel & Higa, 1970). The standard method of transformation of *E. coli* involves DNA binding to the cell surface in the presence of CaCl₂ (50-100 mM) at 0°C and subsequent entry of the DNA to the cell cytosol by a brief heat pulse from 0°C to 42°C (Cohen et al., 1972). Besides Ca²⁺ ions, other frequently used cations include Mn²⁺, Mg²⁺, Rb⁺ for competence generation (Hanahan, 1983). However, the mechanism by which *E. coli* cells take up DNA and why this is stimulated by these treatments is still largely mysterious.

Since the transformation process is a membrane-bound phenomena, authors chose to observe the influence of ethanol, a well-known membrane perturbant, on this process. The intercalation of ethanol molecules, within *E. coli* membrane lipids, causes multidimensional effects, viz. alteration of membrane lipid composition (Ingram & Butlke, 1984; Ingram, 1986) and membrane fluidity (Dombek & Ingram, 1984), perturbation of the assembly of membrane proteins to their exact locations (Enquist et al., 1981; Chen & Tai, 1987; Maneewannakul et al., 1992; Basu & Poddar, 1997), inhibition of bacteriophage 6X 174 infection (Basu & Poddar, 1994a) and influences membrane-bound phenomenon such as DNA replication (Basu & Poddar, 1994b). Any alteration of the (TR) of *E. coli* cell by treatment with ethanol is, therefore, very likely to throw some light on the basic mechanism behind the process of transformation. In this brief communication, the basic results of investigations on the role of ethanol over the transformation of *E. coli* by plasmid DNA are presented.

The bacterial strain used for transformation was XL1-Blue, a genetically engineered highly transformable strain of *E. coli*. The plasmid DNA used was the cloning vector pUC19. Dehydrated ethanol was purchased from Bengal Chemical and Pharmaceutical works Limited, Kolkata, and was used without further purification.

Cells were transformed with the plasmid DNA by the standard method of CaCl₂ treatment (Sambrook et al., 1989). Cells were grown to log phase [up to (O.D.)₆₀₀ = 0.10 i.e. ~5 × 10⁷ cells/ml] in Luria Broth (LB), washed, and concentrated 25 times in ice-cold 100 mM CaCl₂. DNA (no more than 50 ng in a volume of 10 µl) was added to 200 µl of competent cell suspension and was allowed to incubate at 0°C for 30 min. The cell-DNA complex was then transferred to 42°C for exactly 90 sec and was rapidly chilled in ice for 5 min. 800 µl LB was then added and the cells were allowed to incubate at 37°C for 45 min. At the next step, cells were serially diluted with chilled tryptophan broth (1.0 g bactotryptone, 0.5 g NaCl in 100 ml H₂O, pH 7.5). 100 µl cells from properly diluted samples were spread on agar-LB medium, prepared with and without Ampicillin (50 µg/ml), to obtain the countable number of transformants and viable cells, respectively. (TR) was defined as the ratio of the number of transformants to that of the viable cells.

Three sets of experiments were performed with the competent cells. In set I, competent cells were treated...
with different concentrations of ethanol for 30 min at 0°C, DNA was then added and the subsequent steps of transformation were performed to study the (TR)_E. In set II, DNA was first added to the competent cells to allow its adsorption to the cell surface for 30 min at 0°C, different concentrations of ethanol were then added to treat the cell-DNA complex at 0°C for another 30 min followed by subsequent steps of transformation. In set III, after treating the competent cells with different concentrations of ethanol for 30 min at 0°C, ethanol was removed; cells were washed two times by centrifugation and finally suspended in 0.1 M CaCl_2. To these cells, DNA was added and the steps of transformation were continued to observe (TR)_E.

To investigate the leaching of lipopolysaccharide (LPS), if any, cells were taken at a concentration of 1.25 x 10^11 cells/ml, treated with different concentrations of ethanol for 30 min at 0°C, centrifuged and the supernatants were collected to assay for the presence of LPS, which was assayed by quantitation of the sugar acid, 2-keto-3-deoxyoctulosonate (KDO), a unique sugar present in LPS (Raetz, 1990). In the colorimetric method used to estimate KDO (Ashwell, 1966), per-iodate oxidation of KDO yielded β-formyl pyruvate, which then reacted with 2-thiobarbituric acid to form a characteristic chromophore having absorption maxima at 549 nm. From the standard curve [known amount of E. coli K-12 LPS vs. (OD)549 of the chromophore], the amount of leached LPS in the supernatants of ethanol-treated competent cells was calculated.

