Binding of Deoxyribonucleic Acid by Cell Walls of Transformable and Nontransformable Streptococci

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Cell walls isolated from competent streptococci (group H strain Challis) were shown to bind more homologous and heterologous deoxyribonucleic acid (DNA) than noncompetent walls. Heat- and alkali-denatured DNA was not bound by either wall preparation. Pretreatment of cell walls with cetyltrimethylammonium bromide sharply increased the binding of DNA but did not increase transformation of whole cells. Pretreatment of the walls with either sodium dodecylsulfate, deoxyribonuclease and ribonuclease, or with crude competence-provoking factor did not affect the binding of DNA. Antiserum prepared against whole competent cells completely blocked transformation and also inhibited DNA binding to competent cell walls. Adsorption of this antiserum with competent Challis cells removed its blocking action for both binding and transformation. Pretreatment of walls with trypsin and Pronase destroyed their ability to bind DNA. Trypsin treatment also blocked transformation in whole cells. The transforming activity of DNA bound to cell walls was found to be protected from deoxyribonuclease action. Significant differences were observed in the arginine, proline, and phenylalanine content of competent and noncompetent walls. With few exceptions, the amino acids released from competent cell walls by trypsin were several-fold greater than from noncompetent walls. The results indicate that (i) two binding sites exist, one in competent cells only and essential for subsequent transformation, and a second, present in all cells, which is not involved in transformation; (ii) both sites are protein in nature; (iii) the transformation site is blocked by antibody; and (iv) the competent cell wall possesses tryptic-sensitive protein not present in the noncompetent wall.

Studies done with several species of transformable bacteria have shown that competent, whole cells irreversibly bind greater amounts of deoxyribonucleic acid (DNA) than do noncompetent cells (2, 4, 5, 8, 25). The first steps in the transformation process must involve the interaction of DNA with the cell surface. Upon entering the competent state, bacterial cells develop new antigenic surface structures, and antibodies directed against these antigens can block transformation (7, 9). Quantitative differences in the cell wall composition of transformable and nontransformable strains of Bacillus subtilis have been shown (26). The replacement of certain cell wall components with analogues was shown to destroy the transformability of pneumococci (23). Also, cells probably possess mechanisms which alter the cell wall in order for them to undergo transformation (22).

It would thus appear that the cell wall plays a significant part in the initial uptake of DNA by competent cells, perhaps in the form of DNA binding sites on or within the wall structure. Specific knowledge, however, about the nature of these DNA binding sites is lacking.

In the present study, cell walls were isolated from competent and noncompetent streptococcal cells to investigate the binding of DNA to the walls. The function of DNA binding sites and the effects of various reagents on these sites were studied in a system that was free of the effects of membrane transport and other physiological factors involved in DNA uptake by whole cells.

MATERIALS AND METHODS

Bacterial strains. A group H streptococcus (strain Challis) was used as a source of cell wall and as the recipient in transformation. Donor DNA was prepared from a Challis strain resistant to over 1.0 mg of streptomycin per ml (14).

Transformation procedure. The modified transforming medium (PYG) and the transformation procedure have been described (16). Streptomycin resistance was the genetic marker used in all transformation experiments.
DNA preparation. Unlabeled transforming DNA was isolated and purified as described previously (15).

³²P-labeled DNA was isolated by a modification of the method described by Perry (13). The procedure involved dialysis of the DNA against 1 x SSC (0.15 M sodium chloride plus 0.015 M sodium citrate) for 48 hr at 4°C instead of adsorption of the DNA with charcoal. A typical preparation of DNA contained 59.5 μg/ml, less than 2% ribonuclease acid, and 3.7% protein. The DNA contained 96% of the total radioactivity and had a specific activity of 13,326 counts per min per μg.

The ³²P-labeled B. subtilis DNA was kindly supplied by K. Bott. The ³²P-labeled calf thymus DNA was obtained from the Worthington Biochemical Corp., Freehold, N.J.

