Colicin El Induced Depolarization of the Bacterial Inner Membrane

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Summary

When a small pulse of oxygen is added to an anaerobic suspension of logarithmic phase *Escherichia coli* cells, the subsequent acidification of the medium which is observed in the absence of permeant charged ions is slow ($t_{1/2}$ = 10 sec) 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 sec) and its relaxation ($t_{1/2}$ = 10-20 sec) 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 very similar to the El-induced loss of K+ ($t_{1/2}$ = 3 min). Furthermore, the effect of colicin El on the kinetics and extent of H+ efflux is dependent upon the presence of K+, in the medium, with an apparent "K_m" for potassium of about 0.5mM. 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^-. rhus, these experiments provide direct evidence for a rapid, colicin El-induced depolarization of the bacterial membrane.

Introduction

Jacob *et al* (1) reported that colicin El inhibited growth and nucleic acid synthesis in a sensitive *Escherichia coli* strain while not inhibiting respiration. It was also found that this colicin inhibits a range of active transport systems (2). These observations, and that of an oxygen requirement for the action of this colicin, led to the hypothesis that colicin El is an uncoupler of oxidative phosphorylation [Levinthal and Levinthal, quoted in (2)]. This hypothesis was consistent with further studies on colicin inhibition of active transport (3,4) and with the observation of a rapid decrease in intracellular ATP levels caused by colicin (5).

However, it was also found that the efficiency of colicins El and K is not markedly decreased in anaerobic samples, as measured by the inhibition of active transport (5) and the colicin El-induced increase

in fluorescence intensity of a lipophilic probe (6). These observations, together with the report that the effectiveness of colicins K and El was not changed in hemin-less mutants (5) implied that the action of these colicins does not necessarily require respiratory activity. An energized membrane, however, may be required for the action of all colicins (7,8), although the actual degree of required energization may be small (9), and this energization may be achieved through either ATP hydrolysis or respiration.

It has been proposed that the energy level of energy transducing membranes is controlled by the free energy stored in an electrochemical potential consisting of a proton gradient and/or an electrical potential across the membrane (10,11). According to this chemiosmotic hypothesis, charge separation generated through electron transport can generate a transmembrane potential, which can subsequently be relaxed by the movement of a proton through an energy transducing complex (e.g., ATP synthetase, transport proteins). At present there is disagreement with aspects of this model relating to the precise significance of the ion movements observed between the bulk aqueous phases separated by the membrane (12,13), and some of these problems are discussed elsewhere (14). However, the data presented here is not inconsistent with the existence of a local or transmembrane potential in the energized membrane of *E. coli*. In intact *E. coli* it appears that the dominant component of the electro-chemical potential at pH 7 is the membrane electrical potential (15,16). It is reasonable to expect, therefore, that the deenergization of the membrane by colicin would involve dissipation of this potential. In a system in which energy is stored primarily as an electrical potential, the appropriate movement of any charged species, not just protons, can serve to deenergize the membrane. Indeed, an event which appears to be closely related in time to the first biochemical events caused by colicin is an increase in K^+ permeability of the cell resulting in the leakage of intracellular K^+ into the medium (17-19,6,33).

Fluorescence studies with the probe 3,3′-dihexyloxycarbocyanine indicated that colicin K partially depolarized the cytoplasmic membrane (20). However, very similar fluorescence changes induced by colicin El (6), K (21), or Ia (22) have been reported for the lipophilic probe N-phenyl-l-naphthylamine (PhNap), although this probe will not respond directly to a membrane potential, and much of it is localized in the cellular envelope outside of the inner membrane prior to colicin addition (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 El, in the presence of $K+$, 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.

Experimental Methods

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 $(NH_4)_2SO_4$, 0.5 g sodium citrate, 0.1 g $MgSO_4 \cdot 7H_2O$, 7 g K_2HPO_4, 3 g KH_2PO_4, 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 El*), 8 g casamino acids, 5 g yeast extract, 1 g glucose, 5 g NaCl (pH 7.0); strain A_{586} (*tol VIII*), 1 g NH_4SO_4, 10.5 g K_2HPO_4, 4.5 g KH_2PO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 1 mg thiamine, 20 mg proline, 20 mg histidine, 20 mg leucine, 20 mg threonine, 4 g glucose (pH 7.1) (ref.30); strain K12 1100 and strain ML 308-225, same as B/1,5 above.

