Studies on the Depolarization of the \textit{Escherichia coli} Cell Membrane by Colicin El*

J. Michael Gould, and William A. Cramer

From the Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

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When a small pulse of oxygen is added to an anaerobic suspension of logarithmic phase \textit{Escherichia coli} cells, the subsequent acidification of the medium which is observed in the absence of permeant charged ions is slow ($t_{1/2} \approx 10$ s) as is its relaxation ($t_{1/2} = $ at least several minutes). The number of protons extruded for each oxygen atom added ($H^+/O$) is small, varying from about 0.4 to about 1.0 depending upon the carbon source used for growth and the growth phase of the cells. Treatment of the cells with colicin El causes a large increase in the amplitude of the proton extrusion elicited by an oxygen pulse, so that the $H^+/O$ ratio attains values $>2.0$ regardless of the cell growth conditions. In addition, the rate of proton efflux ($t_{1/2} < 1$ s) and its relaxation ($t_{1/2} = 10$ to 20 s) are greatly accelerated in colicin-treated cells. After addition of colicin El, the increase in the $H^+/O$ ratio has a time course which is similar to the El-induced loss of K$^+$. Furthermore, the effect of colicin El on the kinetics and extent of H$^+$ efflux is dependent upon the presence of K$^+$, Na$^+$, or Li$^+$ in the medium, with an apparent $K_m$ for potassium of about 0.5 mM. The properties of the proton pulses measured in the presence of colicin El plus K$^+$ are very much like those measured in cells treated with the permeant anion SCN$^-$. Thus, these experiments provide direct evidence for a rapid, colicin El-induced depolarization of the bacterial membrane. The actual pattern of ion movements leading to this depolarization is at present not known. Proton uptake occurring with the same time course as the El-induced potassium efflux can be detected, but the amount of H$^+$ uptake is too small to balance the observed potassium efflux. The efflux of the organic anions pyruvate and glucose 6-phosphate also seems to be much too small to provide adequate charge balance for the K$^+$ which leaks out after colicin El treatment.

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changes induced by colicin E1 (6), K (21), or Ia (22) have been reported for the lipophilic probe N-phenyl-1-naphthylamine (PhNap), although this probe will not respond directly to a membrane potential, and prior to colicin addition, much of it is localized in the cellular envelope outside of the inner membrane (21). Thus, it is important to study the effect of colicin on the cellular membrane potential by an independent technique.

Mitchell and Moyle (23, 24) observed that addition of an oxygen pulse to an anaerobic suspension of mitochondria causes an increase in the acidity of the suspension. In those experiments, and in similar experiments done with anaerobic bacterial suspensions (25-27), the amplitude and rate of the proton efflux after an oxygen pulse was increased in the presence of mobile, charge-compensating counterions (e.g., SCN-, K+ plus valinomycin). According to Scholes and Mitchell (27), these counterions allow a larger and faster proton movement by dissipating the membrane electrical potential which is formed by the charge separation accompanying respiration-catalyzed proton efflux. In this paper, we report that colicin E1, in the presence of K+, Na+, or Li+, causes an increase in the rate and extent of proton efflux after an oxygen pulse in a manner similar to SCN-, and that this effect closely parallels in time the loss of K+ by the cells. A preliminary account of some of these experiments has been published elsewhere (28).

EXPERIMENTAL PROCEDURES

Bacteria - For most experiments reported in this paper, cells of the Escherichia coli strain B/1.5 were used. These cells were grown on a minimal medium (pH 7.0) containing (per liter): 1 g of (NH4)2SO4, 0.5 g of sodium citrate, 0.1 g of MgSO4.7H2O, 2 g of KH2PO4, 3 g of KHPO4, and trace metals according to Anraku (29). The carbon source was either 1% succinic acid or 1% glycerol (w/v). The growth media for the other E. coli strains used in this study were (per liter): strain JC411 (col E1), 8 g of casamino acids, 5 g of yeast extract, 1 g of glucose, 5 g of NaCl (pH 7.0); strain A946 (col VIII), 1 g of (NH4)2SO4, 10.5 g of KH2PO4, 4.5 g of KHPO4, 0.1 g of MgSO4.7H2O, 1 mg of thiamin, 20 mg of proline, 20 mg of histidine, 20 mg of leucine, 20 mg of threonine, 4 g of glucose (pH 7.1) (Ref. 30); strain K12 1100 and strain ML 308-225, same as B/1.5 above.

