COLICIN IA AND IB BINDING TO ESCHERICHIA COLI ENVELOPES AND PARTIALLY PURIFIED CELL WALLS

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The specific binding of ^{125}Iodine labelled colicin Ia and Ib to *Escherichia coli* cell envelopes and partially purified cell walls is demonstrated. Neither partially purified cytoplasmic membranes isolated from a wild type sensitive strain nor envelopes or cell walls prepared from an *E. coli* mutant known to be defective in the colicin I receptor could bind the colicins. Competition studies suggest that colicins Ia and Ib have a common bacterial receptor which resides in the bacterial cell wall.

INTRODUCTION

To fully understand the mechanism of action of the bactericidal proteins, colicins, will require an elucidation of the processes whereby colicins associate with specific receptors on the surface of the bacterium *Escherichia coli.* As one approach, we have recently studied the interaction of iodinated, yet biologically active, colicin Ia with its cognate receptors (1). Our results showed that sensitive *Escherichia coli* cells have approximately 2–3,000 colicin Ia specific receptors with an average association constant of approximately 10^{10} M^{-1} at 37°C. Mutants selected for resistance to colicin Ia were shown to be defective in their capacity to bind either colicin Ia or the closely related colicin Ib. Furthermore, these colicins were shown to compete for a common receptor.

As seen under the electron microscope, the *E. coli* cell envelope consists of three distinct morphological layers (2). The cell wall consists of an outer membrane on the bacterial surface and an underlying rigid peptidoglycan murein layer. These layers are connected by a lipoprotein recently characterized by Braun and Borsch (3). Interior to the cell wall is the cytomplasmic membrane which, for the most part, is separated from the cell wall layers by the periplasmic space.

Recently, methods have been devised for fractionating the total cell envelope of *E. coli* into component layers by use of sucrose gradient centrifugation (4). This has made possible the localization of colicin receptors within the cell envelope. Recent work with colicin K (5) and colicin E3 (6), showed that, whereas partially purified cell wall fractions isolated from colicin sensitive strains neutralize colicin activity, cell wall fractions isolated from colicin resistant cells do not. Partially purified cytoplasmic membrane fractions isolated from colicin sensitive strains showed little capacity to neutralize colicin activity. These results were interpreted as indicating that the specific receptor for colicins K and E3 resides in the cell wall.

In the present study we have extended our studies on the interaction of iodinated colicins Ia and Ib with their cognate receptors. We have been able to demonstrate specific

binding of ^{125}I-colicin Ia or Ib to both total cell envelope and isolated cell wall fractions, but not to the *E. coli* cytoplasmic membrane. Isolated envelopes and cell walls from a colicin resistant strain do not have the capacity to bind colicin Ia or Ib. Our results show that colicin Ia and Ib adsorb to a common receptor which is localized in the bacterial cell wall.

MATERIAL AND METHODS

Bacterial Strains

Escherichia coli K12 strains W3110 str-r (JK1) and W3110 str-r I^r (BC3) were from our stock collections. BC3 is a colicin I resistant mutant of JK1 which has been shown to be defective in its capacity to bind colicin Ia or Ib (1).

Iodinated Colicin

^{125}I-colicin Ia and Ib were prepared and purified as previously described (1). Iodination of the colicins led to radioactive derivatives exhibiting 85–100% of the original biological activity observed with noniodinated colicin. For quantitation of specific activity, the colicins were assumed to have molecular weights of 80,000 (7).

Preparation of Subcellular Fractions

Fractions were prepared essentially as described by Schnaitman (4). Cells were grown at 37°C in 800 ml of Tris medium (8) containing 0.15% glucose and 0.15% casamino acids to a concentration of approximately 3×10^8 per milliliter, harvested by centrifugation at 4°C, and suspended in 70 ml of 0.05 M Tris-HC1, pH 8.0, containing 0.001 M EDTA. The suspension was next blended in a Sorvall Omni-Mixer for 1 min at a speed setting of 7.5. The suspension was then centrifuged at 4°C at 10,000 g for 10 min, and the pellet resuspended in 25 ml 0.05 M Tris-HC1, pH 8.0–0.001 M EDTA. After addition of 0.7 mg RNase and 0.7 mg DNase, the cells were broken by a single passage through a French pressure cell maintained at 15,000 psi. After addition of $MgC1_2$ to a final concentration of 0.002 M, the mixture was centrifuged at 4°C at 3,000 g for 5 min in order to remove unbroken cells and aggregated debris. The supernatant fraction was next centrifuged at 5°C in a Spinco 50.1 type rotor for 60 min at 42,000 rpm. The pellet (cell envelope fraction) was drained well and then suspended in 3 ml 0.01 M N-2-hydroxyethyl-peperazine-N^1–2 ethane sulfonic acid (HEPES) buffer, pH 7.4. This suspension was then homogenized in a tissue grinder in order to break up large aggregates. Cell envelope fractions prepared in this manner had a protein concentration of 2–5 mg/ml. This fraction contained approximately 1×10^4 viable cells.

