Transformation of *Escherichia coli* with Plasmid Deoxyribonucleic Acid: Calcium-Induced Binding of Deoxyribonucleic Acid to Whole Cells and to Isolated Membrane Fractions

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Plasmid deoxyribonucleic acid (DNA) was tightly bound to cells of *Escherichia coli* at 0°C in the presence of divalent cations. During incubation at 42°C, 0.1 to 1% of this DNA became resistant to deoxyribonuclease. Deoxyribonuclease-resistant DNA binding and the ability to produce transformants became saturated when transformation mixtures contained 1 to 2 μg of plasmid NTP16 DNA and about 5 × 10⁶ viable cells. Under optimum conditions, between 1 and 2 molecule equivalents of ³H-labeled NTP16 DNA per viable cell became deoxyribonuclease resistant. Despite this, only 0.1 to 1% of viable cells became transformed by saturating amounts of the plasmid. The results suggest that transport of DNA across the inner membrane is a limiting step in transformation. After transformation the bulk of labeled plasmid DNA remained associated with outer membranes. However, in vitro assays indicated that plasmid DNA would bind equally well to preparations of inner or outer membranes provided divalent cations were present. Divalent cations promoted differing levels of binding to isolated inner and outer membranes in the order Ca²⁺ > Ba²⁺ > Sr²⁺ > Mg²⁺. This parallels their relative efficiencies in promoting transformation. Binding of plasmid DNA was greatly reduced when outer membranes were treated with trypsin; this suggests that protein components may be required for the binding or transport of DNA (or both) during transformation.

Transformation of bacteria involves DNA binding to the cell surface followed by uptake across the wall-membrane complex into the cytoplasm. The binding and uptake processes have been most studied in *Bacillus subtilis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*, organisms which become naturally competent during growth (13). In contrast, *Escherichia coli* cells can take up plasmid (4, 22), chromosomal (7, 25), or plasmid (6, 10) DNA, but only in the presence of high concentrations of CaCl₂. The overall process is very inefficient, and at best only 1 to 2% of the cells surviving the CaCl₂ treatment are transformed (15, 26). Calcium ions promote DNA binding to the outer cell surface (19), but only a small proportion (<1%) of this bound DNA can be converted to a DNase-resistant form in a subsequent uptake step. It is not known whether this DNase-resistant material is taken solely into transformable cells, or whether all cells can take up the DNA but only a small proportion of these cells are capable of establishing the plasmid as a replicon. Consequently, a direct analysis of the uptake of transforming DNA molecules is difficult. All attempts to isolate the subpopulation of transformable cells have so far failed.

In this paper we have examined the factors which influence the binding of plasmid DNA to whole cells and to isolated inner and outer membrane fractions of *E. coli*. The results are discussed in terms of their relevance to transport of DNA during transformation.

**MATERIALS AND METHODS**

**Bacterial strains.** *E. coli* K-12 C600 was obtained from B. J. Bachmann (3). *E. coli* K-12 UB1139, *met thy leu nalA* (20), was used for the preparation of ³H-labeled plasmid DNA. *E. coli* K-12 W3805, *galE*, incorporated galactose predominantly into lipopolysaccharide and was obtained from J. P. Beard.

**Preparation of plasmid DNA.** Nonradioactive DNA of the nonconjugative plasmid NTP16 (molecular weight, 5.7 × 10⁹), which codes for resistance to ampicillin and kanamycin (2), was extracted from strain C600 (NTP16) by the method of Humphreys et al. (11).

Radioactively labeled NTP16 DNA was prepared.
from strain UB1139 (NTP16). The bacteria were grown overnight in 5 ml of M9 minimal medium (1) enriched with 1 μg of thymine, 40 μg of methionine, and 40 μg of leucine per ml. The cells were diluted 10-fold with fresh medium and incubated at 37°C with aeration for 2 h. At this time 4 mM of [methyl-3H]thymidine (specific activity 24 Ci/mmol; Radiocaltech Centre, Amersham, United Kingdom) was added, and the cultures were incubated until they reached an absorbance at 660 nm of 0.7. Chloramphenicol was then added to a final concentration of 50 μg/ml, and the cells were incubated for a further 2 h to allow amplification of the plasmid DNA.

Transformation. Cells of E. coli K-12 C600 were transformed under optimal conditions by the method previously described (5, 10). Frequencies of transformation are expressed as transformants per viable cell at the end of the expression period.

