Transport of Vitamin B₁₂ in *Escherichia coli*: Common Receptor Sites for Vitamin B₁₂ and the E Colicins on the Outer Membrane of the Cell Envelope

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The first step in the transport of cyanocobalamin (CN-B₁₂) by cells of *Escherichia coli* was shown previously to consist of binding of the B₁₂ to specific receptor sites located on the outer membrane of the cell envelope. In this paper, evidence is presented that these B₁₂ receptor sites also function as the receptors for the E colicins, and that there is competition between B₁₂ and the E colicins for occupancy of these sites. The cell strains used were *E. coli* KBT001, a methionine/B₁₂ auxotroph, and B₁₂ transport mutants derived from strain KBT001. Colicins E1 and E3 inhibited binding of B₁₂ to the outer membrane B₁₂ receptor sites, and CN-B₁₂ protected cells against these colicins. Half-maximal protection was given by CN-B₁₂ concentrations in the range of 1 to 6 nM, depending upon the colicin concentration used. Colicin E1 competitively inhibited the binding of ⁵⁷Co-labeled CN-B₁₂ to isolated outer membrane particles. Functional colicin E receptor sites were found in cell envelopes from cells of only those strains that possessed intact B₁₂ receptors. Colicin K did not inhibit the binding of B₁₂ to the outer membrane receptor sites, and no evidence was found for any identity between the B₁₂ and colicin K receptors. However, both colicin K and colicin E1 inhibited the secondary phase of B₁₂ transport, which is believed to consist of the energy-coupled movement of B₁₂ across the inner membrane.

We have shown previously that the uptake of vitamin B₁₂ by *Escherichia coli* consists of a rapid, initial phase followed by a slower, secondary phase (4, 5). The initial phase of uptake consists of binding of vitamin B₁₂ to specific receptors which are firmly bound to the outer membrane of the cell envelope (14). The subsequent steps required to transfer the B₁₂ from these receptors and move it across the inner membrane and into the interior of the cell constitute the secondary phase of uptake. At least one of these secondary steps is coupled to the energy metabolism of the cell. During the course of studies on the energy coupling processes involved in this transport system, the effects of colicin E1 upon B₁₂ uptake were investigated. It has been shown in other laboratories that colicin E1 can inhibit some energy-coupled transport systems in *E. coli* (1, 6). In our work, it quickly became apparent that colicin E1, in addition to inhibiting the secondary phase of B₁₂ transport, inactivated the initial B₁₂ binding sites. We have followed up this observation and have obtained evidence that the outer membrane receptor sites for vitamin B₁₂ also serve as the receptor sites for the E colicins. These results are presented in this paper. Kadner and Liggins (9) also include some evidence indicative of genetic identity between the determinants for the outer membrane receptors for B₁₂ and those for the colicin E receptors.


MATERIALS AND METHODS

Radioactive compounds. Cyanocobalamin (CN-B₁₂), labeled with ⁵⁷Co, was obtained from the Amer-
sham/Searle Corp., Arlington Heights, Ill. L-proline-U-\(^{14}\)C (200 mCi/mmol) and L-lysine-U-\(^{14}\)C (210 mCi/ mmol) came from ICN, Irvine, Calif., and Schwarz/ Mann, Orangeburg, N.Y., supplied the L-leucine-U-\(^{14}\)C (312 mCi/mmol).

**Organisms.** The bacterial strains used in this work were \(E.\ coli\) KBT001, a methionine/\(B_{12}\) auxotroph of genotype \(F^-\) ( \(leu, pro, lysA, trp, purE, metE, str, lac\) ), and \(B_{12}\) transport mutants derived from this strain. These strains have been described more fully previously (5, 15) and are listed in Table 1. Their genetic properties are presented in the accompanying paper (9). The cells were maintained on nutrient agar and, for experimental purposes, were grown on the minimal medium of Davis and Mingioli (3) supplemented with 0.5% glucose, adenine (40 \(\mu g/\)ml), and the required amino acids (50 \(\mu g/\)ml).