Preparation of antisera. New Zealand red rabbits were injected intravenously with whole, competent Chalilis cells. The vaccine was prepared as described (9), except that the cells were suspended in saline to an absorbancy at 550 nm (A₅₅₀) of 0.40. Rabbits were inoculated every 12 hr. Samples were counted daily for 3 days and then rested 4 days. Test bleedings were made at the end of each resting period. The sera were adsorbed with noncompetent Chalilis cells. Five milliliters of serum was adsorbed with 1 ml of packed cells by incubation with occasional shaking for 3 hr at 37°C followed by standing at 4°C overnight. The sera were centrifuged to remove the cells and then tested for their ability to inhibit transformation and ³²P-DNA binding.

Cell wall preparation. Cell walls were prepared from cells grown in ET 3 medium (11). A 1% inoculum was grown for 18 hr at 37°C and then added to fresh medium, and the incubation was continued. Competent cells were harvested after 3 hr of incubation and noncompetent cells after 6 hr. Cells were harvested by centrifugation at 8,000 x g for 10 min and washed three times with saline. A 50% suspension of cells in saline was sonically treated for 15 min at a setting of 6 on a 20-kc Branson Sonifier. Gram staining indicated greater than 90% breakage of cells. Whole cells were removed by centrifugation at 1,500 x g for 10 min, and the cell walls were then sedimented by centrifugation at 12,000 x g for 10 min. The walls were washed with water and 0.1 M phosphate buffer, pH 7.4 (1), and lyophilized.

DNA binding experiments. Cell walls were used at a final concentration of 0.025 mg per ml of saline in all binding experiments. Labeled DNA was used at a final concentration of 10 μg/ml. Cell walls were exposed to DNA for 15 min at 37°C, 100 μg of pancreatic deoxyribonuclease (Mann Research Laboratories Inc., New York) per ml was added, and the tubes were incubated for an additional 20 min. One-milliliter samples were collected on HA filters (Millipore Corp., Bedford, Mass.), washed with 50 ml of water, and placed in vials for counting. The vials contained 10 ml of scintillation fluid (12). Samples were exposed in duplicate for 20 min in a Beckman LS-100 liquid scintillation counter. All counts were corrected for background, binding of DNA to the filter, and decay of isotope.

Pretreatment of cell walls and whole cells. Cell walls and whole cells were treated with various reagents before being used in binding or transformation experiments. The cells walls (0.025 mg/ml of saline) or whole cells (grown for transformation as described above) were treated at 37°C with one of the following methods: (i) 0.025% cetyltrimethylammonium bromide (Cetab) for 15 min; (ii) 0.4% sodium dodecyl sulfate (SDS) for 30 min; (iii) 10% antiserum (0.1 ml of serum/ml of sample) for 15 min; (iv) 10% crude competence-provoking factor (CPF; 0.1 ml of CPF/ml of sample), prepared as described (16), for 30 min; (v) 50 μg of deoxyribonuclease per ml in 0.02 M MgSO₄ and 50 μg of ribonuclease per ml (Mann) for 1 hr at pH 7.4; (vi) 50 μg of trypsin (two times crystallized, Mann) per ml or Pronase (B grade, Calbiochem, Los Angeles, Calif.) for 1 hr at pH 7.4.

After treatment, cell walls and whole cells were centrifuged at 4°C, washed twice with saline, and re-suspended in the original volume of either saline or transformation medium. The treated samples were then used in either binding or transformation experiments as described above.

Analysis of dialysates of trypsin-treated cell walls. Cell walls were suspended in water (25 mg/2 ml). Trypsin was added to a final concentration of 50 μg/ml, and the cultures were incubated for 1 hr at 37°C. The cell walls were sedimented at 4°C by centrifugation at 12,000 x g for 10 min and then washed twice with water. The supernatant fluid and washings were pooled and dialyzed against water for 24 hr. The dialysates were dried, weighed, and the material was redisolved in 2 ml of water, three dilutions of the amino acids in these dialysates were obtained by chromatographic analysis on a Beckman model 116 amino acid analyzer (19).

Analysis of cell wall hydrolysates. Four-milligram samples of both competent and noncompetent cell walls were hydrolyzed with 6 N HCl for 16 hr at 100°C in sealed tubes, dried under vacuum over P₂O₅ and NaOH at 22°C, and rehydrated and dried three times to remove all traces of HCl. These hydrolysates were then analyzed for amino acid content as described above.

Electron microscopy. Cell wall suspensions were diluted with distilled water to a turbidity just visible to the naked eye. A drop of the diluted suspension was placed on Formvar-coated grids and dried in air. The grids were then shadowed at 21 to 22°C with chromium at a distance of 3 cm from the filament.

