Inhibitory Effect of Colicin E₂ on Transport Systems of 
Escherichia coli in the Presence of the rex Gene of 
λ Prophage

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Purified colicin E₂ was found to cause marked inhibition of the permeation rate of o-nitrophenyl-galactoside (ONPG) in several λ-lysogenic strains of Escherichia coli in the presence of chloramphenicol to prevent prophage induction. The inhibitory effect of colicin E₂ on transport systems was analyzed with cells of E. coli CP78(λ). The dose of colicin E₂ for the half-maximum inhibition of the ONPG-permeation rate was about 9 molecules of the colicin per bacterium under the aerobic condition, which corresponded to about 1 killing unit per bacterium. Kinetics of the transport of [³⁴C]methylthiogalactoside suggested that colicin E₂ began to inhibit the influx rate of β-galactosides within a few minutes after the colicin addition, and the maximum inhibition reached more than 80%. Extensive leakage of intracellular potassium ion and inhibition of L-proline transport also occurred at the same time. Acid solubilization of cellular deoxyribonucleic acid by the colicin was apparently delayed to the initiation of the transport inhibition. The extents of the inhibition of β-galactoside transport and leakage of potassium ion by the colicin were extensive in cells lysogenic for wild λ phage or λind⁻, whereas the extents were slight in the nonlysogenic cells or cells carrying λrex⁻ prophage. It was concluded that the sensitization of membrane transport systems of E. coli cells to colicin E₂ was achieved by the presence of the rex gene product of λ phage.

Colicin E₂ is a protein antibiotic of Escherichia coli with a molecular weight of 60,000. When sensitive E. coli cells adsorb the colicin, damage of cellular deoxyribonucleic acid (DNA) occurs at first, whereas other macromolecular syntheses continue for a relatively long time (18). Colicin E₂ is similar in several points, such as receptor specificities, immunological reactions, and physical parameters, to colicin E₃ (15), although the ultimate biochemical effects of these two colicins are quite different. Studies on the mode of action of colicin E₂ have shown that the colicin has an intrinsic enzymatic activity to cleave 16S ribonucleic acid and cause the inactivation of the ribosome (4, 5, 21). Several attempts to find deoxyribonuclease activity associated with colicin E₂ had been negative (1, 18, 22); however, recent studies by Saxe showed that purified colicin E₂ preparations possess deoxyribonuclease activity to introduce one single-strand scission in supercoiled λ phage DNA (24, 25). Although this finding implies the nucleolytic activity of colicin E₂ as the basis of the in vivo action, the isolation of a mutant in which the colicin stops cell division without DNA degradation indicates that the primary cause of killing by the colicin is not necessarily DNA damage (3). In the case of colicins like E₁ and K, rapid loss of potassium and other cations from the treated cells is observed, and it seems likely that the mode of action involves structural alteration of the cell membrane (10, 17, 18). However, it has been believed that colicin E₂ has no such activity to induce loss of intracellular potassium (19).

In this paper, we reexamined this point with various sensitive E. coli strains and found that colicin E₂ caused strong inhibition of membrane transport systems of the λ-lysogenic cells of E. coli without prophage induction. Marked inhibitory effects on membrane transport systems for β-galactosides, potassium ion, and L-proline were induced by low doses of colicin E₂ in cells of λ-lysogenic cells, although less slight inhibition was also observed in the nonlysogenic cells. Bacteriophage λ has been found to confer the capacity on the lysogenic cells to block the multiplication of rII mutants of T₄ phage, which mainly involves the product of the rex gene of λ (12, 15). Observations had been made that
acid solubilization of DNA by colicin E\textsubscript{2} was partially reduced at late times of the colicin challenge in cells carrying \( \lambda \) prophage (16, 20), and this reduction was due to the \( \beta \) rex gene product (23). This effect may be elucidated by the marked inhibition of membrane transport systems observed here. The sensitization of transport systems of \( E. \ coli \) to colicin E\textsubscript{2} is a newly found phenotypic expression of the \( \beta \) rex gene. This work gives the first characterization of the interaction of colicin E\textsubscript{2} with the \( \beta \) rex gene product and the cell membrane.

