Accessibility of Enterobacterial Common Antigen to Antibodies in Encapsulated and Non-capsulated S and R Forms of *Escherichia coli*

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Antiserum specific for the enterobacterial 'common antigen' (ECA) was obtained by absorbing a rabbit ECA antiserum with an ECA-negative mutant. Accessibility of ECA to antibodies in encapsulated and non-capsulated S and R forms derived from *Escherichia coli* O8 : K27 was studied using the indirect immunoferritin technique (whole-mount electron microscopy). The number of ferritin particles on the bacterial surface decreased in the order, non-capsulated R > encapsulated R > non-capsulated S > encapsulated S form, indicating that both the O and K antigens partly cover ECA on the surface of the outer membrane. Whole-mount and thin-section electron microscopy showed that ferritin was evenly distributed on the surface of R mutants, whereas it formed clusters on the S forms.

**INTRODUCTION**

The enterobacterial 'common antigen' (ECA), discovered in 1962 by Kunin (Kunin et al., 1962; Mayer & Schmidt, 1979), is localized in the outer membrane of the enterobacterial cell envelope. This had first been indicated by the phagocytosis-promoting and bactericidal activities of ECA antibodies (Domingue & Neter, 1966a, b).

Recently, ECA has been directly demonstrated in the outer membrane of R mutants of *Escherichia coli* using monospecific ECA antiserum and fluorescein- or ferritin-conjugated anti-rabbit IgG antibodies (Rinno et al., 1980). The specificity of the reaction was indicated by the failure of this antiserum to label an isogenic ECA-deficient *rfe* mutant (Schmidt et al., 1976). These studies were performed with non-capsulated R mutants of *E. coli* O8.

The accessibility of ECA in enterobacterial S forms has so far been studied only with a fluorescent *E. coli* O14 antiserum containing both anti-O and anti-ECA antibodies (Aoki et al., 1966) and by the indirect immunoferritin technique with a special strain of *Yersinia enterocolitica* (Acker et al., 1981) which showed a marked dependence of O-chain length on growth temperature (Acker et al., 1980). In this study, we have compared the ferritin labelling of ECA in mutants derived from a strain of *E. coli* O8 : K27 and having defects in the synthesis of either the O chain or the K antigen or both, in order to investigate how the O and K polysaccharides interfere with the accessibility of ECA to antibodies.

**METHODS**

*Bacterial strains and culture conditions.* The strains used and their descriptions are given in Table 1. The R mutants have the complete lipopolysaccharide (LPS) core of type R1 which is characteristic of *E. coli* D280 (Schmidt et al., 1970). Strain F782 is a his+ hybrid from a cross of Hfr639 x F470. The donor Hfr639 is an encapsulated R form (O8 : K27+, rfb). The his+ hybrid F782 has acquired the donor genes coding for the K27 antigen which are closely linked to the his operon (Schmidt et al., 1977).
**Table 1. Strains and mutants derived from the wild-type E. coli E56b**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Description</th>
<th>ECA</th>
<th>Reference/source</th>
</tr>
</thead>
<tbody>
<tr>
<td>D280</td>
<td>O8:K27+:H-</td>
<td>Encapsulated S form, derived from E. coli E56b</td>
<td>+</td>
<td>Ørskov, Copenhagen</td>
</tr>
<tr>
<td>F492</td>
<td>O8:K27-:H-</td>
<td>Non-capsulated S form derived from D280</td>
<td>+</td>
<td>Schmidt et al. (1970)</td>
</tr>
<tr>
<td>F470</td>
<td>R:K27-:H-</td>
<td>Non-capsulated R form derived from F492</td>
<td>+</td>
<td>Whang et al. (1972)</td>
</tr>
<tr>
<td>F782</td>
<td>R:K27:H-</td>
<td>Encapsulated R form derived from D280</td>
<td>+</td>
<td>Whang et al. (1972)</td>
</tr>
<tr>
<td>F1283</td>
<td>R:K27-:H-</td>
<td>Non-capsulated R form derived from D280, ECA-negative rfe mutant</td>
<td>-</td>
<td>Kiss et al. (1978)</td>
</tr>
</tbody>
</table>

Rough strains F470 (ECA-immunogenic rfb mutant) and F1283 (ECA-negative rfe mutant) were used, as in a previous study (Acker et al., 1981), for the preparation of ECA-specific antiserum (see below) as well as positive and negative controls for the immunoferritin technique.

For electron microscopy investigations, subcultures of these strains were used. They were grown in Tryptic soy broth (TSB, Difco) at 37 °C and harvested in the exponential phase of growth.

**K27 antisera.** Rabbit OK antisera was obtained as described previously (Whang et al., 1972) after repeated injections of formalin-treated cells of E. coli D280. The O8:K27 antisera was thoroughly absorbed with the K7 mutant F492 to produce the anti-K27 serum.