RESULTS

Cell wall preparations. To minimize the differences in cell wall composition that would result from using different growth media, the streptococci were harvested from identical culture media at the time of maximum competence (3 hr) and noncompetence (6 hr). Figure 1 illustrates the relation between growth and the rate of transformation. Cell walls were prepared from the cells harvested at each of these times.

Cell walls from competent and noncompetent cells appeared similar under the electron microscope and gave no evidence of nonwall material. The preparations resembled those previously reported for cell walls of streptococci (18).

Binding of DNA to cell wall preparations. In
studies of DNA binding to whole cells, heterologous as well as homologous DNA has been shown to be adsorbed by competent cells (5, 20). Table 1 shows the results of binding studies done with DNA from various sources to determine the ability of both competent and noncompetent cell walls to bind DNA such that it cannot be removed by the action of deoxyribonuclease. The competent cell walls bound approximately twice as much DNA as did noncompetent cell walls. This increased binding was evident whether the source of the DNA was Challis cells, *B. subtilis* cells, or calf thymus. It can also be seen from Table 1 that noncompetent cell walls bind significant quantities of DNA.

Studies done with whole cells have shown that native DNA is adsorbed by competent cells to a much greater extent than is denatured DNA (2, 5, 26). In the present experiments, a 1.0-mL volume containing 100 μg of 32P-labeled Challis DNA was denatured by either heating to 98°C for 10 min and cooling in an ice bath or by being brought to pH 11.0 with KOH and then neutralized with HCl. This denatured DNA showed no transforming activity when tested on competent Challis cells. The cell walls, both competent and noncompetent, bound only 5 to 10% as much heat- or alkali-denatured DNA as native DNA.

**Effect of detergents on the DNA binding sites.**

The cationic detergent, Cetab, has been shown to alter the permeability of streptococcal cells in relation to their ability to utilize certain metabolites (17). Thus the effect of this detergent on the ability of cell walls to bind DNA and its effect on transformation of whole cells was studied. Table 2 shows that pretreatment of cell walls with Cetab resulted in a 10-fold increase in the quantity of 32P-labeled DNA bound. This effect was noted for both competent and noncompetent cell wall preparations. Treatment of competent whole cells with Cetab also increased their ability to bind labeled DNA (data not shown) but caused no significant change in the rate of transformation of these cells to streptomycin resistance. At the concentration of Cetab used, the detergent did not alter the viable count of the cultures.

The anionic detergent, SDS, has been shown to solubilize cell membrane particles which may be contaminating cell wall preparations (6). Experiments were performed to determine whether the irreversible binding of DNA to cell walls was the result of either trapping of the DNA between the cell wall and contaminating membrane or adsorption to the membrane. The cell walls were treated with SDS and then used in binding experiments. Table 2 shows that SDS treatment had no significant effect on the binding of DNA by either competent or noncompetent cell walls. These results indicate that the observed irreversible binding is a function of binding sites located in the cell walls.

**Effect of temperature and metabolic inhibitors on DNA binding.** It has been shown that temperature and metabolic inhibitors such as 2,4-dinitrophenol (DNP) and NaN3 can affect the uptake of DNA by viable whole cells (2). The effect of temperature, DNP, and NaN3 on the binding of 32P-DNA by cell walls was determined. Binding experiments were carried out at 0, 4, 25, 37, and 45°C. Immediately after the addition of deoxyribonuclease, all tubes were brought to 37°C, incubated for 20 min, and assayed for binding as described previously. In other binding experiments,
either DNP or NaN₃ was added to a concentration of 1.0 × 10⁻³ M during the period of exposure of cell walls to DNA to observe any indication of their effect on binding. In all cases, neither temperature nor metabolic inhibitors had any significant effect on the DNA bound by the cell walls.

**Effect of antiserum and competence-provoking factor on the binding sites in cell walls.** When competent bacteria are injected into rabbits, antibodies are produced which can block DNA binding and transformation (7, 9). We have used a similar serum to determine whether it blocked binding of DNA by cell walls as well as transformation of whole cells. This antiserum was first adsorbed with whole noncompetent Challis cells. This adsorption was necessary to remove antibodies against cell surface antigens other than competent DNA binding sites. Unadsorbed serum was found to agglutinate the cell walls and whole cells. The specific antiserum reduced the ability of competent cell walls to bind labeled DNA to approximately 13% of the control value (Table 3). The antiserum also effectively blocked transformation of whole cells. When the specific antiserum was adsorbed with competent Challis cells, the ability to block DNA binding was removed. Pooled normal rabbit serum had no significant effect on binding or transformation.

