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# PHOSPHOLIPASE ACTIVITY PLAYS NO ROLE IN THE ACTION OF COLICIN K

JOAN E. LUSK and MYUNG HEE PARK

*Department of Chemistry, Brown University, Providence, R. I. 02912 (U.S.A.)*

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## SUMMARY

1. A mutant lacking both detergent-resistant and detergent-sensitive phospholipase A activities is fully sensitive to colicin K.

2. In the absence of cellular phospholipases A, colicin K does not promote hydrolysis of phosphatidylethanolamine.

3. Cells of the colicin-treated mutant lacking lysophosphatidylethanolamine are as abnormally permeable to $Co^{2+}$ as the wild type is.

4. Increased levels of lysophosphatidylethanolamine in colicin-treated cells are not necessary for the increased sensitivity to sodium dodecylsulfate.

## INTRODUCTION

*Escherichia coli* produces lysophosphatidylethanolamine after treatment with colicins that interfere with energy metabolism, such as colicin E1 and K [1]. The significance of lysophosphatidylethanolamine in the lethal action of these colicins has never been proved, although the consequences of treatment with colicins E1 or K are not inconsistent with plausible roles for such a detergent-like molecule. The colicins uncouple oxidative phosphorylation and active transport systems, lower ATP levels and inhibit synthesis of DNA, RNA and protein [2–4]. Since phospholipase A can uncouple oxidative phosphorylation in mitochondria [5] and submitochondrial particles [6]; phospholipase action might do so in *E. coli*. The detergent properties of lysophosphatidylethanolamine could result in the colicin-promoted increase in permeability to cations [7] or sensitivity to sodium dodecylsulfate [8].

Recently Cramer and Keenan [9] have shown that a doubling of lysophosphatidylethanolamine in cells treated with colicin E1 occurs much later than the response of a fluorescent probe. Increased permeability to ions, however, is not one of the earliest events after treatment with colicin K. Loss of thiomethylgalactoside begins before loss of $Mg^{2+}$ [7]. Loss of $K^+$ or $Mg^{2+}$ also shows a lag that depends on temperature and multiplicity of colicin K [10], which suggests that the colicin initiates a process leading to ion permeability.

Abbreviation: CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

We have examined the colicin-promoted permeability of mutants lacking phospholipase A activities [11]. These strains produce very little lysophosphatidylethanolamine when treated with colicin K, but they are killed as efficiently, they become as permeable to $Co^{2+}$, and they become as sensitive to sodium dodecylsulfate as the wild type.

## MATERIALS AND METHODS

*E. coli* strains S15 and 23 (*F⁻' thi' leu' thr' lac' mel'*) were obtained from Dr Misao Ohki and strain 17 from Dr John Cronan. Strain 23 lacks detergent-resistant phospholipase A and strain 17 lacks both detergent-resistant and detergent-sensitive phospholipase A activities [11, 12]. The F'lac episome was transferred from strain CSH28 (*lac$_\Delta$ pro$_\Delta$ sup*F *trp pyr*F *his str*A *thi*/F'*lac*$^+$ *pro*$^+$), kindly provided by Dr Frank Rothman. The bacteria were grown in 1 % tryptose (Difco) containing $^{32}P_i$ at 1 $\mu$Ci/ml when lipids were to be analyzed.

Colicin K was purified according to Kunugita and Matsuhashi [13] from *E. coli* strain K235 (colK). Multiplicities (*m*) were determined from the fraction of treated cells that were able to form colonies, $e^{-m}$.

Lipids were extracted by the method of Bligh and Dyer [14] from cell pellets that were dispersed with a Vortex mixer. Combined chloroform phases from two extractions were chromatographed on Eastman sheets of silica gel, no. 6061, in chloroform/methanol (65 : 35, v/v). The lipids were located by autoradiography, and the spots cut out and counted in 1 ml water and 10 ml Triton/toluene scintillation fluid [15].

Entry of $^{60}Co^{2+}$ was determined by Millipore filtration as previously described [16]. The cells were grown in tryptose, washed in Buffer N (5 mM KCl, 7.5 mM $(NH_4)_2SO_4$, 1 mM $KH_2PO_4$, 100 mM Tris·HCl, pH 7.4) and resuspended in Buffer N+0.4 % glucose. $^{32}P_i$ and $^{60}CoCl_2$ were purchased from New England Nuclear.