Sterile 250 ml Erlenmeyer flasks containing 50-60 ml minimal medium and the desired carbon source were innoculated from overnight cultures, and incubated at 37° with vigorous shaking for 4.5-5.5 hrs (mid-logarithmic phase growth) or 14-17 hrs (stationary phase growth). Cells were harvested by centrifugation at 4°, washed twice in 150 mM KCl, 0.5 mM MOPS-KOH (pH 7.0) and resuspended in this medium to a final concentration of about 3 x 10^9 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 MOPS-KOH (pH 7.0).

For experiments involving colicin El treatment of cells, the colicin was added to the cell suspension in a small volume (2 μl) to a final concentration of 1 μg protein/ml. When cells grown with succinate as carbon source were used, it was necessary to add the colicins 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 El treatment was $< 0.1\%$.

pH Measurements. A 2 ml aliquot of the final cell suspension was placed in a water-jacketed, glass reaction vessel (3 ml capacity) which contained a small magnetic stirring bar. The top of the reaction chamber was sealed with a rubber stopper through which passed a Sargent miniature combination pH electrode. 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 sufficient to give 0.01 pH units full scale (6 in). At this level of amplification, the noise level of the pH measuring system was $\leq 2 \times 10^{-4}$ pH units. The overall half-response time for the system was on the order of 1 sec 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 5-10 μl) of air-saturated, double distilled water. The time required to inject a 10 μl pulse was 0.1 sec or less. After an experiment the chart paper was calibrated in H^+-ion equivalents by titrating the sample with small aliquots of 0.001 M HCl. The H+/O ratio was calculated from the maximum extent of the pH change after an oxygen pulse, assuming a concentration of 275 μmoles O_2/1 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 mV) was calibrated against known concentrations of KCl. The response was completely linear for K^+ concentrations $> 2.5 \times 10^{-5}$M, with a slope of 56 mV (33°C) per decade change in K^+ concentration.

Miscellaneous. Colicin El 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 lyophilized El was dissolved in M-9 salt medium and had a specific activity of approximately 1:50 on a protein basis. FCCP (*p*-trifluoromethoxy-carbonylcyanide-phenylhydrazone), 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 El*)), H. R. Kaback (ML 308-225), S. E. Luria (A_{586} *tol VIII*), P. W. Postma K12 1000, and S. Silver (B/1,5).

Results

Effects of colicin El and FCCP on oxygen-pulse dependent pH changes.

The addition of a small pulse of oxygen to an anaerobic suspension of *E. 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 resuspension 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 mid-

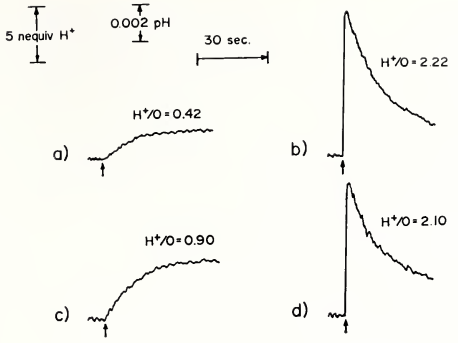

FIGURE 1. *The effect of colicin E1 on the rate and extent of proton efflux in* E. coli *following an oxygen pulse. Cells of* E. coli *strain B/1,5 grown on 1% succinate (a,b) or 1% glycerol (c,d) were harvested during mid-logarithmic phase growth. Colicin E1 (1 μg/ml), sufficient to allow < 0.1% survival,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

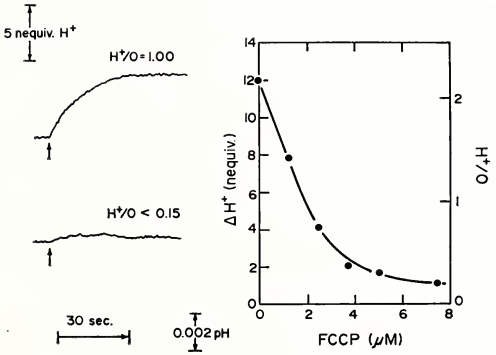

FIGURE 2. *Inhibition of net proton efflux by FCCP.* Left. *Cells of* E. coli *strain B/1,5 were grown on 1% glycerol to mid-logarithmic phase before harvesting. Upper trace, control; lower trace, with 5 μM FCCP. Right. Cells of* E. coli *strain B/1,5 were grown on 1% succinate to mid-logarithmic phase. Colicin E1 (1 μg/ml) was added 5 minutes before the initiation of anaerobiosis. Conditions otherwise as described in Fig. 1.*