For preparation of cell wall-enriched and cytoplasmic membrane enriched-fractions, 1 ml of cell envelope was fractionated per 28 ml discontinuous sucrose gradient exactly as described by Schnaitman (4). After centrifugation at 12°C for 16 hrs at 25,000 rpm in a Spinco SW 25.1 type rotor, two discrete opaque bands could be seen corresponding to the cytoplasmic membrane enriched fraction and the cell wall enriched fraction (4, and below). Each band was carefully removed with a syringe and diluted at least 5-fold with 0.01 M–HEPES buffer, pH 7.4. The isolated fractions were next centrifuged at 12°C at 42,000 rpm for 2 hrs in a Spinco type 50.1 rotor. After draining of the pellets, each fraction was resuspended in 2 ml of 0.1 M Tris-HC1, pH 8.0. Protein concentrations for

TABLE I. Composition of Various Fractions

Fraction	Protein* (% Total)	Lipopolysaccharide** (Specific Activity)	SDH† (Specific Activity)
Envelope	100	0.39	0.35
Wall-enriched	87	0.48	0.43
Membrane-enriched	13	0.16	0.78

*Determined by the method of Lowry et al (9).
**Determined as KDO by the method of Osborn et al (10). Expressed as optical density at 548 nm/mg. protein.
†Succinic dehydrogenase (SDH) determined at the method of Osborn et al. (10). Expressed as Δ optical density at 550 nm/min/mg protein.

membrane-enriched and cell wall-enriched fractions were 0.2–0.3 and 2–3 mg/ml, respectively.

In Table I is shown the distribution of protein, lipopolysaccharide, and succinic dehydrogenase in the various fractions. In agreement with the results of Schnaitman (4) the more dense fraction contains approximately 80% of the total envelope protein and is enriched in cell wall specific lipopolysaccharide while the less dense fraction is enriched in the membrane specific enzyme succinic dehydrogenase. Based on these data and the studies of Schnaitman (4) the less dense fraction and denser fraction are designated cell membrane-enriched and cell wall-enriched fraction, respectively.

Binding Assays

The capacity of various subcellular fractions to bind ^{125}I-colicin Ia or Ib was determined by the filter paper method described previously (1) as well as by sucrose gradient centrifugation. In the filter paper assay ^{125}I-colicin was incubated with an amount of either total cell envelope, cell wall-enriched fraction, or cytoplasmic membrane-enriched fraction for 30 min at 37°C. These reactions were carried out in 0.1 M Tris-HCl, pH 8.0–0.01 M–β–mercaptoethanol containing 1–2 mg bovine serum albumin per milliliter (Buffer A) in a total volume of 1–2 ml. The mixtures were next filtered over Schleichter and Shuell B6 filter paper. Under the conditions used, greater than 90% of the free colicin passes through the filter paper. Preliminary experiments utilizing (^{14}C)–proline labelled subcellular fractions showed that, under the conditions employed, greater than 97% of the envelope, cell wall, or cytoplasmic membrane protein was retained on the filter paper. Thus, after correcting for nonspecific binding (1), the amount of ^{125}I-colicin retained on the filter paper was taken to represent specific binding to the particular fraction present in the incubation mixture.

Alternatively, the colicin-subcellular fraction incubation mixture (0.2 ml) was layered over a discontinuous sucrose gradient of the following composition: (bottom to top) 1.4, 1.7, 1.7 ml of 2.02 M, 1.44 M, 0.77 M sucrose, respectively, all in 0.01 M–HEPES, pH 7.4. The mixtures were next centrifuged for 4 hrs at 40,000 rpm in a Spinco type SW 50.1 rotor maintained at 10–12°C. Upon completion of the run, the rotor was decellerated to 5–10,000 rpm with braking, at which time braking was discontinued. 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 through a needle inserted into the tube bottom. The position of ^{125}I-colicin in the gradient was determined by direct counting of 0.2 fractions in a Nuclear Chicago

gamma counter. For determination of optical densities, 1 ml of 0.1 M Tris-HCl, pH 8.0 was added to each fraction and the absorbancy at 280 nm determined in a Beckman DU spectrophotometer.

RESULTS

Binding of Colicin to Subcellular Fractions

Table II shows that the envelope fraction isolated from colicin sensitive strain JK1 is capable of binding iodinated colicin Ia or Ib. However, the envelope fraction isolated from colicin resistant strain BC3 is defective in this binding capacity. This binding specificity is identical to in vivo studies with whole cells which showed that whole cells of strain BC3 had defective binding capacity (1). When the cell envelope was fractionated into cell wall-enriched and membrane-enriched fractions by a modification of the method of Schnaitman (4), and the capacity of each fraction tested for its ability to bind colicin, it was found that binding was preferentially to the cell wall-enriched fraction (lines 3 and 4). The cell wall-enriched fraction from strain BC3 did not adsorb either colicin. In different preparations the amount of colicin bound per milligram membrane-enriched fraction was 3 to 15% of that bound per milligram cell wall-enriched fraction protein. Similar experiments using (^{14}C)–proline labelled membrane fractions showed that colicin I treated membranes were quantitatively retained on the filter papers. Although the small amount of the binding to the membrane fraction may represent binding to the membranes themselves, such fractions are usually contaminated with some cell wall material which could

TABLE II. Binding of ^{125}I-Ia and ^{125}I-Ib to Subcellular Fractions

		Colicin Adsorbed (cpm)	
Strain	Fraction	^{125}I-Ia	^{125}I-Ib
1. W3110 str-r	envelope	3071	699
2. BC3	envelope	0	0
3. W3110 str-r	wall	1176	625
4. W3110 str-r	membrane	36	72
5. BC3	wall	0	23
6. W3310 str-r	wall (lysozyme)	0	0
7. W3110 str-r	membrane (lysozyme)	0	0
8. None		180	105

Each reaction mixture contained the following components in 2.6 ml of Buffer A:

1 and 2: 70 μg envelopes and 0.06 μg ^{125}I-colicin Ia (4585 cpm) or ^{125}I-Ib (2825 cpm).

