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Evidence for the Direct Action of Colicin K on Aerobic $^{32}P_i$ Uptake in *Escherichia coli* In Vivo and In Vitro

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Pentachlorophenol (PCP)-sensitive incorporation of ^{32}P-labeled orthophosphate ($^{32}P_i$) into nucleotides and nucleic acids by disrupted spheroplasts of *Escherichia coli* was inhibited by addition of colicin K. Incorporation by intact cells was also inhibited by a similar concentration of colicin K. Various colicin K-resistant mutants were isolated, and their ability to incorporate $^{32}P_i$ was tested. When *T6^r-colK^r* mutants (T6 phage-resistant) and *tol I* mutants (T6-sensitive, colicin E-sensitive) were converted to disrupted spheroplasts, their $^{32}P_i$-incorporation became sensitive to colicin K. On the contrary, incorporation by disrupted spheroplasts from *tol II* mutants (T6-sensitive, colicin E-resistant) was fairly resistant to colicin K like that of intact cells. A modification of the cell surface of *T6^r-colK^r* mutants, caused by mutation to novobiocin-permeable, T4 phage-resistant cells, restored the sensitivity of the cells to colicin K. The modified *T6^r-colK^r* cells did not adsorb T6 phage or colicin K, indicating that the receptors for T6 phage or colicin K are not reactivated by this modification. Similar treatment of *tol I* mutants did not have this effect. These observations strongly suggest that colicin K can act on its target on the cell membrane if it can penetrate the cell surface to reach this target. The receptor for colicin K on the cell surface, which may be part of the T6 phage-receptor, may have some unknown function in relation to the action of colicin K in normal cells, but tends to become dispensable if the cells become permeable to colicin K.

Preparations of disrupted spheroplasts of *Escherichia coli* cells can incorporate externally added, ^{32}P-labeled orthophosphate ($^{32}P_i$) into nucleotides and nucleic acids under aerobic conditions (13, 14). These biosynthetic reactions consist of a sequence of reactions involving: (i) formation of γ-^{32}P-labeled adenosine triphosphate by oxidative phosphorylation and then of β,γ-^{32}P-labeled nucleoside oligophosphates, (ii) formation of α-^{32}P-labeled ribose and deoxyribose nucleoside oligophosphates (precursors of nucleic acids), and (iii) polymerization reactions to form ^{32}P-labeled nucleic acids. All these reactions can occur in disrupted spheroplasts of *E. coli* (14).

In the present work, we studied the mode of action of colicin K on cell membranes in vitro by examining its effect on disrupted spheroplasts of *E. coli*. Colicin K is a simple protein with a molecular weight of about 70,000 (10), and it is known to become attached to the cell surface of *E. coli* and to cause death of the cells by inhibiting energy metabolism on the cell membranes (4, 17). Thus, by using the above complex in vitro system, it should be possible to obtain information on the mode of action of colicin K on cell membranes and, conversely, on the role of the cell membrane in the action of colicin K.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The properties of the bacterial strains used are summarized in Table 1.

The following media were used: nutrient broth medium containing, in 1 liter of deionized water, 10 g of polypeptone (Daigo Eiyo Kagaku, Osaka, Japan), 10 g of Ehrlich meat extract (Kyokuto Seiyaku, Tokyo, Japan), 2 g of NaCl and NaOH to adjust the *p*H to 7.4; Fraser's medium (5) containing, in 1 liter of

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TABLE 1. *Bacterial strains*[a]

Strain	Relevant characteristics	Origin
Escherichia coli		
MK1	F⁻ *leu pro*	W3110
W2252	(Col E2)	
K235	(Col K, Col X⁻)	
CA38	(Col E3, Col I)	
Salmonella typhimurium		
cysD36	(Col E1)	

[a] Symbols: *leu*, leucine; *pro*, proline; Col, colicinogenic. *E. coli* strain MK1 was isolated in this laboratory. Colicinogenic *E. coli* strain W2252 was received from T. Watanabe, strain CA38 from A. Maeda, and *Salmonella typhimurium* strain cysD36 from T. Fukasawa.

deionized water, 15 g of vitamin-free Casamino Acid (Difco), 30 g of glycerol, 4.5 g of KH_2PO_4, 10.5 g of Na_2HPO_4, 1.0 g of NH_4Cl, and 0.3 g of $MgSO_4 \cdot 7H_2O$; and L-broth medium containing, in 1 liter of deionized water, 10 g of polypeptone, 5 g of yeast extract (Ebiosu, Tokyo, Japan), 5 g of NaCl, 1 g of glucose, and NaOH to adjust the *p*H to 7.0. For preparing plates, medium was solidified by adding 1.5% agar (Wako Pure Chemical Co., Osaka, Japan).