**MATERIALS AND METHODS**

Organisms. \( E. \ coli \) K-12 CP78 (\( F^{-}, \ thr^{-}, \ leu^{-}, \ arg^{-}, \ his^{-}, \ thi^{-}, \ mal^{-}, \ supE, \ rel^{-}, \ \lambda^{-} \)) was mainly used as sensitive strain. \( E. \ coli \) K-12(\( \lambda \)) wild, W2255T (\( Hfr, \ met^{-}, \ thy^{-}, \ \lambda^{-} \)), and B were also used. Nonlysogenic derivatives of CP78 including strain 353 were isolated by plating the ultraviolet-irradiated CP78 cells with \( \lambda \)-antisera. Spontaneous \( \lambda^{e} \) revertants of the strain 353 were obtained by selecting \( \lambda^{+} \) colonies (29). Series of lysogens for wild \( \lambda \) phage and its mutants were reconstructed by reinfecion of the strain 353A, and their lysogens were confirmed by their immunities against \( \lambda cl \) and ultraviolet inducibilities.

Wild \( \lambda \) phage was obtained by induction of K-12(\( \lambda \)). Several mutants of \( \lambda \) phage, i.e., \( \lambda^{ind^{-}}, \lambda^{imm434} \), and \( \lambda cl \), were supplied by Y. Sakakibara, National Institute of Health of Japan. Two Rex mutants, \( \lambda^{rex^{-}5a} \) and \( \lambda^{rex^{-}Qam} \), were originally isolated and supplied by G. N. Gussin, the University of Iowa (12). Bacteriophage T4B and its \( \eta r \) mutant, \( \eta r221 \), were supplied by H. Uchida, the University of Tokyo.

Growth media and treatment with colicin E\textsubscript{2}. Nutrient broth (Eiken, Tokyo) and C medium containing 0.76 g of Na\textsubscript{2}HPO\textsubscript{4}·2H\textsubscript{2}O, 3.87 g of NaH\textsubscript{2}PO\textsubscript{4}·2H\textsubscript{2}O, 0.2 g of MgSO\textsubscript{4}·7H\textsubscript{2}O, 4.0 g of Casamino Acids (Difco, vitamin free), 5 mg of thiamine-hydrochloride, and 4.0 g of glycerol in 1 liter of distilled water were used for almost all experiments. To induce \( \beta \)-galactosidase and \( \beta \)-galactoside peraease, 0.25 mM isopropylthio-\( \beta \)-D-galactoside (IPTG) or 1 mM methylthio-\( \beta \)-D-galactoside (TMG) was added to these media. For the experiment of L-proline transport contained: 8.8 g of NaH\textsubscript{2}PO\textsubscript{4}·2H\textsubscript{2}O, 3.0 g of KH\textsubscript{2}PO\textsubscript{4}, 1.0 g of NH\textsubscript{4}Cl, 20 mg of MgSO\textsubscript{4}·7H\textsubscript{2}O, 0.8 mg of FeCl\textsubscript{3}·6H\textsubscript{2}O and 2.0 g of glucose in 1 liter with supplements of required amino acids to 20 \( \mu \)g/ml, and thiamine-hydrochloride to 5 \( \mu \)g/ml. Cells were grown aerobically in 10 ml of medium contained in 30-ml L-shaped tubes with shaking at 37 \( ^{\circ} \)C, and growth was monitored by measurements of the optical density at 550 nm or total cell counting by Coulter counter (Coulter Electronics Inc.). Chloramphenicol (100 \( \mu \)g/ml) was added to the culture at a cell density of \( 5 \times 10^{9} \) to \( 8 \times 10^{9} \) cells/ml. Then the cultures treated with chloramphenicol were divided into 5-ml portions in the L-shaped tubes and treated with different amounts of colicins 15 min after the addition of the antibiotic under the aerobic condition with constant shaking at 37 \( ^{\circ} \)C. Nutrient broth containing 1.5% agar was used for counting viable cells.

