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Effect of Colicin K on a Membrane-Associated, Energy-Linked Function

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The purpose of this work was to investigate the capability of cell extracts of *Escherichia coli* and *E. coli* treated with colicin K to catalyze the following energy-dependent, reverse transhydrogenase reaction: NADP + NADH + ATP ⇆ NADPH + NAD + ADP + P_i. Under anaerobic conditions this reaction requires the presence of a specific portion of the electron transport chain, a functional energy coupling system, including an adenosine triphosphatase enzyme, and ATP as energy source. The ATP-linked reaction was partially inhibited in French press extracts of *E. coli* K-12 C600 cells that had been pretreated with colicin K but not in extracts from similarly treated cells of a colicin-tolerant mutant. Ultracentrifugation of extracts yielded particulate fractions competent in catalyzing the reaction; this reaction is substantially inhibited in fractions from colicin-treated cells. The extent of inhibition increased with increasing concentration of colicin. Supernatants also supported ATP-linked formation of NADPH, but this reaction was insensitive to the colicin effect. A comparison between the requirement of the reaction in supernatant and particulate fractions suggests that the reaction in the supernatant is different from the one inhibited by colicin. The ATP-hydrolyzing ability of particulate fractions from control or treated bacteria was identical. Likewise, the electron transport chain was not affected by colicin treatment, as evidenced from lack of effect on NADH oxidase, succinic dehydrogenase, and NADPH-NAD transhydrogenase. It is concluded that colicin K interferes with the coupling of ATP hydrolysis with the generation of a required high-energy intermediate or with the utilization of the intermediate for the ATP-linked transhydrogenase reaction.

Colicin K, acting on susceptible *Escherichia coli* cells, causes several physiological changes: lowering of adenosine 5'-triphosphate (ATP) levels, arrest of most macromolecular syntheses, and inhibition of active transport of amino acids and β-D-galactosides (12). A recent study (15) utilizing an adenosine triphosphatase (ATPase)-deficient *E. coli* mutant has led to the proposal that colicin K acts primarily by energy uncoupling of membrane transport systems and that this uncoupling leads, in ATPase-containing cells, to a lowering of the ATP levels, which in turn is responsible for the arrest in synthesis of nucleic acids and protein.

It is evidently important to attempt to demonstrate some effects of colicin K on energetic coupling in the *E. coli* membrane that can be recognized in subcellular fractions. It has been reported that in membrane vesicles active transport is blocked by colicin K (2). The present study, aimed at detecting abnormalities in the function of a number of enzyme systems in fractions extracted from colicin-treated bacterial cells, is an initial effort toward the objective of characterizing colicin K effects in purified cytoplasmic membranes. The results reported here indicate that the reduction of nicotinamide adenine dinucleotide phosphate (NADP) by NADH (reverse transhydrogenase), which under anaerobic conditions is activated by ATP, is inhibited in particulate fractions of colicin K-treated bacteria. This reaction, first discovered in mitochondria (5), was later demonstrated in sonically disrupted *E. coli* cells (13) and characterized in particulate fractions (18). Particles from sonically disrupted bacteria appear to be unsuitable for fractionation on sucrose gradients into the cell wall and the cytoplasmic membrane components. Hanson and Kennedy (7) noted that the cell wall fraction from sonically treated membranes contained a large peak of ATPase, which is a membrane enzyme. This might reflect a hybridization of cell wall and cytoplasmic membrane.

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The methods and conditions of cell disruption described in this investigation, therefore, were developed to yield particulate fractions that could be effectively separated on sucrose gradients into cell wall and cytoplasmic membrane components. The inhibition observed in the present study consisted of a reduced response to exogenous ATP by particulate fractions of cells treated with colicin K. This inhibition is interpreted as an impairment of an energy coupling mechanism in the membrane.

MATERIALS AND METHODS

Materials. All the reagents and enzymes were purchased from Sigma Chemical Co. General lab chemicals were obtained from Mallinckrodt Scientific Co. and Fisher Scientific Co. All reagent solutions for enzyme assays were prepared fresh before use.