Sterile 250-m1 Erlenmeyer flasks containing 50 to 60 ml of minimal medium and the desired carbon source were inoculated from overnight cultures and incubated at 37°C with vigorous shaking for 4/ to 5 1/2 h (midlogarithmic phase growth) or 14 to 17 h (stationary phase growth). Cells were harvested by centrifugation at 4°C, washed twice in 150 mM KCl, 0.5 mM 3-(N-morpholino)propanesulfonic acid/KOH (pH 7.0), and resuspended in this medium to a final concentration of about 3 x 109 cells/ml. For experiments using the permeant anion SCN-, the cells were washed twice and resuspended in a medium containing 100 mM KCl, 50 mM KSCN, 0.5 mM 3-(N-morpholino)propanesulfonic acid/KOH (pH 7.0).

For experiments involving colicin E1 treatment of cells, the colicin was added to the cell suspension in a small volume (2 ml) to a final concentration of 1 µg of protein/ml. When cells grown with succinate as carbon source were used, it was necessary to add the colicin 5 min before the initiation of anaerobiosis, presumably because of an energy requirement for colicin action. When cells grown with glycerol as carbon source were used, colicin could be added either before or after anaerobiosis with no difference in its effects. Cell survival after colicin E1 treatment was ≤0.1%.

pH Measurements - A 2-ml aliquot of the final cell suspension was placed in a glass-jacketed, glass reaction vessel (3-m1 capacity) which contained a small magnetic stirring bar. The top of the reaction chamber was sealed with a rubber stopper through which a Sargent miniature combination pH electrode was passed. Small Teflon tubes also passing through the stopper allowed a continuous stream of water-saturated nitrogen gas to be passed across the surface of the cell suspension. Another small hole in the stopper permitted the insertion of the needle of a microliter syringe, which was used to make additions to the sample. The temperature of the reaction chamber was maintained at 33°C by a constant temperature circulating water bath.

The output from the pH electrode was amplified by a Corning model 14 pH meter operated on the expanded scale. The electrometer output was recorded on a strip chart recorder with a scale expansion of 10 1/2 units to give 0.01 pH units full scale (6 inches). At this level of amplification, the noise level of the pH measuring system was >2 x 10-14 pH units. The overall half-response time for the system was approximately 1.2 s as measured by the recorded response to a rapid injection of HCl.

After the cell suspension had become anaerobic (approximately 15 min), small pulses of oxygen were added by the rapid injection of a small volume (usually 5 to 10 µl) of air-saturated, double distilled water. After an experiment, the chart paper was calibrated in pH equivalents by titrating the sample in the presence of 5 to 10 µM FCCP with small aliquots of 0.001 M HCl. The H+ /O ratio of the suspending medium was calculated from the maximum extent of the pH change after an oxygen pulse, assuming a concentration of 275 µmol of O2/liter for distilled water at 23°C. No correction was made for any decay of the pH change which may have occurred during the proton efflux.

Changes in the concentration of K+ in the suspending medium were monitored similarly using a glass Beckman cation electrode. The reference portion of the Sargent miniature combination pH electrode served as the reference electrode, except that the saturated KCl solution within the reference electrode was replaced with saturated NaCl. The cation electrode potential (in millivolts) was calibrated against known concentrations of KCl. The response was linear for K+ concentrations >2.5 x 10-4 M, with a slope of 56 mV (33°) per decade change in K+ concentration.

Miscellaneous - Colicin E1 was prepared from the colicinogenic E. coli strain JC411 according to the methods of Schwartz and Helinski (31) except that the last CM-cellulose step was omitted. The lophophidis E1 was dissolved in M-9 salts medium and had a specific activity of approximately 1:50 on a protein basis. FCCP (carbonyl cyanide p-trifluoromethoxyphenylhydrazone), kindly supplied by Dr. P. G. Heytler, was dissolved in ethanol. The cell strains used in this study were the generous gifts of Drs. D. Helinski (JC411 (col E1)), H. R. Kaback (ML 608-225), S. E. Luria (Astel tol VIII), P. W. Postma (K12 1000), and S. Silver (B/1.5).

