Solubilization and Partial Characterization of the Colicin I Receptor of Escherichia coli

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SUMMARY

Treatment of the cell envelope of a colicin I-sensitive strain of Escherichia coli with the nonionic detergent Triton X-100 solubilizes an envelope component which forms a complex with 125I-colicin Ia and Ib. A similar component could not be shown in a strain known to be lacking active colicin I receptors. On this basis, this component is assumed to be the colicin I receptor.

The colicin I receptor was found to be sensitive to trypsin, but insensitive to DNase, RNase, periodate, and phospholipases. Its capacity to bind colicin I was inhibited by salt.

Sedimentation analysis in 5 to 20% sucrose gradients containing 0.05% Triton X-100 revealed that the colicin I receptor complex has a sedimentation constant of 12.4 S. Based on its behavior in gel filtration on Sepharose 4B columns containing detergent, the complex was shown to have a Stokes radius of 73.5 Å. On the basis of these experiments, the colicin I receptor complex has a molecular weight of 387,000 and a frictional ratio of about 1.5 and an axial ratio of about 8 or 9 for prolate or oblate ellipsoids, respectively.

The colicin Ia-receptor complex is assumed to be the colicin I receptor.

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Sensitivity of Escherichia coli to the action of the bactericidal proteins, colicins, requires the presence of a specific bacterial receptor on the cell surface (1, 2). Although information is available concerning the effects of colicin treatment on various metabolic functions, little is known concerning the nature of the interaction between a particular colicin and its cognate receptor, nor the events between the time of adsorption and observation of biochemical effects.

Recent studies utilizing fractionated E. coli cell envelopes have shown that whereas the cell wall fraction isolated from sensitive cells has the ability to neutralize the biological activity of colicins K (3) and E3 (4), a similar fraction from resistant strains does not. In addition, we have demonstrated the specific adsorption of iodinated colicin Ia and Ib to partially purified cell walls but not to the cytoplasmic membrane (5). Thus, in studies of four unique colicins, it has been shown that their receptor specificities reside in components of the bacterial cell wall.

In a recent study, Sabet and Schnaitman (6) have used Triton X-100 to solubilize a trypsin-sensitive envelope component which they have identified as the receptor for colicin E2 and E3. In the present study we report on the similar solubilization of the colicin Ia, Ib receptor, as well as on partial characterization of the colicin Ia-receptor complex.

MATERIALS AND METHODS

Bacterial Strains—E. coli K-12 strains W3110 str-r (JK1) and W3110 str-r 7 (BC3) were from our stock collection. BC3 is a colicin I-resistant mutant of JK1 which has been shown to be defective in its capacity to adsorb colicin Ia or Ib (7).

Colicin—Purified colicins were prepared as previously described (8) and iodinated by reaction with iodine monochloride (7). Colicin concentrations were determined from their extinction coefficients (9).

Protein Assay—Protein determinations were performed by the method of Lowry et al. (10) with bovine serum as a standard.

Reagents—Triton X-100, tris(hydroxymethyl)aminomethane (Trizma), EDTA, EGTA (ethylene glycol bis[β-aminoethyl ether]-N,N'-tetraacetic acid), RNase (bovine pancreas), phospholipase A (Vipera russellii), phospholipase C (Clostridium welchii), phospholipase D (cabbage), ß-galactosidase (E. coli), thyroglobulin (bovine), ovalbumin, and trypsin inhibitor (soybean) were purchased from Sigma. DNase (bovine pancreas) was obtained from Nutritional Biochemicals, and serum albumin (bovine, Fraction V) from General Biochemicals. Trypsin (pancreatic) was obtained from Worthington. Purified γ-globulin (rabbit) was a gift from D. Lopatin, University of Illinois.

Envelope Extracts—Cell envelopes prepared from 10 liters of cell culture as previously described (5) were suspended in 50 ml of 0.1 M Tris buffer (pH 8.0) containing 0.005 M EDTA and Triton X-100, 1% (v/v), and stored overnight in the cold. The suspension was next homogenized at room temperature by several passages through a 23-gauge needle before being centrifuged in a Spinco type 65 rotor for 1 hour at 50,000 rpm at 15°. Two volumes of cold acetone were slowly stirred into the chilled supernatant fraction, and the resulting precipitate collected by centrifugation at 10,000 rpm for 10 min in a Sorvall type SS34 rotor. The acetone precipitate was dissolved in 15 ml of 0.1 M Tris (pH 8.0) containing Triton X-100, 0.05% (v/v) (Buffer A), and dialyzed overnight against 2.5 liters of Buffer A in the cold. The dialysate was next clarified of insoluble material by cen-

* The abbreviation used is: EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid.
trifugation at 10,000 rpm for 10 min before being concentrated to a desired volume by ultrafiltration under nitrogen pressure with an Amicon PM 10 filter membrane. The extract was stored either at 10°C or frozen at -30°C.