Preparations of colicins and assay in their killing activities. Colicin E\textsubscript{2} was produced by \( E. \ coli \) W3110(E\textsubscript{2}) and purified mainly according to the method of Herschman and Helinski (13), except for slight modifications described below. Extraction of the colicin from cells was carried out with 0.01 M phosphate buffer (pH 8.0) containing 0.03 M ethyl-endiaminetetraacetae and 1 M NaCl instead of 1 M NaCl. The colicin was precipitated from the extract with 0.3 to 0.6 saturation of ammonium sulfate. After successive chromatographies of diethylaminoethyl-cellulose and CM-Sephadex C-50 columns, the final preparation was concentrated by ultrafiltration with Diaflo UM-10 membrane (Amicon) to give a solution of 2 mg of colicin protein per ml and stored at -80 \( ^{\circ} \)C. Almost all experiments were carried out using this preparation after appropriate dilution with 1 mg of bovine serum albumin solution per ml. The specific activity of this preparation was \( 1.09 \times 10^{10} \) killing units/mg under the aerobic condition described above.

Fractionation of the purified colicin E\textsubscript{2} preparation by isoelectric focusing electrophoresis was performed in a 100-ml Ampholine column containing 0.5% Ampholite (pH 7–9) stabilized with sucrose density gradient. The dialyzed preparation containing 14 mg of the colicin protein was applied in the column and electrophoresis was continued for 72 h. Fractions of 50 drops were collected and monitored for killing activity, permease inhibiting activity, optical density at 280 nm, and pH.

Colicin E\textsubscript{2} was produced by \( E. \ coli \) JC411(E\textsubscript{2}) and purified as described by Schwartz and Helinski (27) except for omitting the final chromatograph with CM-Sephadex. The specific activity of the preparation was about \( 8 \times 10^{14} \) killing units/mg.

Colicin E\textsubscript{2} was produced by \( E. \ coli \) W3110(E\textsubscript{2}) and purified according to the method of Herschman and Helinski (13). The specific activity of E\textsubscript{2} preparation was not assayed exactly but it seemed to be comparable with that of E\textsubscript{2} preparation judging from titration by the spot test.

Killing of the bacterium was measured by titrating viable cells on nutrient agar plates. To determine killing units of colicins, cells of CP78 which had been treated with chloramphenicol were incubated with different dilutions of colicin preparation for 30 to 40 min at 37 \( ^{\circ} \)C. Each sample was then assayed for viable bacteria. The number of killing units was calculated from the killing multiplicity obtained from the equation \( m = \ln(S/S_{0}) \) where \( S/S_{0} \) is the survival ratio of sensitive bacteria. The presence of 1 mg of bovine serum albumin per ml in dilution medium for the colicin and the aerobic condition of cells by shaking were essential to obtain the higher killing activity of colicin E\textsubscript{2}.

In the case of fractionation of colicin E\textsubscript{2} by Ampholine electrophoresis, killing activities of fractions were assayed by serial dilution and by spotting on nutrient agar plates freshly seeded with about 10\textsuperscript{5} cells of CP78 as an indicator.

**Assay of \( \beta \)-galactoside peraease.** Galactoside
permease was assayed by two types of experiments. In the first of these, the rate of hydrolysis of o-nitrophenyl-\(\beta\)-d-galactoside (ONPG) by intact cells was measured as follows. Cells were grown with 0.25 mM IPTG for more than three generations at 37°C to a cell density of about 8 \times 10^8 cells/ml and 100 \(\mu\)g of chloramphenicol per ml was added to stop the growth. Then colicin E\(_2\) was added 15 min after the addition of the antibiotic. Samples (0.3 ml) were removed at intervals and mixed with 3.5 ml of ONPG solution (0.5 mg of ONPG per ml in 0.1 M phosphate buffer, pH 7.4) at 37°C. After incubation for 3 min, the reaction was stopped by adding 1 ml of 1 M Na\(_2\)CO\(_3\), and the production of o-nitrophenol was measured at 410 nm after the removal of cells by centrifugation at 3,000 \(\times\) \(g\) for 15 min. Almost all incubations with the colicin for the assay except for those of Ampholine electrophoresis were performed under the aerobic condition.