Preparation and assay of colicins. Preparations of colicin K from the colicinogenic strain *E. coli* K-235 were chromatographed on CM-Sephadex before use in most experiments. Colicin E1 was prepared from *Salmonella typhimurium cysD36* (Col E1) by the same processes as colicin K (10). Colicin E2 and colicin E3 were prepared by the method of Herschman and Helinsky (7) from the appropriate colicinogenic strains described in Table 1. One killing unit (KU) was determined as described by Fields and Luria (4).

Phages and their adsorption tests. Phage T6 was obtained from T. Watanabe, phage T4 from Y. Masamune, and phage BF23 from K. Imahori. For adsorption tests, overnight cultures of cells in nutrient broth were mixed with phages (about 0.5×10^{-4} multiplicity of infection), incubated at 37 C for 10 min, and then centrifuged. The resulting supernatant fluids were treated with chloroform, and the remaining plaque-formers were counted.

Colicin K adsorption test. Cells in the middle of the logarithmic phase of growth were washed with and suspended in 0.85% NaCl containing 5 mM $MgCl_2$ and 0.5 mM $CaCl_2$ (5×10^9 cells per ml). Various amounts of purified colicin K preparation were added to 0.8-ml portions of this cell suspension, and, after incubation at 37 C for 20 min, the cells were centrifuged and the amount of colicin remaining in the supernatant fluid was determined by a spot test.

Isolation of colicin K-resistant mutants. Spontaneous colicin K-resistant mutants were isolated from nutrient agar plates containing two concentrations of colicin K. For selection with a low concentration of colicin K, the nutrient agar plate was pretreated overnight at 37 C with ca. 10^7 colicinogenic cells and then exposed to chloroform vapor. Cells of strain MK1 were cultured overnight in nutrient broth at 30 C. Then, a 0.1-ml sample (ca. 2×10^9 cells per ml) was mixed with 2.5 ml of nutrient broth containing 0.75% agar (soft nutrient agar) and layered on the pretreated nutrient agar plate. The plate was incubated overnight at 37 C, and the resistant colonies which appeared were purified by the single-colony isolation technique. Only one resistant mutant strain was selected from each parent clone. For selection with a high concentration of colicin K, 0.1 ml of an overnight culture of the parent cells was mixed with soft nutrient agar containing 4×10^{10} KU of purified colicin K preparation and layered on a nutrient agar plate, and the mutants were isolated as described above. The resistances of the isolated mutants to colicins and to phages were checked by the cross-streaking technique on nutrient agar plates.

Preparation of cells and disrupted spheroplasts for assay of $^{32}P_i$ incorporation. Cells of strain MK1 or its mutant strains were cultured in Fraser medium at 37 C with shaking and harvested in the middle of the logarithmic phase of growth (about 8×10^8 cells per ml). Cells were washed twice and suspended in deionized water at room temperature at a concentration of 10% (wet weight per volume) (see "intact cells" in Tables 3 and 4 and Fig. 2 and 4). To prepare disrupted spheroplasts, the suspension was diluted with five volumes of solution containing 1.5 mM ethylenediaminetetraacetic acid (EDTA), 36 μg of lysozyme per ml (Eizai Co., Tokyo, Japan), 0.45 M sucrose, and 0.03 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (*p*H 8.0) at 30 C. The mixture was incubated at 30 C for 30 min, cooled in an ice bath, and then centrifuged at 15,000 × *g* for 5 min. The pellet was suspended at a concentration of 5% (wet weight of original cells per volume) in 0.05 M Tris-hydrochloride (*p*H 7.4) containing 5 mM $MgCl_2$ and 10 μg of deoxyribonuclease I per ml (EC 3.1.1.3, Sigma Chemical Co.) and homogenized in an ice-cold, Potter-Elvehjem type glass homogenizer by 10 manual strokes of a loosely fitting Teflon piston. The homogenate was centrifuged at 15,000 × *g* for 20 min, and the pellet was suspended in cold 0.05 M Tris-hydrochloride (*p*H 7.6) containing 5 mM $MgCl_2$ at a concentration of 5% (wet weight of original cells per volume). The viable cell count of this suspension was less than 0.01% of the original cell count, and the preparation was designated as "disrupted spheroplasts."