Bacterial strains and culture conditions. All strains used were from the stock collection of this laboratory. These included: *E. coli* K-12 C600, *E. coli* K-12 C600 *tol II*, *E. coli* K_{235}, and *E. coli* G6 *uncA*. *E. coli* K-12 C600 *tolA* is a colicin-tolerant strain that binds colicin K but is refractory to its killing effect. The colicinogenic strains *E. coli* K_{235}, *E. coli* CA38, *E. coli* QR47, and *E. coli* CR34 were used as the source for colicins K, E3, E2, and E1, respectively. Bacteria were grown in LB broth containing in grams per 100 ml: tryptone, 1.0; yeast extract, 0.5; and NaCl, 0.5. The pH was adjusted to neutrality with 0.4 ml of 1 N NaOH. When so stated, bacteria were grown in M-9 minimal salts medium containing in grams per 100 ml: Na_2HPO_4, 0.58; KH_2PO_4, 0.3; NH_4Cl, 0.1; NaCl, 0.05; and $MgSO_4$, 0.0012. This was supplemented with succinate, 0.5 g/100 ml; Casamino Acids, 0.1 g/100 ml; 1 μg of thiamine per ml; and 20 μg each of L-leucine and L-threonine per ml of culture.

Colicin preparation. Colicin K induction and purification were carried out as described by Kunugita and Matsuhashi (10). As judged by sodium dodecyl sulfate gel electrophoresis, the purity of the colicin K preparation was above 90%. Colicins E1, E2, and E3 were used as crude extracts, and colicin survival assays were carried out as described previously, except for the addition of trypsin at the end of incubation (200 μg per 1 ml of incubation mixture) and 10 min before plating (16).

Preparation of cell extracts and particulate fractions. Log-phase cultures were divided into two equal parts: one part was used as a nontreated control; the other part was incubated with the colicin at 37 C without aeration for intervals that varied between 1 and 15 min. At the end of incubation, the cell density of the two cultures did not differ significantly. All operations thereafter were carried out at 0 to 4 C. The cultures were harvested by centrifugation at 15,000 $\times$ *g* for 15 min and were washed once with 100 ml of 0.01 M HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 7.4, which contained 0.001 M ethylenediaminetetraacetate and 8.5% sucrose. During washing, the suspended cells were mixed in a Lourdes omnimixer for 1 min before centrifugation to remove flagella and pili from the bacterial surface. The washed pellets were suspended in 40 ml of 0.01 M HEPES buffer at pH 7.4 with 0.005 M ethylenediaminetetraacetate and disrupted by two passages through an AMINCO French pressure cell at 10,000 lb/in^2. The unbroken cells were removed by centrifugation at 3,000 $\times$ *g* for 15 min. The supernatant fluids obtained from this step are designated as the cell extracts. A portion of the extracts was kept and the remaining part was centrifuged at 60,000 $\times$ *g* for 2 h. The pelleted particulate material was suspended in 0.01 M HEPES buffer of pH 7.4 with 0.005 M ethylenediaminetetraacetate. The suspended particulate fraction, the supernatant fraction, and the remaining portion of the cell extract were dispensed into aliquots, frozen in acetone dry ice, and stored at -30 C. Samples were thawed before assay. Protein was assayed on all fractions by the method of Lowry et al. (11).

Assays. (i) NADH oxidase and succinic dehydrogenase. The assay system described by Osborn et al. (14) was employed. A Zeiss spectrophotometer was used to measure, at room temperature, the decrease in absorbance at 340 nm due to the NADH oxidase and the increase in absorbance at 550 nm due to succinic dehydrogenase.

(ii) Energy-independent NADPH-NAD transhydrogenase. The assay was carried out essentially as described by Bragg and Hou (3). The reaction mixture contained 0.3 μmol of NADPH, 5 μmol of $MgCl_2$, and about 1 mg of sample protein in a final volume of 1.0 ml of 0.1 M tris(hydroxymethyl)-aminomethane-hydrochloride buffer at pH 7.5. The reaction was started by the addition of 0.44 μmol of NAD^+, and the change in absorbance at 340 nm was recorded.