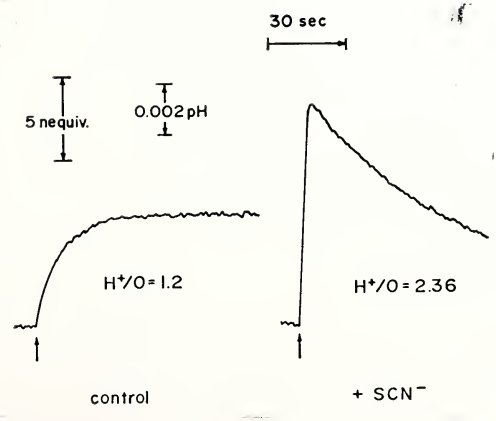

FIGURE 3. *Effect of the permeant anion SCN− on proton efflux and the H+/O ratio in* E. coli. *Cells of* E. coli *strain B/1,5 were grown on 1% succinate to stationary phase. Conditions otherwise as described in Fig. 1.*

logarithmic 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 ($t_{\frac{1}{2}} = 5\text{-}15$ sec). The pH change decays very slowly, with only a small fraction of the change reversed in 10 minutes. 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 O_2 pulse results in a large change in the properties of the proton extrusion (Figure 1b). The rate of proton efflux is greatly stimulated, the pH change reaching a maximum ($\triangle H^+$) in about 4-6 seconds. The halftime for the pH change ($t_{\frac{1}{2}} = 1$ sec) 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 O_2-induced increase in medium acidity is also much faster after the cells have been treated with colicin E1 ($t_{\frac{1}{2}} = 10\text{-}20$ sec). The apparent H^+/O ratio is increased to values > 2.0 after colicin addition, and this 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_{586} and the colicinogenic strain JC411 are unaffected by 1 μg/ml 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 Figure 1. Strain ML 308-225,

TABLE 1. *The effect of colicin El on proton extrusion ($\Delta H+$) and the H+/O ratio in various* E. coli *strains.*

Cells were harvested from either mid-logarithmic phase growth (ML 308-225, K12 1100, B/1,5, A_{586}) or stationary phase growth (JC411). The carbon sources used for growth were 1% succinate (ML 308-225, K12 1100, B/1,5), 0.4% glucose (A_{586}) or 0.1% glucose (JC411). The concentration of colicin E1 was 1 μg/ml, which was sufficient to allow 0.1% survival of susceptible strains. The oxygen pulse contained 5.5 ng atoms O. Note that colicin does not cause any marked increase in the H^+/O ratio in cells of the colicinogenic strain JC411 or the colicin tolerant strain A_{586}, and only a small effect in cells of strain ML 308-225, where survival was ~50% after treatment with 1 μg/ml E1.

E. coli strain	$\Delta H+$ (nequiv.)		H+/O	
	−El	+El	−El	+El
A_{586} *(tol VIII)*	3.85	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	1.95	5.60	0.35	1.02

which is relatively insensitive to colicin E1 (survival = 50%) also showed an increase in the H+/O ratio in the presence of 1 μg/ml 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 (2-5 μM) (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 reentry of extruded protons into the cell (Table II). Thus, FCCP appears to act by increasing the permeability of the membrane to H^+ ions—a conclusion consistent with much data already in the literature [e.g., ref. (32)]. Colicin E1, over a multiplicity range of 1-100 causes an increase in the extent of the

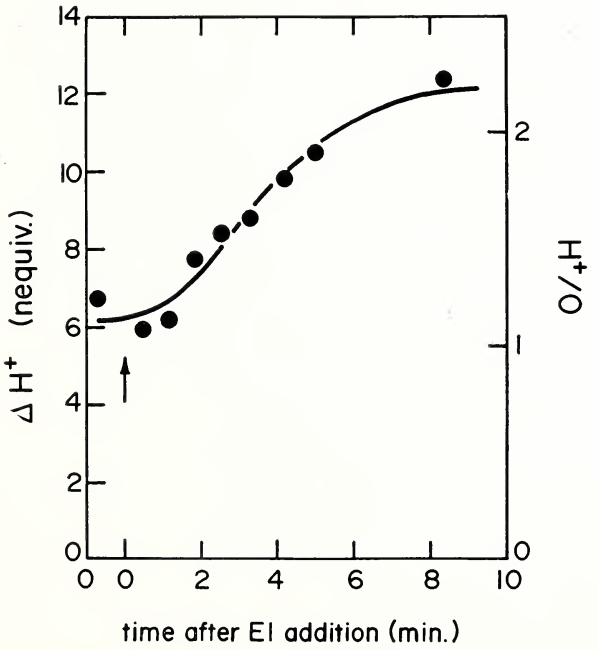

FIGURE 4. *(a) Time course of the increase in proton efflux (ΔH+) and the H+/O ratio by colicin E1. Cells of* E. coli *strain B/1,5 were grown on 1% glycerol to mid-logarithmic phase before harvesting. After the cell suspension had become anaerobic, colicin E1 was added (upward arrow) to a final concentration of 1 μg/ml. Oxygen pulses containing 5.5 ng atoms O per pulse were added at the indicated times after the addition of colicin E1.*