Assay of $^{32}P_i$ incorporation into nucleotides and nucleic acids in intact cells and disrupted spheroplasts. Incorporation of $^{32}P_i$ into nucleotides and nucleic acids was measured as described by Nagata et al. (13, 14). Before use, $^{32}P_i$ (Institute of Atomic Energy, Japan, carrier-free) was diluted with 0.01 M HCl and heated to 100 C for 30 min to decompose labeled impurities. The reaction mixture for measuring $^{32}P_i$ incorporation contained, in a final volume of 2 ml in 20-ml, L-shaped tubes, 50 mM Tris-hydrochloride (*p*H 7.6), 7.5 mM $MgCl_2$, 1.25 mM sodium phosphate (*p*H 7.6), 1 mg of Casamino Acids (Difco, vitamin-free), 1 μCi of ^{32}Pi, 50 mg (wet weight) of intact cells, or the equivalent amount of disrupted spheroplasts, and the inhibitors indicated in the text.

Tubes were shaken in a water bath at 30 C for 15 min. Then, 40 μliters of 1 M sodium phosphate buffer was added, and the mixture was chilled in an ice bath. The reaction was stopped by adding an equal volume of ice-cold 10% trichloroacetic acid. Nucleotides and nucleic acids were fractionated by a modification (13) of the method of Schmidt and Thannhauser (19). Radioactivity was counted in a Geiger-Mueller counter. In this procedure, the processes of pretreatment of $^{32}P_i$ with HCl and the addition of phosphate to the reaction mixture before addition of trichloroacetic acid consistently resulted in very low, zero-time control counts (less than 130 counts per min with an input of ca. 400,000 counts per min). Protein was assayed by a standard method (11).

RESULTS

Isolation of T6^r-colK^r and tol mutants of strain MK1. A number of genetically independent mutants were isolated from strain MK1 by selection with two concentrations of colicin K (see Materials and Methods). From the pattern of resistance of the mutant strains to phage T6 and colicins E1 to E3, as checked by the cross-streak test (not by genetical identifications), the mutants were classified by the procedures of Nomura and Witten (16) and Nagel de Zweig and Luria (15), as shown in Table 2. *T6^r-colK^r* and *tol I* mutants were exclusively isolated from plates containing low concentrations of colicin K, whereas *tol II* and *tol III* mutants were isolated from plates containing high concentrations of colicin K (Table 2). Adsorption experiments showed that *T6^r-colK^r* mutants were the only strains which did not adsorb T6 and colicin K.

The survival counts of the parent and mutant strains were plotted against the concentrations of colicin K added to the nutrient broth (Fig. 1). The colicin K-sensitive parent and the partially resistant *T6^r-colK^r* strains gave so-called single-hit curves for the lethal effect of colicin K. That is, the logarithm of the decrease in viable cell count was proportional to the concentration of colicin K. The slope was steeper for the sensitive parent strain than for the partially

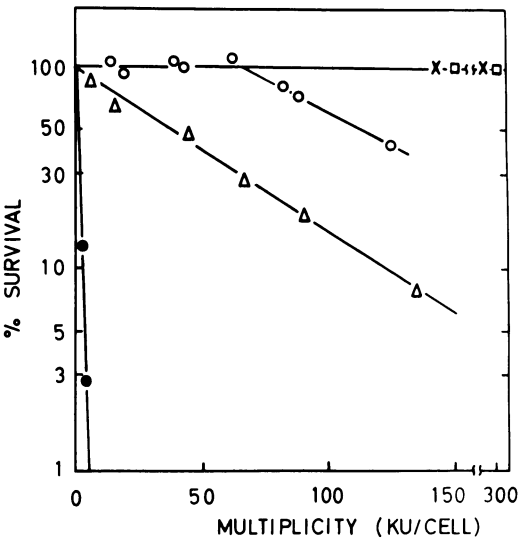

FIG. 1. *Survival curves of strain MK1 and its derivative colicin K-resistant strains plotted against colicin K concentrations. Cells in the logarithmic phase in nutrient broth were mixed with purified colicin K and incubated at 37 C for 10 min with shaking. Then, survivors were counted as colonies on nutrient agar plates. Symbols: ●, strain MK1 (colicin K-sensitive parent strain); Δ, strain CKR 9 (T6^r-colK^r); O, strain CKR 7 (tol I); □, strain CKT 72 (tol II); ×, strain CKT 61 (tol III); KU, killing unit.*

TABLE 2. *Classification and number of colicin K-resistant mutants obtained*[a]

Class	Colicin				Phage		No. isolated by selection in:		Adsorption of colicin K[b]
	K	E1	E2	E3	T6	BF23	Low concn of colicin K	High concn of colicin K	
Parent	s	s	s	s	s	s			+
T6^r-colK^r	pr	s	s	s	r	s	31	0	−
tol I	r	s	s	s	s	s	4	0	+
tol II	r	r	r	r	s	s	0	14	+
tol III	r	s	r	r	s	s	0	11	+
Others[c]							10	24	

[a] Symbols: s, sensitive; pr, partially resistant; r, resistant.

