Study of mechanisms of electric field-induced DNA transfection I

DNA entry by surface binding and diffusion through membrane pores

Ting-Dong Xie, Lei Sun, and Tian Yow Tsong
Department of Biochemistry, University of Minnesota College of Biological Sciences, St. Paul, Minnesota 55108 USA

ABSTRACT A study of mechanisms of electrotransfection using *Escherichia coli* (JM105) and the plasmid DNA pBR322 as model system is reported. pBR322 DNA carries an ampicillin resistance gene: *E. coli* transformants are conveniently assayed by counting colonies in a selection medium containing 50 μg/ml ampicillin and 25 μg/ml streptomycin. Samples not exposed to the electric field showed no transfection. In the absence of added cations, the plasmid DNA remains in solution and the efficiency of the transfection was $2 \times 10^6/\mu$g DNA for cells treated with a 8-kV/cm, 1-ms electric pulse (square wave). DNA binding to the cell membrane greatly enhanced the efficiency of the transfection and this binding was increased by millimolar concentrations of $CaCl_2$, $MgCl_2$, or NaCl ($CaCl_2 > MgCl_2 > NaCl$). For example, in the presence of 2.5 mM $CaCl_2$, 55% of the DNA added bound to *E. coli* and the transfection efficiency was elevated by two orders of magnitude ($2 \times 10^8/\mu$g DNA). These ions did not cause cell aggregation. With a low ratio of DNA to cells (<1 copy/cell), transfection efficiency correlated with the amount of DNA bound to the cell surface irrespective of salts. When the DNA binding ratio approached zero, the transfection efficiency was reduced by two to three orders, indicating that DNA entry by diffusion through the bulk solution was <1%. Square pulses of up to 12 kV/cm and 1 ms were used in the electrotransfection experiments. When cell concentration was 1×10^{10} cell/ml and DNA was added before the pulse, a transfection efficiency of up to $5 \times 10^8/\mu$g DNA was obtained under optimum conditions (a single pulse of 8 kV/cm, 1 ms, in the presence of 5 mM $CaCl_2$). When DNA was added to *E. coli* after the electric pulse, the efficiency of the transfection was dramatically reduced owing to the resealing of pores. Transfection was reduced to zero when DNA was added 2 h after the electroporation. However, transfection as high as $5 \times 10^4/\mu$g DNA was still recorded when DNA was added 10 min after the electric field was turned off. Because DNA entry took place in the absence of an electric field it could not be driven by the electrophoretic forces. DNA entry was facilitated by surface binding followed by lateral diffusion of the bound DNA into the cells through the field-induced membrane pores.

INTRODUCTION

Cell membranes are charged and are highly sensitive to electric perturbations (1). In 1967 and 1968, Sale and Hamilton reported that when suspensions of yeast, bacteria, and erythrocytes were treated with intense electric pulses in the range of kilovolts per centimeter and of microsecond duration, their membranes were ruptured and these cells were instantly killed (2, 3). Neumann and Rosenheck (4), using milder conditions, found that the permeability of chromaffin granules could be reversibly modified by discharging the electrical energy stored in a capacitor into the suspension of these organelles. Independently, Kinosita and Tsong studied effects of intense, pulsed electric fields (PEF) on human erythrocytes and presented evidence that a primary effect of the electric field was the implantation of aqueous pores of limited size into the cell membranes (5–7). It was shown that electric field-induced pores could reseal under some conditions and the cytoplasmic macromolecular contents could be retained (5–7). Several laboratories have focused their attention on the study of mechanisms of pore formation and characterization of the electric field modified cell membranes (5–16). As electrically induced pores are relatively stable at temperatures below 15°C and can readily reseal at higher temperatures, e.g., 37°C, it has been suggested that electroporation could be used to introduce normally impermeant molecules into living cells (6, 17, 18). Tracer molecules loaded into mouse erythrocytes were shown to be retained in the mouse circulation, indicating that electrically treated erythrocytes were surviving and electric fields did not substantially alter the properties of the cell membranes (17). In 1982, Wong and Neumann (19) described transfection of a foreign gene into eukaryotic cells by the electroporation method. They observed that the transfected gene was expressed in the host cells.