**Colicin preparation.** Colicins E1, E3 and K were prepared from cells of \(E.\ coli\) strains ML(COL E1\(^{+}\)), CA38, and K235, respectively. The methods followed those described by Herschman and Helinski (8) for colicin E3, and included induction with mitomycin C (0.2 \(\mu g/\)ml), extraction of the cells with 1 M NaCl, and collection of the protein which precipitated between 15 and 50% saturated ammonium sulfate. The colicins were dissolved and diluted in colicin diluent, which was filter sterilized and which contained bovine serum albumin (2 mg/ml), 0.85% NaCl, 5 mM MgCl\(_2\), and 0.5 mM CaCl\(_2\).

**Colicin assays.** The colicins were assayed routinely by means of a plate assay (10). Plates of nutrient agar (containing 50 \(\mu g\) of streptomycin per ml) were overlaid with 0.7% nutrient agar (3 ml) containing about 10\(^6\) cells from a sensitive strain of \(E.\ coli\) (usually KBT001). Samples (10 uliters) of 10-fold dilutions of the colicins were placed on the soft agar, and the plates were incubated overnight. The reciprocal of the highest dilution which gave a distinct inhibition of growth was used as a measure of the colicin titer.

**Assay of CN-\(B_{12}\) uptake.** The methods used to measure uptake of CN-\(B_{12}\) by whole cells of \(E.\ coli\) have been described in detail previously (4) and consisted basically of the inclusion of radioactive CN-\(B_{12}\) in the reaction mixtures, filtration of samples through membrane filters (0.45 \(\mu m\) pore size, Millipore Corp.), and liquid scintillation counting.

**Amino acid uptake.** Amino acid uptake methods were essentially the same as those described for CN-\(B_{12}\) uptake, except that the reaction mixtures contained a \(^{14}\)C-labeled amino acid instead of CN-\(B_{12}\). Inhibition of amino acid uptake in whole cells of \(E.\ coli\) was used as another assay of activity of colicins E1 and K.

**Assay of protein synthesis.** In addition to the plate assay method, the activity of colicin E3 was also determined by measuring its ability to inhibit protein synthesis in cells of \(E.\ coli\). Overnight cultures of an appropriate strain (usually KBT001) were used to inoculate final cultures in the minimal medium described above which was further supplemented with yeast extract (0.5 mg/ml). These cultures were grown to mid-log phase, then samples (5 ml) were transferred to small flasks in a water bath shaker at 37 C. After 10 min various amounts of colicin E3 were added and, after a further 10 min of incubation, 1 ml of the growth medium containing 0.25 \(\mu g\) of \(l-[\(^{14}\)C]\) leucine was added to each flask. Shaking was continued for 10 min, and protein synthesis was stopped by pouring the samples into iced tubes which contained 0.5 mg of chloramphenicol in 0.5 ml of water. The samples were mixed and allowed to stand in ice for several minutes. Duplicate 2.5-ml samples were taken from each tube, added to 2.5 ml of 10% trichloroacetic acid, and incubated in boiling water for 20 min. The precipitated protein was collected on glass fiber filters (2.5 cm), which were then washed twice with 10 ml of 5% trichloroacetic acid (containing 0.2 \(mg\) of \(l\)-leucine per ml) and once with 10 ml of 95% ethanol. The filters were dried and counted in a liquid scintillation counter. Control experiments (experiments without colicin E3) showed that the incorporation of \(^{14}\)C into precipitable protein was linear with time for about 20 min in this system.

**Isolation of cell envelope particles from \(E.\ coli\) cells.** These methods have been described previously (11, 14). Cells of the desired \(E.\ coli\) strain, from 1 liter of a late log-phase culture, were suspended in 200 ml of 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride buffer at pH 7.8, containing 1 mM ethylenediaminetetraacetic acid, and were mixed in a Sorvall Omnimixer. The cells were recovered by centrifugation at 0 C and were resuspended in 50 ml of the same buffer. After addition of about 1 mg each of pancreatic ribonuclease and deoxyribonuclease, the suspension was passed twice through an Aminco French pressure cell at 20,000 lbs/in\(^2\). Magnesium chloride was added to give a final concentration of 2 mM, and any intact cells were removed by centrifugation at 3,000 \(\times\) \(g\) for 5 min and at 6,000 \(\times\) \(g\) for 7 min. The cell envelope particles were sedimented by centrifugation for 1 h at 2 C and 144,000 \(\times\) \(g\). Outer membrane particles were derived from these whole envelope preparations by selective solubilization of the inner membranes by using 10 mM KPO\(_4\), pH 6.6, containing 2% Triton X-100 and 1 mM MgCl\(_2\), and recentri-