(iii) ATPase. The assay mixture was similar to the one described by Hanson and Kennedy under method C (7). The ATPase incubation mixture contained in a final volume of 1 ml: 40 μmol of tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.8), 10 μmol of $MgCl_2$, 100 μmol of KCl, 2 μmol of phosphoenol pyruvate, 20 μg of pyruvate kinase, and sample protein in the range between 100 and 500 μg. The reaction was initiated by the addition of 0.1 μmol of ATP. Control incubation mixtures received all components except ATP. The reaction mixtures were incubated at room temperature for 1 to 15 min. At the end of each incubation period, the tubes were immediately immersed in ice and received 0.1 ml of cold 55% trichloroacetic acid. After 15 min in ice, the flocculated material was removed by centrifugation and the supernatants were used to determine inorganic phosphorus by Bartlett's modification of the method of Fisk and Subbarow (1). In some experiments, ATPase activity was assayed under the conditions described for energy-dependent formation of NADPH.

Formation of NADPH. The method used was adapted from those described by Sweetman and Griffiths (18) and Fisher and Sanadi (6). The reaction mixture contained, in addition to the sample under test, 219 μmol of sucrose, 1.2 mg of bovine serum albumin, 12 μmol of $MgCl_2$, 45 μmol of tris-

(hydroxymethyl)aminomethane-hydrochloride (pH 7.8), and 3 μmol of Na_2S. After 20 min of incubation at room temperature, the following components were added to this basic mixture to bring the total volume to 1 ml: 3 μmol of dithiothreitol, 0.03 μmol of NAD, 204 μmol of ethanol, 120 μg of alcohol dehydrogenase, and 0.6 μmol of NADP. The reaction was started by the addition of 3.6 μmol of ATP. The increase in absorbance at 340 nm was recorded at room temperature in a Zeiss spectrophotometer for 30 to 60 min. Control cuvettes received all components of the reaction mixtures in the same amounts, except that buffer was added instead of ATP. The formation of NADPH was verified by the addition of 7.5 μmol of glutathione and 7.5 μg of glutathione reductase to the incubation mixtures. This coupled reaction caused a rapid decline in absorbance at 340 nm to initial levels.

RESULTS

Formation of NADPH catalyzed by cell-free extracts, particulate fractions, and supernatant fractions. The reduction of NADP to NADPH linked to the oxidation of NADH (reverse transhydrogenase) was measured in the presence of an NADH-regenerating system, as described above, and under anaerobic conditions provided by the addition of sodium sulfide. Under such conditions the reaction is stimulated by ATP. Figure 1A through C shows that cell-free extracts, particulate fractions, and supernatant fractions from *E. coli* treated and not treated with colicin K catalyze the reduction of NADP to NADPH. The reaction, in the presence of sodium sulfide, proceeded at a low rate and the addition of ATP stimulates the rates and extents of the reactions.

Examination of the response to ATP in fractions from *E. coli* treated or not treated with colicin K reveals that ATP stimulation in total extracts from colicin-treated cells is markedly lower than in extracts of untreated cells (Fig. 1A). That this effect is due to colicin action is confirmed by its absence in extracts of similarly treated cells of *E. coli tol II*, a mutant that adsorbs colicin but does not respond to it (Fig. 1E).

When different fractions from sensitive cells were tested for stimulation of NADPH formation by ATP, a colicin-induced reduction of ATP stimulation was observed in the particulate (Fig. 1B), but not the supernatant, fraction (Fig. 1C). Mixtures of particulate and supernatant fractions in proportions similar to that in cell-free extracts yield reconstituted fractions that catalyzed formation of NADPH (Fig. 1D) in a manner similar to that of the total extracts. The rate of the ATP-stimulated reaction in the particulate fraction is about 30% of that reported by Sweetman and Griffiths (18). The lower rate in our experiments may be due to the lower temperature used in our assays. It is also possible that the method of cell disruption we used may have caused the release of some factors required for optimum activity.