[b] One strain each tested.

[c] Three partially resistant *tol I* mutants and 31 temperature-sensitive mutants (resistant at 42 C, sensitive at 30 C).

resistant *T6*^{r}-*colK*^{r} mutant strains. In contrast, *tol II* and *tol III* mutants were completely stable at all concentrations of colicin K tested. However, *tol I* mutants were less resistant and showed some decrease in the viable cell count at higher concentrations of colicin K. These mutants were more resistant to these concentrations of colicin K when Fraser medium was used instead of nutrient broth. Our *tol II* and *tol III* mutants grew slowly, became fragile during growth, and were sensitive to deoxycholate, as described by others (15).

Incorporation of $^{32}P_i$ into nucleotides and nucleic acids in intact cells and in disrupted spheroplasts and its inhibition by colicin K. Disrupted spheroplasts of *E. coli*, when suspended in buffer solution containing Tris-hydrochloride (*p*H 7.6), $^{32}P_i$, $MgCl_2$, and Casamino Acids, show aerobic, pentachlorophenol (PCP)-sensitive incorporation of $^{32}P_i$ into nucleotides and nucleic acids (14). In the same buffer and under similar conditions, the amounts of $^{32}P_i$ incorporated into both nucleotides and nucleic acids in disrupted spheroplasts were comparable with those in intact cells. Therefore, the effect of colicin K could be examined in vitro by measuring $^{32}P_i$ uptake by disrupted spheroplasts of the parent and mutant strains in the presence of colicin K. $^{32}P_i$ incorporation into both nucleotides and nucleic acids continued for over 60 min at 30 C and was linear for at least 15 min, so all experiments were performed for 15 min at 30 C.

Table 3 shows results with the parent strain, MK1. Addition of 4.2 to 50 μg of purified colicin K per ml strongly inhibited $^{32}P_i$ incorporation into nucleotides and nucleic acids, both in intact cells and in disrupted spheroplasts. In intact cells, however, inhibition of $^{32}P_i$ uptake by addition of colicin K was only partial (62% inhibition on addition of 50 μg of colicin K per ml) and inhibition by PCP was also incomplete (46% inhibition on addition of 10^{-4} M PCP). This suggests that there was sufficient glycolytic phosphorylation in intact cells under the present incubation conditions (see Materials and Methods) to support 40 to 50% of the total phosphorylation. Alternatively, it is possible that extremely thick suspensions of cells, such as those used in the present experiments (about twice the cell concentration obtained on maximum growth in Fraser medium at 37 C), were much less sensitive to addition of antimetabolites than cells growing normally.

By contrast, $^{32}P_i$ incorporation in disrupted spheroplasts of strain MK1 was inhibited 84% by addition of 50 μg of colicin K per ml and 93% by 10^{-4} M PCP. These results showed that most of the inhibition of $^{32}P_i$ incorporation by colicin K was due to inhibition of an oxidative process.

For comparison, the effects of other colicins, E1, E2, and E3, were tested on the sensitive strain MK1 (Table 4). Colicin E1, at a concentration of 25 μg per ml, caused 39% inhibition of $^{32}P_i$ incorporation into intact cells and 85% inhibition of that into disrupted spheroplasts. Colicin E2, at a concentration of 10 μg per ml, caused 74% inhibition of $^{32}P_i$ incorporation into intact cells and 90% inhibition of that into disrupted spheroplasts. Colicin E1 is generally supposed to have a similar or identical action to colicin K, but colicin E2 is known to act primarily on deoxyribonucleic acid metabolism, with a secondary affect on ribonucleic

TABLE 3. *$^{32}P_i$ incorporation in vivo and in vitro by E. coli strain MK1 (colicin K-sensitive parent strain) and inhibition by colicin K*