## RESULTS

### *Sensitivity of phospholipase mutant to colicin K*

If activation of phospholipase A and perturbation of the membrane by lysophosphatidylethanolamine are essential to the killing action of colicin K, mutants lacking phospholipase A should be tolerant to the colicin. However, strain 17 was as sensitive to colicin K as its parent strain S15, although it lacks both the most active, detergent-resistant phospholipase A and a detergent-sensitive phospholipase A activity [12].

### *Lysophosphatidylethanolamine content of colicin-treated cells*

Cells treated with colicin K converted pre-existing phosphatidylethanolamine to lysophosphatidylethanolamine (Table I). The cellular phospholipases, particularly the detergent-resistant enzyme, appear to be responsible for the hydrolysis of phosphatidylethanolamine, rather than any intrinsic phospholipase activity of the colicin. Mutants lacking one or two phospholipase A activities (strains 23 and 17) produced only 0.2–0.4 % lysophosphatidylethanolamine. This small amount represents an upper

TABLE I

LYSOPHOSPHATIDYLETHANOLAMINE CONTENT OF CELLS AFTER TREATMENT WITH COLICIN K

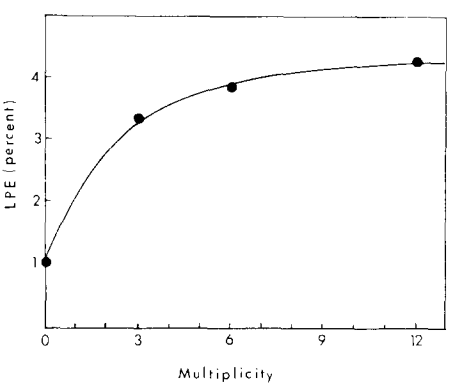
Cells labeled with $^{32}P_i$ for several generations were resuspended in 0.1 M sodium phosphate, pH 7.0, with or without 0.2 mM $CaCl_2$. They were incubated with or without colicin K ($m = 10$) for 1 h at 37 °C. The lipids were extracted and analysed as described in Materials and Methods.

| Strain | Lysophosphatidylethanolamine (percent of lipid $^{32}P$) | |
|---|---|---|
| | −colicin K | +colicin K |
| S15 | 0.4 | 1.6 |
| 23 | 0.2 | 0.2 |
| 17 | 0.2 | 0.35 |
| S15+$CaCl_2$ | 0.45 | 3.4 |
| 17+$CaCl_2$ | 0.3 | 0.4 |
| S15 F'lac | 0.4 | 3.4 |
| 17 F'lac | 0.2 | 0.35 |

limit, since autoradiography detected some tailing from the spot of phosphatidylethanolamine instead of any distinct spot of lysophosphatidylethanolamine.

The addition of $CaCl_2$, an activator of *E. coli* phospholipase A [17], stimulated the production of lysophosphatidylethanolamine in the wild type but not in the phospholipase mutant.

In preliminary experiments we had observed that the HFr strain 3300 produced more lysophosphatidylethanolamine than several $F^-$ strains. In order to test whether a specific phospholipase activity is associated with maleness, a F'lac episome was introduced into strains S15 and 17. Strain S15 F'lac produced twice as much lyso-



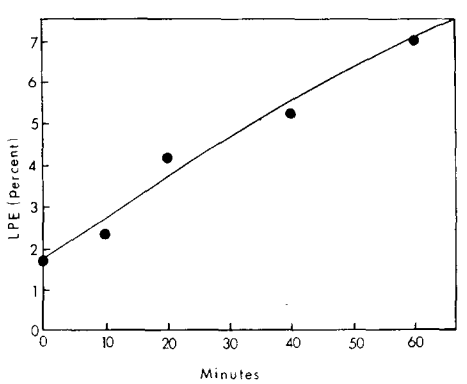




Fig. 1. Multiplicity dependence of formation of lysophosphatidylethanolamine (LPE) in strain S15. The bacteria were grown in 1 % tryptose with $^{32}P_i$, washed in Buffer N and resuspended in Buffer N+ 0.4% glucose. Colicin K was added, at the indicated multiplicities. After 1 h at room temperature, the lipids were extracted and analysed.