To examine the nature of the ATP-stimulated NADP reduction, preparations of the ATPase-deficient mutant *E. coli* G6 *uncA* were tested. The original N144 *uncA*$^-$ mutant, from which G6 *uncA* was constructed (15), is known to lack the ATP-linked reverse transhydrogenase activity, although it carries out the aerobic reverse transhydrogenase reaction (4, 9). As shown in Fig. 1F, both particulate and supernatant fractions of *E. coli* G6 *uncA*, whether treated or untreated with colicin K, generated NADPH in the presence of sodium sulfide in a reaction that was not significantly stimulated by ATP. Thus, the ATP-stimulated reactions in both the supernatant and the particulate fractions require ATPase activity.

All fractions catalyzed the formation of NADPH to a certain extent in the absence of ATP, as seen in Fig. 1A through E. The rates and extents of the non-ATP-linked reaction did not vary significantly between fractions from *E. coli* treated or not treated with colicin K. The presence of such a non-ATP-linked formation of NADPH has been reported (18) in particulate fractions of sonically disrupted *E. coli*. Since, under aerobic conditions, the energy required to drive the reverse transhydrogenase reaction is generated via oxidation processes of the electron transport chain, the activity observed in the absence of ATP might represent a residual aerobic reaction that was not completely blocked by sodium sulfide. This view would fit with the presence of this reaction in ATPase-less mutants and the finding that the concentration of sodium sulfide used did not abolish NADH-oxidase activity (see Table 2).

Particulate fractions from ATPase-positive bacteria treated with colicin E1 exhibited an uncoupling of the reverse transhydrogenase reaction from ATP similar to that described above for colicin K. Particulate fractions from bacteria treated with colicins E2 or E3 did not show any significant effect (unpublished data).

Requirements for the formation of NADPH by supernatant and particulate fractions. The puzzling finding that the ATP-stimulated formation of NADPH catalyzed by the particulate fractions was sensitive to cell treatment with colicin K, whereas the supernatant-catalyzed reaction was only slightly affected, was clarified by the observation that the two reactions had different requirements and, thus, are probably of different natures. As shown in Table 1, the ATP-linked NADP reduction catalyzed by