### Table 1. Bacterial strains used

<table>
<thead>
<tr>
<th>E. coli strains</th>
<th>Genotype*</th>
<th>B(_{12}) transport characteristics</th>
<th>Outer membrane - B(_{12}) binding sites</th>
<th>Energy-coupled secondary phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBT001</td>
<td>F(^{+}), leu, pro, lysA, trp, purE, metE, str, lac</td>
<td>Present</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>KBT026</td>
<td>As KBT001, but also btuB</td>
<td>Present</td>
<td>Present*</td>
<td></td>
</tr>
<tr>
<td>KBT041</td>
<td>As KBT001, but also btuA</td>
<td>Present</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>KBT069</td>
<td>As KBT001, but also btuB</td>
<td>Absent</td>
<td>Present*</td>
<td></td>
</tr>
</tbody>
</table>

* For further details see Kadner and Liggins (9).

* Presumably present, but in the absence of the outer membrane B\(_{12}\) binding sites, the secondary phase does not have access to the CN-\(B_{12}\) in the medium.
fugation at 144,000 × g for 60 min, as described by Schnaitman (12).

Other methods. Protein was determined primarily by the method of Waddell (13).

RESULTS

Effects of colicins E3, E, and K upon the uptake of CN-B₁₂. Figure 1 shows the effects of various amounts of colicin E1 upon the uptake of ⁵⁷Co-CN-B₁₂ by whole cells of E. coli KBT001. In the absence of the colicin the normal biphasic pattern of B₁₂ uptake was observed, which consisted of a rapid initial phase which was complete within the first minute, followed by a slower, secondary phase which was linear for about 30 min. The smallest amount of colicin E1 used, in the range of 5 to 50 killing units per cell, gave about 65% inhibition of the rate of the secondary phase of B₁₂ uptake, with no detectable effect upon the initial phase. The largest amount of the colicin E1 used (50 to 500 killing units/cell), however, eliminated the initial phase of uptake; i.e., it inhibited the binding of CN-B₁₂ to the B₁₂ receptor sites on the outer membrane.

The effects of colicins E3 and K upon the uptake of ⁵⁷Co-CN-B₁₂ were also examined in the same way, and these results are summarized in Table 2. The amounts of CN-B₁₂ taken up in the first minute and between 1 and 20 min have been used to obtain estimates of the initial and secondary phases, respectively. Colicin K inhibited the energy-dependent, secondary phase of B₁₂ uptake only, whereas colicin E3, in large amounts, inhibited both phases of uptake. We believe that the primary effect of colicin E3 in this system was to inhibit the initial B₁₂ uptake, resulting in a concomitant decrease in the secondary phase. White et al. (14) have shown previously that an active initial phase is apparently necessary to provide B₁₂ for the secondary uptake.

Sensitivity of E. coli strains KBT001, KBT026, KBT041, and KBT069 to colicins E1, E3, and K. The plate assay method was used to determine the sensitivity of the B₁₂ transport mutant strains to the colicins E1, E3, and K (Table 3). Those strains (KBT001 and

![Figure 1. Effects of colicin E1 upon the uptake of ⁵⁷Co-CN-B₁₂ by cells of E. coli KBT001.](http://jb.asm.org/)

<table>
<thead>
<tr>
<th>Amt of colicin (µg/5 ml)</th>
<th>Initial phase of B₁₂ uptake</th>
<th>Secondary phase of B₁₂ uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Picomoles of B₁₂ taken up</td>
<td>Percentage of total B₁₂ taken up</td>
</tr>
<tr>
<td>E3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.75</td>
<td>100</td>
</tr>
<tr>
<td>0.13</td>
<td>0.73</td>
<td>97</td>
</tr>
<tr>
<td>1.3</td>
<td>0.54</td>
<td>72</td>
</tr>
<tr>
<td>13</td>
<td>0.02</td>
<td>3</td>
</tr>
<tr>
<td>K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.53</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>0.49</td>
<td>92</td>
</tr>
<tr>
<td>200</td>
<td>0.51</td>
<td>96</td>
</tr>
</tbody>
</table>