Electroporation has now been successfully applied to the transfection of mammalian cells, plant protoplasts, yeast spheroplasts, and bacteria (20–29). Electrotransfection has certain advantages over the conventional chemical methods, e.g., the use of calcium chloride to facilitate DNA entry into cells. First, it is convenient and requires

Address correspondence to Dr. Tsong.

little special skills when a commercial instrument is available. Second, sometimes mammalian cells or plant cells that are difficult to transfect by the chemical methods can be more easily transfected by the electroporation method. Third, transfection efficiency is generally higher with the electroporation method than with chemical methods. However, mechanisms of electrotransfection remains unclear. Because of the lack of mechanistic information in this area, success or failure of an electrotransfection experiment depends on trial, error, and luck. Moreover, despite the fact that transfection efficiencies as high as 0.1% have been regularly obtained, improvement beyond this value has been difficult to achieve and the benefits of this method are limited. Further improvement in the transfection efficiency is essential if electrotransfection is to be applicable to gene therapy in a clinical setting. *Esecherichia coli* and the plasmid DNA pBR322 have been chosen as a model system because of the relative ease in growing cells and in assaying for the transformants.

MATERIALS AND METHODS

Instruments

Cober 605P high voltage generator was employed. This instrument can deliver up to 2.2 kV. It can produce rectangular pulses with a width from 50 ns to 10 ms at a repetition rate of up to 1 MHz. The chamber for the cell suspension was made of two hollow cylindrical stainless steel blocks, which were tightened into a plexiglass cylinder. Two platinum sheets, which were in contact with the steel blocks, served as electrodes (5, 20). The distance between the two electrodes was 0.15 cm and the total volume of cell chamber was 50 μl. The temperature of the sample was maintained at ~ 4°C by circulating water through the hollow steel blocks. After applying an electric pulse, the sample temperature was measured by a microthermistor which was placed directly in the cell chamber. Temperature changes due to pulsed electric fields were < 10°C in all cases and the temperature of a sample returned to its initial value within 20 s. The voltage drop between the two electrodes was measured directly with a circuit box and displayed on a storage oscillocope (model 5103; Tektronix, Inc., Beaverton, OR).

Media for DNA binding and electroporation experiments (BP medium)

Three media of different ionic compositions were used. The first contained 0.1 mM Tris buffer pH 7.4, varying concentrations of $MgCl_2$ from 0.05 to 10 mM and a suitable concentration of sucrose to make the solution isotonic. The second was identical to the first, except that $MgCl_2$ was replaced with $CaCl_2$. The third was also identical to the first, except that up to 40 mM NaCl was substituted for $MgCl_2$.

Cell cultures and preparations

E. coli strain JM105 was grown in Luria-Bertani (LB) medium which contained 10 g Bacto-tryptone, 5 g Bacto-yeast extract, and 5 g NaCl per liter, with vigorous shaking at 37°C. The overnight culture grown to stationary phase was diluted 1:100 into LB medium and grown to mid log phase (specific optical density OD_{600nm} of 0.5–1.0). The cells were harvested and centrifuged at 4,000 *g* for 10 min at 4°C and washed twice with the appropriate Binding and Pulsing Medium (BP medium), and resuspended to desired cell concentrations. The cells were maintained on ice before use.

Preparation of ^{32}P-labeled plasmid DNA

Plasmid pBR322 DNA which contains the ampicillin resistant gene was radioactively labeled with ^{32}P-dCTP by the nick translation method, using a Du Pont Nick Translation System (NE004). A mixture containing 20 μl of translation buffer, 16 μl of dNTP, 40 μl of [^{32}P-α]dCTP (3,000 Ci/mmol, 10 mCi/ml), 20 μl of water, 8 μl of pBR322 DNA (250 μg/ml), 8 μl of DNA polymerase I, and 8 μl of DNAse I was incubated at 12°C for 1 h. 5 μl of stop buffer was added to terminate the reaction. The labeled DNA was treated with 4 μl of T4 DNA Ligase and 25 μl 5X Ligation Buffer and incubated at 12°C overnight to ligate the nicks. Labeled plasmid pBR322 DNA was separated from free nucleotides using a Sephadex G-50 column with a TE buffer.

DNA binding protocol

E. coli suspensions (10^6–10^{11} cells/ml) were prepared in appropriate BP media. For each 100-μl sample, 1 ng of labeled pBR322 DNA (140 k CPM) at a concentration of 0.01 μg/ml was added. The mixture was incubated on ice for 5 min and centrifuged at 4,000 *g* for 10 min. The radioactivity of the supernatant and the pellet were measured by a 1600CA liquid scintillation analyzer (1600CA; Packard Instrument Co. Inc., Downers Grove, IL). The binding ratio was defined as the ratio of CPM's of the pellet and total CPM's of the sample. To investigate time course of DNA binding, plasmid DNA and *E. coli* were incubated for different lengths of time before centrifugation. Pelleting of cells took 10–15 s and we assumed negligible DNA binding after the cells were tightly packed.