Galactoside permease was also tested by measuring the accumulation rate of \(1^4\)CTMG by short period incubation followed by rapid filtration. Cells were grown with 1 mM TMG in C medium to a cell density of about 5 \times 10^8 cells/ml. Colicin challenge was carried out similarly to the above experiments in the presence of chloramphenicol. Samples (0.25 ml) were rapidly mixed with 0.05 ml of the growth medium containing 1 mM radioactive TMG (2.5 \(\mu\)Ci) at 37°C. After 30-s incubation, 0.2 ml of the incubation mixtures was removed to filter cells on membrane (Millipore Corp.) filters (0.45-\(\mu\)m pore size), and cells were rapidly washed twice with 2 ml of the fresh medium prewarmed at 37°C. Radioactivity accumulated in cells on the membrane filters was counted in a dioxane scintillator solution by a liquid scintillation counter (Aloka LSC-655, Nihon Musen Co.).

To measure leakage of intracellular TMG by colicin E\(_2\), cells of CP78 were grown in C medium containing 1 mM TMG and treated with chloramphenicol as described above. Then \(1^4\)CTMG (0.5 \(\mu\)Ci/ml) was added and the culture was divided into 5-ml portions. Colicin E\(_2\) was added 15 min after the addition of radioactive TMG and samples (0.2 ml) were taken to filter at intervals, washed, and counted as described above.

Measurements of intracellular potassium ion. About 10 to 1 \(\mu\)Ci of \(\text{KCl}\) per ml was added to the cells growing in C medium supplemented with 1 mM KCl at 37°C. After growth for two or three generations, radioactive KCl in the medium was removed by filtration and cells were resuspended into the fresh potassium-free medium. Chloramphenicol was added 10 min after the resuspension, and the colicin challenge was made with 5 ml of the chloramphenicol-treated cultures. Samples (0.3 ml) were filtered on membrane (Millipore) filters and cells were washed with 2 ml of cold C medium. Radioactivity on the filter membrane was counted by a GM counter.

Measurement of L-proline transport. Cells of CP78 grown in M medium were treated with 100 \(\mu\)g of chloramphenicol per ml. Colicin E\(_2\) was added 10 min after the addition of the antibiotic and \(1^4\)C]proline (final 20 \(\mu\)M, 20 mCi/ml) was subsequently added 1 min after the colicin challenge. Radioactivity incorporated into the cells was measured by the rapid filtration of 0.3-ml samples and washing as described above.

Measurement of DNA degradation by colicin E\(_2\). DNA of E. coli CP78 was labeled by growing for two generations in C medium containing 1.7 \(\mu\)Ci of \(1^4\)C]thymine (2.5 mCi/ml) per ml and 0.2 mM deoxyadenosine. IPTG (0.25 mM) was also added to induce \(\beta\)-galactosidase and its permease. Radioactive thymin in the medium was removed by filtration and resuspension of cells in the fresh medium, and the colicin challenge was carried out as described above. Samples (0.5 ml) were taken into equal volume of 10% trichloroacetic acid and cells were filtered by membrane (Millipore) filters. Radioactivity in the cold acid-insoluble materials on the filter membranes was measured by liquid scintillation counting.

Chemicals. IPTG, TMG, and thiogalactoside were products of Sigma Chemical Co. (St. Louis). ONPG was purchased from Wako Pure Chemicals Co. (Osaka, Japan). [Methyl-\(1^4\)C]TMG was obtained from New England Nuclear Corp. (Boston). KCl was supplied by the Japan Atomic Energy Institute (Tokai, Japan). [2-\(1^4\)C]Thymine and L-[\(1^4\)C]proline were purchased from the Radiochemical Centre (Amersham, U.K.). Crystalline trypsin was from Mochida Pharmaceutical Co. (Tokyo, Japan).

**RESULTS**

Inhibition of ONPG permeation by colicin E\(_2\). When purified colicin E\(_2\) was added to actively growing E. coli K-12(\(\lambda\)) previously induced with IPTG in nutrient broth, a distinct drop of the ONPG permeation rate occurred after a short lag period. In the presence of 100 \(\mu\)g of chloramphenicol per ml to avoid prophage induction and net increase of the permeation activity due to growth, a more rapid decrease was observed and final inhibition by about 50% was obtained 20 min after the colicin addition. Various strains of colicin E\(_2\)-susceptible E. coli were tested for the inhibition. The ONPG permeation system of E. coli CP78, which was lysogenic for \(\lambda\) phage, was found to be the most susceptible to the colicin (Fig. 1). On the other hand, transport in E. coli B and several strains of K-12 such as W2252T\(^{-}\) was considerably less susceptible to colicin E\(_2\), although their cells were susceptible to the lethal effect of the colicin. Most of the further experiments on this permeation inhibitory effect of colicin E\(_2\) were performed with E. coli CP78 in the presence of chloramphenicol.