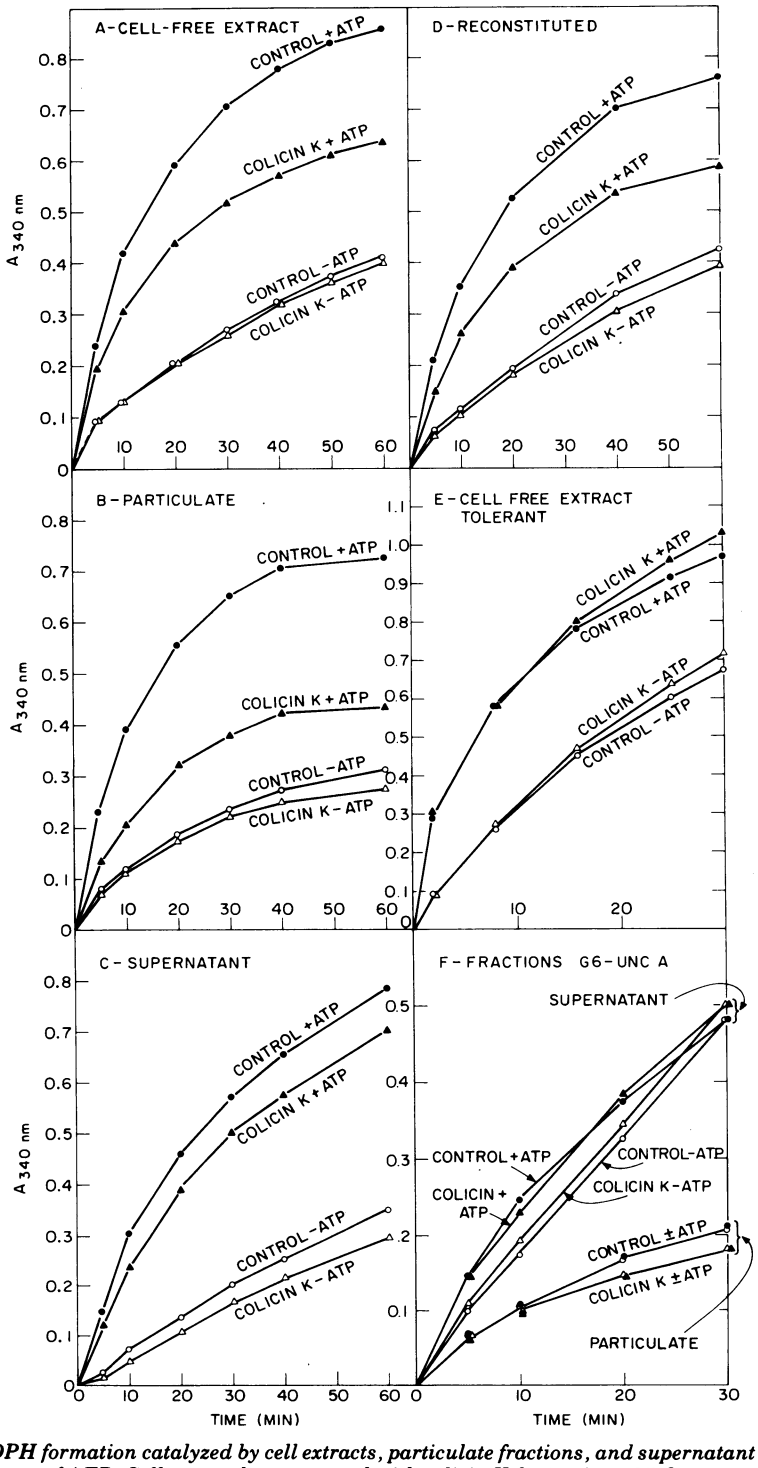

FIG. 1. *NADPH formation catalyzed by cell extracts, particulate fractions, and supernatant fractions in the presence or absence of ATP. Cells treated or untreated with colicin K for 5 min at 37 C were extracted, and the extracts were fractionated as described in the text. The formation of NADPH was measured in the presence of* Na_2S. *The results are given as increases in absorbance at 340 nm* (A_{340}) *as a function of time. (A) Total French press extract of E. coli C600, 1.35 mg of protein. (B) Particulate fraction from above, 1.5 mg of protein. (C)*

TABLE 1. *Requirements for formation of NADPH*[a]

Determination	Supernatant				Particulate			
	−ATP		+ATP		−ATP		+ATP	
	U/mg of protein	%	U/mg of protein	%	U/mg of protein	%	U/mg of protein	%
Complete System	0.097	100	0.21	100	0.014	100	0.044	100
−NADP		0		0		14		3
−NAD		111		101		80		24
−Alcohol dehydrogenase		104		104		16		5
−Ethanol		100		102		23		10
$-MgCl_2$		24		3		100		44
$-Na_2S$				127		31		57

[a] Fractions were prepared and assayed as described in the text. The activity was measured after 10 min from the start of the reaction, and units of activity were calculated as Δ absorbance (at 340 nm) per minute.

the supernatant fraction requires neither NAD or a NADH-regenerating system. The possibility that NADH or a utilizable NAD plus an endogenous NADH-generating system already existed in this fraction was excluded by the finding that supernatant samples displayed no decrease in absorbancy at 340 nm upon addition of the NADH-consuming system, alcohol dehydrogenase (10 μg), and acetaldehyde (5 μmol). Additional differences between the ATP-linked transhydrogenase reactions in particulate versus supernatant fractions are the different requirements for Mg^{2+} and the response to sodium sulfide. Sodium sulfide stimulated the ATP-linked reaction, a result that is consistent with an anaerobic reaction. Sodium sulfide also stimulated the ATP-unlinked reaction, a result that does not fit the interpretation that this is a residual aerobic reaction. Sodium sulfide, however, may have protected the product NADPH against NADPH oxidases.