Electroporation protocol

50 μl of cell suspension and 0.5 ng of pBR322 DNA (0.01 μg/ml) were incubated on ice for 5 min. Single electric pulses (square wave) of amplitudes of up to 12 kV/cm and of widths of up to 8 ms were applied at 4°C. Cells were immediately transferred to a 1.5-ml test tube which contained 500 μl of LB medium. The cells were then incubated at 37°C for 30 min and 100-μl aliquots were subsequently plated. Transformants were determined, 12 h later, by counting colonies on LB-Agar selective plates which contained 15 g agar, 10 g Bacto-tryptone, 5 g Bacto-yeast extract, and 5 g NaCl, 50 mg ampicillin, and 25 mg streptomycin per liter.

RESULTS

Effects of cations on DNA binding to *E. coli*

In the presence of millimolar concentrations of cations, either Ca^{2+}, Mg^{2+}, or Na^+, pBR322 DNA rapidly bound to *E. coli*. Time courses of DNA binding in the presence

of 2.5 mM $MgCl_2$ or 2.5 mM $CaCl_2$ are shown in Fig. 1 *A*. At 25°C, binding was completed within 30 s. The equilibrium binding in the presence of different concentrations of $MgCl_2$, $CaCl_2$, and NaCl are shown in Fig. 1 *B*. In these experiments, each sample (100 μl) contained 1×10^9 cells and 1 ng pBR322 DNA (2.1×10^8 copies of DNA/ml). Under these conditions the maximum percentage of cells that could bind DNA at one copy/cell was 21%. A low DNA ratio was chosen for studying transfection efficiency to avoid binding of multiple copies of DNA to a single cell where multiple equilibria of DNA binding would complicate the analysis. $CaCl_2$ appeared to be the most effective in facilitating DNA binding. The binding constant, K_b, of DNA in the presence of 10 mM $CaCl_2$ was $\sim 6.7 \times 10^{10}$ M^{-1}. $MgCl_2$ (10 mM) was slightly less effective (K_b of 4.0×10^{10} M^{-1}) than $CaCl_2$ but was much more effective than the monovalent ion NaCl (K_b of 1.47×10^{10} M^{-1}). The binding curves in the presence of 2.5 mM $CaCl_2$ and $MgCl_2$ with varying cell concentrations are shown in Fig. 1 *C*.

Transfection efficiency correlated with DNA binding to the cell surface

$CaCl_2$ facilitated DNA binding and also dramatically enhanced the efficiency of the electrotransfection. For example, with a 6-kV/cm, 1-ms electric pulse, the transfection efficiency was $5 \times 10^6/\mu$g DNA in the presence of 0.5 mM $CaCl_2$, but increased to $2 \times 10^8/\mu$g DNA in the presence of 2.5 mM $CaCl_2$. Similarly, $MgCl_2$ and NaCl (millimolar concentrations) improved transfection efficiency although not as effectively as $CaCl_2$. Transfection efficiency vs. salt concentration for $CaCl_2$, $MgCl_2$, and NaCl is shown in Fig. 2 *A*. By extrapolating these three curves to very low cation concentrations, a transfection efficiency of $<1 \times 10^6/\mu$g DNA may be obtained. Fig. 2 *B* plots transfection efficiency as a function of the binding ratio of DNA in the presence of different concentrations of NaCl, $CaCl_2$, and $MgCl_2$. This figure clearly demonstrates that transfection efficiency correlated with the quantity of DNA bound to the cell surface but not with the chemical property of salts.