The inhibitory effect of colicin E\(_2\) on the ONPG permeation system of CP78 in nutrient broth was compared with those of other E-group colicins, E\(_1\), and E\(_3\). As shown in Fig. 2,
colicin $E_2$ induced a gradual decrease of the permeation rate which started immediately after the colicin addition, but colicin $E_1$ caused a rapid drop without an induction period. The inhibition of the permease by colicin $E_2$ was not reversed at all by adding trypsin (0.5 mg/ml) to the inhibited culture 10 min after the colicin challenge. Colicin $E_3$ did not show a distinct inhibition but a slight temporary increase of the permeation rate. Addition of a larger amount of colicin $E_3$ (18 $\mu$g/ml) showed almost the same profile.

Dose effect of colicin $E_2$ on the inhibition of the ONPG permeation. Dose effect of colicin $E_2$ on the killing and the inhibition of ONPG permeation was measured under the two conditions, that is, relatively anaerobic and aerobic ones. Under the former condition, a culture of CP78 grown aerobically in nutrient broth (8 $\times$ 10^8 cells/ml) and treated with chloramphenicol was divided into several 0.5-ml portions in small test tubes (13-mm diameter) and then incubated with various amounts of colicin $E_2$ without shaking. The aerobic condition was achieved by shaking 5.0 ml of the similar cultures in L-shaped tubes. As shown in Fig. 3, the killing effect of colicin $E_2$ was remarkably stimulated by aeration. The single hit dose of the colicin calculated from the amount to give 36% survival was 9.1 molecules of colicin $E_2$ per bacterium under the aerobic condition and 158 molecules per bacterium.

![Graph](http://aac.asm.org/)
under the anaerobic condition. The dose of colicin E₂ necessary to obtain the half-maximum inhibition was 9 and 338 molecules per cell, respectively, which corresponded to about 1 and 2 killing units of the colicin per bacterium under each condition. The maximum inhibition on ONPG permeation was about 67% in both conditions, and the addition of the larger amounts of the colicin did not increase the extent of the inhibition. The residual activity to transport ONPG at the higher colicin concentrations was effectively inhibited by 5 mM thiodigalactoside with 2 mM KCN. This indicates that the residual transport is still mediated by the specific carrier, M protein (8), and not a nonspecific diffusion due to destruction of the membrane structure.

Confirmation of colicin E₂ protein as the inhibitory effector to the ONPG permeation system. When colicin E₂-resistant mutants of CP78 selected for resistance to BF23 phage were examined, their ONPG permeation systems were found to be unsusceptible to the colicin. This suggested that the inhibition of permeation by our colicin preparation was due to the colicin itself and not to some other contaminants. To confirm this further, the colicin preparation was fractionated by Ampholine electrophoresis and fractions were assayed for the killing activity and the permeation inhibitory activity. As shown in Fig. 4, both activities coincided completely with protein peaks. The main two peaks had isoelectric points of pI 7.63 and 7.43, which showed good coincidence with the data of Herschman and Helsinki (13). The third small peak of pI 7.25 also showed both activities. This component seemed to be a third conformer of colicin E₂, hitherto unknown.

Effect of colicin E₂ on transport of TMG. The inhibitory effect of colicin E₂ on the β-galactoside permease system of CP78 was further analyzed by measuring transport of [14C]TMG. β-Galactosidase and its permease of CP78 cells were induced by the growth in C medium containing TMG instead of IPTG so as to avoid competitive interference of IPTG with accumulation of radioactive TMG. The addition of colicin E₂ caused a rapid decrease in the influx rate starting within a few minutes (Fig. 5). About 20% of the transport activity remained at the maximum inhibition with the larger amounts of the colicin.