The requirements of the reaction in the particulate fractions are similar to those reported by Sweetman and Griffiths (18) for particulate fractions prepared from sonically disrupted cells. It appears, therefore, that the reaction in the particulate fraction that is partially blocked by colicin K is the ATP-linked reverse transhydrogenase.

Effect of colicin K multiplicity. The inhibition of ATP-stimulated reduction of NADP to NADPH catalyzed by particulate fractions depends on the concentration of colicin used to treat the bacteria (Fig. 2). In these experiments, large amounts of the colicin preparation were used because bacteria grown on minimal media are less sensitive to colicin K killing. Evidently, the observed inhibition depends upon the efficiency of colicin killing, not on colicin mass. In other experiments, carried out with fractions from bacteria grown in LB broth instead of minimal medium, inhibitions up to 80% were observed at high concentrations of the colicin.

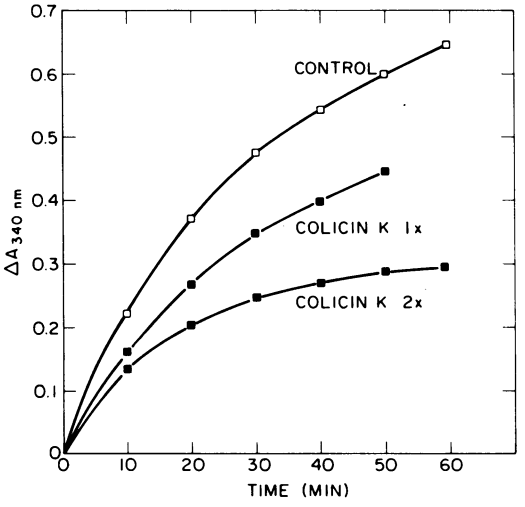

FIG. 2. *ATP stimulated formation of NADPH by particulate fractions as a function of the amount of colicin K. Cells of E. coli C600 from succinate medium treated or untreated with colicin for 15 min at 37 C were extracted, and the particulate fractions were tested for NADPH in the presence or absence of ATP. The reaction mixtures contained 0.95 mg of protein in a 1-ml volume. The results are recorded as the increase in absorbance at 340 nm in the ATP-containing samples after subtracting the reading in the corresponding samples without ATP (ΔA_{340}). The lower colicin K concentration (5 μg of colicin protein per 10^{10} bacteria) gave a survival of 58%; the higher concentration (30 μg of colicin protein per 10^{10} bacteria) gave a survival of 11%.*

Supernatant fraction from above, 1.12 mg of protein. (D) Reconstituted particulate, 0.3 mg of protein, plus supernatant, 1.12 mg of protein. (E) Total extract from E. coli C600 tol II (insensitive to colicin K), 1.65 mg of protein. (F) Fractions from E. coli G6 uncA ATPase deficient, 1.35 mg of particulate protein and 1.93 mg of supernatant protein. For E. coli C600 the survival after colicin K treatment was 20% (1 μg of colicin protein per 10^{10} bacteria); for C600 tol II the survival was 100%. The amount of colicin K used (4 μg of colicin protein per 10^{10} bacteria) gave a survial of 0.5% with sensitive cells. The survival of G6 uncA was 12% after colicin K treatment (1.8 μg of colicin protein per 10^{10} bacteria). In each experiment NADPH formation was measured in the presence and the absence of ATP.

Effect of ATP concentration on the formation of NADPH catalyzed by particulate fractions, and the hydrolysis of ATP. The ATP-stimulated reduction of NADP to NADPH was measured in particulate fractions from *E. coli* at different concentrations of ATP. The release of inorganic phosphorus was also measured to compare the ATP affinity of membrane ATPase enzymes under the conditions of these experiments. Figure 3 shows that, at low ATP concentrations, an increase in ATP results in an increase in both the rates of formation of NADPH and of release of inorganic phosphorus. At high concentration of ATP, however, the rate of ATP hydrolysis continued to increase when the NADPH formation had reached its maximum velocity. The important point is that the particulate fractions from *E. coli* treated with the colicin K, though they exhibited the colicin effect on the ATP-linked formation of NADPH, were identical to the controls with regard to their hydrolytic capacity toward ATP. It is concluded that the inhibition of NADP reduction is not due to a reduced activity of the ATPase.