*Each reaction mixture contained 0.1 M KPO₄, pH 6.8, 1% glucose, 4.5 nM ⁵⁷Co-CN-B₁₂, approximately 10¹⁰ cells, and various amounts of colicin E₁, in a final volume of 10 ml. The reaction mixtures were preincubated at 37°C in a water bath shaker for about 15 min before addition of the colicin, and then incubated 5 min longer before addition of the ⁵⁷Co-CN-B₁₂. Samples (1 ml) were removed after different time intervals. The amounts of colicin E₁ used per reaction mixture were: none, ○; 8 µg, ●; 24 µg, □; 80 µg, ■. From plate assays, the titer of the colicin E₁ preparation was 10⁹ per 16 µg of protein, giving an estimated 10¹⁰ to 6 × 10¹⁰ killing units per microgram of protein.

Table 2. Effects of colicins E₃ and K upon uptake of ⁵⁷Co-CN-B₁₂ by cells of E. coli KBT001
KBT041) which possessed an intact initial phase of B₁₂ uptake (i.e., possessed functional outer membrane B₁₂ receptor sites) were sensitive to colicins E₁ and E₃, whereas those strains (KBT026 and KBT069) which lacked functional B₁₂ receptors were not sensitive to the E colicins. All of the strains were apparently equally sensitive to colicin K. Of particular interest was the observation that inclusion of colicin E₃, the presence and absence of CN-B₁₂.

Table 3. Sensitivity of various E. coli strains to colicins E₁, E₃, and K, in the presence and absence of CN-B₁₂

<table>
<thead>
<tr>
<th>Test strain</th>
<th>Colicin titer*</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E₁</td>
<td>E₃</td>
<td>K</td>
<td>-B₁₂</td>
<td>+B₁₂</td>
<td>-B₁₂</td>
</tr>
<tr>
<td>KBT001 . . .</td>
<td>10⁴</td>
<td>10³</td>
<td>10³</td>
<td>10⁴</td>
<td>10³</td>
<td>10³</td>
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<tr>
<td>KBT026 . . .</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>10⁴</td>
<td>10³</td>
<td>10³</td>
</tr>
<tr>
<td>KBT041 . . .</td>
<td>10⁴</td>
<td>10³</td>
<td>10³</td>
<td>10⁴</td>
<td>10³</td>
<td>10³</td>
</tr>
<tr>
<td>KBT069 . . .</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>10⁴</td>
<td>10³</td>
<td>10³</td>
</tr>
</tbody>
</table>

* Determined by means of the plate assay. Duplicate plates were set up, and one series contained 1 μM CN-B₁₂ in the agar. The other series had no added B₁₂.

Fig. 2. Sensitivity of E. coli strains KBT001, KBT026, and KBT041 to colicin E₃. Inhibition of protein synthesis was used as a measure of the activity of colicin E₃. The procedure is described fully in Materials and Methods. The results are plotted as counts per minute of L-[1⁴C]leucine incorporated into protein at 37 C for 10 min versus the amount of colicin E₃ per 5 ml of sample. The colicin E₃ titer was 10⁴ per mg of protein.

Fig. 3. Effects of colicin E₁ and CN-B₁₂ upon the uptake of L-proline by cells of E. coli strains KBT041 and KBT026. Each reaction mixture contained 0.1 M KPO₄, pH 6.8, 1% glucose, approximately 3.3 × 10⁶ cells, and 10 μM L-[1⁴C]proline in a final volume of 5 ml. There was an aerobic preincubation at 37 C for 20 min before addition of the [¹⁴C]proline. Where necessary, other additions included 0.5 μM CN-B₁₂ (added at the beginning of the preincubation) and 1.6 μg of colicin E₁ (added 5 min before the [¹⁴C]proline). Samples (1 ml) were removed for assay of proline uptake. Additions: none, • colicin E₁, • CN-B₁₂ and colicin E₁, O. The amount of colicin E₁ used was estimated to be in the range of 5 to 30 killing units per cell.