To analyze the effect of colicin E₂ on the transport system of TMG further, the change in amounts of intracellular [14C]TMG due to the colicin action was measured. The culture of CP78 grown in C medium induced with TMG was added with [14C]TMG in the presence of chloramphenicol. It was incorporated rapidly into the cells and reached complete equilibrium about 15 min after the addition at 37 C. Colicin E₂ was added to this equilibrated cultures, and amounts of intracellular TMG were sequentially measured by the rapid filtration technique. A rapid decrease in the level of intracellular TMG occurred within a few minutes after the colicin addition. The exit rate of intracellular TMG was accelerated about 5 min after the colicin addition and finally reached less than 17% of the initial level (Fig. 6). Considerably good coincidence of this profile with that of the influx rate obtained by measuring ONPG hydrolysis described above suggested that colicin E₂ inhibited the influx of TMG without significant acceleration or inhibition of the exit velocity.

Although these experiments were performed in C medium to compare with the following experiments about potassium leakage, similar
effects were also observed with cells grown in nutrient broth.

Leakage of potassium ion from cells by colicin E₂. Experiments using radioactive $^{40}$KCl showed that almost complete loss of the intracellular potassium ion was caused by colicin E₂. Cells preloaded with $^{40}$KCl and resuspended in fresh potassium free C medium were challenged with colicin E₂ in the presence of chloramphenicol. Extensive loss of the intracellular potassium ion began within 2 or 3 min after the addition of the colicin, which was almost the same time as the initiation of the inhibition of the $\beta$-galactoside permease (Fig. 7). The mode of the leakage by colicin E₂ was different from that by colicin E₁ which showed no lag period; however, almost 99% of the intracellular potassium ion was released after a prolonged incubation with 27.5 killing units of colicin E₂ per bacterium. No significant lysis of cells was observed during the experimental periods by turbidimetry or total cell counting.

Effect of colicin E₂ on transport of L-proline. The inhibitory effect of colicin E₂ on the transport of L-proline was also observed. The colicin challenge was performed with cells of CP78 which were grown in M medium and treated with chloramphenicol. Radioactive L-proline was added 1 min after the addition of the colicin, and the radioactivity incorporated into the cells was measured sequentially. The inhibition of uptake velocity appeared 2 min

**Fig. 5. Inhibition of the influx rate of $^{14}$C/TMG by colicin E₂.** Rates were indicated by the amounts of radioactivity incorporated for 30 s of incubation under the condition described in the text. Colicin E₂ was added at time 0. Symbols: ○, control; ●, E₂ (0.02 μg/ml, 29 killing units/cell).

**Fig. 6. Leakage of the intracellular $^{14}$C/TMG by colicin E₂.** The colicin was added at time 0. Symbols: ○, control; ●, E₂ (0.02 μg/ml, 29 killing units/cell); ●, E₂ (0.066 μg/ml, 95.7 killing units/cell).

**Fig. 7. Leakage of the intracellular potassium ion by colicins E₂ and E₁.** Symbols: ○, control; ▲, E₂ (0.008 μg/ml, 11 killing units/cell); ●, E₂ (0.02 μg/ml, 27.5 killing units/cell); ×, E₁ (0.1 μg/ml, about 5 killing units/cell).
after the colicin challenge, and distinct leakage of the incorporated radioactivity started 5 to 6 min after the addition of colicin E2 (Fig. 8).

Degradation of DNA by colicin E2. Degradation of DNA into acid-soluble materials by colicin E2 was measured with cells of CP78 in C medium. Simultaneous measurements of the inhibition of the ONPG permeation rate were also carried out. As shown in Fig. 9, the extensive degradation of DNA began 10 min after the colicin challenge. The initiation of DNA degradation was apparently delayed compared to the initiation of the permease inhibition; however, it seems possible that limited endonucleolytic cleavage of DNA occurs at the earlier stage.

Sensitization of the transport systems to colicin E2 by λ-lysogenization. Among various strains of E. coli treated, all the strains which showed marked susceptibility to the permeation inhibitory activity of colicin E2 were lysogens for λ phage. In contrast, transport systems of the nonlysogenic strains, W2252T- and B, were considerably less susceptible. These results suggested the possibility that the presence of λ prophage affected the susceptibility of the membrane transport systems to the colicin. To investigate this possibility, the prophage was cured to derive the isogenic nonlysogenic strains, and susceptibilities of their ONPG permeation systems to colicin E2 were compared with that of the parent. The permeation systems of all the nonlysogenic derivatives, e.g., strain 353 (Table 1), showed distinct decreases in the susceptibility to the colicin.