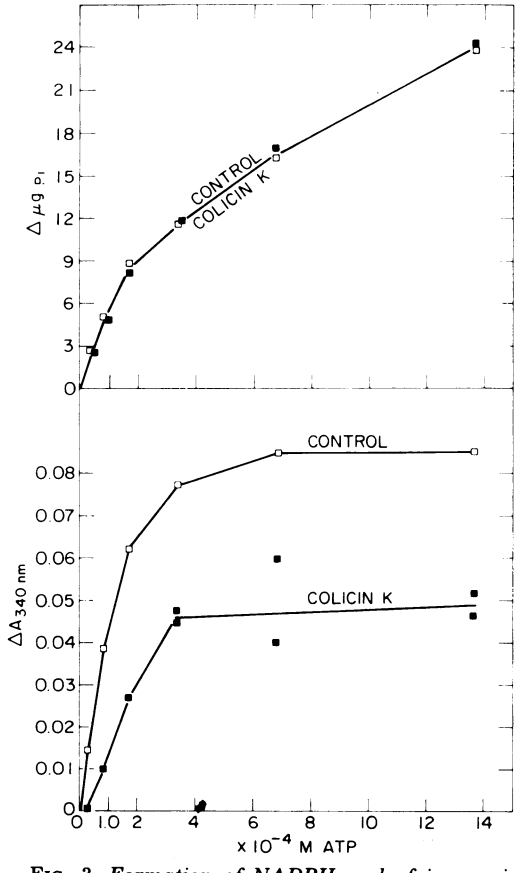

FIG. 3. *Formation of NADPH and of inorganic phosphate by particulate fractions as a function of ATP concentration. E. coli K-12 C600 cells with or without colicin K treatment were extracted, and the particulate fractions (at 1.2 mg of protein per ml) were tested after 10 min for NADPH formation and for inorganic phosphate (P_i). ΔA_{340} are recorded as described in the legend to Fig. 2. ΔP_i are measured as inorganic phosphorus released in the presence of ATP minus background (very low) inorganic phosphorus in samples without ATP. In this experiment, the bacteria were grown in LB and exposed to the colicin (12.5 μg of colicin protein per 10^{10} bacteria) for 1 min at 37 C and gave a survival of 3%.*

Distribution of proteins and enzymes in fractions of cell-free extracts. Inhibition of ATP-linked transhydrogenase, caused by colicin K, may be a result of an effect on respiratory chain enzymes, ATPase enzyme, or energy coupling of membranes. To investigate these possibilities, activities of ATPase, NADH oxidase, succinic dehydrogenase, and NADPH-NAD transhydrogenase were measured in subcellular fractions of bacteria treated or not treated with colicin K. Table 2 illustrates a typical result of the distribution of protein and enzyme activities in supernatant and particulate fractions. It is evident that treatment of cells with colicin K before extraction had no significant effect on the specific activities or the distribution pattern of the assayed enzymes.

Under the conditions used, about 80% of the total cellular protein was released in the supernatant fraction. The majority of the activities of the NADH oxidase, succinic dehydrogenase, and NADPH-NAD transhydrogenase was retained in the sedimented particulate fractions, as expected from previous experience (14, 17). The NADH oxidase activity was completely blocked in 3.3 mM NaCN (Table 2).

Both supernatant and particulate fractions catalyzed the oxidation of NADPH in the absence of NAD. The supernatant reaction was not stimulated by the addition of NAD, but the particulate fraction was stimulated onefold. The NAD-stimulated reaction represents NADPH-NAD transhydrogenase (Table 2).

Although ATPase is a membrane-bound enzyme (7), about 50% of its total activity was found in the supernatant. This enzyme may be loosely bound to the membrane, and its distribution between the particulate and the supernatant fractions may depend upon the method and conditions of cell disintegration.