Dependence of transfection efficiency on electric field strength

When the width of the pulsed electric field (single square wave pulse) was fixed at 1 ms, the logarithm of the transfection efficiency increased with the increasing field strength up to 6 kV/cm. Beyond this value the efficiency plateaued and began to decline above ~8 kV/cm. The survival rate of *E. coli* subjected to the electric field also declined above this field strength. Fig. 3 *A* gives an example of such an experiment in the presence of different concentrations of $MgCl_2$ and Fig. 3 *B* compares results of

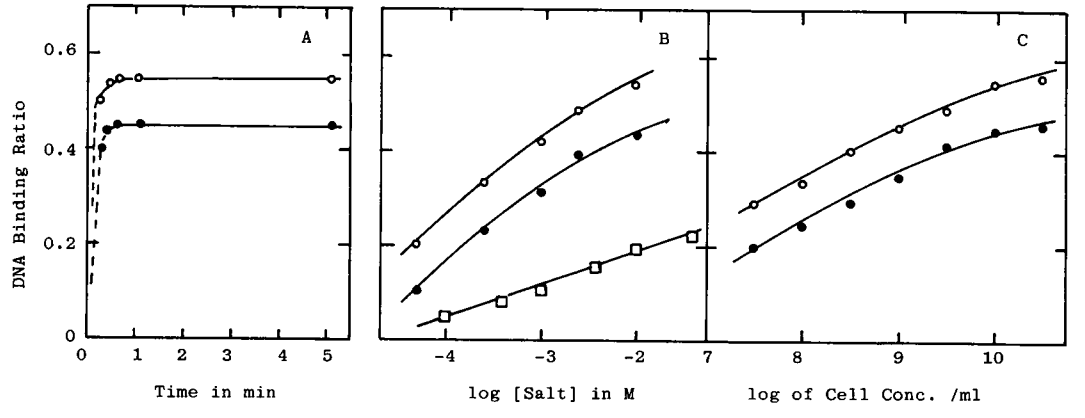

FIGURE 1 Effects of $CaCl_2$, $MgCl_2$, and NaCl on the DNA binding to the cell surface of *E. coli*. (*A*) Time course of DNA binding. Cells were suspended in a BP medium containing 0.1 mM Tris buffer at pH 7.4, and 2.5 mM of $MgCl_2$ (●) or 2.5 mM $CaCl_2$ (○). 1 ng of labeled pBR322 DNA (concentration was 0.01 μg/ml [140 K CPM]) was added to 100 μl of cell suspension (cell concentration was 1×10^{10}/ml). At different time intervals (from 5 s to 5 min), aliquots were assayed for binding by centrifugation as described in Methods. Pelleting of cells took ~10 s, although centrifugation was continued for 10 min. Results using a filtration method to separate the supernatant and the cells was similar to the data shown here. (*B*) Dependence of DNA binding on salt concentration. Experimental conditions were the same as in (*A*) except that salt concentrations ranging from 0.1 to 10 mM for $CaCl_2$ and $MgCl_2$ and to 40 mM for NaCl were used. After the addition of DNA, cells were incubated on ice for 5 min before centrifugation. The fraction bound, or DNA binding ratio, was experssed as CPM's in the pellet divided by the combined CPM's of the pellet and the supernatant. Data obtained with NaCl are given in (□). Other symbols are the same as those in (*A*). (*C*) Dependence of DNA binding on cell concentration. Experimental conditions were the same as in (*A*) except that cell concentration was varied in the presence of 2.5 mM $MgCl_2$ (●) or 2.5 mM $CaCl_2$ (○).

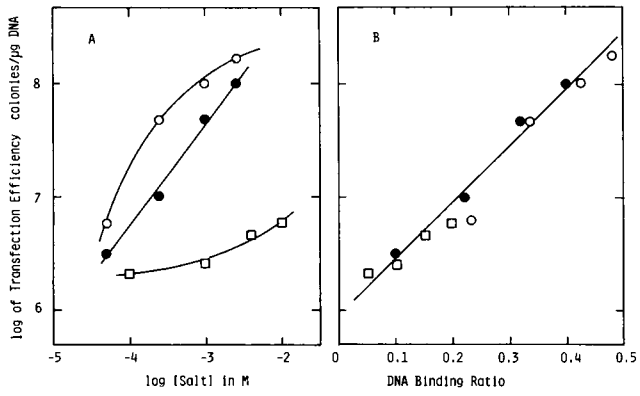

FIGURE 2 Effects of $CaCl_2$, $MgCl_2$, and NaCl on the efficiency of transfection of *E. coli* by plasmid DNA pB322. (*A*) Dependence on salt concentration. Experiments were done in the BP medium containing different concentrations of $CaCl_2$ (○), $MgCl_2$ (●), and NaCl (□). Each sample contained 0.5 ng DNA and 50 µl of *E. coli* suspension (1.1×10^{10} cells/ml). A single pulse of 8 kV/cm and of 1-ms duration was applied at 4°C. Thereafter, the cell suspension was transferred to 500 µl of LB medium and plated as described in Methods. The logarithm of the transfection efficiency is plotted against the logarithm of the salt concentration. (*B*) Data in (*A*) are plotted as transfection efficiency vs. the DNA binding ratio. Symbols are identical to those used in (*A*).

such an experiment in the presence of $MgCl_2$ and NaCl. A field strength of 11 kV/cm resulted in 50% cell death.