3: 20 μg cell walls and 0.06 μg ^{125}I-colicin Ia (6040 cpm) or ^{125}I-Ib (2300 cpm).

4: Same as 3 except containing 20 μg membrane.

5: Same as 3.

6: Same as 3 except cell wall were incubated with lysozyme (2.5 mg) at 37° for 30 min before addition of colicin.

7: Same as 6 except that reaction mixture contained 20 μg membranes.

8. Same as 3 except that reaction mixture contained neither walls nor membranes.

Each reaction mixture containing the various fractions was incubated for 30 min at 37° (± lysozyme). ^{125}I-colicin I was next added and incubation continued for an additional 30 min before the amount of bound colicin was determined by the filter paper method. The amount of colicin bound in 1–7 has been corrected for nonspecific binding. Lysozyme has no effect on colicin Ia or Ib activity.

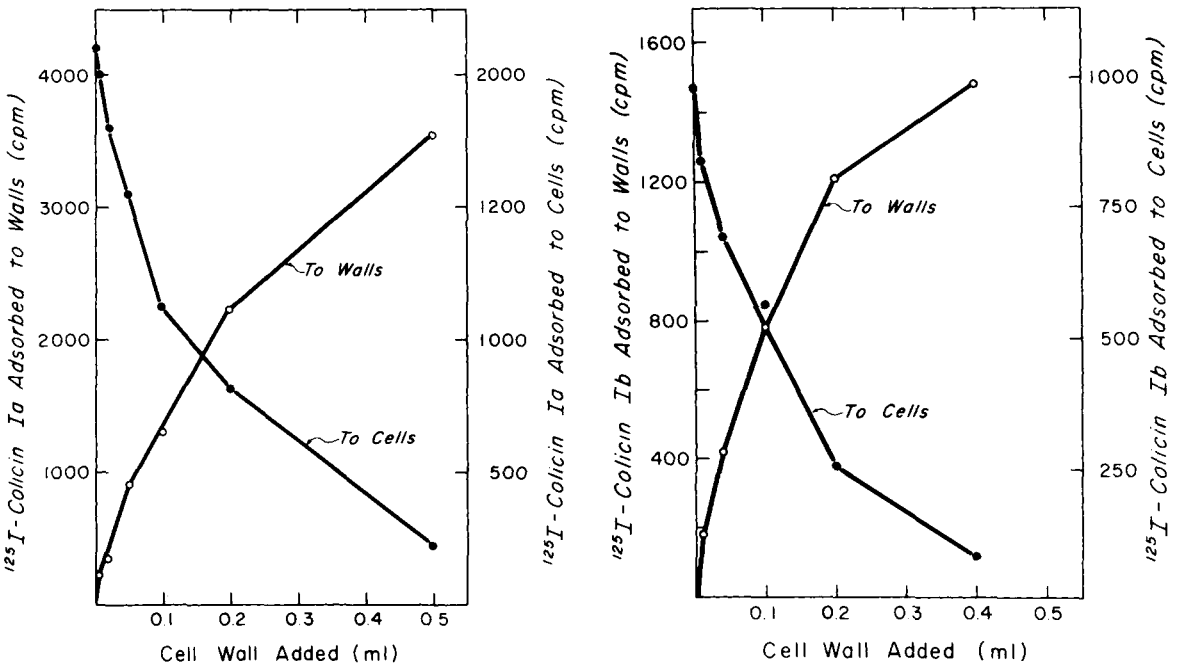

Fig. 1. Specific binding of ^{125} I-colicin Ia or Ib to cell wall fraction as a function of cell wall concentration. Various amounts of cell wall (0–20 μg) were incubated in a total volume of 1.9 ml of buffer A containing 60 μg ^{125} I-Ia (5296 cpm) for 30 min at 37°C. The mixture was next centrifuged twice at 15,000 rpm in a Sorvall SE 12 rotor. The final pellet was resuspended in 1.0 ml of buffer A, and the amount of Ib bound to cell wall material was determined by filtration (–0–0–). The combined supernatant fractions were added to 1.3 ml of buffer A containing 2 × 10^8 cells of strain JK1 and the amount of ^{125} I-Ia bound to cells (–●–●–) determined as previously described (1).

be responsible for the binding observed to this fraction. Lysozyme treatment of the cell wall-enriched fraction of strain JK1 (Table II, line 6) leads to complete loss of binding capacity. It should be noted that lysozyme sensitivity does not necessarily indicate receptor sensitivity per se, since any treatment which results in a loss of ability to retain the receptor on the filter paper would give a similar result. Table II, line 7, shows that treatment of the membrane-enriched fraction with lysozyme leads to inability to bind colicin. The simplest interpretation is that our membrane-enriched fraction is contaminated with small amounts of cell wall material whose binding capacity is lysozyme sensitive.