TABLE 2. *The effect of FCCP on proton efflux (ΔH+), proton influx, and the H+/O ratio in colicin treated* E. coli

Cells of *E. coli* strain B/1,5 were grown on 1% succinate to mid-logarithmic phase before harvesting. The cells were treated with 1 μg/ml colicin E1 before the initiation of anaerobiosis. The indicated concentration of FCCP was added approximately one minute before the addition of any oxygen pulse containing 5.5 ng atoms O. Note that FCCP decreases the extent of the observed proton efflux while increasing the rate of subsequent proton influx.

FCCP (uM)	ΔH+ (efflux) (nequiv.)	H+/O	ΔH+ (influx) ($t_{1/2}$ in sec)
0	12.05	2.19	10
1.25	7.87	1.43	6.6
2.5	4.07	0.74	5
5.0	1.71	0.31	2

proton efflux even though it also causes an increase in the rate at which extruded protons reenter the cell (Fig. 1) (28). These findings are therefore consistent with the idea that colicin E1 causes an increase in the

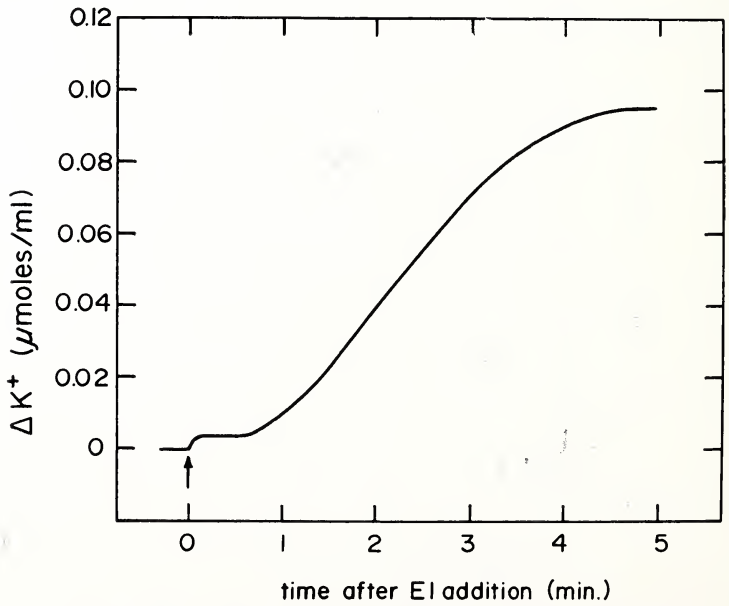

FIGURE 4. *(b) Time course of colicin E1-induced K+ efflux from* E. coli *cells. The cells were washed twice and resuspended in a medium containing 150 mM choline chloride, 0.5 mM MOPS- tris (hydroxymethyl)aminomethane (pH 7.0). Colicin E1 (dissolved in distilled H_2O) was added (upward arrow) to a final concentration of 1 μg/ml. No difference in the rate or extent of K+ efflux was observed between aerobic or anaerobic cell suspensions. Conditions otherwise described as in Fig. 1.*

permeability of the bacterial membranes to charge-compensating counter-ion(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^- in the reaction medium. In the presence of SCN^- the kinetics of H^+ efflux, H^+ influx, and the H^+/O ratio are all increased (Fig. 3), much as they are in the presence of colicin E1 (Fig. 1).

Time Course of the Increase in H^+/O Ratio. 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 KCl is shown in Figure 4a. The initial H^+/O ratio in the glycerol-grown cells is $\sim$ 1.0 as in Fig. 1. After an initial lag of about 1 minute, the H^+/O ratio increases over a period of about 5-6 minutes ($t_{1/2} \sim 3$ min.) to a value approximately twice that measured before colicin addition. 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 of the change in the H^+/O ratio is very similar to the time-course for K^+ efflux after colicin addition, measured under identical conditions (Fig. 4b).

Counterion requirement for the 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 KCl medium (Figure 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 Figure 6, where it is clear that in order to observe the effects of colicin E1 on the kinetics of proton efflux, proton influx, and on the H^+/O ratio, K^+ ion must be present in the medium. The smaller increases in the H^+/O ratio observed in Figures 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 0.3-0.5 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_m" for potassium required for colicin E1 effects is approximately 0.3-0.5 mM.