System	Addition	Nucleotides		RNA		DNA	
		Counts/min	%	Counts/min	%	Counts/min	%
Intact cells	None	8,827	(100)	6,638	(100)	842	(100)
	Colicin K						
	4.2 μg/ml	8,031	91	6,088	92	754	90
	8.3 μg/ml	6,738	76	5,947	90	689	82
	25 μg/ml	4,640	53	2,789	42	240	29
	50 μg/ml	4,171	47	1,881	28	199	24
	PCP, 10^{-4} M	5,470	62	2,985	45	313	37
Disrupted spheroplasts	None	3,366	(100)	1,688	(100)	149	(100)
	Colicin K						
	4.2 μg/ml	1,676	50	568	34	106	71
	8.3 μg/ml	1,404	42	429	25	18	12
	25 μg/ml	871	26	62	4		
	50 μg/ml	750	22	65	4	41	28
	PCP, 10^{-4} M	343	10	3	0	41	28

acid synthesis. Here, also, inhibition of intact cells by colicin E1 and E2 was incomplete, probably for the reasons suggested previously. It is uncertain why ribonucleic acid synthesis in intact cells was not completely inhibited by colicin E2, whereas deoxyribonucleic acid synthesis was completely inhibited. Colicin E3 had no effect in either experiment.

TABLE 4. *Effect of colicins E1, E2, and E3 on $^{32}P_i$ incorporation by E. coli strain MK1 in vivo and in vitro*

System	Addition	Nucleotides		RNA		DNA	
		Counts/min	%	Counts/min	%	Counts/min	%
Intact cells	None	4,022	(100)	2,767	(100)	483	(100)
	Colicin E1, 25 μg/ml	2,483	62	1,715	62	247	51
	Colicin E2, 10 μg/ml	975	24	703	25	8	2
	Colicin E3, 25 μg/ml	3,705	92	2,544	94	452	94
Disrupted spheroplasts	None	1,489	(100)	752	(100)	404	(100)
	Colicin E1, 25 μg/ml	246	17	22	3	130	32
	Colicin E2, 10 μg/ml	193	13	76	10	0	0
	Colicin E3, 25 μg/ml	1612	108	936	124	374	93

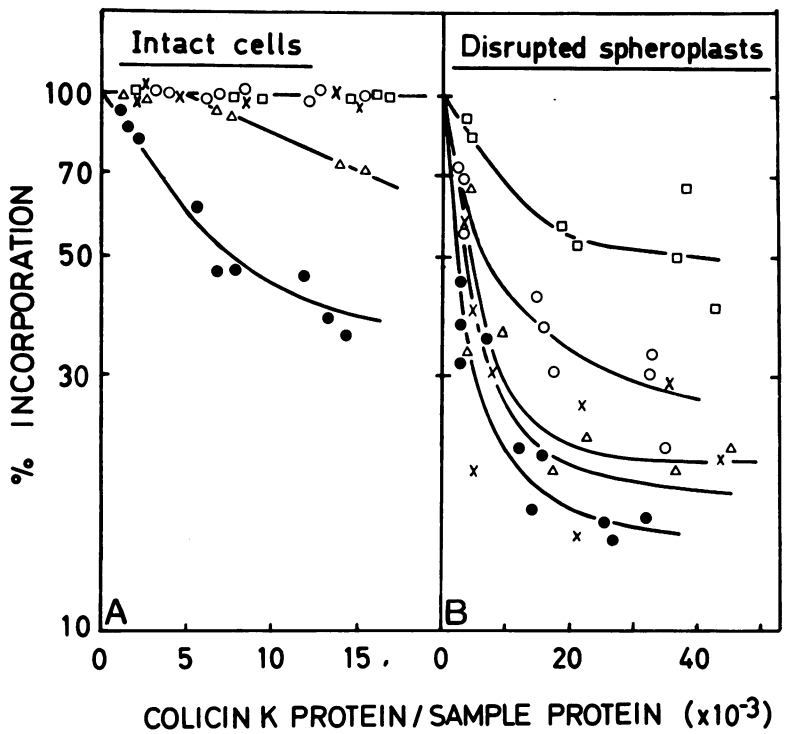

FIG. 2. *Summary of experiments showing colicin K sensitivity of $^{32}P_i$ incorporation (sum of nucleotides and nucleic acids) in vivo and in vitro. Symbols: ●, strain MK1 (colicin K-sensitive parent strain); △, strains CKR 5, CKR 9, CKR 47 (T6r-colK+); ○, strains CKR 7, CKR 20, CKR 45 (tol I); □, strains CKT 52, CKT 72, CKT 82 (tol II); ×, strains CKT 51, CKT 61, CKT 77 (tol III). Abscissae: microgram of colicin K per milligram of protein of intact cells (A) or disrupted spheroplasts (B).*