RESULTS

Effects of Colicin E1 and FCCP on Oxygen Pulse-dependent pH Changes - The addition of a small pulse of oxygen to an anaerobic suspension of Escherichia coli B/1.5 cells results in a brief period of electron transport which is accompanied by an acidification of the medium. The efficiency of coupling between this proton efflux into the external medium and the reduction of oxygen (H+/O ratio) is low in the absence of any additions to the ressuspension medium, and is dependent upon both the growth phase of the cells and the carbon source used for growth (Fig. 1 and Ref. 14). Cells grown on succinate and harvested in midlogarithmic phase growth typically exhibit H+ /O ratios of 0.5 or less, whereas succinate-grown cells harvested in stationary phase or glycerol-grown cells harvested in either log phase or stationary phase exhibit H+ /O ratios around 1.0 (Fig. 1). In all cases, the kinetics of proton efflux are rather slow (t1/2 = 5 to 15 s). The pH change decays very slowly, with only a small fraction of the change reversed in 10 min. West and Mitchell (25) have previously observed similar small H+ /O ratios for E. coli cells grown with glucose as carbon source and resuspended in a buffer/salts medium.

Treatment of the cells with colicin E1 before addition of the O2 pulse results in a large change in the properties of the proton extrusion (Fig. 1b). The rate of proton efflux is greatly stimulated, the pH change reaching a maximum (ΔH+*) in about 4 to 6 s. The half-time for the pH change (t1/2 = 1 s) is very close to the half-response time of the electrode system determined by the addition of calibrated acid pulses to the medium. The rate of decay of the O2-induced increase in medium acidity is also much faster after the cells have been treated with colicin E1 (t1/2 = 10 to 20 s). The apparent H+ /O ratio is increased to values >2.0 after colicin addition, and this
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high H+/O ratio is no longer dependent upon either the growth phase of the cells or on the carbon source used for growth (Fig. 1). Furthermore, these effects of colicin E1 are only observed when colicin-sensitive cell strains are used (Table I). The H+/O ratios obtained with cells of the colicin-tolerant strain A\textsubscript{m} and the colicinogenic strain JC411 are unaffected by 1 \( \mu \)g/ml of colicin E1, whereas cells of the colicin-sensitive strains B/1,5 and K12 1100 (cell survival <0.1%) show the effects described above and shown in Fig. 1. Strain ML 308-225, which is relatively insensitive to colicin E1 (survival = 50%) also showed an increase in the H+/O ratio in the presence of 1 \( \mu \)g/ml of colicin E1, although the increase was not as large as that seen with the colicin-sensitive strains and is predictable on the basis of a 50% survival level in this experiment.

The effects of the uncoupler FCCP on the oxygen-dependent proton pulse are very different from those described above for colicin E1. With concentrations of FCCP in the minimal range needed to inhibit active transport under aerobic conditions (2 to 5 \( \mu \)M) (21), the proton extrusion following an oxygen pulse is almost completely eliminated by FCCP in both normal cells and in cells treated with colicin E1 (Fig. 2). At concentrations of FCCP which give a partial inhibition of the extent of the proton extrusion, FCCP causes a marked increase in the decay of the pH change, due to the re-entry of extruded protons into the cell (Table II). Thus, 1 to 5 \( \mu \)M FCCP appears to act by increasing the permeability of the membrane to H\textsuperscript{+} ions, a conclusion consistent with much data already in the literature (e.g. Ref. 32). Colicin E1, over a multiplicity range of 1 to 100, causes an increase in the extent of the proton efflux even though it also causes an increase in the rate at which extruded protons re-enter the cell (Fig. 1) (28). These findings, therefore, are consistent with the idea that colicin E1 causes an increase in the permeability of the bacterial membranes to charge-compensating counterion(s). This notion is further supported by the finding that the effects of colicin E1 on the proton pulse can be mimicked by including the permeant anion SCN\textsuperscript{-} in the reaction medium. In the presence of SCN\textsuperscript{-}, the kinetics of H\textsuperscript{+} efflux, H\textsuperscript{+} influx, and the change in the H+/O ratio are increased (Fig. 3), as they are in the presence of colicin E1 (Fig. 1).

**FIG. 1.** The effect of colicin E1 on the rate and extent of proton efflux in Escherichia coli following an oxygen pulse. Cells of E. coli strain B/1,5 grown on 1% succinate (a and b) or 1% glycerol (c and d) were harvested during midlogarithmic phase growth. Colicin E1 (1 \( \mu \)g/ml), sufficient to allow <0.1% survival, was added to b and d 5 min before the initiation of anaerobiosis. The oxygen pulse (upward arrows) contained 5.5 ng atoms of oxygen. An upward deflection of the pH trace represents an acidification of the medium.