**ECA antisera.** ECA-specific antisera was obtained as described previously (Acker et al., 1981) by immunizing rabbits with the ECA-immunogenic strain F470 and absorbing the resulting sera at a dilution of 1:5 with the ECA-negative strain F1283. The passive haemagglutination test (Neter, 1956; Mannel & Mayer, 1978) showed that agglutinins against alkali-treated LPS of E. coli F1283 had been eliminated from the absorbed antisera, whereas the titre (1280) against ECA of Salmonella montevideo SH94 remained essentially unchanged. The specificity of the absorbed ECA antisera was tested by the indirect immunoferritin technique (see below); cells of the ECA-immunogenic strain F470 became heavily labelled (Fig. 3a), whereas most cells of the ECA-negative mutant F1283 were not labelled at all and only few cells showed a weak, probably non-specific, labelling (<20 ferritin particles per cell). For some experiments the ECA antisera was fractionated on an Ultrogel AcA34 (LKB) column (12.5 x 100 cm) into the IgM and IgG fractions; each fraction was pooled and concentrated in collodion bags (Sartorius, Göttingen, F.R.G.) to the original volume of the absorbed ECA antisera (1:5 dilution).

**Indirect immunoferritin technique.** All labelling experiments were done using the indirect immunoferritin technique as described previously (Acker et al., 1981). Two volumes of cell suspension in TSB medium (standardized to an A560 of 0-15) were mixed with one volume of the absorbed ECA antisera (heated 30 min at 56 °C) or with the isolated IgM or IgG fractions and incubated for 30 min at 10 °C. Subsequently, the cells were washed twice (15 min at 10000 g), resuspended in 2 ml of the same medium and incubated (60 min at 0 °C) with ferritin-conjugated goat anti-rabbit IgG (heavy and light chains) purchased from Cappel Laboratories, Cochranville, Pa., U.S.A. Unbound antiserum components were removed by two washes in cold TSB (5000 g at 4 °C). For controls, cells were incubated with serum taken from rabbits before immunization instead of with ECA antisera.

The pellets were examined by whole-mount or thin-section electron microscopy. In some experiments, the labelled pellets of the encapsulated strains D280 and F782 were resuspended in 3 ml cold TSB medium and anti-K27 serum was added (0.75 ml to each sample) to preserve the capsule (Bayer & Thurow, 1977; Acker et al., 1981). The samples were incubated for 30 min at 0 °C, washed twice in cold TSB medium (15 min at 5000 g), and then examined by the thin-section technique.

**Electron microscopy.** (a) Thin-section technique. The cells were fixed with glutaraldehyde–OsO4, and embedded in Epon 812 as described previously (Traub et al., 1976). Thin-sections were cut with an LKB ultramicrotome (Ultratome III) and contrasted with 2% (w/v) aqueous uranyl acetate and lead citrate (Reynolds, 1963). (b) Whole-mount technique. Pellets of the treated samples were resuspended in distilled water and adsorbed to carbon films according to the procedure described by Valentine et al. (1968). They were then observed in the electron microscope as whole mounts. All preparations were examined with a JEOL JEM-100B electron microscope.

**RESULTS**

**Thin-section electron microscopy**

In a first series of experiments cells were examined by thin-section electron microscopy. The outermost layer of the cells of all strains examined was the outer membrane (Fig. 1a).
Fig. 1. Thin-section micrographs of cells of the encapsulated wild-type strain D280 (O8:K27): (a) untreated; (b) treated with anti-capsule (K27) serum before preparation of thin section. Note the thick capsule (K) that appears on the cell surface after treatment with anti-capsule serum. OM denotes the outer membrane. The bar markers represent 0.2 μm.

When cells were incubated with the homologous anti-capsule (K27) serum prior to their preparation for electron microscopy, a thick capsule was observed on cells of the encapsulated wild-type strain D280 (Fig. 1b) and on cells of the encapsulated R mutant F782 (not shown). As expected, cells of the non-capsulated S and R forms F492 and F470 treated in the same way did not show a capsule on their surface (not shown).

In order to study the accessibility of ECA to antibodies in the encapsulated and non-capsulated strains, the cells were incubated with rabbit ECA antiserum and subsequently with ferritin-conjugated goat anti-rabbit antibodies to identify the attached ECA antibodies on the cells. Washed cells of the encapsulated strains were further incubated with anti-capsule serum to visualize the bacterial capsule. Thin-section micrographs showed that cells of strains F470, F782, F492 and D280 were all labelled with ferritin, but the degree of labelling differed from strain to strain (Fig. 2a–d). The ECA-negative strain F1283 was not labelled at all (not shown) despite the fact that it was a non-capsulated R form.