**Gel Filtration**—Gel filtration chromatography was performed on agarose (Sepharose 6B, Pharmacia) columns (2.5 x 95 cm) equilibrated at 4°C with Buffer A containing 0.2 M NaCl and having a flow rate of 25 ml per hour. The gel filtration data are expressed in terms of $K_{AV}$, the parameter defined by Laurent and Killander (11), as follows:

$$K_{AV} = \frac{V_e - V_v}{V_t - V_v}$$

where $V_e$ is the elution volume of the solute, $V_v$ is the void volume of the column as determined by the appearance of blue dextran 2000 (Pharmacia), and $V_t$ is the total volume of the gel bed as determined by the elution volume of $^{125}$I.

**Density Gradient Centrifugation**—Density gradient centrifugation on 5 to 20% (w/v) sucrose linear gradients was performed by the procedures described by Martin and Ames (12). Except where noted the solvent was Buffer A. Samples were incubated with $^{125}$I-colicin I for 30 min at 37°C and cooled in ice before layering on top of the gradients. In cases where analysis was to be carried out in gradients containing NaCl, NaCl was added to a final concentration of 0.2 M after incubation. Centrifugation was performed at 5°C at 37,500 rpm for 16 to 19 hours in a Spinco SW 50.1 rotor. The gradients were fractionated at room temperature in an ISCO automatic fractionator. The contents of the tube were pumped out the top of the centrifuge tube by introduction of Fluorinert (3M Company) through a needle inserted into the tube bottom. The position of $^{125}$I-colicin in the gradient was determined by direct counting of fractions (0.2 ml) in a Nuclear Chicago gamma counter. For determination of optical densities, 1 ml of 0.1 M Tris-HCl (pH 8.0) was added and the absorbance at 280 nm determined in a Beckman DU spectrophotometer.

**RESULTS**

**Neutralization of Colicin**—Treatment of isolated cell envelopes under the conditions described under “Materials and Methods” with Triton X-100, 1% (v/v), leads to solubilization of 30% of the envelope protein. When the resulting Triton X-100-insoluble material was collected by centrifugation and examined for its capacity to bind $^{125}$I-colicin I, it was found that Triton X-100 treatment had reduced its binding capacity by 90%. After precipitation with cold acetone and dissolution and dialysis in a 0.1 M Tris-HCl (pH 8.0)-Triton X-100, 0.05% (v/v) buffer, the Triton X-100-soluble supernatant fraction from the extraction mixture was tested for its capacity to neutralize colicin activity. As can be seen in Table I, incubation of either colicin Ia or Ib with the Triton X-100 extract of cell envelopes prepared from the colicin I-sensitive strain, JK1, leads to colicin inactivation. In contrast, a similar extract prepared from strain BC3, known to be defective in its ability to adsorb colicin Ia and Ib, has no inhibitory effect. These results are consistent with the notion that Triton X-100 treatment had solubilized the colicin I receptor. Colicin inactivation by JK1 extracts is presumably due to an essentially irreversible association of the colicin with this receptor.

**Sucrose Gradient Centrifugation**—When $^{125}$I-colicin Ia was incubated with the Triton X-100 extract of strain JK1, and the mixture sedimented through 5 to 20% sucrose gradients, the results shown in Fig. 1 were obtained. The $^{125}$I-colicin Ia is neutralized by the Triton X-100 extract of strain JK1 in a manner consistent with its inability to adsorb colicin Ia or Ib. In contrast, a similar extract prepared from strain BC3, known to be defective in its ability to adsorb colicin Ia and Ib, has no inhibitory effect. These results are consistent with the notion that Triton X-100 treatment had solubilized the colicin I receptor.