Figure 1 shows that the amount of ^{125} I-Ia or ^{125} I-Ib present on the filters is proportional to the amount of enriched-cell walls present in the reaction mixtures. Furthermore, cell walls compete with whole cells for binding of free colicin. When the various ^{125} I-colicin-cell wall complexes are removed from the solution by centrifugation and the amount of free colicin remaining in the supernatant fraction determined by adsorption to whole cells, there is a progressive decrease in amount of supernatant colicin as the amount of colicin bound to walls increases.

When increasing amounts of ^{125} I-Ia or ^{125} I-Ib are incubated with a fixed concentration of the cell wall fraction, and the amount of colicin bound at each concentration determined, results of the kind shown in Fig. 2 are obtained. Thus binding to the cell wall fraction is a saturable process with respect to colicin concentration. Furthermore, this

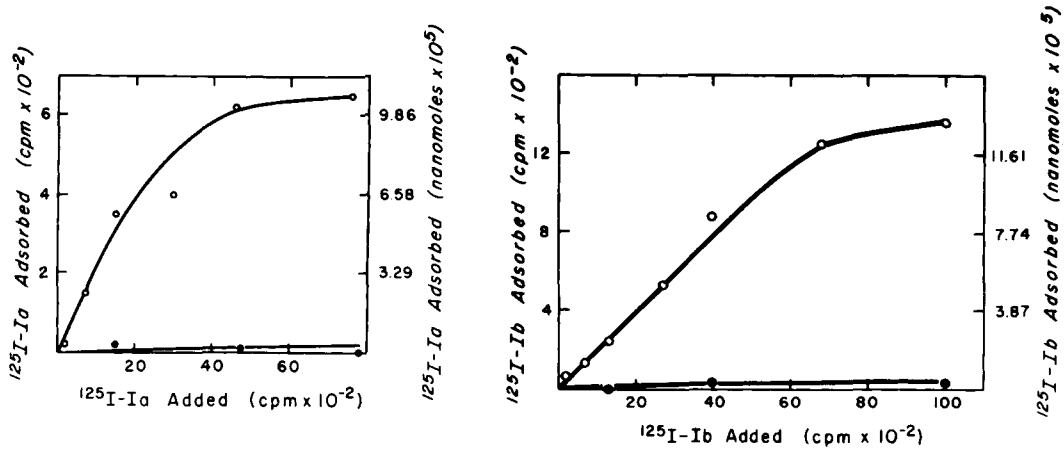

Fig. 2. Adsorption of ^{125}I-Ia or ^{125}I-Ib to isolated cell walls as a function of colicin concentration. Various amounts of ^{125}I-Ia or ^{125}I-Ib were incubated with 8 μg cell wall for 30 min at 37°C with shaking. Under the assay conditions employed, adsorption was complete by 20 min. –○–○–, JKI; –●–●–, BC3.

fraction can bind near equal amounts of either colicin Ia or Ib. When cell walls prepared from resistant strain BC3, which does not bind ^{125}I-Ia or Ib in vivo (1), replace walls from sensitive strain JK1 in the reaction mixture, little radioactivity is found associated with filter discs. Thus, the binding specificity observed in vivo is reflected in the binding capacities of isolated cell wall fractions.

Competition of Ia and Ib for Binding Sites

When increasing amounts of purified noniodinated colicin Ia or Ib are included in the ^{125}I-Ia or ^{125}I-Ib-cell wall incubation mixture, there is a progressive decrease in the amount of radioactive colicin bound to cell walls (Fig. 3a,b). Thus competition exists between radioactive colicin and either homologous or heterologous nonradioactive colicin. It is interesting to note that, in the experiment described in Fib. 3a, b, colicin Ia is a better competitor than is Ib. This result was also noted in previous studies dealing with adsorption of radioactive Ia to whole cells, and may indicate that colicin Ia binds more avidly to colicin I receptors than does colicin Ib.

Kinetics of Adsorption

We have previously shown that the binding of ^{125}I-colicin Ia to whole cells is inhibited by anti-colicin Ib Fab' fragments. This finding was used to study the kinetics of the binding reaction (1). We have carried out a similar analysis here. As shown in Fig. 4, incubation of a constant amount of ^{125}I-Ia or ^{125}I-Ib with increasing amounts of anticolicin Ia Fab' prepared from anti-Ia purified gamma globulin by pepsin digestion followed by mild reduction with cysteine (11) leads to a progressive decrease in the amount of colicin adsorbed. Under the assay conditions employed, anti-Ia Fab' did not displace bound colicin. In order to determine the kinetics of colicin adsorption, the ^{125}I-colicin was incubated with cell wall preparations at either 0°C or 37°C. At various times adsorption was terminated by dilution of an aliquot of the reaction mixture into buffer containing Fab' and the amount of colicin adsorbed to walls determined. As can be seen in Fig. 5, the binding of the colicins is a time dependent process whose rate is temperature dependent.