CN-B₁₂ (at a final concentration of 1 μM) in the nutrient agar protected sensitive strains against colicins E₁ and E₃, but not against colicin K.

Essentially the same results were obtained when other, more specific assays of colicin action were made. Figure 2 shows the effects of colicin E₃ upon incorporation of L-[¹⁴C]leucine into protein by whole cells of three E. coli strains. No inhibition was obtained with cells of strain KBT026, which lack outer membrane B₁₂ receptors. Similarly, Fig. 3 shows the effects of colicin E₁ upon the uptake of L-[¹⁴C]proline by cells of strains KBT041 and KBT026. Again, there was no effect upon cells of strain KBT026. The uptake of proline was inhibited in cells of strain KBT041, but complete protection against the colicin E₁ was obtained by inclusion of 1 μM CN-B₁₂ in the reaction mixture.

Vitamin B₁₂ was unable to protect cells from the action of colicin K. Colicin K inhibited the uptake of L-[¹⁴C]lysine by cells of strain KBT001, but CN-B₁₂ had no detectable effect upon this inhibition (Fig. 4).

Assay of colicin receptors in cell envelopes from E. coli strains KBT001, KBT026, KBT041, and KBT069. The presence of colicin receptors in cell envelopes of the various E. coli strains was assayed by measuring the ability of such envelope preparations to neutralize the colicins. Tenfold dilutions of the colicins were incubated with cell envelope particles for 15 min at 37 C prior to assay by the plate tech-
nique (Table 4). Neutralization of the E colicins occurred with cell envelopes only from those strains which possessed functional B₁₂ receptor sites on the outer membrane (i.e., KBT001 and KBT041, but not KBT026 or KBT069).

The neutralization of colicins E₁ and K is also shown in Fig. 5. In these experiments, the envelope particles were removed by centrifugation (144,000 × g at 2 C for 30 min) after neutralization at 37 C, and the colicin content of the supernatant solutions was assayed by measuring their ability to inhibit the uptake of L-[14C]lysine by cells of E. coli KBT001. Cell envelopes from strain KBT026, which lack the outer membrane B₁₂ binding sites, were again shown to lack functional receptors for colicin E₁. All of the strains tested possessed cell envelopes which were able to neutralize colicin K.

Interactions of vitamin B₁₂ with the colicin systems. The ability of CN-B₁₂ to protect sensitive cells against the action of the E colicins has been studied. Inhibition of lysine uptake was used as an index of colicin E₁ activity. The amount of colicin E₁ used was that which gave about 70% inhibition of the rate of lysine uptake by cells of E. coli KBT041. This strain was selected because it has no secondary phase of B₁₂ uptake, and the B₁₂ taken up remains associated with the cell surface. The degree of protection conferred by varying levels of CN-B₁₂ against colicin E₁ in this system was

![Fig. 4. Effects of colicin K and CN-B₁₂ upon the uptake of L-[14C]lysine by cells of E. coli KBT001. Each reaction mixture contained 0.1 M KPO₄, pH 6.8, 10 mM MgSO₄, 1% sodium succinate, 10 μM L-[14C]lysine, and approximately 3 × 10⁸ succinate-grown cells in 5 ml final volume. The incubation was aerobic at 37 C, and there was a 20-min preincubation before addition of the [14C]lysine. Where necessary, 1.2 μM CN-B₁₂ (added at the beginning of the preincubation) and 200 μg of colicin K (added 10 min before [14C]lysine were included in the reaction mixtures. Samples (1 ml) were removed at different times. The colicin K titer was about 2 × 10⁸ per mg of protein. Additions: none, ○; colicin K, ●; CN-B₁₂ and colicin K, □.