Isolation of inner and outer membranes. Membranes were isolated by the method of Osborn et al. (17). Cells of E. coli K-12 C600 were grown overnight in nutrient broth at 37°C without shaking. The overnight culture (50 ml) was diluted with 1 liter of prewarmed nutrient broth and incubated at 37°C with aeration until the cells reached an absorbance at 660 nm of 0.2. The cells were then harvested by centrifugation at 0°C, and all subsequent manipulations were carried out at this temperature. The cells were suspended in 0.75 M sucrose-10 mM Tris-hydrochloride (pH 7.8) to a final absorbance at 600 nm of 10. Lysosome was added to a final concentration of 100 μg/ml, and the mixture was incubated on ice for 2 min. The suspension was diluted twofold with the addition of 1.5 mM EDTA-0.2 mM dithiothreitol (DTT) at a rate of 1 ml/min. The suspension was then sonicated by using an MSE sonicator in 30-μ bursts at 4°C for a total of 2 min. The remaining intact cells and debris were removed by centrifugation (3,000 × g for 10 min in a Sorvall RC-5 centrifuge). The membranes were pelleted (40,000 × g for 90 min) and washed in 0.25 M sucrose-3 mM Tris-hydrochloride-1 mM EDTA-0.2 mM DTT (pH 7.5). Inner and outer membranes were separated on a 25-mL linear sucrose gradient (25 to 60%, wt/wt) by centrifugation at 4°C (95,000 × g for 17 h in an MSE swing-out rotor with three 25-mL tubes). Inner and outer membrane fractions were harvested by syringe, washed, and finally suspended in 25% (wt/vol) sucrose-5 mM EDTA-0.2 mM DTT (pH 7.5). The protein concentration of membrane fractions was determined by the method of Lowry et al. (14).

The purity of outer membrane fractions was checked by assaying succinic dehydrogenase activity (8) as an inner membrane marker. When D-[1-14C]glucose was added to growing cells of a K-12 strain lacking galactose-4-epimerase, all of the incorporated label was located in the outer membrane fractions, confirming the lack of cross-contamination between the membranes (see below). Membrane fractions were stored at 4°C for use in DNA-binding studies and were used within 1 week of preparation.

Binding of [3H]DNA to whole cells. E. coli K-12 C600 was grown overnight in nutrient broth at 37°C. The culture was diluted 1:20 in fresh, prewarmed broth, and incubation was continued for 90 min. The cells were harvested by centrifugation, washed once in 10 mM CaCl2 at 0°C, and finally suspended in 1/10 of the original culture volume of 75 mM CaCl2. This procedure is known to produce cells of optimal transformation efficiency (5, 10). Various amounts of [3H]labeled NTP16 DNA were added to transformation mixtures. The mixtures were kept at 0°C for 45 min and then transferred to 4°C for 10 min. The cells were washed three times by centrifugation in a Burhak microfuge type 320 (14,000 × g for 1 min) with 20% (wt/vol) sucrose-0.15 M NaCl. The total [3H]DNA bound to the cells was measured by suspending the cells in distilled water, treating with 50 mM Tris buffer (pH 8.0) and 50 μg of lysozyme per ml and counting directly in aqueous Triton-toluene scintillant (300 ml Triton X-100, 100 ml distilled water, 600 ml of toluene containing 6 g of 2(4'-tert-butylphenyl)-5(4''-biphenylyl)-1,3,4-oxadiazole (butyl-PBD; Koch-Light)).

[3H]DNA bound to cells in a DNase-resistant form was measured after the first wash by adding 20 μl of bovine pancreatic DNase I (Sigma Chemical Co.; 2 mg/ml in 10 mM MgCl2-0.2 M sodium acetate buffer, pH 5.0) and incubating at 0°C for 5 min. After a further wash the cells were lysed, and radioactivity was assayed as described above.