The results of similar experiments with the colicin K-resistant mutants, *T6^r-colK^r*, *tol I*, *tol II*, and *tol III*, are summarized in Fig. 2. For the results in Fig. 2, the experiment was repeated three times with the parent strain and once each with three different strains of each mutant (*T6^r-colK^r*: CKR 5, CKR 9, CKR 47; *tol I:* CKR 7, CKR 20, CKR 45; *tol II:* CKT 52, CKT 72, CKT 82; *tol III:* CKT 51, CKT 61, CKT 77).

Figure 2A represents the inhibition by colicin K of $^{32}P_i$ incorporation in the parent and mutant cells. These figures resemble those of their survival curves in Fig. 1 plotted against the concentration of colicin K. Figure 2B represents the inhibition in disrupted spheroplasts.

In general, phosphorylation was more sensitive to added colicin K in disrupted spheroplasts than in intact cells. This could be partly due to colicin K-insensitive anaerobic phosphorylation, which may be only operative in intact cells under the present conditions (PCP-insensitive part, see Table 3). However, the increase in sensitivity of phosphorylation to colicin K caused by converting intact cells to disrupted spheroplasts was much greater than that calculated from the decrease of anaerobic, PCP-insensitive phosphorylation. About two-thirds of the protein in intact cells was removed during preparation of disrupted spheroplasts. If the amount of colicin K added to disrupted spheroplasts is calculated from the original amount of cell protein, the scale of the amount of colicin K added to disrupted spheroplasts (abscissa of Fig. 2B) must be multiplied by about 3, and the scales of the abscissae in Fig. 2A and 2B then become comparable. Even after these corrections, disrupted spheroplasts from the colicin K-resistant mutant strains are much more sensitive to added colicin K than intact cells. The increase in sensitivity to colicin K was greater with *T6^r-colK^r* strains, followed by *tol I* and *tol III* mutant strains. These results suggest several possibilities with regard to the mechanism of attachment of colicin K to the cell surface, as discussed below.

Recovery of colicin K sensitivity of T6^r-colK^r mutants by T4 phage-resistant mutation. On mutation to colicin K resistance, *T6^r-colK^r* mutants simultaneously become resistant to phage T6. This double resistance suggested that colicin K might share its receptor on the cell surface with T6 (6). However, the results strongly suggest that colicin K molecules can act directly on cell membranes on which oxidative phosphorylation and synthesis of nucleotides and nucleic acids occur and that the primary attachment of colicin K molecules to the cell surface may be unimportant. Therefore, the so-called colicin K receptor may only have an auxiliary rather than an essential function in the action of colicin K.

Accordingly, we attempted to modify the cell surface to facilitate the direct attachment of colicin K molecules on their target on the cell membranes without reactivating the T6 receptor. Previously, it was found in our laboratory that removal of phosphate and several sugar residues which are covalently linked to the heptose-polysaccharide region of lipopolysaccharides in the cell wall of *E. coli* facilitates the penetration of certain macromolecular (S. Tamaki and M. Matsuhashi, *in press*) as well as low-molecular compounds (21). These phosphate or sugar residues also form part of the structure of the receptor for phage T4 (21). Thus, T4-resistant mutants showing increased penetration of external compounds could be isolated.

Therefore, a number of T4-resistant mutants were isolated from three *T6^r-colK^r* strains (strains CKR 5, CKR 9, CKR 47), and, from each of them, three novobiocin-hypersensitive mutants were selected which did not grow on L-broth agar plates containing 30 μg of novobiocin per ml (21). These mutant cells were all T6-resistant and, as far as tested, (strain CKR 9 derivatives) did not adsorb colicin K, like their parent *T6^r-colK^r* strain, indicating that their receptors for colicin K and phage T6 were still inactive. However, they were all sensitive to colicin K (Fig. 3 and 4). The cross-streak tests in Fig. 3 show that strain MK-1 (parent) was colicin K-sensitive and phage T4- and T6-sensitive, whereas strain CKR 9 (*T6^r-colK^r*) was partly resistant to colicin K, T4-sensitive, and T6-resistant. On the contrary, the T4-resistant strains CKR 9-1, CKR 9-2, and CKR 9-3 (derived from strain CKR 9) were T6-resistant but colicin K-sensitive. Identical results were obtained with the other two *T6^r-colK^r* strains (CKR 5 and CKR 47) and the three strains derived from each of them. The sensitivity of the phage T4-resistant strains to colicin K was also checked by measuring $^{32}P_i$ incorporation into the intact cells. The results (Fig. 4) show that $^{32}P_i$ incorporation by T4-resistant cells (strain CKR 9-1, CKR 9-2, and CKR 9-3, none of which adsorbed T4) was inhibited by colicin K to the same extent as that of the colicin K-sensitive strain MK1, whereas that of the parental *T6^r-colK^r* cells (strain CKR 9) was virtually not inhibited under the same conditions. These results strongly suggest that colicin K molecules can reach their targets on the cell membrane and act on them without becoming attached to T6 receptors. These results are