DISCUSSION

These studies show that ATP-linked transhydrogenase is partially inhibited in membranous fractions from colicin K-treated cells. This reaction requires the participation of the electron

TABLE 2. *Distribution of proteins and enzymes in fractions of cell-free extracts*[a]

Determination	Particulate fraction		Supernatant fraction	
	U/mg of protein	% of total	U/mg of protein	% of total
Protein content				
Controls		20.4		78.6
K-treated		19.8		80.2
NADH oxidase				
Controls	4.25 (0)[b] (1.19)[c]	94.3	0.07	5.7
K-treated	4.25	95.7	0.05	4.3
Succinic dehydrogenase				
Controls	2.05	94.6	0.03	5.4
K-treated	1.75	93.6	0.03	6.4
NADPH-NAD transhydrogenase				
Controls	(0.069 − 0.033)[d] = 0.036	100	(0.026 − 0.026)[d] = 0	0
K-treated	(0.064 − 0.027)[d] = 0.037	100	(0.026 − 0.026)[d] = 0	0
ATPase				
Controls	0.255	51	0.063	49
K-treated	0.265	54	0.055	46

[a] Cells of *E. coli* were incubated with colicin K for 15 min at 37 C and gave a survival of 4.3%. Fractions were prepared and enzymes were assayed as described in the text. A unit of activity was calculated as a unit change in optical density per minute measured from initial rates, at 340 nm for NADH oxidase and NADPH-NAD transhydrogenase and at 550 nm for succinic dehydrogenase. The ATPase units were calculated as micromoles of inorganic phosphate released per minute.

[b] Activity tested in the presence of NaCN at a final concentration of 3.3 mM.

[c] Activity tested in the presence of Na_2S at a final concentration of 3.0 mM.

[d] In parentheses, specific activities calculated from measurements carried out in the presence and the absence of NAD.

transport system, the activity of ATPase enzyme, and a functional energy coupling system. Neither the electron transport system, as tested by the activity of NADPH-NAD transhydrogenase, of succinic dehydrogenase, and of NADH oxidation, nor the activity of the ATPase enzyme appeared to be altered in fractions from colicin K-treated cells. Yet the particulate fractions from the treated bacteria are impaired in their ability to couple ATP hydrolysis to the reverse transhydrogenase reaction. The energy provided to the reaction from ATP is presumably made available through the generation of a high-energy intermediate since the reaction is known to be inhibited by chemical uncouplers (3, 13). Thus, colicin K apparently interferes with the coupling of ATP hydrolysis with the generation of a required high-energy intermediate or with the utilization of the intermediate for the reverse transhydrogenase reaction.

The supernatant fractions catalyze an ATP-stimulated formation of NADPH that was not affected by colicin K treatment of bacteria. This reaction is not the reverse transhydrogenase since it does not utilize NADH as the electron donor, but it may reflect the presence of endogenous dehydrogenases and substrates for NADPH reduction.

That the observed effects of colicin K on the energy-linked reactions are truly biochemical effects and are not merely a reflection of membrane alteration due to the bound colicin is suggested by the absence of colicin effect in fractions from tolerant mutants treated with the colicin. The effect is specific since fractions from cells treated with colicin E2 or colicin E3 did not exhibit the effect, whereas treatment with colicin E1, which like colicin K affects energy-linked functions in intact cells, altered the ATP-linked reverse transhydrogenase in the same way colicin K did.

The observation that colicin K affects only ATP-linked reactions associated with particulate (membrane) fractions is consistent with the idea that cell membranes are the primary

target of colicin K action, especially since the inhibition of the membrane reaction increases with increasing colicin concentration. Complete inhibition of the ATP-stimulated reactions was never observed in particulate fractions, even under conditions when more than 99% of the cells were killed. It is possible that the membranous vesicles present in the particulate fraction consist of two populations, one derived from membrane stretches that were affected by colicin and the other derived from membrane stretches that had escaped colicin action.

The present results demonstrate for the first time a specific effect of colicin K that is recognizable as inhibition of an energy-linked reaction in bacterial membranes isolated from colicin K-treated bacteria. This system may lend itself to an analysis of what classes of membrane-associated components are effected and thereby may narrow the search for a primary action of colicin K and colicin E1.

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