Cell-free filtrates of competent streptococcal cultures have the ability to induce competence in noncompetent cells (11). Experiments were carried out to determine if crude CPF had any further effect on the activation of cell wall binding sites. Although the CPF did induce transformation in noncompetent whole viable cells, it had no effect on the binding of labeled DNA by either competent or noncompetent cell walls (Table 3).

**Table 2. Effect of detergents on the DNA binding sites in streptococcal cell walls**

<table>
<thead>
<tr>
<th>Cell wall prepn</th>
<th>Pretreatment</th>
<th>%P-DNA bound</th>
<th>Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competent None</td>
<td>2,049</td>
<td>12.3 × 10⁰</td>
<td></td>
</tr>
<tr>
<td>Noncompetent None</td>
<td>700</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competent 0.025% Cetab for 15 min</td>
<td>11,220</td>
<td>11.5 × 10⁶</td>
<td>9,630</td>
</tr>
<tr>
<td>Noncompetent None</td>
<td>700</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competent 0.4% SDS for 30 min</td>
<td>2,190</td>
<td></td>
<td>756</td>
</tr>
</tbody>
</table>

*Expressed as counts per minute per cubic centimeter.
*Figures indicate percentage of transformation of cells similarly treated.
*Cetyltrimethylammonium bromide.
*Sodium dodecyl sulfate.

**Table 3. Effect of antiserum and CPF on the DNA binding sites in streptococcal cell walls**

<table>
<thead>
<tr>
<th>Cell wall prepn</th>
<th>Pretreatment</th>
<th>%P-DNA bound</th>
<th>Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competent None</td>
<td>2,140</td>
<td>15.0 × 10⁰</td>
<td>585</td>
</tr>
<tr>
<td>Noncompetent None</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competent 0.2 ml of specific antiserum for 15 min</td>
<td>268</td>
<td>301</td>
<td></td>
</tr>
<tr>
<td>Noncompetent None</td>
<td>468</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competent 0.2 ml of adsorbed specific antiserum for 15 min</td>
<td>2,058</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noncompetent None</td>
<td>384</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competent 0.2 ml of normal rabbit serum for 15 min</td>
<td>1,970</td>
<td>11.0 × 10⁶</td>
<td>384</td>
</tr>
<tr>
<td>Noncompetent None</td>
<td>1,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competent 10% crude CPF for 30 min</td>
<td>2,358</td>
<td>10.0 × 10⁶</td>
<td>864</td>
</tr>
<tr>
<td>Noncompetent None</td>
<td>864</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Expressed as counts per minute per cubic centimeter.
*Figures indicate percentage of transformation of cells similarly treated.
*Rabbit antiserum against whole competent Challis cells adsorbed with whole noncompetent Challis cells.
*Same serum as c, also adsorbed with whole competent Challis cells.

**Effect of enzymes on DNA binding sites.** Pretreatment of cell walls with both ribonuclease and deoxyribonuclease had no effect on their ability to bind labeled DNA (Table 4). These results indicate that the cell walls were not contaminated with nucleic acids which may have been contrib-