Whole-mount electron microscopy

With the non-capsulated R form F470 a very dense labelling was observed by the whole-mount technique (Fig. 3a). Cells of the encapsulated R form F782 were also labelled but somewhat less densely than cells of strain F470 (not shown). The non-capsulated S form F492 showed a weaker labelling, and the ferritin was found in clusters (Fig. 3b). The encapsulated S strain D280 was only weakly labelled (not shown).

Similar differential labelling was observed when the IgM fraction of ECA antiserum was used instead of the ECA antiserum. The number of ferritin particles per μm² of whole-mount micrographs was found to decrease in the same order (non-capsulated R > encapsulated R > non-capsulated S > encapsulated S; Table 2). In experiments with the IgG fraction of ECA antiserum, ferritin labelling was denser, but the results were essentially the same (not shown).
Fig. 2. Thin-section micrographs of cells after incubation with rabbit ECA antiserum and labelling with ferritin-conjugated goat anti-rabbit IgG; in (b) and (d) the cells were further treated with anti-capsule serum to preserve the capsule. (a) Non-capsulated R form, F470 (O8⁻:K27⁻): the outer side of the
Fig. 3. Whole-mount micrographs of cells after incubation with rabbit ECA antiserum and labelling with ferritin-conjugated goat anti-rabbit IgG. (a) Non-capsulated R form, F470 (O8⁻:K27⁻): the cell shows a dense and uniform distribution of ferritin (positive control). (b) Non-capsulated S form, F492 (O8⁻:K27⁻): the ferritin particles are clustered. The bar markers represent 0.2 μm.

outer membrane shows many ferritin particles (dark dots), indicating ECA. (b) Encapsulated R form, F782 (O8⁻:K27): the ferritin particles indicating ECA are located beneath the capsule on the surface of the outer membrane. (c) Non-capsulated S form, F492 (O8⁻:K27⁻): clusters of ferritin particles are visible (arrowed). (d) Encapsulated S form, D280 (O8⁻:K27): ferritin particles are located on the outer membrane beneath the capsule (arrowed). The bar markers represent 0.2 μm.
Table 2. Numbers of ferritin particles attached to the surface of bacteria

Cells were treated with rabbit ECA antiserum (IgM fraction) and subsequently with ferritin-conjugated goat anti-rabbit antibodies. Whole-mount micrographs were prepared and the numbers of ferritin particles per µm² of micrograph were counted. The results show the mean values (± standard deviation) for seven determinations.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>No. of ferritin particles per µm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>F470</td>
<td>O8⁻:K27⁻</td>
<td>943 (± 82)</td>
</tr>
<tr>
<td>F782</td>
<td>O8⁻:K27⁻</td>
<td>148 (± 62)</td>
</tr>
<tr>
<td>F492</td>
<td>O8:K27⁻</td>
<td>66 (± 18)</td>
</tr>
<tr>
<td>D280</td>
<td>O8:K27</td>
<td>23 (± 11)</td>
</tr>
</tbody>
</table>

DISCUSSION

The presence of ECA and its accessibility to antibodies could be demonstrated by the indirect immunoferritin technique in all the strains and mutants of E. coli O8 : K27 examined with the exception of the ECA-negative strain F1283.

The average number of ferritin particles attached to the surface of the bacteria (particles counted per µm² of micrograph) was used as a quantitative measure of the accessibility of ECA. These values varied greatly, ranging from 943 for the non-capsulated R strain (F470) to only 23 for the encapsulated S strain (Table 2), suggesting that both the O and K antigens partly cover ECA on the surface of the outer membrane. However, since only one representative of each mutant type was studied this conclusion must be taken with some reservation, especially since also only one O : K type was studied.

The extent of the shielding effect of the K antigen on the accessibility of ECA can be seen by comparing the number of ferritin particles counted for the K⁺ and K⁻ strains (Table 2). For the R strains the number was reduced to 15% when comparing the K⁺ with the K⁻ form, whereas a reduction to 35% was obtained for the S strains. The K27 antigen, which belongs to the thermostable A type (Ørskov et al., 1977), does not apparently form such a dense net that antibodies cannot penetrate. Further studies with other strains of E. coli and with different K antigens are needed to reveal whether this is a general characteristic.

ECA can occur in two modifications, one the ubiquitous free ECA, and the other the LPS-bound immunogenic form (Kiss et al., 1978). The O8⁺ strains contain only the former type of ECA, whereas the O8⁻ strains contain both forms (Mäkelä & Mayer, 1976; Mayer & Schmidt, 1979). The presence of two forms of ECA in the R strains and of only the first form in S strains may also account for the observation that ferritin in the S strains showed a clustered distribution (Figs 2c, d and 3b), whereas it was evenly distributed in the R strains. One can assume that the lateral mobility of free ECA is higher than that of the LPS-bound form. Since R mutants of E. coli O8 : K27 with incomplete R cores are available which lack the immunogenic form of ECA (Whang et al., 1972), this assumption can be tested.

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REFERENCES


Accessibility of ECA to antibodies in E. coli