**FIG. 1.** Sedimentation analysis of $^{125}$I-colicin Ia incubated with Triton extracts on 5 to 20% sucrose gradients. Sedimentation is from left to right. Top, the reaction mixture contained in a volume of 0.2 ml of Buffer A-0.02 μg of $^{125}$I-colicin Ia. Center, and bottom, same as top except containing 0.02 μg of JK1 extract and 600 μg of BC3 extract, respectively. The mixtures were incubated at 37°C for 30 min, chilled, and subjected to sucrose gradient centrifugation under conditions described under “Materials and Methods.”

### Table I

Neutralization of colicin activity by Triton extracts

<table>
<thead>
<tr>
<th>Additions</th>
<th>Killing activity units per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. None</td>
<td>0</td>
</tr>
<tr>
<td>2. Colicin Ia</td>
<td>12</td>
</tr>
<tr>
<td>3. Ia + JK1 extract</td>
<td>&lt;1</td>
</tr>
<tr>
<td>4. Ia + BC3 extract</td>
<td>18</td>
</tr>
<tr>
<td>5. Colicin Ib</td>
<td>48</td>
</tr>
<tr>
<td>6. Ib + JK1 extract</td>
<td>&lt;1</td>
</tr>
<tr>
<td>7. Ib + BC3 extract</td>
<td>48</td>
</tr>
</tbody>
</table>

**Fig. 1.** Sedimentation analysis of $^{125}$I-colicin Ia incubated with Triton extracts on 5 to 20% sucrose gradients. Sedimentation is from left to right. Top, the reaction mixture contained in a volume of 0.2 ml of Buffer A-0.02 μg of $^{125}$I-colicin Ia. Center, and bottom, same as top except containing 0.02 μg of JK1 extract and 600 μg of BC3 extract, respectively. The mixtures were incubated at 37°C for 30 min, chilled, and subjected to sucrose gradient centrifugation under conditions described under “Materials and Methods.”
found in a fraction (Fig. 1, center) well separated from the position of free colicin (Fig. 1, top). When the Triton X-100 extract is derived from the colicin I-resistant strain, BC3, 125I-Ia is found in a position close to that of free colicin (Fig. 1, bottom). The slightly faster sedimentation of 125I-Ia in Fig. 1, bottom, is quite reproducible and is discussed below. When 125I-Ib replaces 125I-Ia in the experiment described in Fig. 1, essentially the same results are obtained.

As shown in Fig. 2, when a large excess of either nonradioactive purified colicin Ia or Ib is present in a reaction mixture containing the Triton X-100 extract of JKI and 125I-colicin Ia, the radioactive colicin is found at a position close to free colicin. Similar results are seen in the reciprocal experiment utilizing 125I-colicin Ib. The results described in Figs. 1 and 2, suggest that Triton X-100 treatment of envelopes isolated from strain JKI solubilizes a component which interacts with colicin Ia or Ib to form a stable complex. A component that this complex is not obtained from cells known to be deficient in the colicin I receptor, strongly suggests that this component is the colicin I receptor.

**Sensitivity of Complex Formation to Various Treatments**

Treatment of the Triton X-100 extract with the following substances for 60 min at 37°C before addition of 125I-colicin Ia had no effect on either complex formation or on the sedimentation properties of the resulting complex: DNase (200 μg per ml), RNase (200 μg per ml), sodium metaperiodate (0.4 m), glycerol (5%, v/v), phospholipase C (250 μg per ml), and phospholipase D (250 μg per ml). Although phospholipase A treatment (250 μg per ml) does not inhibit complex formation, it does render the complex insoluble in buffers containing 0.05% (v/v) Triton X-100. It should be noted that experiments utilizing phospholipase treatment were complicated by the fact that the concentration of CaCl₂ (0.005 M) required for enzyme activity was partially inhibitory to complex formation. Attempts at neutralizing the effects of CaCl₂ by addition of EGTA were unsuccessful. Although the formation of a stable 125I-Ia-receptor complex was inhibited by the presence of 0.2 m NaCl in the incubation mixture, once formed in solutions containing no NaCl, the complex was not dissociated by subsequent NaCl (0.2 m) addition (see Figs. 4 and 5). The presence of the following salts (0.2 m) were also inhibitory to the formation of a stable Ia-receptor complex: CsCl, KCl, MgCl₂, NaBr, and NH₄Cl. Incubation of the JKI Triton X-100 at 90° for 10 min inactivated its ability to form a complex with 125I-colicin Ia.