Time and sequence of DNA addition and electroporation

Transfection efficiency was nearly constant regardless of the incubation time if DNA had been added before the application of the electric pulse. The transfection efficiency reached its maximum as short as 10 s after DNA addition. This is not surprising because DNA binding was fast and was completed in the 10-s time range as shown in Fig. 1 *A*. In Fig. 4, a negative value on the time axis indicates that DNA was added before the PEF treatment of cells and a positive value indicates the opposite sequence of DNA addition and PEF treatment. It was technically difficult to perform the experiment for times shorter than 10 s. The most remarkable results of this experiment is the finding that transfection still occurred when DNA was added to *E. coli* 10 min after these cells were treated with an electric pulse (width of 1 ms). There was a dramatic reduction in the transfection efficiency from 2×10^8 to 2×10^4/µg DNA if DNA was added 10 s after the PEF treatment. Yet transfection of DNA could still occur 10 min after the electric field was turned off. However, DNA added 2 h after electroporation failed to transfect above control values.

DISCUSSION

Efficiency of electrotransfection

DNA transfection efficiency is commonly expressed by counting the number of transformants formed per microgram of transfecting DNA using a low DNA/cell ratio. In our study, a value of 5×10^8 transformants/microgram DNA was obtained under optimizing conditions. Another way of expressing transfection efficiency might be the percentage of cells transfected in the presence of excess DNA. Although our experiments were

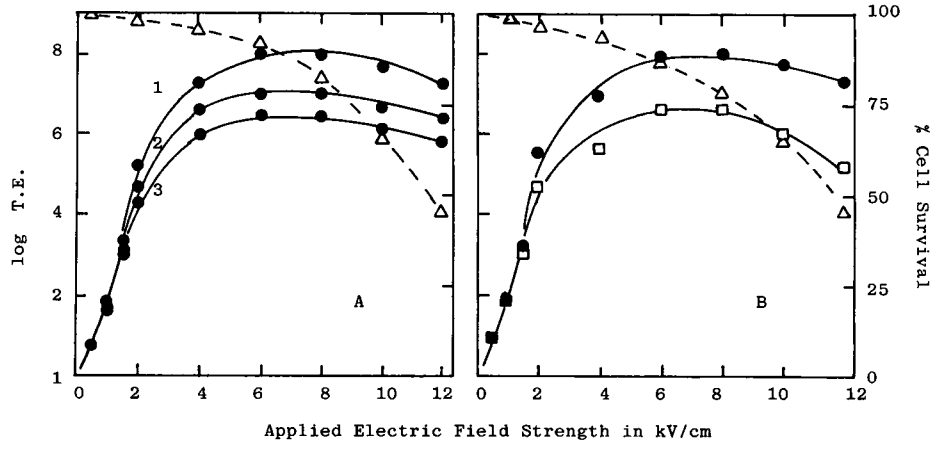

FIGURE 3 Cell survival and transfection efficiency as functions of applied field strength. (*A*) Experiments with varied $MgCl_2$ concentration. Electroporation was done in the BP medium containing 0.05 mM (*curve* 3), 0.25 mM (*curve* 2), and 2.5 mM (*curve* 1) of $MgCl_2$. A single electric pulse of 1-ms duration with varied pulse width was applied and transfection efficiency was determined as described in Methods. Logarithm of the transfection efficiency (●) and percentage of cell survival (△) as a function of the applied electric field strength are shown. Cell survival was done in the presence of 2.5 mM $MgCl_2$ but survival was similar for other concentrations of $MgCl_2$. (*B*) Comparison of transfection efficiency in $MgCl_2$ (●) and NaCl (□). Experimental procedures are identical to Fig. 3 *A* and to Fig. 1. The concentration of $MgCl_2$ was 2.5 mM and of NaCl was 10 mM.