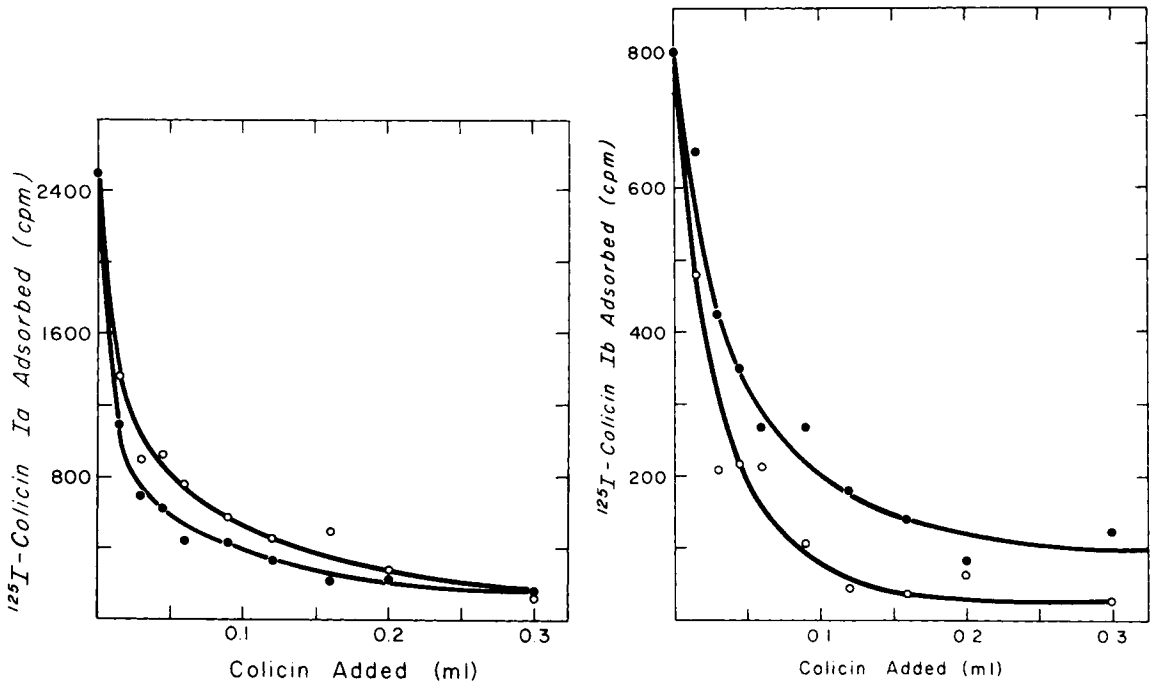

Fig. 3. Competition of nonradioactive colicins Ia or Ib for adsorption of ^{125}I-Ia or ^{125}I-Ib to cell walls. (A) to 1.6 ml of buffer A containing 0.06 μg of ^{125}I-Ia (6060 cpm) and various amounts of non-iodinated Ia or Ib was added 0.4 ml buffer A containing 16 μg cell wall. After incubation for 30 min at 37°C, the amount of ^{125}I-Ia adsorbed was determined. —●—●—, Ia; —○—○—, Ib, (B) Same as (A) except that ^{125}I-Ib (3580 cpm) replaced ^{125}I-Ia. —○—○—, Ia; —●—●—, Ib.

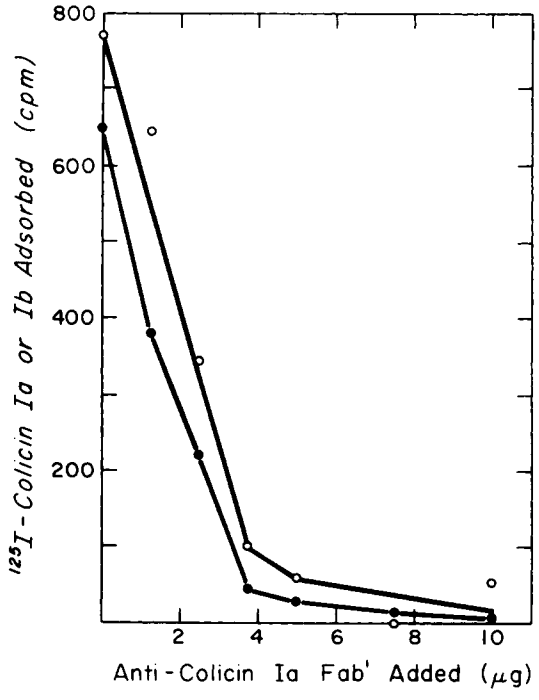

Fig. 4. Effects of anti-Ia Fab' on adsorption of ^{125}I-Ia or ^{125}I-Ib to cell walls. To 0.06 μg of ^{125}I-Ia (3096 cpm) or ^{125}I-Ib (5460 cpm) was added various amounts of anti-Ia Fab'. After incubation at 37°C for 30 min, 8 μg of cell wall was added and incubation was continued for an additional 30 min before the amount of colicin adsorbed was determined. —●—●—, Ia; —○—○—, Ib.