Further analyses of the effect of λ prophage were made by comparing susceptibilities of the reconstructed series of strains lysogenic for various λ mutants. As a starting nonlysogenic strain, the λ-susceptible derivative of strains 353 and W2252T- was used. Table 1 shows potassium leakage by colicin E2 in these strains. Slight leakage of the intracellular potassium ion was induced in the nonlysogenic strains by the colicin; however, lysogenization for wild λ phage and λind" caused marked sensitization to the colicin. Since expression of almost all λ genes except for the cl and rex are prevented completely in cells carrying λind", it may be concluded that the sensitization is due either to the cl or to the rex gene of λ prophage. On the other hand, cells carrying λimm434 in which the λ-specific immunity region, including the cl and rex genes, was displaced by the comparable region of prophage 434 showed no increased susceptibility of the potassium transport system. Then, it seemed highly probable that the rex gene of λ prophage, responsible for the specific exclusion of λ11 mutants of T4 phage of the λ-lysogenic cells, was involved in the observed sensitization to colicin E2. This was really confirmed with the transport systems for TMG and potassium ion in cells carrying λrex". As shown in Table 2, the sensitization of both transport systems to colicin E2 was enhanced by λlysogenization.
systems by λ prophage failed to occur with λ rex-5a, which caused no effective exclusion or T4rII, whereas λ rex-Qam, whose Rex- property was suppressed in cells of 353λ*, behaved like wild λ concerning the sensitization of the transport systems. It is reasonably concluded that the sensitization to colicin E₂ by λ prophage depends solely on the rex gene product.

**DISCUSSION**

The transport inhibitory effect of colicin E₂ on the λ-lysogenic cells described here was similar to that of colicin E₁; however, the presence of an induction period in the action of colicin E₂ discriminates these two types of colicins. The inhibition of membrane transport systems by colicin E₂ takes place in two stages sequentially. The first slowly proceeding inhibition stage begins immediately after the colicin adsorption, and after 4 or 5 min the second stage starts to cause a marked inhibition of the influx rate and rapid leakage of the metabolites. Since chloramphenicol was added to the system to prevent induction of λ and the lysogen for the induction-negative mutant of λ phage (λind⁻) showed distinct susceptibility to the colicin, the possibility is negated that the progressive inhibition of the transport systems is an indirect result of the prophage induction triggered by the colicin action. Lusk and Nelson (17) reported that colicin K had an induction period depending upon the temperature and the multiplicity, whereas colicin E₁ did not. Experiments at a different temperature and multiplicity of E₂ would establish how similar its induction period is to that of colicin K.

The remarkable sensitization of the membrane transport systems to colicin E₂ by λ-lysogenization is caused by the rex gene function of the prophage. The addition of colicin

| Table 1. Effect of λ-lysogenization on leakage of potassium ion from cells by colicin E₂ |
|---------------------------------|----------------|----------------|----------------|
| **Strains** | **Counts/min** | **%** | **Counts/min** | **%** | **Counts/min** | **%** |
| CP78(λ) | 8,270 | 100 | 219 | 2.38 | 113 | 1.36 |
| 353λ* | 9,641 | 100 | 7,885 | 81.6 | 7,281 | 76.5 |
| 353λ(λi) | 9,131 | 100 | 749 | 8.20 | 811 | 8.89 |
| 353λ(λind⁻) | 7,886 | 100 | 405 | 5.13 | 398 | 4.45 |
| 353λ(λimm434) | 1,994 | 100 | 1,749 | 87.6 | 6,497 | 100 |
| 353λ(λrex-5a) | 15,325 | 100 | 12,876 | 84.0 | 287 | 2.88 |
| W2252T⁻ | 7,618 | 100 | 585 | 7.68 | 519 | 6.22 |

* Colicin E₂ solution (20 μg/ml) was added to 5-ml cultures of each strain (5 × 10⁶ to 8 × 10⁷ cells/ml) preloaded with ⁴⁰KCl and incubated for 20 min at 37 C aerobically in the presence of chloramphenicol. The residual intracellular radioactivity was measured.