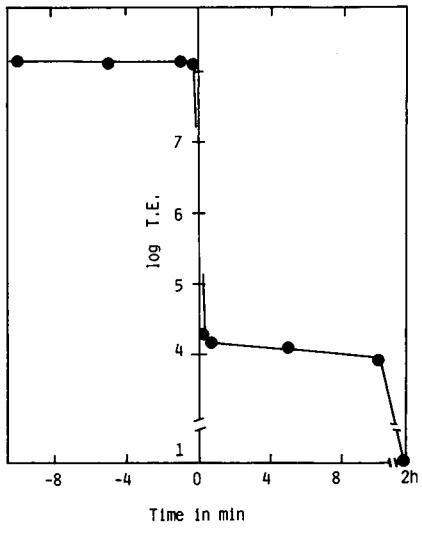

FIGURE 4 Dependence of transfection efficiency on the time and sequence of DNA addition and electroporation. Experiments were done in the BP medium containing 2.5 mM $MgCl_2$. Each sample contained 0.5 ng DNA and 50 μl *E. coli* suspension (1.1×10^{10} cells/ml). Cells were treated with a single electric pulse of 8 kV/cm and of 1-ms duration. Logarithm of the transfection efficiency is plotted against the time of DNA addition. A negative value on the time axis indicates that DNA was added before the application of the electric pulse and a positive value indicates that the electric pulse was applied before the addition of DNA. No transfection was observed for control samples that were not treated with the electric field. Likewise, when DNA was added 2 h after treatment with the electric field, no transfection was observed.

done using conditions with low DNA/cell ratios, just to get an estimate, let us consider a typical experiment. A sample has a volume of 100 μl and contained 1×10^9 cells to which 1 ng of DNA (molecular weight of 2.88×10^6) was added. 1 ng contains 2.1×10^8 copies of pBR322 DNA, 50% of which bound to the cells (Fig. 1 *B*). Therefore, of the 1×10^9 cells in the sample, only 1×10^8 or 10% of the cells had a copy of plasmid DNA bound to the plasma membrane. With a transfection efficiency of $5 \times 10^8/\mu$g DNA, the number of colonies counted for a sample was 5×10^5. This gives a 0.5% transfection for cells that carried at least one copy of the plasmid DNA. If we also considered that under these conditions only 70% of the cells were surviving, the actual transfection would be 0.7%. It is possible that a fraction of cells that had taken up one copy or more of DNA through electroporation was much greater than 0.7%. In such a case, not every cell transfected with the plasmid DNA would end up a transformant.

Mechanisms of electrotransfection

Several possible mechanisms may be considered. The first one assumes that a DNA molecule in solution will diffuse through the "aqueous pores" induced by the pulsed electric field (Fig. 5 *A*). Data in Fig. 2, *A* and *B* showed that in the absence of DNA binding (zero binding ratio), transfection efficiency remained high ($1 \times 10^6/\mu$g DNA) when cells were treated with one pulse of 8-kV/cm, 1-ms electric field. However, a millimolar concentration of salts that facilitated DNA binding also greatly improved the transfection efficiency by two to three orders of magnitude. These data suggest that transfection by the first mechanism is relatively unimportant and point to the second mechanism, that is, DNA entry by the surface binding after lateral diffusion and penetration of the DNA through the membrane pores (Fig. 5 *B*). Our data indicate that <1% of the transfection was by the diffusion of DNA through the bulk solution. More than 99% of the transfections occurred by the second mechanism. Because the experimental uncertainty of assaying DNA binding was ~1%, it may be concluded that the electrotransfection was exclusively by the second mechanism.

Electrophoresis vs. surface diffusion

Another question pertains to whether the DNA entry into a cell is by the electrophoretic movement of DNA