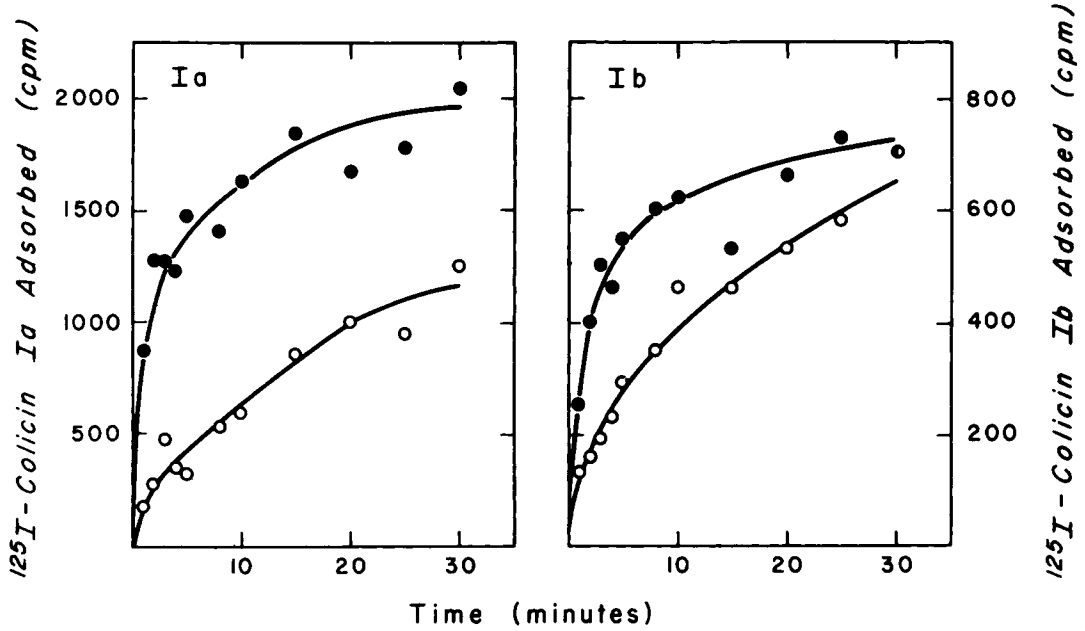

Fig. 5. Kinetics of ^{125}I-Ia or ^{125}I-Ib adsorption to cell walls. To 10 ml of buffer A containing 240 μg cell walls maintained at 5°C or 36.5°C in a jacketed reaction flask was added 4 ml buffer A containing either 1.2 μg of ^{125}I-Ia (6.4 × 10^3 cpm) or ^{125}I-Ib (4.9 × 10^3 cpm) previously equilibrated to 5°C or 36.5°C. Stirring was maintained by a stir bar. At various times 0.9 ml aliquots were removed and added to 1 ml buffer A containing 25 μg anti-Ia Fab'. The amount of colicin adsorbed to cell walls was determined by filtration —○—○—, 5°C; —●—●—, 36.5°C.

Under the conditions employed, adsorption at 37°C was approximately 80% complete in 10 min.

Sucrose Gradient Centrifugation

That colicin ^{125}I-Ia or ^{125}I-Ib binds to the cell wall component of the *E. coli* cell envelope can be demonstrated by discontinuous sucrose gradient centrifugation. As mentioned above, this procedure resolves the total envelope into cell wall and cell membrane-enriched components. This is shown in Fig. 6 in which fractions 7–10 and 16–19 correspond to cell wall and cytoplasmic membrane material, respectively. The material in fractions 2–4 is an artifact of our fractionation procedure and results from the adherence of cell wall material to the sides of the centrifuge tube which is pushed out by the dense fluorinert.

When colicin ^{125}I-Ib is incubated with the unfractionated cell envelope fraction and the mixture subjected to sucrose gradient centrifugation, results of the kind shown in Fig. 7 and 8 are obtained. As can be seen (Fig. 7B), approximately 33% of the added colicin is found at a position identical to that of cell walls. No colicin was found to cosediment with membrane material (fractions 16–20). This is in marked contrast to the situation in which colicin alone is subjected to centrifugation (Fig. 7A). When a similar analysis is carried out using the cell envelope fraction derived from the colicin resistant strain BC3 (Fig. 7C), one finds a reduction in the amount of colicin cosedimenting with cell wall material. In some experiments in which the amount of colicin per milligram mutant envelope was lower, essentially no colicin was found in the cell wall region of the gradient,

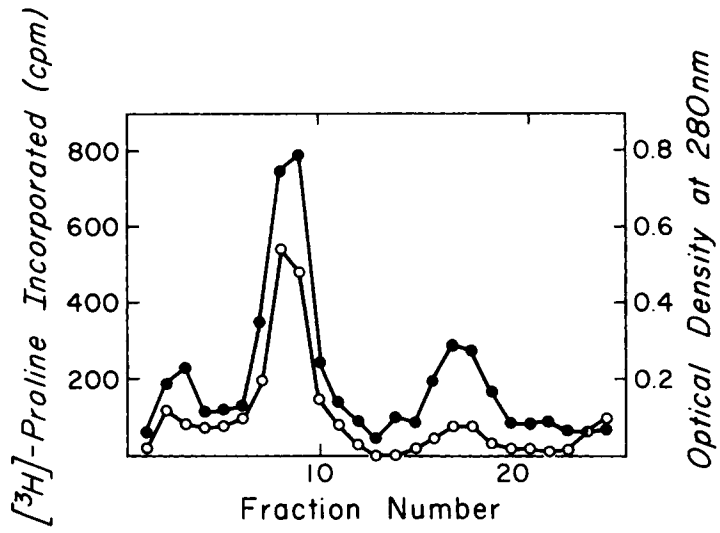

Fig. 6. Sucrose gradient centrifugation of (^{3}H)–proline labelled envelope. A 500 ml cell culture was grown at 37°C in Tris-medium (8) containing 0.15% glucose, and 500 μg/ml L–proline. At a cell concentration of 2 × 10^7, 125 μg of (^{3}H)–proline (6.4 mc/μmole) was added and the culture grown to a concentration of 4 × 10^8. Preparation of envelopes was as described in the Materials and Methods Section. 0.2 ml was applied to a 4.8 ml discontinuous sucrose gradient and centrifuged in a Spinco SW 50.1 rotor for 4 hrs at 40,000 rpm at 10–12°C. Each fraction was assayed for optical density at 280 nm, —○—○—; and trichloroacetic acid insoluble counts, —●—●—, top of gradient is at the right.