As shown in the experiments described in Fig. 3, the colicin I receptor is sensitive to trypsin. In Fig. 3C, the Triton X-100 extract was incubated with trypsin before addition of trypsin inhibitor and 125I-colicin. In the experiment shown in Fig. 3B, the trypsin inhibitor was added to trypsin prior to addition of the Triton X-100 extract and 125I-colicin. The procedure resulting in Fig. 3A was the same as in Fig. 3B except that the extract was omitted from the incubation mixture. These experiments show that trypsin treatment leads to the absence of the fast sedimenting complex, showing that complex formation requires intact protein or peptide to bind colicin. Identical results were observed using 125I-colicin Ia.

Studies with "Resistant" or Inactivated "Sensitive" Extracts

As mentioned above, incubation of 125I-colicin I with a Triton X-100 extract of envelopes isolated from colicin-resistant strain BC3 leads to a slightly faster sedimentation of the colicin (compare Fig. 1, top, with Fig. 1, bottom). Neither trypsin treatment, heat treatment, nor incubation with phospholipase A of such "resistant" extracts prevented this altered rate of sedimentation. Examination of several experiments utilizing "sensitive" extracts reveals a similar situation. Thus, either the presence of competing nonradioactive colicin in the reaction mixture (Fig. 2), or heat (data not shown), or trypsin (Fig. 3C) inactivation of the "sensitive" extract prior to colicin addition leads to sedimentation profiles similar to those seen in Fig. 1, bottom; that is, the

![Fig. 2](http://www.jbc.org/)  
**FIG. 2.** Inhibition of 125I-colicin Ia complex formation by excess nonradioactive colicin Ia or Ib. Reaction mixtures contained the following in a total volume of 210 μl of Buffer A. (1) 0.62 μg of 125I-colicin Ia, ○ ○ ○; (2) 0.62 μg of 125I-Ia and 470 μg of JKI Triton extract, ■ ■ ■; (3) same as (2) except containing 47 μg of nonradioactive Ia, △—△; (4) same as (2) except containing 47 μg of nonradioactive Ib, ▲—▲. Sucrose gradient centrifugation was as in Fig. 1.

![Fig. 3](http://www.jbc.org/)  
**FIG. 3.** Sensitivity of colicin I receptor to trypsin. Reaction mixture in a total volume of 0.17 ml of Buffer A contained the following: (A) trypsin, 200 μg; (B) trypsin, 200 μg; trypsin inhibitor, 200 μg; Triton X-100 extract of strain JKI, 440 μg; (C) trypsin, 200 μg; Triton X-100 extract of strain JKI, 440 μg. After incubation for 90 min at 37°, trypsin inhibitor (200 μg) was added to A and C and incubation carried out an additional 20 min. 125I-Colicin Ib (0.85 μg) was then added to A, B, and C and after incubation for 30 min at 37°, the mixtures were subjected to sucrose gradient analysis as described in Fig. 1. Sedimentation is from left to right.
The Stokes radius of the \textsuperscript{125}I-colicin Ia receptor complex was determined by agarose filtration chromatography (Fig. 4). In these experiments, complexes were pooled from sucrose gradients and dialyzed against column buffer before being applied to the columns. The same results were obtained when the colicin-Triton X-100 extract reaction mixture was applied directly to the column. In either case the radioactivity eluted as a single peak with a $K_{av}$ of 0.31. It should be noted that it was necessary to include 0.2 M NaCl in the column buffer to prevent adsorption of the colicin to the resin. From a comparison of the gel filtration behavior of the Ia-receptor complex to that of several standard proteins whose Stokes radius is known or can be calculated, a Stokes radius of 73.5 Å was determined.

As shown in Fig. 5, the sedimentation relationships originally described by Martin and Ames (12) are valid in sucrose gradients containing 0.05% Triton. From a comparison of the sedimentation behavior of the colicin-receptor complex with that of several standard proteins, an extrapolated $s_{20,w}$ of 12.4 is obtained. As shown in Fig. 6, the sedimentation behavior of the complex is dependent upon buffer composition. When NaCl is omitted from the gradients, an $s_{20,w}$ of 8.1 is obtained, suggesting either ionic dependent conformational states of the complex or changes in stoichiometric relationships between colicin and receptor molecules or the complex and other molecules present in the extract. When detergent is omitted from the sucrose gradients, the radioactivity is found at the bottom of the centrifuge tube presumably due to highly aggregated forms of the complex. It should be noted that under all of the conditions of sedimentation analyses examined, free colicin sedimented as a single peak with an $s_{20,w}$ of 3.7.