Binding of [3H]labeled plasmid DNA to isolated membrane fractions. The method used to estimate DNA binding was based on that of Joenje et al. (12). Binding to outer membranes was measured in incubation mixtures (200 μl) containing 2.6 μg of [3H]labeled NTP16 DNA and outer membranes in a final concentration of 2.5% (wt/vol) sucrose-0.5 mM EDTA-0.02 mM DTT (pH 7.5). The protein content of membranes added was usually 40 to 50 μg. Various concentrations of CaCl2 were added as described below. The binding mixtures were incubated at 20°C for 10 min. Similar results were obtained when the binding and centrifugation were performed at 0, 20, or 40°C. Each mixture was then layered onto a discontinuous sucrose gradient (2.7 ml of 15% [wt/vol] sucrose overlaid with 0.8 ml of 5% [wt/vol] sucrose in 1.5 mM EDTA-0.2 mM DTT; pH 7.5) in a 4.2-ml polypropylene centrifuge tube and centrifuged (155,000 × g) for 20 min at 20°C in an MSE swing-out rotor with six 4.2-ml tubes. After centrifugation the supernatant fluids were decanted, the inside walls of the tubes carefully wiped, and the membrane pellets were dissolved in 200 μl of 10% (wt/vol) sodium dodecyl sulfate. Duplicate samples (50 μl) were removed and counted directly in Triton-toluene scintillant.

Binding of [3H]DNA to inner membranes was carried out as for outer membranes. Binding mixtures contained 3.0 μg of [3H]labeled NTP16 DNA and inner membranes (40 to 50 μg of membrane protein) in a final concentration of 0.5 mM CaCl2.

Trypsin treatment of outer membranes. Outer membranes (138 μg of membrane protein) were mixed with 50 μl of trypsin solution (1 mg/ml) in a final volume of 250 μl of 25% (wt/wt) sucrose-5 mM EDTA-0.2 mM DTT (pH 7.5) and incubated at 37°C for 1 h. CaCl2 (final concentration, 75 mM) and 5.5 μg of [3H]labeled NTP16 DNA (specific activity, 7.7 × 106 cpm/μg) were then added, and the mixture was kept at 0°C for 30 min. Controls were treated in the same manner without the addition of trypsin. Binding mixtures were
loaded on to 25 to 60% (wt/wt) sucrose gradients as described above and centrifuged at 95,000 × g for 17 h. Fractions were collected and counted directly in Triton-toluene scintillant.

RESULTS

Binding and uptake of plasmid DNA by whole cells. The transformation process consists of an initial stage when cells are incubated with DNA at 0°C in the presence of Ca²⁺ ions and a subsequent stage when the transformation mixture is subjected to a heat pulse at 42°C (6, 7, 10). The DNA responsible for the production of transformants only became resistant to DNase after the heat pulse (Fig. 1). Between 90 and 100% of the DNA in a transformation mixture becomes cell associated (10). However, only about 10% of this loosely bound DNA remained associated after three washes with saline-sucrose (tightly bound DNA), and only about 1% was DNase resistant after the heat pulse. The relationship between the amount of [³H]DNA added to transformation mixtures and the amount bound to cells is illustrated in Fig. 2. The capacity to bind plasmid DNA in a DNase-resistant form was saturated by 2 μg of DNA per transformation mixture, and the slope of the line at nonsaturating DNA concentrations was approximately 1. A similar curve was obtained for transformants (Fig. 2 and reference 10), but in this case saturation occurred at approximately 0.8 μg of DNA per mixture. The curve for [³H]DNA tightly bound to the cells also had a slope of approximately 1, but saturation was not reached even at 10 μg of DNA per transformation mixture.

The concentration of CaCl₂ present in transformation mixtures had a considerable effect on the quantity of [³H]-labeled NTP16 DNA bound to intact cells of E. coli. The amount of DNA bound in a DNA transformation mixture showed a marked increase with increasing Ca²⁺ concentration up to a maximum at 100 mM and thereafter decreased dramatically (Fig. 3). Total DNA binding was also dependent on Ca²⁺ concentration (there being none in the absence of divalent

![Graph](https://example.com/graph.png)

**Fig. 1.** The effect of DNase on yields of transformants. E. coli K-12 C600 was grown and rendered transformable as described in the text. Transformation mixtures contained a saturating amount (2 μg of DNA per 2 × 10⁸ cells) of NTP16 DNA; 20 μl of a solution containing 2 μg of pancreatic DNase 1 per ml, 10 mM MgCl₂, and 50 mM sodium acetate (pH 5.0) was added to each transformation mixture at the times shown. Mixtures were subsequently treated as in the normal transformation protocol. Results are expressed as a percentage of the transformation frequency (2.3 × 10⁻⁴ transformants per viable cell) obtained with a control mixture where DNase was not added.