**Table 4. Effect of various enzymes on DNA binding sites in streptococcal cell walls**

<table>
<thead>
<tr>
<th>Cell wall prepn</th>
<th>Pretreatment</th>
<th>%P-DNA bound</th>
<th>Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competent None</td>
<td>1,647</td>
<td></td>
<td>963</td>
</tr>
<tr>
<td>Noncompetent None</td>
<td>1,615</td>
<td></td>
<td>817</td>
</tr>
<tr>
<td>Competent RNase and DNase</td>
<td>1,625</td>
<td>11.0 × 10⁶</td>
<td>455</td>
</tr>
<tr>
<td>Noncompetent RNase and DNase</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Competent Trypsin</td>
<td>2,000</td>
<td>15.0 × 10⁶</td>
<td>364</td>
</tr>
<tr>
<td>Noncompetent Trypsin</td>
<td>234</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Expressed as counts per minute per cubic centimeter.
*Figures indicate percentage of transformation of cells similarly treated.
*Ribonuclease (RNase) and deoxyribonuclease (DNase) each at 50 μg/ml for 1 hr.
*At 50 μg/ml for 1 hr.
utung to the binding of DNA. Tomasz (Bacteriol. Proc., 1967, p. 52) reported that treatment of competent pneumococcal cells with relatively high concentrations of trypsin led to loss of transformability. Thus, studies were undertaken to determine the effect of proteolytic enzymes on the binding of DNA to cell walls and whole cells and their effect on transformation of whole cells. As seen in Table 4, pretreatment of cell walls with 50 µg of either trypsin or Pronase per ml reduced their ability to bind labeled DNA. Similar treatment of competent whole cells destroyed their ability to undergo transformation. The treatment with either enzyme did not significantly alter the viable count of the cultures.

Figure 2 shows the results of a kinetic study of the binding of labeled DNA and the transformation of cells treated with 50 µg of trypsin per ml. Challis cells were inoculated into transformation medium (PYG) and, at appropriate times, samples were removed and treated with trypsin. The first sample was removed after 1 hr of growth, trypsin was added, and incubation continued for 60 min, i.e., the time required for competence to develop in the absence of trypsin. Subsequent samples were removed from the culture after 1.25, 1.5, 1.75, and 2 hr of growth. Trypsin was added to each, and the mixture was held until the total elapsed time from inoculation was 2 hr. At this point, i.e., the time of maximum competence in the absence of trypsin, 32P-DNA was added to each and held 15 min, and the reaction was terminated with deoxyribonuclease. The cells were then assayed for binding and transformation; both binding and transformation decreased with increasing time of exposure of cells to trypsin. It is interesting to note that the curves of binding and transformation are not parallel and that trypsin treatment appears to have a greater initial effect on transformation than on binding of DNA.

Release of 32P DNA from cell walls by trypsin. Pretreatment of cell walls with trypsin destroys their ability to bind DNA (Table 4). Thus it was considered of interest to determine if trypsin could release DNA after it had been bound by the cell walls. In these experiments cell walls were allowed to bind DNA and were then treated with 50 µg of trypsin per ml for either 30 or 60 min (Table 5). The trypsin treatment removed approximately 40% of the bound DNA after 30 and 60 min of treatment.

Biological activity of DNA released by trypsin. To determine if DNA released from cell walls by trypsin retained biological activity, 25 mg of competent cell walls in 1 ml of saline was added to 500 µg of transforming DNA in 0.2 ml of SSC, incubated 15 min at 37 C, and then treated with 100 µg of deoxyribonuclease per ml for 30 min. The cell walls were washed three times with SSC, suspended in saline, and treated with 100 µg of trypsin for 1 hr. The concentration of the released DNA was determined, and this DNA was tested for transforming activity with normal transforming DNA as a control. Both DNA preparations were used at a concentration of 5 µg/ml. The released DNA possessed 60% of the

### Table 5. Release of 32P-DNA from cell walls by trypsin

<table>
<thead>
<tr>
<th>Cell wall preparation</th>
<th>Treatment</th>
<th>32P-DNA remaining bound</th>
<th>Per cent DNA removed by trypsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competent</td>
<td>None</td>
<td>2,110</td>
<td></td>
</tr>
<tr>
<td>Noncompetent</td>
<td>None</td>
<td>1,208</td>
<td></td>
</tr>
<tr>
<td>Competent</td>
<td>Trypsin¹</td>
<td>1,320</td>
<td>37</td>
</tr>
<tr>
<td>Noncompetent</td>
<td>Trypsin¹</td>
<td>723</td>
<td>40</td>
</tr>
<tr>
<td>Competent</td>
<td>Trypsin²</td>
<td>1,281</td>
<td>39</td>
</tr>
<tr>
<td>Noncompetent</td>
<td>Trypsin²</td>
<td>677</td>
<td>44</td>
</tr>
</tbody>
</table>

* Cell walls were allowed to bind DNA for 15 min and were treated with deoxyribonuclease for 20 min, and then with trypsin for the times indicated.

* Expressed as counts per minute per cubic centimeter.

* At 50 µg/ml for 30 min.