![Fig. 5. Neutralization of colicins E₁ and K by cell envelope preparations from various strains of E. coli. Cell envelope particles were obtained from cells of E. coli strains KBT001, KBT026, and KBT041. Approximately 80 μg of colicin E₁ or 200 μg of colicin K were incubated with protein (2 mg) of cell envelopes in colicin diluent reaction mixtures (1 ml) at 37 C for 15 min, and were then centrifuged at 144,000 × g for 30 min at 2 C. The supernatant solutions were assayed for colicin E₁ and K by measuring the inhibition of [14C]lysine uptake in cells of E. coli KBT001, exactly as described for Fig. 4. Additions: none, ○; untreated colicins, △; colicin which had been treated with cell envelopes from E. coli strains KBT001, O; KBT026 ■, and KBT041 □.

<table>
<thead>
<tr>
<th>Source of cell envelope</th>
<th>E₁</th>
<th>E₉</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10⁸</td>
<td>10⁸</td>
</tr>
<tr>
<td>KBT001</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>KBT026</td>
<td>10⁸</td>
<td>10⁸</td>
</tr>
<tr>
<td>KBT041</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>KBT069</td>
<td>10⁸</td>
<td>10⁸</td>
</tr>
</tbody>
</table>

*Tenfold dilutions of the colicins were made in colicin diluent or in colicin diluent containing 0.6 mg of cell envelope protein per 0.1 ml. After a preincubation at 37 C, 10 μlitters of each dilution was spotted on an agar plate seeded with about 10⁶ cells of E. coli KBT001, and colicin assays continued in the usual way.

TABLE 4. Neutralization of colicins E₁ and K by cell envelope preparations from various strains of E. coli*
measured. The results are plotted in Fig. 6 as the percentage of protection versus the CN-B12 concentration in the reaction mixtures. The rate of lysine uptake in the presence of colicin E1 and without any B12 was 0%, and the rate of lysine uptake in the absence of the colicin was 100%. The inset (Fig. 6) shows a double reciprocal plot of the same data, and an apparent \( K_a \) for CN-B12 of about 1 nM was obtained. This value is only slightly above that which was previously determined for the B12 receptor sites (14).

In a similar experiment the effects of the CN-B12 concentration upon colicin E3 activity were studied. Inhibition of the rate of protein synthesis by cells of E. coli KBT001 was used as a measure of colicin E3 activity. In Fig. 7 the percentage of protection against colicin E3 is plotted against the concentration of CN-B12. Half-maximal protection was given by about 6 nM CN-B12.

Further evidence for the interaction of vita-

min B12 and colicin E1 at the same outer membrane sites is presented in Fig. 8. In this experiment the effects of colicin E1 upon the binding of \( ^{35}\text{Co-CN-B}_{12} \) to outer membrane particles from E. coli KBT001 were measured at 0 C, by using the membrane filtration assay. The concentrations of colicin E1 and of CN-B12 were both varied. The lines of best fit in the double reciprocal plot (inset, Fig. 8) were found by means of linear regression analyses of the data. The results are consistent with the view that colicin E1 is a competitive inhibitor of CN-B12 binding by the outer membrane particles. A similar experiment at 4 C, in which the binding of \( ^{35}\text{Co-CN-B}_{12} \) was measured by equilibrium dialysis, gave essentially the same results (data not shown).

**DISCUSSION**

Previous studies have shown that the uptake of vitamin B12 by cells of E. coli consists of two distinct, sequential phases (4). The first of these is rapid binding of the B12 to specific receptor sites which are firmly attached to the outer membrane of the cell envelope (14). There are approximately 200 such sites per cell. These sites have an apparent physiological function in enabling B12-methionine auxotrophs to grow more rapidly than mutants which lack these
sites (media containing low B₁₂ concentrations and no methionine) (9). This initial binding of B₁₂ to these outer membrane receptors is not dependent upon coupled energy metabolism (4). The secondary phase of B₁₂ uptake, however, is coupled to the cell’s energy metabolism, and consists of the transfer of the B₁₂ from the outer membrane receptors across the inner membrane and into the cell’s interior. Colicins E₁ and K are known to inhibit the energy metabolism of aerobically grown cells of E. coli and to inhibit the energy-coupled transport of amino acids and of β-galactosides (1, 6).