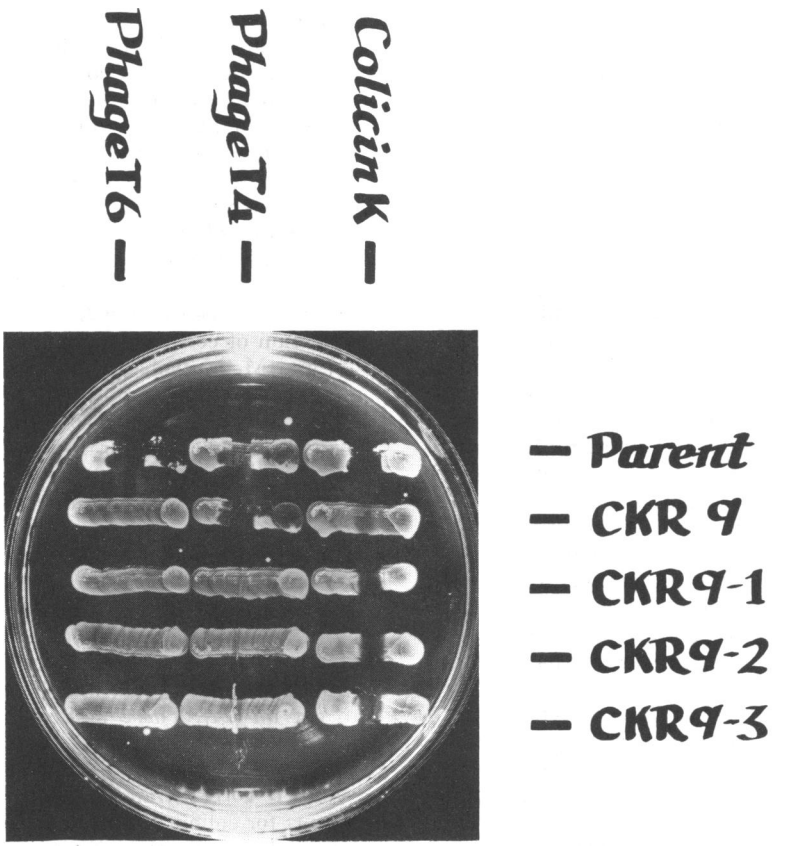

FIG. 3. *Cross-streak tests showing that conversion of strain CKR 9 (T6r-colKr) to T4 resistance was coupled with restoration of colicin K sensitivity. Overnight cultures of the strains indicated were cross-streaked with phages and colicin K on nutrient agar plates and incubated at 37 C.*

compatible with those obtained with the in vitro systems which showed that disrupted spheroplasts from *T6r-colKr* strains were colicin K-sensitive.

Similar experiments were performed with three *tol I* strains. However, no restoration of colicin K-sensitivity was observed on isolation of T4-resistant, novobiocin-hypersensitive mutants. This suggests that the mechanism of resistance to colicin K in these mutants is different from that in *T6r-colKr* mutants.

DISCUSSION

The results obtained in the present study clearly showed that colicin K acts directly on disrupted spheroplasts. Its effect on this system is rather more efficient than that on intact cells. The disrupted spheroplast preparations used consisted of cytoplasmic membranes and other cell envelope components, with no appreciable number of intact cells, judging from the viable cell counts and essential absence of PCP-insensitive ^{32}P incorporation. When spheroplasts are prepared by adding lysozyme solution in the cold (0 C) and then quickly warming the mixture to 30 C, they were practically free from outer membranes (12), which are supposed to be located outside the peptidoglycan saccules (23). These disrupted spheroplast preparations lacking outer membranes were devoid of the typical protein components of the latter (S. Tamaki, Y. Takagaki, and M. Matsuhashi, *unpublished results*). These preparations could not incorporate $^{32}P_i$ into nucleotides or nucleic acids under any conditions tested, and therefore it was not possible to examine the action of colicin K on them by using this technique. Bhattacharyya et al. (1), by using the subcellular membrane vesicles described by Kaback (8), showed that colicin E1 inhibits ^{14}C-proline uptake, which is coupled to respiratory chain reactions. Their preparation also did not incorporate $^{32}P_i$, and so their results cannot be compared with ours. Our system seems to