Knowledge of the independently determined Stokes radius ($a$) and sedimentation coefficient ($s$) allows a calculation of the molecular weight ($M$) and frictional ratio ($f/f_0$) of the \textsuperscript{125}I-colicin I-receptor complex according to the following equations (14, 15).

$$M = \frac{60\pi N}{1 - \frac{a^2}{s}}$$

$$\frac{f}{f_0} = a - \frac{41N}{3M(\theta + b/\rho)}$$

where $\rho$ is the solvent density in grams per cm$^3$, $N$ is Avogadro's number, and $\theta$ is the solvation factor in grams of solvent per g of protein. The values obtained are shown in Table II. The data indicate that the colicin I-receptor complex is asymmetrical and has a molecular weight of 387,000. The validity of these calculations depends on an assumption of $\theta$. Unfortunately a direct determination of $\theta$ by several common methods proved difficult. For example, a calculation of $\theta$ by comparison of the sedimentation properties of the complex in sucrose gradients containing H$\textsubscript{2}$O and D$\textsubscript{2}$O were impossible due to the instability of the complex in D$\textsubscript{2}$O. Furthermore, methods based on sedimentation in sodium bromide or cesium chloride were unsatisfactory for a similar reason.

The rather high $f/f_0$ is probably not due to unusual solvation

**Fig. 4 (left).** Stokes radius of \textsuperscript{125}I-colicin Ia-receptor complex calculated from gel filtration chromatography on Sepharose 6B. The Stokes radius of the standard proteins was calculated according to the equation, $a = kT/6\pi ND$, where $k$ is the Boltzman constant, $T$ is absolute temperature, $\eta$ is viscosity of the medium, and $D$ is the diffusion coefficient. Physical data were taken from Sober (20). The column was equilibrated and run in Buffer A containing 0.2 M NaCl. In this experiment the standard proteins were run simultaneously with the Ia-receptor complex. The presence of the standard proteins had no effect on the $K_{AV}$ of the complex.

**Fig. 5 (right).** Sedimentation coefficient of \textsuperscript{125}I-colicin Ia-receptor complex determined on 5 to 20% (w/v) sucrose gradients prepared in Buffer A. The experiment is as described in Fig. 5.
Molecular parameters of $^{125}$I-colicin Ia-receptor complex

<table>
<thead>
<tr>
<th>Physical parameter</th>
<th>Value</th>
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<tr>
<td>$K_A$ *</td>
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</tr>
<tr>
<td>Stokes radius, $a$ ($\lambda$)</td>
<td>73.5</td>
</tr>
<tr>
<td>$K_M$</td>
<td>12.4</td>
</tr>
<tr>
<td>Molecular weight *</td>
<td>387,000</td>
</tr>
<tr>
<td>$f_i/f_o$ ($\delta = 0$)</td>
<td>1.51</td>
</tr>
<tr>
<td>$f_i/f_o$ ($\delta = 0.2$)</td>
<td>1.40</td>
</tr>
</tbody>
</table>

* Distribution coefficient (11), determined by gel filtration.

** Determined as described in Fig. 4.

** Determined as described in Fig. 5.

** Determined as described in text.

* Calculated according to the method of Schachman (16).

Discussion

Triton X-100 has proved to be a useful detergent for solubilization of both cytoplasmic and outer membranes of E. coli (17, 18). We have previously shown that the colicin I receptor is a component of the outer membrane (5) and in the present study describe its solubilization and partial characterization. Our results show that treatment of E. coli cell envelopes with 1% Triton X-100-EDTA solubilizes a component which has the capacity to form a complex with $^{125}$I-colicin Ia or Ib. This component cannot be shown in the envelope of an E. coli mutant known to lack functional colicin I receptors. These results strongly suggest that this component is the colicin I receptor.

The detergent-soluble colicin I receptor is inactivated by boiling or trypsin digestion showing that intact protein or peptide is necessary for its activity. It is insensitive to nuclease or lipase treatment. Its nuclease resistance is important since it is known that $^{125}$I-colicin Ia binds to DNA isolated from both sensitive and resistant cells.