**TABLE I**

| Escherichia coli strain | \( \Delta H^+ \) (nanoequivalents) | H+/O |
|-------------------------|-----------------------------------|------|
|                         | -E1                               | +E1  |
| A\textsubscript{m} (col VIII) | 2.85 | 4.02 | 0.70 | 0.73 |
| JC411 (col E1)          | 2.10 | 2.24 | 0.38 | 0.41 |
| B/1,5                   | 2.30 | 12.20 | 0.42 | 2.22 |
| K12 1100                | 5.25 | 12.90 | 0.95 | 2.35 |
| ML 308-225              | 1.96 | 5.60 | 0.35 | 1.02 |

**FIG. 2.** Inhibition of net proton efflux by FCCP. Left, cells of Escherichia coli strain B/1,5 were grown on 1% glycerol to midlogarithmic phase before harvesting. Upper trace, control; lower trace, with 5 \( \mu \)M FCCP. Right, cells of E. coli strain B/1,5 were grown on 1% succinate to midlogarithmic phase. Colicin E1 (1 \( \mu \)g/ml) was added 5 min before the initiation of anaerobiosis. Conditions otherwise as described in Fig. 1.

**TABLE II**

| FCCP (\( \mu \)M) | \( \Delta H^+ \) (efflux) | H+/O | \( \Delta H^+ \) (influx) |
|-------------------|--------------------------|------|--------------------------|
| 0                 | 12.05                    | 2.19 | 10                       |
| 1.25              | 7.87                     | 1.43 | 6.6                       |
| 5.0               | 4.07                     | 0.74 | 5                        |
| 10                | 1.71                     | 0.31 | 2                        |
Time Course of Increase in H+/O Ratio—A typical experiment showing the time course for the colicin E1-induced increase in the H+/O ratio for a suspension of cells grown in glycerol and resuspended in 150 mM KC1 is presented in Fig. 4a. The initial H+/O ratio in the glycerol-grown cells is -1.0 as in Fig. 1. After an initial lag of about 1 min, the H+/O ratio increases over a period of about 5 to 6 min (t_{1/2} = 3 1/2 min) to a value approximately twice that measured before the colicin addition. The half-time for the increase in the H+/O ratio was generally about 3 min. This time course is similar to that observed previously under anaerobic conditions for the colicin-induced change in the fluorescence intensity of the probe N-phenyl-1-naphthylamine (6). More importantly, the time course for the change in the H+/O ratio is also similar to the time course for K+ efflux after colicin addition (t_{1/2} = 2 1/3 min) measured under the same conditions (Fig. 4b). The data shown here seem to indicate that, although the change in H+/O ratio reflects a relatively early event caused by colicin E1, the time course of the H+/O change may lag slightly behind the K+ efflux. However, it is not possible to clearly separate the kinetics of the two events, since the t_{1/2} for K+ efflux can vary by ±1/4 min (five measurements).

Counterion Requirement for Colicin E1-dependent Increase in the H+/O ratio—If the cells are treated with colicin in a choline chloride medium which is free of metal cations (K+, Na+, etc.) a similar time course for the effects of colicin is observed, except (a) the H+/O ratio prior to the addition of colicin is lower and (b) the H+/O ratio does not attain the same high level (>2.0) seen in the KCI medium (Fig. 5). Addition of...
KCl to a final concentration of 7.5 mM results in an immediate and abrupt increase in the H+/O ratio to the maximum value observed in the KCl medium. This same effect of KCl is shown in a different way in Fig. 6, where it is clear that in order to observe the effects of colicin E1 on the kinetics of proton efflux, and on the H+/O ratio, K+ ion must be present in the medium. The smaller increases in the H+/O ratio observed in Figs. 5 and 6 before the addition of potassium can be attributed to the colicin E1-induced loss of K+ from the cells (see below), which results in a final K+ concentration in the medium of approximately 0.3 to 0.5 mM. This concentration of K+ increases the H+/O ratio to approximately one-half of the maximum value which can be obtained at higher external K+ concentrations, indicating that the K_ion for potassium required for colicin E1 effects is approximately 0.3 to 0.5 mM. In a similar experiment, the effects of some other cations on the rates of H+ efflux and H+ influx were tested (Fig. 7). The final concentration of K+ in the medium after colicin addition was evidently sufficient to allow a larger increase (Fig. 7b) in the rates of H+ efflux (t_1/2 = 3 s), H+ influx (t_1/2 = 60 s), and the H+/O ratio in this experiment compared with the experiment shown in Fig. 6b. The addition of 5 mM Na+ (Fig. 7c) caused a further increase in the rate of H+ efflux (t_1/2 = 1 s) and H+ influx (t_1/2 = 12 s). The addition of 5 mM Li+ (Fig. 7d) also caused an increase in the rates of H+ movement, although Li+ was less effective in stimulating H+ influx (t_1/2 = 25 to 30 s). The addition of 5 mM Mg2+ (Fig. 7e) caused very little further increase in H+ efflux or H+ influx rates above those in the control (Fig. 7b).