In set I, the (TR)_E of E. coli cells was found to decrease with the increasing concentrations of ethanol (Fig. 1, curve A). At 2.5, 5.0 and 10% v/v ethanol concentrations, with reference to control cells, (TR)_E reduced by 10, 70 and 95%, respectively despite no cell death by the presence of 2.5 and 5.0% v/v ethanol and only 20% cell death by 10% v/v ethanol (data not shown). Assuming that ethanol could affect the DNA binding process to the cell surface, when previously bound DNA-cell complex was treated with the different concentrations of ethanol as in the case of set II, almost similar reduction of (TR)_E was observed (Fig. 1, curve B).

By one hypothesis (Micklos & Freyer, 1990), DNA passes through any one of the hundreds of channels formed at adhesion zones, where the outer and inner cell membranes fuse to pores in the cell wall. The zones of adhesion are rich of LPS molecules (Osborn & Wu, 1980). Since, both DNA and LPS are negatively charged molecules, DNA cannot enter the cell easily as the two negative polarities repel each other. The divalent cation such as calcium, forming stable co-ordination complexes with phosphates, may facilitate the otherwise unlikely association of the two phosphate rich structures like DNA and LPS. The CaCl_2-mediated reorientation of LPS on the E. coli cell surface might have a role on the artificial transformation of E. coli by plasmid DNA (Hanahan, 1983). It was further reported that ethanol caused partial leaching of LPS from E. coli cell surface (Basu & Poddar, 1994a). Whether the release of LPS also took place from the surface of the competent cells, the supernatants of the ethanol-treated cells were checked for the presence of leached LPS. The result (Fig. 2) indicated that the amount of leaching was directly proportional to the concentration of ethanol and approximately 110, 137 and 240 μg of LPS leached out to the suspending medium by the presence of 2.5, 5 and 10% v/v ethanol, respectively. Assuming the dry weight of an E. coli cell to be of the order of 10^{12} g (Setlow & Pollard, 1962) and 3.5% of E. coli dry weight is due to LPS (Luberitz et al, 1968), the above amounts of leached LPS were estimated to be 25, 40 and 55% of total cellular LPS. Thus, it can be stated that the binding of a part of DNA to the free LPS leached by ethanol (set I) and leaching of a part of DNA-bound LPS by ethanol (set II) decreased the effective concentration of DNA for entry into the cells, thereby reduced the (TR)_E. Similar reduction of bacteriophage φX174 infectivity in E. coli occurred due to ethanol-induced leaching of LPS from cell surface (Basu & Poddar, 1994a). The results also
indicated that the release of up to 25% of cellular LPS, due to treatment with 2.5% v/v ethanol, had no considerable effect on (TR)_E.

In contrast, curve C of Fig. 1 indicates that when competent E. coli cells were treated with different concentrations of ethanol for 30 min at 0°C and then the ethanol was removed by centrifugation (set III), the addition of DNA to these cells and subsequent transformation steps increased the (TR)_E. The increment was found to be 15, 115 and 70%, by the pretreatment of the cells respectively, with 2.5, 5.0 and 10% v/v ethanol, over the untreated control cells. As, in this case, the ethanol-leached LPS from the cell surface was removed together with ethanol, no loss of plasmid DNA due to binding with the free leached LPS took place and thus no inhibition of (TR)_E was observed. However, for 10% v/v ethanol-treated cells, (TR)_E reduced nearly by 20% from that of the 5% v/v ethanol-treated cells; this was because 10% v/v ethanol caused approximately 20% cell killing (data not shown). The investigation to understand increase of (TR)_E is going on. Partial results (unpublished) of this study on the ethanol-induced release of the periplasmic protein alkaline phosphatase from the competent cell membrane to the extra-cellular medium, indicated membrane disintegration, which might be responsible for increase in transformation. This improved method of plasmid DNA uptake by E. coli cells, pre-treated with ethanol, will obviously have its application in recombinant DNA technology to obtain more transformed clones.

Acknowledgement
Authors are indebted to the Department of Science & Technology, Government of India, New Delhi, for financial assistance.

References