<p>| Table 2. Effect of the rex gene on leakages of intracellular TMG and potassium ion |
|---------------------------------|----------------|----------------|----------------|</p>
<table>
<thead>
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<th><strong>Strains</strong></th>
<th><strong>EOP * of T4rII</strong></th>
<th><strong>Intracellular TMG %</strong></th>
<th><strong>Intracellular K⁺ %</strong></th>
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<tr>
<td>353λ*</td>
<td>100</td>
<td>16,161</td>
<td>14,902</td>
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<td>353λ(λ)</td>
<td>0</td>
<td>15,586</td>
<td>4,207</td>
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<td>353λ(λrex⁻5a)</td>
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<td>14,432</td>
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<td>353λ(λrex⁻Qam)</td>
<td>&lt;5</td>
<td>9,357</td>
<td>153</td>
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</tbody>
</table>

* EOP, Efficiency of plating.
* Radioactivities (counts per minute) of [¹⁴C]TMG or ⁴⁰K⁺ remaining in cells after the incubation with colicin E₂ (20 μg/ml) for 20 min at 37 C were indicated. Incubation condition was the same as that in Table 1. Values in parentheses are percentages of the amounts in the absence of the colicin.
* Only extremely faint plaques are visible.
E₂ to cells carrying λrex⁺ phage seems to inhibit the oxidative phosphorylation accompanying with the inhibition of the active transport systems of the cell membrane (unpublished data). Inhibition of ³²P incorporation by colicin E₂ has also been reported in a strain not stated to be λ lysogenic (28). Since initiation and maintenance of the DNA degradation directed by colicin E₂ require energy (14), the rex gene-dependent reduction of the DNA degradation appearing in later periods of the colicin challenge (23) may be explained by this membrane attacking effect of the colicin stimulated by the rex gene.

The mechanism of the apparent sensitization of the cell membrane to the colicin action by the rex gene product cannot be decided from the results presented here. Although the primary function of the rex gene product has not yet been identified, several observations seem to suggest its close relationship with the cell membrane. It has been reported that rII exclusion by the Rex function is suppressed by the presence of higher concentrations of magnesium ion (11) or sucrose with calcium ion (7), and abnormalities of cation transport and leakage of pyridine nucleotides occur during the rII infection of a lysogen for λrex⁺ (27).

Premature lysis after infection by T1 phage was also observed to be due to the rex gene (9). These experiments imply that the Rex function may affect cell membrane stability and permeability in the cases of the several infectious events. It may be assumed that colicin E₂ itself has an intrinsic activity to affect the structure of the cell membrane, and the rex gene product has modified the cell membrane to make it susceptible to the colicin action in some way. However, several alternative possibilities should also be considered. For instance, it is possible that colicin E₂ is activated by the rex gene product to produce the membrane attacking activity in a similar manner to the case of colicin E₈, whose intrinsic ribonucleolytic activity is unmasked by endolytic cleavage to produce an active fragment (21). On the other hand, it seems also possible that the rex gene product is activated by the colicin to reveal its latent membrane attacking activity. In this context, it should be noted that activation of periplasmic endonuclease I by colicin E₂ has been postulated (1, 2). Further experiments are needed to settle these problems; however, this system may be useful to analyze not only the mode of action of colicin E₂ but also the rex gene function which has hitherto been unknown.

The strong inhibition of transport systems by colicin E₂ may be a principal cause for the killing of λ-lysogenic cells. In contrast, non-lysogenic cells such as E. coli B are susceptible to the lethal effect of the colicin, whereas their transport systems show considerable resistance (1 killing unit of colicin E₂ for E. coli B is about 18 molecules per bacterium under the aerobic condition). This fact poses a question whether the membrane attacking activity of the colicin plays a primary role for the killing of Rex⁻ cells. However, the partial sensitivity of the transport systems, especially for β-galactosides, occurs in nonlysogenic cells. This indicates that a similar reaction of the colicin against the membrane occurs to a smaller extent even in the absence of the rex gene product. It may be assumed that a minor alteration in the membrane of nonlysogenic cells induced by colicin E₂ is sufficient to cause the effective killing. Although the demonstration of an intrinsic nuclease activity in colicin E₂ protein (25) suggests direct action of the colicin molecules in the DNA degradations, possible role of the membrane alteration induced by colicin E₂ for the killing and the DNA degradation should be carefully examined further.

LITERATURE CITED