![Graph](https://example.com/other-graph.png)

**Fig. 2.** Dose-response curves for [³H]-labeled NTP16 DNA bound to whole cells. Competent cells (approximately 10⁸ per mixture) suspended in 75 mM CaCl₂ were mixed with [³H]-labeled NTP16 DNA (specific activity, 8.4 × 10⁶ cpm/μg) in a total volume of 0.5 ml. Mixtures were incubated at 0°C for 45 min and subsequently at 42°C for 10 min. Samples were removed from the mixtures and treated as described in the methods section: ○, total DNA binding; □, DNase-resistant binding; ■, transformants per viable cell.
cations), but it was efficient over a much wider range of concentrations (20 to 100 mM) than was DNase-resistant binding (Fig. 3). Total DNA binding and viability of cells were reduced significantly at concentrations of Ca\(^{2+}\) above 100 mM.

Isolation of \(^3\)H-labeled NTP16 DNA attached to inner and outer membranes after transformation. Competent cells of E. coli K-12 C600 were transformed with \(^3\)H]DNA and washed to remove unbound DNA, and the inner and outer membranes were isolated by sucrose gradient centrifugation (17). The bulk of the labeled DNA was attached to the outer membrane fraction with smaller amounts attached to inner membranes and as free DNA (Fig. 4). Little radioactivity was associated with the membranes when the cells were treated with DNase before membrane isolation, but most of this (>90%) gave a defined peak banding with inner membranes. DNA did not band at the position of inner or outer membranes in the absence of membranes or Ca\(^{2+}\) ions. Furthermore, when the membranes were treated at any stage with concentrations of EDTA above 5 mM, all of the DNA was removed from membrane material and sedimented in the position indicated for free DNA.

DNA binding to isolated membrane fractions. Experiments were carried out to determine the conditions under which DNA was bound to isolated membranes. Approximately 1.0 \(\mu\)g of DNA was bound per 54 \(\mu\)g of outer membrane protein or 36 \(\mu\)g of inner membrane protein. For subsequent experiments the amounts of membranes employed were adjusted so that they would bind approximately half of the \(^3\)H]DNA present. Maximum DNA binding to outer and inner membranes, respectively, was obtained at Ca\(^{2+}\) concentrations of 5 mM and 0.5 to 1.0 mM (Fig. 5). All subsequent experiments with inner membranes were carried out in 0.5 mM CaCl\(_2\); at concentrations higher than 1 mM the membranes aggregated and adhered to glassware.

All divalent cations tested facilitated binding of labeled plasmid DNA to both inner and outer membranes, but calcium was the most effective (Table 1). No binding to either membrane was

![Graph](http://jb.asm.org/)

**Fig. 3.** Effects of CaCl\(_2\) concentration on binding of \(^3\)H-labeled plasmid DNA to whole cells. E. coli cells were prepared for transformation as described in the text, except that after washing in 10 mM CaCl\(_2\), the cells were suspended in the concentrations of CaCl\(_2\) indicated. Transformation mixtures (0.5 ml) contained 3 \(\mu\)g of \(^3\)H-labeled NTP16 DNA (specific activity, 8.4 \(\times\) 10\(^4\) cpm/\(\mu\)g) and approximately 10\(^8\) total cells of E. coli K-12 C600. Amounts of DNA bound were determined as described in the legend to Fig. 2.

![Figure 4](http://jb.asm.org/)

**Fig. 4.** Isolation of \(^3\)H-labeled plasmid DNA attached to membrane fractions. Bacteria (10\(^6\) cells in 2 ml of 75 mM CaCl\(_2\)) were transformed with 10 \(\mu\)g of \(^3\)H-labeled NTP16 DNA (specific activity, 8.4 \(\times\) 10\(^4\) cpm/\(\mu\)g). The cells were washed immediately after the heat-pulse step and lysed, and the membranes were separated by centrifugation on a 25 to 60% (wt/wt) sucrose gradient (95,000 \(\times\) g for 17 h) at 20°C (17). Fractions were collected by piercing the bottoms of the centrifuge tubes, and samples (180 \(\mu\)l) were counted directly. The positions of outer membrane and free DNA and inner membrane (arrows) were determined in tubes centrifuged in parallel.
observed when divalent cations were omitted from the mixtures or when Na\(^+\) was substituted. Binding in the presence of high NaCl concentrations is apparently nonspecific since it also occurs with whole cells, but without inducing transformation (10).

In all binding mixtures the concentration of EDTA was 0.5 mM. This concentration was necessary for DNA binding to inner membranes regardless of the Ca\(^{2+}\) concentration. Concentrations of EDTA above 1 mM inhibited DNA binding to both inner and outer membranes. The reason for this rather strict requirement for EDTA to allow binding to isolated membranes is not yet clear.