* At 50 µg/ml for 60 min.

Figure 2. Effect of trypsin pretreatment on 32P-DNA binding and transformation of whole Challis cells. Cells were grown in transformation medium. Samples of cells were removed after 1, 1.25, 1.5, 1.75, and 2 hr of growth and were treated with 50 µg of trypsin per ml. 32P-DNA was added at 2 hr of growth. Cells were then assayed for binding and transformation. Symbols: O, 32P-DNA bound to cells; ●, per cent transformation.
transforming activity of the control DNA. These results indicate that the biological activity is partially protected from deoxiribonuclease action while the DNA is bound to the cell wall, and the 32P counts are a reliable indication of the presence of DNA.

Analysis of material released by trypsin from cell walls. The results of the amino acid analysis of the dialyzable material released from cell walls by trypsin are shown in Table 6. As can be seen, quantitative differences exist between the amino acids released from competent and noncompetent cell walls. In general, greater amounts of amino acids were released from the competent cell walls. No muramic acid, glucosamine, or glycerol was detected. Glucose and rhamnose amounted to less than 1.0% of the dialysates.

Analysis of cell wall hydrolysates. The amino acid analysis of HCl hydrolysates of competent and noncompetent cell walls is shown in Table 6.

**DISCUSSION**

In the present study, we have shown that isolated cell walls from competent and noncompetent streptococci bind DNA and that competent walls bind significantly more native DNA than do noncompetent walls (Table 1). This bound DNA is not removed by deoxiribonuclease. In general, cell walls bind DNA in significant amounts, although competent walls bind more than noncompetent walls. It is unlikely that the counts bound to noncompetent cell wall can be attributed to impurities in the DNA preparations (10). It would appear, then, that the noncompetent walls possess some DNA binding sites.

Experiments with whole cells have shown that competent bacteria bind both homologous and heterologous DNA (5), and our results indicate that the cell wall binding sites are nonspecific in relation to the homology of the donor DNA (Table 1). However, the wall binding sites have considerable specificity for native rather than denatured DNA. The fact that competent cell walls bound only 5 to 10% as much denatured DNA as native DNA indicates that much of the specificity for uptake of native DNA shown by whole cells (2, 5, 25) resides in the cell wall binding sites.

The effect of Cetab in increasing the binding of DNA by cell walls seems to be nonspecific in nature. Although binding was increased for both competent and noncompetent cell walls, no increase in transformation was observed (Table 2). The increased binding of DNA is possibly due to either a net reduction of negative charge in the walls or to structural alterations caused by the cationic detergent.

The treatment of cell walls with SDS (Table 2), deoxiribonuclease, and ribonuclease (Table 4) was performed to determine whether contaminating cell membrane and nucleic acids were contributing significantly to the observed binding. It appears, however, that such contaminants, if present, do not affect the binding and that the binding sites for DNA are part of the wall structure.

The effects of temperature and metabolic inhibitors on the binding of DNA indicate that in isolated cell walls the binding is not dependent on enzymatic action. The inhibitory effect of these treatments on whole cells (2, 5) may be due to inhibition of a step or steps in uptake of DNA subsequent to binding to the walls.

The results in Table 3 indicate that specific antiseraum contains antibodies that are specific for the DNA binding structures within the competent cell walls. It is doubtful that any extracellular CPF remained associated with the competent cell walls after the extensive washings employed in their preparation. Thus the action of the antibodies in these experiments cannot be attributed to the inactivation of CPF or the blockage of access of CPF to the cell surface.

The fact that added CPF has no effect on the
binding of DNA by either competent or noncompetent walls (Table 3) is in good agreement with studies showing the necessity for protein synthesis for the conversion to competence in cells during exposure to CPF (24). Our results support the suggestion that, during the action of CPF, a cell must synthesize other proteins in order to become competent. Thus, in the competent cell walls used in these studies, only those binding sites that were active at the time of isolation of the walls bound DNA, and no new sites could be activated by CPF once the walls had been isolated.

The effect of proteolytic enzymes on the binding of DNA by cell walls and whole cells (Table 4) indicates the protein nature of the DNA binding sites. Both trypsin and Pronase destroyed the ability of cell walls to bind DNA and consequently the ability of whole cells to be transformed.