Based on its sedimentation coefficient and Stokes radius, as determined in buffer containing 0.05% Triton X-100, the colicin I-receptor complex has a molecular weight of 387,000 and an $f_i/f_o$ of 1.5. Although these data suggest that the colicin I-receptor complex is elongated (axial ratio, 0.9 to 1.1), such a conclusion should be approached cautiously. The validity of the determination of the sedimentation constant by the method of Ames and Martin used in this study depends on the assumption that the $\delta$ of the component under study is close to that of the standards (12). In the present study, we have taken the $\delta$ of the colicin-receptor complex to be 0.734 which is an average value for proteins. This assumption was necessitated by our inability to determine $\delta$ experimentally. Although receptor activity was resistant to lipase or periodate treatment, we cannot rigorously exclude the possibility of lipid or carbohydrate content, or even bound Triton X-100 which could cause the $\delta$ to differ substantially from this assumed value. There also exists the possibility that receptor-bound detergent leads to anomalous behavior of the receptor in gel filtration chromatography leading to an apparent Stokes radius higher than one expected for the receptor per se. As discussed above, it is unlikely that the high $f_i/f_o$ value can be explained by unusual hydration of the complex.

The stoichiometric relationship between colicin molecules and receptor units is not known. Assuming a 1:1 ratio and a molecular weight of 80,000 for colicin Ia (9), the non-colicin component of the complex under study would have a molecular weight of 307,000. Clearly, receptor purification is a desirable prerequisite for further characterization.

We have found that reaction mixtures containing 290 µg of Triton X-100 extract protein leads to at most 1 µg (1.25 x 10$^{-11}$ moles) of colicin Ia found in a complex with receptor. Assuming a 1:1 colicin receptor stoichiometry, this corresponds to 1.25 x 10$^{-11}$ moles of receptor units per 290 µg of extract protein. Our preparation, thus, yields 1000 receptor units per cell in good agreement with our previous in vivo studies which showed that cells of strain JK1 have approximately 2000 to 8000 specific receptors per cell (7).

Recent in vivo experiments have shown that the interaction of colicin I molecules with their receptors is very much dependent on ionic conditions. A similar situation is seen in the present study and suggests that ionic considerations are important in either maintaining colicin and receptor in active conformation, or that ionic bonds are involved in electrostatic attractions between the colicin and its receptor, or both. Consistent with this suggestion was the finding that the colicin I-receptor complex was not stable in D$_2$O. Since hydrophobic interactions are thought to be strengthened when D$_2$O substitutes for H$_2$O as a protein solvent (19), the presence of D$_2$O may favor interactions between receptor and Triton X-100 rather than between receptor and colicin.

The altered sedimentation property of $^{125}$I-colicin Ia or Ib when incubated with the Triton extract obtained from colicin I-resistant cells is not understood. These results are also obtained in experiments in which the extract is treated with trypsin, phospholipase A, or heat treatment prior to colicin addition. This altered sedimentation rate of the colicin I is identical with that seen in experiments utilizing JK1 extracts in which receptors were inactivated by either trypsin or heat treatment prior to colicin addition or in experiments in which the incubation mixtures contained an excess of nonradioactive colicin Ia or Ib. These observations can be explained in several ways. It is possible that the altered rate of sedimentation is due to an extract-induced conformational change in the colicin molecule. It is unlikely that such an alteration would be caused by a possible higher Triton X-100 concentration in the extracts due to our inability to effectively lower its concentration from 1 to 0.05% by the procedures which we have employed, since free colicin migrates as 3.7 $S$ in gradients containing 0.05 to 1% Triton X-100. Another possibility is that there exists in both JK1 and BC3 extracts a component having the capacity to form a complex with colicin I. Since incubation of JK1 extracts with limiting concentrations colicins leads to preferential formation of colicin-receptor complexes, this nonspecific "binder" must have a colicin affinity less than that of receptors. This would be consistent with our results showing that incubation of colicin I with the BC3 extract does not neutralize its killing activity. It is

$^3$ R. Cihlar and J. Konisky, unpublished results.
unlikely that this nonspecific binder is lipopolysaccharide (LPS) since purified LPS has been found not to bind $^{125}$I-colicin Ia or Ib. Our results dictate that this "binder" be refractory to trypsin, heat, and phospholipase A. It is interesting to note that our studies on the interaction of colicin Ia with whole cells led us to propose two classes of colicin binding sites on the cell surface. One class of sites (the specific colicin receptor) was observed only on sensitive cells and had an affinity constant an order of magnitude higher than the second class of sites which are found on both sensitive and resistant cells (7). Although it is possible that the nonspecific binding component suggested above is, in fact, identical with the one suggested on studies with whole cells, further work is required for clarification of this point.

REFERENCES
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