**Fluxes of Other Ions**—As described earlier, the addition of colicin E1 to a suspension of sensitive cells results in the loss of cellular potassium with a time course similar to that observed for the change in the H+/O ratio (Fig. 4b). Colicin-induced K+ leakage with similar kinetics has also been reported for colicin K (18) and for colicin E1 (6). It can be calculated that such a potassium efflux, if electrically uncompensated by simultaneous cation influx or anion efflux, would hyperpolarize the membrane to a potential in excess of 100 V, an event which cannot occur. Indeed, as the cell is losing internal K+, the membrane is simultaneously losing its ability to become electrically polarized by proton translocation. This suggests that, in fact, the efflux of internal K+ is electrically compensated and gives no net polarization to the membrane. At the moment, however, there is little data to indicate the nature of the ion or ions which compensate K+ efflux. In the absence of oxygen, a colicin-induced H+ influx with kinetics similar to those of the K+ efflux can be observed (Fig. 8), but to date the highest H+/K+ ratio observed is ~0.1. Although it has previously been suggested that sodium can act as a counterion for potassium leakage caused by colicin K (33), the kinetic data of K+ efflux after the addition of colicin E1 were found to be the same in the presence or absence (Fig. 4b) of 10 mM NaCl. It has been suggested (20) that chloride efflux, or the efflux of organic anions originally observed by Fields and Luria (5), could account for the apparently electroneutral K+ efflux. However, the intracellular Cl− levels are much less than the intracellular K+ levels (33a), and the amounts of pyruvate and glucose 6-phosphate lost by the cells during the time course of K+ efflux appear to be very small. In a typical experiment, the concentration of K+ in the medium after a 36-min incubation...
of B(1,5 cells with 1 μg/ml of colicin E1, was about 0.4 mM while the pyruvate and glucose 6-phosphate concentrations were <2 μM (Table III).

**DISCUSSION**

The increase in H+/O ratio caused by colicin E1 has been shown previously to occur at colicin multiplicities as low as one (28) and to require the presence of potassium ion. The time course for the increase in the H+/O ratio (Fig. 4a) is similar to the time course for other early biochemical events initiated by colicin E1, such as the leakage of intracellular K+ (Fig. 4b), the decrease in intracellular ATP levels, and the structural changes in the cell envelope monitored by fluorescence probes (6). In colicin-treated cells, the time course of the change in H+/O ratio (Fig. 4a), the dependence of the amplitude of the H+/O ratio on externally added monovalent cations (Figs. 5 to 7), and the similarity of the proton pulses obtained in the presence of colicin E1 to those obtained in the presence of SCN⁻ (Fig. 3) or valinomycin plus potassium (25), imply that the larger H+/O ratios measured in these colicin-treated cells result from the fact that the efflux of H⁺, which would normally generate a membrane electrical potential, can now be balanced by a counter flow of potassium or other ions. That is, the cell membrane seems to be freely permeable to potassium movement in either direction in the presence of colicin E1, and to be permeable as well to external sodium and lithium ions. We know little at this time about the mechanism of this increase in ionic conductance across the membrane, whether it is due to (a) an ion channel created by the colicin itself or (b) induced in the inner membrane through structural changes. While the former possibility seems somewhat easier to visualize, it has been inferred from experiments with colicin immobilized on Sephadex beads that colicin E1 may not need to move from the neighborhood of its surface receptor in order to exert its effects (36). Concentrations of colicin E1 up to 3.6 μg/ml also did not increase the conductance of a diphytanoylphosphatidylcholine planar bilayer separating aqueous phases containing 0.1 M potassium chloride. In any case, the consequence of free potassium movement and entry of other monovalent cations is that the cell should no longer be able to maintain a state of charge separation across the inner membrane. In other words, colicin E1 in the presence of potassium or other monovalent cations should cause a cellular membrane potential to be dissipated.

Feingold (19) observed that addition of the uncoupler CCCP after an acid pulse caused alkalization of the medium if the cells had first been treated with colicin E1. This was attributed to compensating potassium movement potentiated by the presence of the colicin. The rate of alkalization was low in the absence of colicin and no pH change was observed upon addition of colicin E1 alone. The main inference from these experiments, and from measurements of the colicin-induced K⁺ leakage and decrease in the intracellular ATP level, was that colicin E1 causes an increase in membrane permeability to potassium ions, but not to protons.