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 ($t_{1/2} = 3$ min., Fig. 4a) is very 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

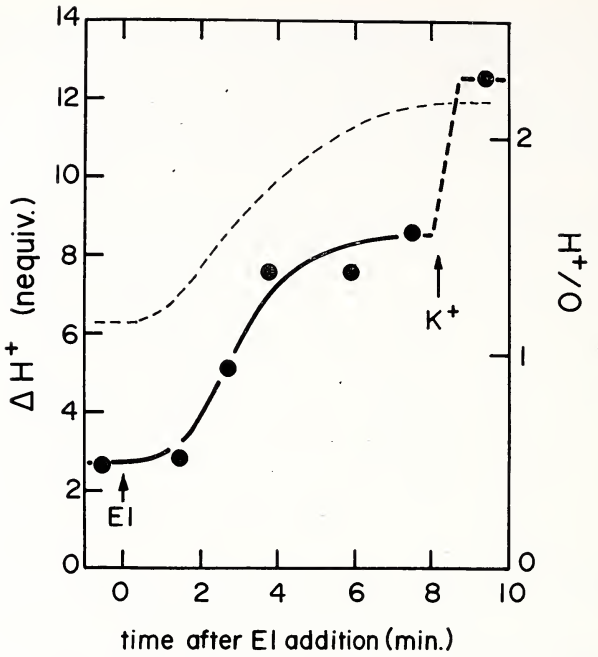

FIGURE 5. *Time-course of the increase in proton efflux (ΔH+) and the H+/O ratio in the absence of external K+ ions. Reaction conditions were as described in the legend to Figure 4b. After the cell suspension had become anaerobic, colicin E1 (dissolved in distilled water) was added to a final concentration of 1 μg/ml. Oxygen pulses containing 5.5 ngm atoms O were given at the indicated times. After the apparent H+/O ratio had reached a maximum, about 8 minutes after the addition of E1, oxygen-free HCl was added in a small volume to give a final concentration of 7.5 mM. The thin dashed line (taken from Figure 4a) represents the time course and extent observed in the presence of 150 mM K+. Conditions otherwise as described in Fig. 1.*

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, 6), 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 results 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. 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 ionic channel created by the colicin itself or (b) induced in the inner

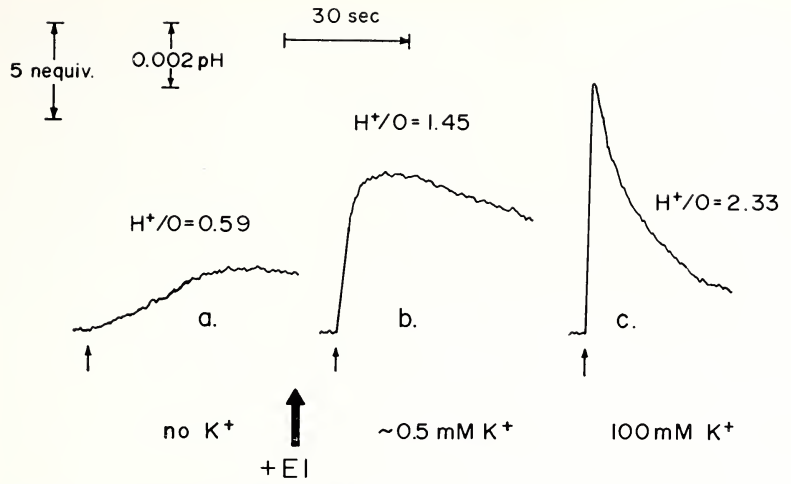

FIGURE 6. *K+ requirement for the increase in the rates of H+ efflux, H+ influx and in the H+/O ratio by colicin E1. Reaction conditions were as in Figure 5. (a) Control. (b) Cells were incubated for 8 minutes with 1 μg/ml colicin E1. (c) Same as b except 7.5 mM K+ was added prior to the oxygen pulse. Conditions otherwise as in Fig. 5.*

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). 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 [but see (41)].

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 deenergization (37). This work has shown that deenergization 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 the hydrophobic probe is decreased upon deenergization of the envelope by colicin E1 and FCCP, resulting in some increased binding of the probe 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 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 do 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 electro-strictive effects on membranes (39), and it is clear from structural studies on the *E. coli* envelope that there are specific connections between the peptido-glycan layer and outer membrane (40).

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 *S. marcescens* and bacteriocin 1580 acting on gram-positive bacteria (44) also seem to resemble E1. These 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.

Acknowledgments

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