Pretreatment of outer membranes with trypsin drastically reduced the amount of \(^{3}H\)-labeled NTP16 DNA bound (Fig. 6a). This reduction was not the result of gross disruption in membrane structure because trypsin treatment did not alter the buoyant density of the outer membranes (Fig. 6b).

**DISCUSSION**

Transformation of *E. coli* by DNA involves at least three stages. First, DNA molecules are adsorbed to the outside of the cell at 0°C in the presence of high concentrations of divalent cations. This is followed by a heat pulse during which the DNA becomes insensitive to DNase (6, 10, 19; this paper) and is presumably transported to a site within the periplasm or inside the inner membrane (or both). Finally, internalized DNA must be established either by forming a stable replicon itself or by recombining with a resident replicon. Experiments using plasmid, phage, or chromosomal DNA have demonstrated that much more DNA is bound to the outside of the cells than is actually taken up (10, 16, 19). Furthermore, under the conditions employed in our experiments the capacity of *E. coli* to bind DNA tightly on the outside of cells does not become saturated (Fig. 2). In contrast, the ability to internalize DNA, as judged by the level of DNase-resistant binding, and the production of transformants both showed saturation (Fig. 2). It is probable that a proportion of the DNase-resistant DNA is located in the periplasmic space. This might account for the slightly higher concentration of DNA required to saturate the DNase-resistant DNA binding (2 µg per mixture) when compared with that required for saturation of the transformation frequency (0.8 µg per mixture). Concentrations of CaCl\(_2\) (75 to 100 mM) which give optimum

**TABLE 1. DNA binding to membrane fractions**

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<thead>
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<th>Divalent cation</th>
<th>Concentration (mM)</th>
<th>Membrane fraction</th>
<th>DNA bound*</th>
</tr>
</thead>
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<tr>
<td>None</td>
<td></td>
<td>Inner</td>
<td>1.04</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
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<td>Inner</td>
<td>100.0</td>
</tr>
<tr>
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<td>Inner</td>
<td>27.3</td>
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<tr>
<td>Sr(^{2+})</td>
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<td>Inner</td>
<td>21.6</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>0.5</td>
<td>Inner</td>
<td>11.8</td>
</tr>
<tr>
<td>Na(^{+})</td>
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<td>Inner</td>
<td>0.8</td>
</tr>
<tr>
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<td></td>
<td>Outer</td>
<td>1.3</td>
</tr>
<tr>
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<td>Outer</td>
<td>100.0</td>
</tr>
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<td>Outer</td>
<td>22.0</td>
</tr>
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<td>19.0</td>
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<tr>
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</tr>
<tr>
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<td></td>
<td>Outer</td>
<td>1.6 (0)(^b)</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>75</td>
<td>Outer</td>
<td>100.0 (100)</td>
</tr>
<tr>
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<td>Outer</td>
<td>45.0 (6.0)</td>
</tr>
<tr>
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<td>Outer</td>
<td>42.7 (6.7)</td>
</tr>
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<td>75</td>
<td>Outer</td>
<td>14.4 (0.3)</td>
</tr>
<tr>
<td>Na(^{+})</td>
<td>75</td>
<td>Outer</td>
<td>5.0 (0)</td>
</tr>
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</table>

\(^a\) The binding assays were carried out as described in the text. Results are expressed relative to the values for calcium (100%).

\(^b\) The numbers within parentheses refer to the relative efficiency of divalent cations in inducing competence of *E. coli* K-12 C600 for transformation (10).
transformation frequencies (10) also produced maximal DNase-resistant binding (Fig. 3). This suggests that transport of DNA across the cell envelope, rather than external binding, is a limiting step in producing transformants.

When membranes were separated by the method of Osborn et al. (17) immediately after transformation the bulk of bound DNA was attached to the outer membrane fraction with a small amount bound to the inner membranes (Fig. 4). This result is not surprising as DNA binding to the outside of the cell is considerably more efficient than uptake via the inner membrane into the cell. Treatment of intact heat-pulsed cells with DNase before separation of the membranes removed most of the bound DNA, leaving only that which was attached to the inner membrane (see also reference 19). However, isolated inner membranes were capable of binding amounts of DNA equivalent to those bound by outer membranes. The DNA-binding capacity of inner membranes was saturated in the presence of 0.5 to 1 mM CaCl₂, and the capacity of outer membranes was saturated in the presence of 5 mM CaCl₂ (Fig. 5). This contrasts with an optimum of 75 to 100 mM for both DNase-resistant DNA binding to intact cells and the production of transformants. Whole cells of E. coli are known to maintain low intracellular Ca²⁺ levels by active extrusion of this cation (18, 21). Thus, high external concentrations of Ca²⁺ might be required to maintain the relatively low Ca²⁺ concentration necessary to promote DNA binding to the inner membranes of intact cells. Furthermore, high external calcium ion levels might promote transport of Ca²⁺-DNA complexes down a Ca²⁺ concentration gradient into the cytoplasm as suggested by Grinius (9). Some DNA which is DNase resistant may be bound in the periplasmic space, but we have not investigated such binding in this study. The precise location of DNase-resistant DNA in transformed cells is under further study with DNA radioactively labeled to much higher specific activity.