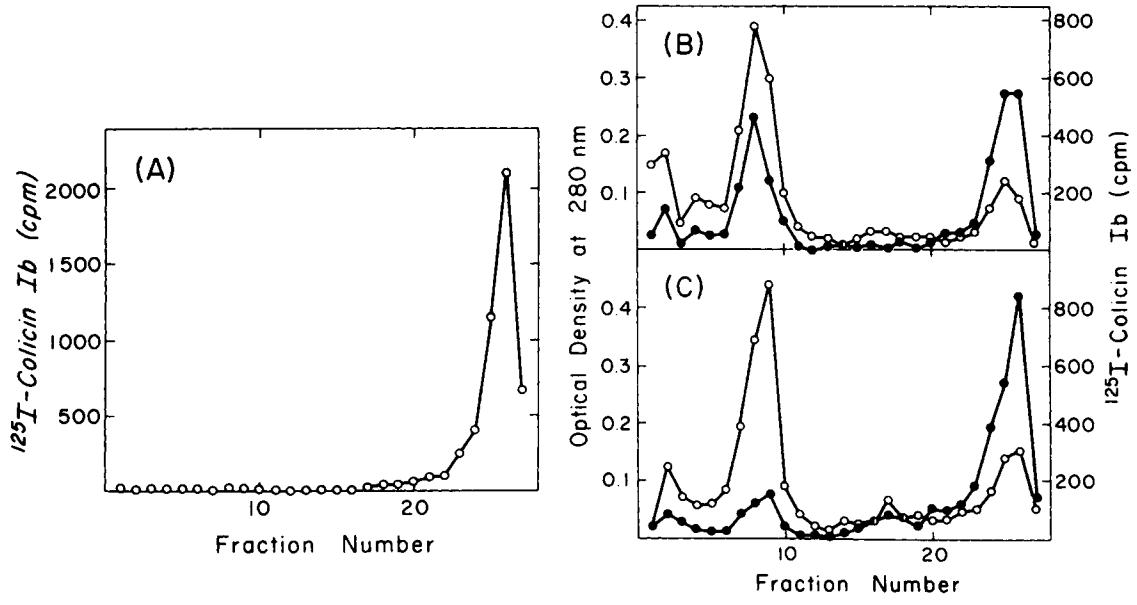

Fig. 7. Sedimentation of ^{125}I-Ia and cell envelopes in sucrose gradients. 200 μl of buffer A containing 500 μg cell envelope and 0.030 μg ^{125}I-Ib was incubated 30 min at 37°C. The mixture was chilled and then layered on a 4.8 ml discontinuous sucrose gradient. After centrifugation the distribution of ^{125}I-Ib was determined as described in the Materials and Methods Section. (A) ^{125}I-Ib alone. (B) ^{125}I-Ib plus JK1 envelope. (C) ^{125}I-Ib plus BC3 envelope. OD^{280}, —○—○—; ^{125}I-Ib, —●—●—. Top of the gradient is at the right.

whereas substantial amounts of colicin were found in this region in the case of the wild type strain (JK1). Figure 8 shows that preadsorbed colicin ^{125}I-Ia also sediments specifically at a position corresponding to cell wall material. Table III shows that the amount of ^{125}I-Ia or ^{125}I-Ib bound to the envelope fraction as assayed by the filter paper method agrees very closely to the amount of colicin found in the cell wall region of the sucrose gradients. We conclude, therefore, that the addition of colicin to cell envelopes leads to adsorption of the colicin to a specific receptor which resides in the cell wall fraction of the cellular envelope.

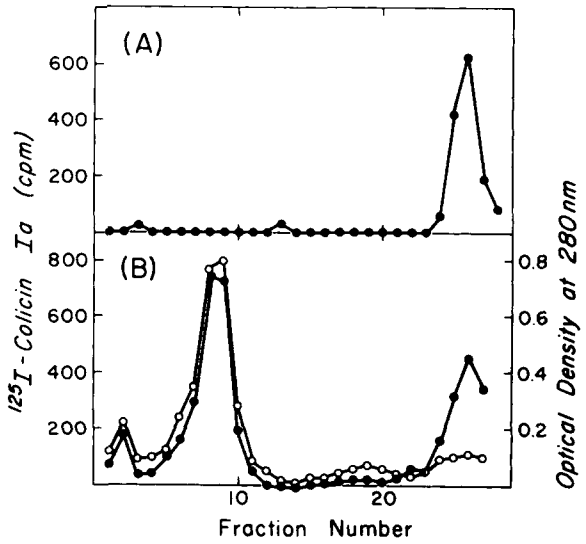

Fig. 8. Sedimentation of ^{125}I-Ia and cell envelopes. Procedure as in Fig. 7. (A) ^{125}I-Ia alone. (B) ^{125}I-Ia plus JK1 envelope. OD^{280}, —○—○—; ^{125}I-Ia —●--●—. Top of the gradient is to the right.