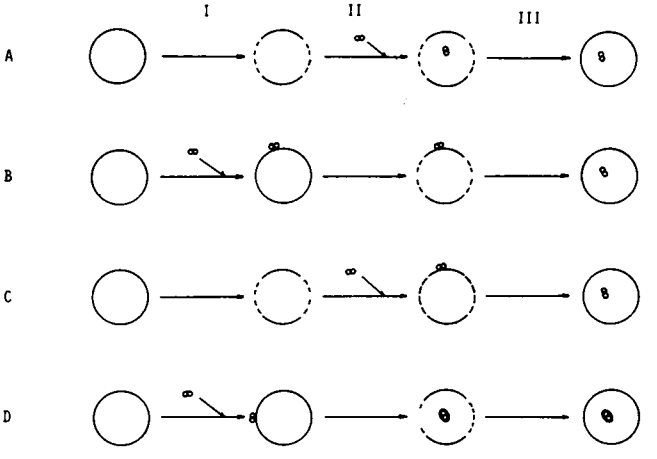

FIGURE 5 Mechanisms of DNA transfection by the electroporation method. Four different models are considered. In (*A*), after the electroporation of a cell (Step I), circular and superhelical pB322 DNA (indicated by ∞) diffuses into the cell through the bulk solution (Step II). Cell membranes reseal (Step III). In (*B*), DNA binds to the cell surface and then enters the cell through surface diffusion. In (*C*), an experiment shown in Fig. 4 is illustrated. Here electroporation is done before the addition of DNA (Step II). DNA binds to the cell surface and diffuses into the cell. Because the size of the pores is reduced during the incubation time (the time between electroporation and addition of DNA), the transfection efficiency is much smaller than that of (*B*). In (*D*), DNA is assumed to enter the cell by the electrophoretic force of the applied field. When DNA enters, it carries with it a piece of cell membrane and is protected by a shell of lipid. See text for details.

(Fig. 5 *D*). This idea would be consistent with the electroosmosis phenomenon reported by Dimitrov and Sowers (14). Cations are electrophoretically driven toward the cathode in the electroporation experiments. Likewise, highly charged DNA would be electrophoretically driven across a cell membrane at the locus where the membrane is positively polarized. Also, Chernomordik et al. (31) reported that DNA taken by lipid vesicles resulting from electroporation was inaccessible for binding by ethidium bromide. These authors suggested that a DNA molecule might be electrophoretically driven across the lipid bilayer and in the process the DNA molecule became enclosed by a layer of lipid. Our data in Fig. 4 cannot be explained by the electrophoretic movement of DNA because transfection of *E. coli* was observed in the absence of an electric field. Although the transfection efficiency was much lower in this case compared with that using the first protocol, i.e., addition of DNA before electric pulse, it was several orders of magnitude greater than that of the background, which was usually zero. One must conclude that electrophoresis is not required for electrotransfection. The reduced transfection efficiency for data in the right half of Fig. 4, where DNA was added after the PEF treatment of cells, was due to resealing of pores (Fig. 5 *C*). In human erythrocytes, electrically induced pores are known to shrink in size in milliseconds and reseal in minutes to hours (16, 32). In our experiments, DNA added 2 h after the electroporation was unable to transfect and the efficiency of transfection decreased to control levels. Presumably, after the 2 h incubation, electric pores had resealed, preventing further DNA entrance into the *E. coli*. *E. coli* has a peptidoglycan/lipopolysaccharide cell wall that precedes the cell membrane. It is not known whether lateral diffusion of bound DNA can take place on the surface of the cell wall or in the space between the cell wall and the cell membrane before it enters the cell. Thus, Fig. 5 *B*, while consistent with our data, must be considered tentative.

This work was supported by a grant from the Office of Naval Research.

Received for publication 14 December 1989 and in final form 8 March 1990.

