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PERMEABILITY TO COBALT IONS AND ACTION OF COLICIN K IN A MUTANT THAT OVERPRODUCES CARDIOLIPIN

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Summary

A mutant of *Escherichia coli* that produces excess cardiolipin becomes less capable of transporting Co^{2+}. Cardiolipin therefore does not act as an ionophore under these conditions. Colicin K brings about the typical increase in permeability to Co^{2+} in the mutant.

Tyson et al. [1] have recently demonstrated that phospholipids can function as ionophores, transporting cations across an organic phase. Cardiolipin and phosphatidic acid were the most active phospholipids tested. A variety of divalent cations and Rb^+ were transported at comparable rates. Whether the phospholipids in biological membranes function as ionophores has not been determined.

In *Escherichia coli*, transport of Mg^{2+} and Co^{2+} is energy dependent, sensitive to sulfhydryl reagents [2,3] and absent in mutants that retain normal transport of other substrates [4,5]. These properties suggest that any ionophore activity of the endogenous phospholipids must be low. After treatment with colicin K or E1, however, the cells become more permeable to Mg^{2+} and Co^{2+}. The colicin-promoted flux of ions has a high K_m and V and is insensitive to sulfhydryl reagents or to uncouplers [6]. Coincidentally, the phospholipid composition of the cells changes. Phosphatidylethanolamine is hydrolysed to lysophosphatidylethanolamine and cardiolipin increases [7]. Either of the phospholipids could perturb the membrane, as a detergent or as an ionophore, respectively. Lysophosphatidylethanolamine has been shown not to be responsible for increased permeability, since mutants lacking phospholipase A become more permeable to Co^{2+} after colicin treatment without an increase in lysophosphatidylethanolamine [8]. The potential role of cardio-

Abbreviation: CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

lipin as an ionophore increasing permeability to Co^{2+} is examined in this report.

The growth of strain A324-1 is inhibited by low concentrations of Na^+ [9] Upon the addition of NaCl to a growing culture, the mutant continues to synthesize protein, nucleic acids and lipids for an hour before growth stops. The phospholipid made is predominantly cardiolipin. In contrast to the normal proportions of phospholipids, which are 75 mol% phosphatidylethanolamine, 21% phosphatidylglycerol and 5% cardiolipin, the Na^+-inhibited mutant attains 57% phosphatidylethanolamine, 12% phosphatidylglycerol and 32% cardiolipin after an hour [9].

Table I shows the transport of Co^{2+} in the parental strain A324 and the Na^+-sensitive mutant A324-1. After growth in the presence of inhibitory concentrations of NaCl, the energy-dependent uptake of Co^{2+} decreased. The rate of entry in cells uncoupled by CCCP did not increase, showing that the increased proportion of cardiolipin could not function as an ionophore catalysing the entry of Co^{2+} at more than 1% of the energy-dependent rate. Treatment with colicin K brought about the same energy-independent rate of Co^{2+} entry in the Na^+-inhibited mutant as in the wild type. The typical colicin-promoted permeability thus occurred in spite of the abnormal phospholipid composition of the mutant.

The cause of lower rates of Co^{2+} entry in the Na^+-inhibited cells is not known. Entry of *o*-nitrophenylgalactoside was previously reported to be inhibited under similar conditions [9]. Both transport systems could have been inactivated directly. Alternatively, lack of energy could have been responsible for the inhibition of either transport system, since even the entry of galactosides down a concentration gradient [10] and their binding to the transport system [11] require energy. If energy levels were reduced, enough energy must have remained to support colicin action [6].

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TABLE I

PERMEABILITY TO Co^{2+}

The bacteria were grown in medium N [8] containing $5 \cdot 10^{-5}$ M $MgSO_4$. Strain A324-1 was inhibited by the addition of 10 mM NaCl 30 or 90 min before the cells were harvested. Growth was not noticeably slower 30 min after the NaCl, but had nearly stopped after 90 min. The cells were washed, and resuspended in medium without $MgSO_4$ or proline, at 37° C. Colicin K (multiplicity of 10) was added 16 min before $^{60}CoCl_2$ and CCCP was added 8 min before $^{60}CoCl$. After the addition of $^{60}CoCl_2$ (0.1 Ci/mol, 0.1 mM), samples were filtered, washed with medium N containing 1 mM $MgSO_4$, dried, and counted by liquid scintillation.

Strain and growth conditions	Rate of entry of $^{60}Co^{2+}$ (nmol·mg^{-1}·min^{-1})			
	Control	+CCCP	+Colicin	+Colicin+CCCP
A324, no NaCl	18.2	0.7	10.5	6.2
A324-1, no NaCl	11.0	0.8	10.5	6.5
A324-1, NaCl 30 min	4.1	0.9	11.5	6.8
A324-1, NaCl 90 min	1.6	0.8	11.0	6.5

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