TABLE III. Comparison of Filter Paper and Sucrose Gradient Assays

Assay	^{125}I-Ia added (cpm)	^{125}I-Ia bound (cpm)	^{125}I-Ib added (cpm)	^{125}I-Ib bound (cpm)	bound / added
Filter paper	1887	817			0.43
Sucrose gradient	1994	932			0.47
Filter paper			4238	1275	0.30
Sucrose gradient			3236	1152	0.36

400 μg of envelope and 0.060 μg of ^{125}I-Ia or ^{125}I-Ib contained in a total volume of 0.180 ml of Buffer A was incubated at 37° for 30 min. The mixtures were next chilled. For the filter assay 2 ml of Buffer A was added to the mixture before filtration. For the sucrose gradient assay, the total reaction mixture was applied to a 4.8 ml 5–20% sucrose gradient. The amount of colicin bound represents the total radioactivity cosedimenting with the cell wall fraction.

DISCUSSION

Treatment of sensitive cells with colicins Ia or Ib was shown to lead to near complete inhibition of RNA, DNA, and protein systhesis (8). These colicins caused partial inhibition of assimilation of radioactive phosphate into total nucleotide and phospholipid fractions. Although treatment of cells with either colicin does not lead to an inhibition of respiration, there is a partial inhibition of ^{32}P incorporation into ATP (8). These results suggested that the primary target of the I colicins was the cells' energy supplying reactions localized in the cell membrane (8).

Recent studies on the colicin K and colicin E3 receptors have localized these colicin adsorption sites to the cell wall component of the *E. coli* cell envelope (5, 6). Using iodinated, yet biologically active, colicin Ia and Ib, we show here, that the I colicins also adsorb to specific receptors which reside in the bacterial cell wall. Using a simple filter paper assay for colicin adsorption, it is shown that whereas the unfractionated *E. coli* cell envelope as well as partially purified cell wall (4) adsorb ^{125}I-colicin Ia or ^{125}I-Ib, the partially purified cytoplasmic membrane fraction shows little binding capacity. The fact that this small amount of membrane binding was sensitive to lysozyme treatment, suggests that it was due to cell wall contamination. The binding of ^{125}I-colicin I specifically to cell wall material was also demonstrated by sucrose gardient analysis. That the binding reaction represents the adsorption of colicin Ia or Ib to the specific colicin I-receptor and is not due to nonspeciific adsorption was demonstrated by the fact that cell envelopes or cell wall fractions isolated from a bacterial mutant known to be defective in the colicin I receptor do not bind radioactive colicin. It should be noted that, although our results would seemingly rule out colicin I receptors as components of the cytoplasmic membrane per se, membrane involvement in the adsorption process cannot be rigorously excluded. Since our cell wall fraction is probably contaminated with membrane material, the possibility exists that the colicin receptor is composed of both membrane and wall components.

The binding of ^{125}I-colicin I to the cell wall receptor is a saturable temperature sensitive process. Furthermore, there exist approximately equal numbers of colicin Ia and Ib receptors per milligram of cell wall fraction protein. These studies confirm previous suggestions that colicins Ia and Ib have identical or cross-reactive receptors (1). With either colicin, the presence of either nonradioactive colicin Ia or Ib inhibited the binding of ^{125}I-colicin. Furthermore, the cell walls isolated from a colicin I resistant mutant selected with colicin Ia, adsorbed neither Ia nor Ib.

Any explanation for the mode of action of the I colicins must explain how the biological activity of these colicins is transmitted from the receptor in the cell wall to the sensitive target, presumably the cell membrane. The cell wall (outer membrane plus murein) of *E. coli* is approximately 10–12 nm in width and, in general, is separated from the cytoplasmic membrane by a space (periplasmic space) of 4.0–4.5 nm. Physical studies (7) as well as electron microscopic examination (12) of colicins Ia and Ib have suggested that these molecules are oblate in shape with approximate diameters of 20 nm. Thus, it is conceivable that colicin I molecules can span the cell envelope and directly cause membrane alterations while remaining adsorbed to their receptors in the cell wall. It should be noted that, where physical characterization has been possible, all of those colicins which are thought to affect membrane associated functions are highly elongated molecules with dimensions sufficient to span the cell envelope (12).

The action of diptheria toxin on cells is thought to be a two-step mechanism in which

the adsorption of toxin molecules to a surface receptor is followed by cleavage of the toxin molecule releasing an active fragment into the cell interior (13). A similar mechanism may be operative in the case of the I-colicins. Enzymatic cleavage could result in the release from the receptor of an active fragment which would then be released into the periplasmic space where it could interact with the outer layers of the cytoplasmic membrane.

It is also possible that the adsorption of I-colicins to the specific cell wall receptors is an intermediate step in the transport of the colicin molecules to some membrane target. Perhaps, the colicin I-receptor complex in toto is dissociated from the cell wall and transported to the membrane. On the other hand, the colicin I molecule may be transported in a series of steps via intermediate transport molecules. It should be clear that these kinds of mechanisms can be generalized to explain the action of other colicins (see, for example, ref. 14).

Contrary to the examples discussed above are possible mechanisms in which the colicin molecule itself does not interact with the final membrane target. For example, the interaction of I-colicin with receptor may trigger an activation of membrane modifying enzymes such as phospholipases in the cell wall or lipases, proteases, ATPases, etc., in the periplasmic space or membrane itself. On the other hand, it is possible that colicin induced conformational changes in the membrane could affect energy producing reactions.

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