REFERENCES

1. Cole, K. S. 1968. Membranes, Ions and Impulses. University of California Press, Berkely, CA.
2. Sale, A. J. H., and W. A. Hamilton. 1967. Effects of high electric fields on microorganisms. I. Killing of bacteria and yeasts. *Biochim. Biophys. Acta.* 118:781–788.
3. Sale, A. J. H., and W. A. Hamilton. 1968. Effects of high electric fields on microorganisms. III. Lysis of erythrocytes and protoplasts. *Biochim. Biophys. Acta.* 163:37–43.
4. Neumann, E., and K. Rosenheck. 1972. Permeability changes induced by electric impulses in vesicular membranes. *J. Membr. Biol.* 10:279–290.
5. Kinosita, K., and T. Y. Tsong. 1977*a*. Hemolysis of human erythrocytes by transient electric field. *Proc. Natl. Acad. Sci. USA.* 74:1923–1927.
6. Kinosita, K., and T. Y. Tsong. 1977*b*. Formation and resealing of pores of controlled size in human erythrocyte membrane. *Nature (Lond.).* 268:438–441.
7. Kinosita, K., and T. Y. Tsong. 1977*c*. Voltage induced pore formation and hemolysis of human erythrocytes. *Biochim. Biophys. Acta.* 471:227–242.
8. Benz, R., F. Beckers, and U. Zimmermann. 1979. Reversible electrical breakdown of lipid bilayer membranes: a charge-pulse relaxation study. *J. Membr. Biol.* 48:181–204.
9. Teissie, J., and T. Y. Tsong. 1981. Electric field induced transient pores in phospholipid bilayer vesicles. *Biochemistry.* 20:1548–1554.
10. Sowers, A. E., and M. R. Lieber. 1986. Electropore diameters, lifetimes, numbers, and locations in individual erythrocyte ghosts. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 205:179–184.
11. Chernomordik, L. V., M. M. Kozlov, S. L. Leikin, G. I. Abidor, and Y. A. Chizmadzhev. 1983. Breakdown of lipid bilayer membranes in an electric field. *Biochim. Biophys. Acta.* 812:643–655.
12. Chizmadzhev, Y. A., V. B. Arakelyan, and V. F. Pastushenko. 1979. Electric breakdown of bilayer lipid membranes. III. Analysis of possible mechanisms of defect origination. *Bioelectrochem. Bioenerg.* 6:63–71.
13. Chang, D. C. 1989. Cell poration and cell fusion using an oscillating electric field. *Biophys. J.* 56:641–652.
14. Dimitrov, D. S., and A. E. Sowers. 1990. Membrane electroporation-Fast molecular exchange by electroosmosis. *Biochim. Biophys. Acta.* In press.
15. Powell, K. T., A. W. Morgenthaler, and J. C. Weaver. 1989. Tissue electroporation. Observation of reversible electric breakdown in viable frog skin. *Biophys. J.* 1163–1171.
16. Serpersu, E. H., K. Kinosita, and T. Y. Tsong. 1984. Reversible and irreversible modification of erythrocyte membrane permeability by electric field. *Biochim. Biophys. Acta.* 812:779–785.
17. Kinosita, K., and T. Y. Tsong. 1978. Survival of sucrose-loaded erythrocytes in circulation. *Nature (Lond.).* 272:258–260.
18. Neumann, E., A. E. Sowers, and C. A. Jordan. 1989. Electroporation and Electrofusion in Cell Biology. Plenum Publishing Corp., New York. 436.
19. Wong, T.-K., and E. Neumann. 1982. Electric field mediated gene transfer. *Biochem. Biophys. Res. Commun.* 107:584–587.
20. Neumann, E., M. Schaefer-Ridder, Y. Wang, and P. H. Hofschneider. 1982. Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:841–845.
21. Potter, H., L. Weir, and P. Leder. 1984. Enhancer-dependent expression of the human kappa immunoglobulin genes introduced into mouse pre-B lymphocytes by electroporation. *Proc. Natl. Acad. Sci. USA.* 81:7161–7165.
22. Chu, G., H. Hayakawa, and P. Berg. 1987. Electroporation for the efficient transfection of mammalian cells with DNA. *Nucl. Acids Res.* 15:1311–1326.
23. Fromm, M. E., L. P. Taylor, and V. Walbot. 1986. Stable transformation of maize after gene transfer by electroporation. *Nature (Lond.).* 319:791–793.

24. Karube, I., E. Tamiya, and X. Matsuoka. 1985. Transformation of saccharomyces cerevisiae spheroplasts by high electric pulse. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 182:90–93.

25. Colvin, N. M., and P. C. Hanawalt. 1988. High-efficiency transformation of bacterial cells by electroporation. *J. Bacteriol.* 170:2796–2801.

26. Dower, W. J., J. F. Miller, and C. W. Ragsdale. 1988. High efficiency transformation of *E. coli* voltage electroporation. *Nucl. Acids Res.* 16:6127–6145.

27. Fiedler, S., and R. Wirth, 1988. Transformation of bacteria with plasmid DNA by electroporation. *Anal. Biochem.* 170:38–44.

28. Taketo, A. 1988. DNA transfection of *Escherichia coli* by electroporation. *Biochim. Biophys. Acta.* 949:318–324.

29. Cymbalyuk, E. S., L. V. Chernomordik, N. E. Broude, and Yu. A. Chizmadzhev. 1988. Electro-stimulated transformation of *E. coli* cells pretreated by EDTA solution. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 234:203–207.

30. Tsong, T. Y. 1987. Electric modification of membrane permeability for drug loading into living cells. *Methods Enzymol.* 149:248–259.

31. Chernomordik, L. V., A. V. Sokolov, and V. G. Budker. 1989. Electrostimulated uptake of DNA by liposomes. *Biologicheskie Membrany.* 6:212–217.

32. Kinosita, K., and T. Y. Tsong. 1979. Voltage induced conductance in human erythrocyte membranes. *Biochim. Biophys. Acta.* 554:479–494.