In the experimental work described in this paper, we were initially interested in determining whether colicins E₁ and K inhibited the energy-dependent phase of B₁₂ transport in E. coli. Colicin E₁ was found to inhibit the energy-coupled secondary phase of B₁₂ uptake (Fig. 1). Of some interest, however, was the observation that this colicin also inhibited the binding of CN-B₁₂ to the outer membrane B₁₂ receptors. From the results of further experimentation, presented here and by Kadner and Liggins (9), we have concluded that the outer membrane sites, which bind vitamin B₁₂ and function as part of the B₁₂ transport system, also serve as the receptors for the E. coli colicins. The evidence that supports this view is summarized below. Those strains of E. coli that lacked outer membrane B₁₂ binding sites also lacked receptors for colicins E₁ and E₃. Kadner and Liggins (9) have shown that approximately 90% of the cell strains, which were isolated on the basis of a lack of sensitivity to colicin E₁, lacked functional B₁₂ receptors on their outer membranes. The other 10% of these mutant strains were presumably tolerant, rather than resistant, to this colicin. Kadner and Liggins also showed that the B₁₂ receptor locus, btuB, is at the same position on the E. coli chromosome as that previously shown for the locus bfe of the colicin E receptors. The number of B₁₂ binding sites per cell (180–220) of E. coli KBT001 (14) is essentially the same as the number (220) of colicin E₃ receptor sites which Sabet and Schnaitman (10a) found per cell of E. coli K-12 C600. We have also shown that vitamin B₁₂ and the E. coli colicins apparently compete for the same sites on the E. coli cell surface. Thus, vitamin B₁₂ was able to protect sensitive cells against colicins E₁ and E₃. Half-maximal protection was given by CN-B₁₂ concentrations which were within one order of magnitude higher than the Kᵢ for CN-B₁₂ of the B₁₂ receptor sites. Colicin E₁ competitively inhibited B₁₂ binding by outer membrane preparations from E. coli KBT001 (Fig. 8). The same correlations between sensitivity to the E. coli colicins and the presence of functional B₁₂ receptors were also observed in experiments which measured the inhibition of amino acid uptake by colicin E₁, and the inhibition of protein synthesis by colicin E₃. There was no correlation between the presence of B₁₂ receptors and sensitivity of cells to colicin K, although colicin K did inhibit the energy-coupled secondary phase of B₁₂ transport. The results of some control experiments, in which we were unable to detect any direct interaction between vitamin B₁₂ and the E. coli colicins, are consistent with our view that B₁₂ and these colicins interact via competition for common receptor sites. Thus CN-B₁₂ did not inactivate colicins E₁ and E₃, and these colicins did not bind ⁵⁷Co-CN-B₁₂ (data not shown).

Although vitamin B₁₂ and the E. coli colicins use the same outer membrane receptors, it seems likely that they do not share a common system for crossing or interacting with the inner membrane. This conclusion is based on the observation that cells of E. coli KBT041 (a strain which lacks the energy-coupled secondary phase of B₁₂ transport but possesses functional outer membrane B₁₂ binding sites) are sensitive to colicins E₁ and E₃.

The colicin E receptors on the E. coli cell envelope apparently also serve as receptor sites for the bacteriophage BF23 (2, 7). It might be
expected, therefore, that vitamin \(B_{12}\) would protect sensitive cells against this bacteriophage, but we have not yet investigated this possibility. The utilization of the same receptor sites by vitamin \(B_{12}\), bacteriophage BF23, and the E colicins does not necessarily mean that all of the components of these receptors are shared. Sabet and Schnaitman (10a) have obtained some evidence which indicates that the colicin E1 receptors require some additional constituent which is not required by the E3 receptors.

The possession of \(B_{12}\) receptors in the outer membrane of the \(E. coli\) cell envelope evidently confers a physiological advantage upon these cells, whereas colicin receptors per se are clearly potentially dangerous. The association of these disparate functions within a single structural element offers a possible rationale for the evolutionary retention of the disadvantageous component. It will be of interest to see whether the receptors for other colicins and other bacteriophages are necessary components of some other physiologically advantageous systems.

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**LITERATURE CITED**