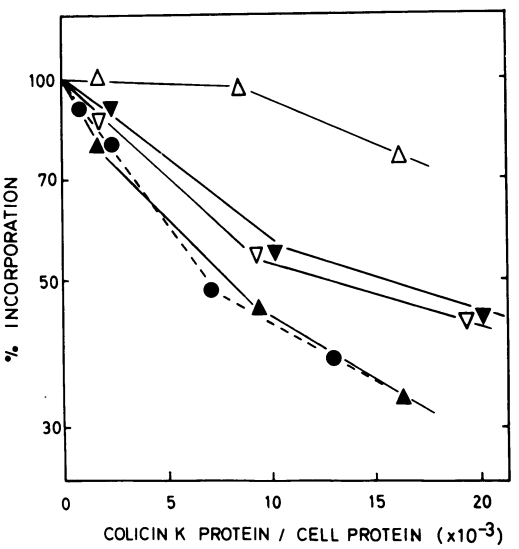

FIG. 4. *Colicin K sensitivity of $^{32}P_i$ incorporation (sum of nucleotides and nucleic acids), in intact cells of the sensitive parent strain MK1, partially resistant strain CKR 9 (lacking a colicin K receptor), and T4-resistant mutants derived from it. Symbols: ●, strain MK1; △, strain CKR 9 (T6^r-colK^r); ▲▽▼, three T4-resistant mutants of strain CKR 9 (CKR 9-1, CKR 9-2, CKR 9-3). As a control, the sensitive strain MK1 was used and results with it are indicated by the dashed line.*

be more complex and therefore has the merit of providing more integral reactions on the membranes for study of the action of colicin K in vitro. However, the simpler system of Bhattacharyya et al. (1) enabled them to show that one of the actions of colicin E1 was on a PCP-sensitive reaction which does not necessarily involve phosphorylation.

In *T6^r-colK^r* strains, the receptor for colicin K was inactivated and the cells could not adsorb colicin K. This suggests that the so-called colicin K receptor, which is presumably a part of the T6 receptor (6, 18, 22), has a function of concentrating colicin K molecules on the cell surface. These molecules are probably rod-shaped, single protein (9) with a molecular weight of 70,000 (10) or 75,000 (3). The colicin K receptor probably allows adequate assembly of molecules or gives the molecule a configuration suitable for attack on its target. Modification of the cell surface results in conversion of the cells to T4 receptor-deficient and novobiocin-hypersensitive cells. This modification is probably due to removal of some lipopolysaccharide structure from the cell surface (21) which prevents the free penetration of compounds like novobiocin, lysozyme (S. Tamaki and M. Matsuhashi, *in press*), and presumably, colicin K into the cells. After this modification of the cell surface, colicin K molecules can penetrate and reach their targets without being trapped on the cell surface by their receptor. The structure and function of this receptor are unknown.

On the other hand, *tol I* mutants presumably have an intact receptor and can adsorb colicin K molecules. They become sensitive to colicin K on conversion to disrupted spheroplasts but not on modification of the cell surface in a way to make it more permeable to colicin K. Presumably *tol I* mutations involve a lyzozyme-sensitive barrier structure (peptidoglycans and adjacent structures) inside the cell surface on which the colicin K receptor and phosphate polysaccharide region (a possible colicin K barrier in normal cells) of cell wall lipopolysaccharide are located. Furthermore, disrupted spheroplasts of *tol II* mutants were the most resistant to colicin K among those of all the resistant strains investigated. Their disrupted spheroplasts seem to have a lysozyme-insensitive structure which is resistant to colicin K. This possibility is compatible with the finding of Burman and Nordström (2), that colicin E2 tolerance was induced in *E. coli* by mutation to ampicillin resistance. Smarda and Taubeneck (20) reported that the L-forms of *E. coli* and *Proteus mirabilis* strains, which are practically devoid of cell wall peptidoglycan, are more sensitive to colicins than normal bacteria.

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