In previous work from this laboratory dealing with the mechanism of action of colicin E1, we have considered the nature of structural changes in the cell envelope (inner and outer membrane) associated with the primary process of membrane de-energization (e.g., 21, 37). This work has shown that de-energization of the cell by colicin E1, or by the uncoupler FCCP, causes a change in the rotational motion of the amphiphilic fluorescence probe ANS and the hydrophobic probe PhNap. An effective permeability barrier in the outer membrane to these probes is decreased upon de-energization of the envelope by colicin E1 and FCCP, resulting in increased binding of the probes to the cells (21). The increase in the binding of PhNap after colicin treatment was first reported by Nivea-Gomez et al. (22) for cells treated with colicin Ia. The time course of these structural changes for colicin E1 (6) and colicin Ia (22) is very similar to that of the earliest biochemical changes, and raised the question as to whether such structural changes caused by colicin E1 could in fact be a primary event in the transmission of the lethal effect of this colicin (37). This hypothesis has the conceptual problem of explaining how a single protein added to the cell envelope can cause such an extensive structural change in the cell envelope. Degradative enzyme activity with rapid kinetics associated with early biochemical events following colicin E1 addition has not yet been detected (38). Since changes in the H+/O ratio and dissipation of the membrane potential occur as rapidly as the first detectable biochemical changes, it would seem that the structural changes and the change in the effective permeability barrier of the outer membrane could be an immediate consequence of the decrease in cytoplasmic membrane potential. One cannot specify at this time how a collapse in electrical potential across the inner membrane could cause immediate structural changes in the envelope other than to say that the local electric field density across the membrane is, of course, very high. There is precedent for electrostrictive effects on membranes (39), and it is clear from structural studies on the Escherichia coli envelope that there are specific connections between the peptidoglycan layer and outer membrane (40).

The concentration of potassium required in the external medium for an increase in H+/O ratio and dissipation of the membrane potential is not large (Fig. 5) and can be partly supplied by the intracellular K⁺ lost to the medium. The data of Figs. 5 and 6 imply that the dissipation of the membrane potential should be even more complete in the presence of high external potassium levels. On the other hand, it has been found that the survival of sensitive cells treated with colicin K or E1 is actually enhanced on agar plates containing high (~100 mM) potassium, relative to those made with low potassium concentrations (41). The latter experiments suggest that

**Table III**

| Assay            | Concentration | mM |
|------------------|---------------|----|
| K⁺               | 0.35          |    |
| Glucose 6-phosphate | 0.002        |    |
| Pyruvate         | 0.002         |    |
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the main, if not the only, cause of cell death is the decrease of the intracellular potassium concentration below the physiological levels needed to sustain protein synthesis and glycolysis. The experiments reported here, in conjunction with the general ideas discussed above on membrane de-energization caused by colicins E1 and K, imply that restoration of high intracellular potassium levels should only be sufficient to restore cell viability in the presence of active, bound colicin if the membrane is not required to do work (i.e. ATP synthesis or active transport) or if the effect of colicin on the membrane is reversed during the incubation.

There are a variety of other colicins which seem to have a mode of action similar to that of E1. These include colicin K, mentioned above, colicin Ia (42), colicin A (43, 44), and possibly S8 (45). Bacteriocins JF246 from Serratia marcescens and bacteriocin 1580 acting on gram-positive bacteria (44) also seem to resemble E1. The conclusions reached in this paper about the mode of action of E1 have been shown to apply to colicin K (28), and possibly apply as well to the above listed colicins and bacteriocins.

Finally, it should be noted that the conclusions reached in this paper on the mechanism of colicin E1 action are based largely on the model for proton efflux and membrane energization in bacteria proposed by Scholes and Mitchell (27) based on the chemiosmotic hypothesis of Mitchell (10, 11). However, some aspects of the proton efflux from bacterial membranes induced by an oxygen pulse are not easily explained by the chemiosmotic hypothesis (14). This of course does not change the empirical similarity between the effects of colicin E1 (plus M+) and permeant ions such as SCN⁻ or valinomycin plus K⁺, or the conclusion that these conditions all lead to a loss of the ability of the membrane to become electrically polarized by vectorially oriented proton transfer reactions, and therefore to the de-energization of the bacterial cell membrane.

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