It appears that trypsin may have multiple effects on the transformation system in whole cells, in that transformation is more sensitive to the enzyme than is DNA binding (Fig. 2). It is possible that in whole cells trypsin may inactivate cell wall DNA binding sites and also destroy the membrane transport system for DNA. Thus, the decrease in binding would be due to loss of cell wall sites, whereas the loss in transformation would be due to loss in binding sites and loss of the transport system. In this system, the initial treatment with trypsin decreased the correlation between binding of labeled DNA and transformation. After 15 min of trypsin treatment, transformation was decreased by half, whereas DNA binding was essentially unchanged. Thus, care should be taken in equating DNA binding and transformation, especially in experiments in which the effects of reagents on the system are being studied. Similar lack of correlation has been noted in certain cases with B. subtilis (25).

Pretreatment of cell walls with trypsin completely destroyed their ability to bind DNA. However, trypsin treatment of the walls after they had bound labeled DNA released only 40% of the label from the walls (Table 5). This released DNA had approximately 60% of the biological activity of an equal amount of normal transforming DNA, indicating that it had not been extensively degraded by deoxyribonuclease action. Thus, when DNA is on the binding sites within the walls, it is partially protected from deoxyribonuclease action and in turn protects the binding sites from complete destruction by trypsin. These results support previous observations that, during uptake, transforming DNA becomes insensitive to deoxyribonuclease at some point before passage through the cell membrane (3, 21).

Table 6 shows that arginine and proline are 35% higher in competent as compared to noncompetent walls, whereas phenylalanine is 45% lower. No significant increase in amino sugar or alanine was seen in competent walls in contrast to walls from B. subtilis (26). The higher quantity of arginine might be sufficient to reduce the net negative charge on the wall and thereby promote the binding of DNA.

The amount of amino acids released by trypsin in the great majority of cases from competent cell walls was 1.4 to 2.7 times greater than that released from noncompetent walls (Table 6). Aspartic acid, however, showed a fivefold increase. It should be noted that arginine is present in a small quantity in the dialysates and that proline is not present. However, a material is present which occupies the same position on the chromatogram as proline. This material adsorbs at 570 nm rather than 440 nm. Citrulline has the same position and adsorption characteristics. It appears, therefore, that competent cell walls possess proteins that are more susceptible to tryptic action. During the competent state, the cell wall may be more loosely structured in certain areas, allowing DNA penetration into the wall and access to the areas responsible for DNA binding. This same loose wall structure would allow trypsin to penetrate into the wall and to act on proteins which are less exposed in noncompetent walls. Mild lysozyme treatment of B. subtilis has been shown to enhance transformation (22), thus indicating that a partial breakdown of wall structure may be involved in the uptake of DNA. Another possibility is that a new protein(s) is synthesized at the cell surface, as indicated by the requirement for protein synthesis during acquisition of competence (24). This protein may be part of the DNA binding site and, because of its surface location, may be particularly susceptible to tryptic digestion.

No sugars, amino sugars, glycerol, or phosphate were found in the tryptic digest. However, it is possible that a polysaccharide or a teichoic acid, or both, may be a part of the binding site and not released during digestion.

Competent cell walls showed a twofold increase in DNA binding over noncompetent cell walls (Table 1) and, yet, the competent whole cells had a many-fold greater rate of transformation. These results raise the question as to the existence of binding sites of differing character in competent as compared to noncompetent cell walls. It is clear that an antigenic specificity is possessed by the competent cell wall, indicating that changes have occurred during the conversion from noncompetence. Has a second binding site been formed during this period in the competent
cell wall? The present results indicate that such is the case. Antiserum containing antibodies only to the binding site on competent cell walls is not able to eliminate binding completely, although transformation was blocked (Table 3). Also, tryptic digestion, acting on both sites, completely eliminated the binding of DNA (Table 4). It follows that the nature of the specific binding site of the competent cell wall is such that transport of the DNA into the cell can proceed with the resulting integration and expression of the genetic marker. The DNA bound by the nonspecific site is not taken into the reaction chain which results in transformation. The differences between competent and noncompetent cell walls do not appear to be a matter of an increased number of the same binding site, otherwise antiserum against noncompetent cell walls would interfere in transformation (9), and the adsorption of competent antiserum with noncompetent cell walls would have eliminated the ability of the serum to prevent binding and transformation (Table 3).

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