Calcium or other divalent cations were absolutely essential for DNA binding to inner and outer membranes and whole cells. The following order of effectiveness was obtained: Ca²⁺ > Ba²⁺ > Sr²⁺ > Mg²⁺ (Table 1). The relative efficiencies of these ions in promoting DNA binding are paralleled by their abilities to produce transformants (10).

Very little plasmid DNA was bound to outer membranes treated with trypsin (Fig. 6). Treatment of whole cells with trypsin or pronase in the presence of Ca²⁺ also reduces the transformation frequency (M. Brown, Ph.D. Thesis, University of Liverpool, 1980; and unpublished observations). Furthermore, pronase treatment is known to reduce the ability of isolated cell envelope preparations to inhibit transfection of E. coli (23). These results suggest that integrity of the protein portion of the outer membrane is essential for DNA binding. van Alphen et al. (24) have described outer membrane particles in E. coli whose generation is stimulated by Ca²⁺ and to a lesser extent by Mg²⁺ ions. These particles consist of aggregates of lipopolysaccharide and protein, and it has been proposed that they function as aqueous pores (24). CaCl₂ treatment may therefore either expose proteins which bind DNA or create pores for the binding or inward passage (or both) of DNA molecules.
The DNA-binding data presented in this paper support previous observations that transformation is a very inefficient process in E. coli. Transformants only represent between 0.1 and 1% of cells surviving the CaCl₂ treatment (6, 10). It is not known whether all of the cells in a population of E. coli treated with CaCl₂ or merely those fated to become transformants can take up DNA molecules to sites protected from the action of deoxyribonuclease. Under the conditions used in our experiments transformation mixtures contained approximately 5 × 10⁶ viable cells and 10¹¹ molecules of NTP16 DNA (1 μg) when the system was just saturating (see Fig. 2). Thus, mixtures contained approximately 200 plasmid DNA molecules per viable cell or 200,000 per transformant, assuming that 0.1% of viable cells became transformed. Washing the cells removed much of this DNA, so that only 10 to 20 molecules per viable cell or 10,000 to 20,000 per transformant remained tightly bound. After treatment of cells with DNase only 1 to 2 molecule equivalents per viable cell or 1,000 to 2,000 per transformant remained attached. It seems inherently unlikely that only transformable cells would bind and internalize such large numbers of DNA molecules. It is more likely that all cells in the population can bind DNA, but that only a small minority are successful in transporting intact plasmid molecules to the interior of the cell where they might establish themselves as replicons. There were sufficient plasmid molecule equivalents (1 to 2 per viable cell) bound in a DNase-resistant form to transform all of the viable cells (Fig. 2). However, any molecules which had only partially traversed the cell envelope after the heat pulse would be damaged by DNase treatment. Therefore, measurement of DNase-resistant molecule equivalents will certainly give an overestimate of potential transformants. We have previously shown by simultaneous transformation of E. coli with pairs of plasmids that even under saturating conditions, the majority (probably >80%) of transformable cells can only take up one DNA molecule, whereas only 10 to 20% can take up two (26) and <1% can take up three (J. R. Saunders, unpublished observations) separate plasmid molecules. These findings taken together indicate that the transition from externally bound DNA molecule to established plasmid replicon occurs with low probability.

We conclude from the data presented that the interaction of externally added DNA with intact cells and isolated membranes is highly specific. However, even under optimal conditions very few cells in a population of E. coli are productively transformed. It must be emphasized that because such a small proportion of cells are transformed, the results of binding experiments with isolated membranes from the total cell population may not directly reflect steps in the transformation process. However, the parallels are intriguing. The reasons for the low transformation efficiency and the mechanisms of transmembrane transport of DNA are under further study.

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LITERATURE CITED