Fig. 2. Rate of formation of lysophosphatidylethanolamine. Conditions were the same as in Fig. 1, except that strain S15 was treated with colicin K at a multiplicity of 30. Lipids were extracted at various times after the addition of colicin.

TABLE II

PERMEABILITY TO $Co^{2+}$ OF COLICIN-TREATED CELLS

Colicin K ($m = 10$) was added 15 min before $^{60}CoCl_2$ and CCCP ($4 \cdot 10^{-5}$ M) 5 min before $^{60}CoCl_2$ (0.04 mM, 1 Ci/mol).

| | Rate of $^{60}Co^{2+}$ entry (nmol · $mg^{-1}$ · $min^{-1}$) | |
|---|---|---|
| | S15 | 17 |
| Control | 4.4 (100 %) | 3.9 (100 %) |
| +colicin K | 2.2 (50 %) | 1.7 (45 %) |
| +colicin K+CCCP | 1.0 (22 %) | 1.0 (26 %) |
| +CCCP | 0.1 (< 2 %) | 0.1 (< 2 %) |

phosphatidylethanolamine in response to colicin K as did S15. The episome, however, did not increase the lysophosphatidylethanolamine found in mutant 17.

The formation of lysophosphatidylethanolamine with increasing multiplicity and time is shown in Figs 1 and 2. At the high multiplicity and temperature of the experiment in Fig. 2 the colicin acts rapidly [18], making detection of any lag phase unlikely. The experiments in Figs 1 and 2 were performed in the same buffer used for measurement of permeability to $Co^{2+}$ (Table II), resulting in formation of somewhat more lysophosphatidylethanolamine than in phosphate buffer (Table I). Strain 17 produced no significant lysophosphatidylethanolamine (0.3 %) under the same conditions.

*Permeability of colicin-treated cells lacking lysophosphatidylethanolamine*

Entry of $Co^{2+}$ catalysed by the normal transport system is completely inhibited by uncouplers of oxidative phosphorylation such as carbonylcyanide *m*-chlorophenyl-

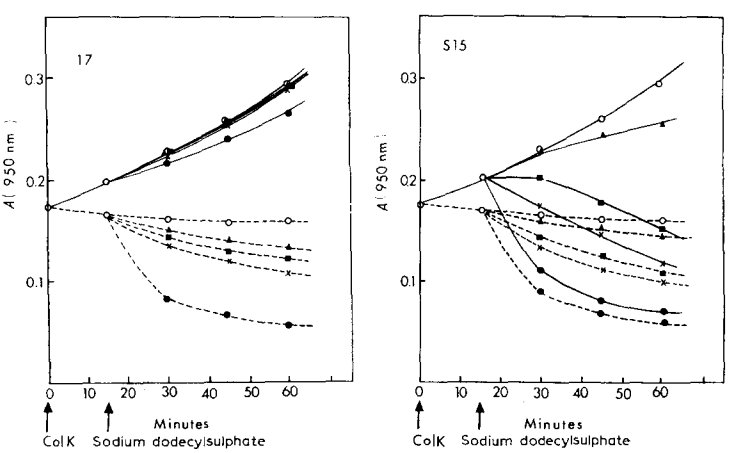



Fig. 3. Lysis by sodium dodecylsulfate. Strains S15 and 17 were grown in 1 % tryptose. One half of each culture was treated with colicin K ($m = 50$) and incubation continued at 37 °C. After 15 min, sodium dodecylsulfate was added to portions of each culture. Turbidity was measured in a Spectronic 20 spectrophotometer. Solid lines, no colicin; dashed lines, with colicin. The following concentrations of sodium dodecylsulfate were used: ○, none; ▲, 0.25 mg/ml; ■, 0.5 mg/ml; ×, 1 mg/ml; ●, 2 mg/ml.

hydrazone (CCCP). Colicin-treated cells have a defective permeability barrier, allowing $Co^{2+}$ to enter in spite of the presence of an uncoupler [7]. Table II shows that the abnormal, CCCP-insensitive colicin-promoted permeability of strain 17 was equivalent to that of the wild type. Lysophosphatidylethanolamine therefore cannot be responsible for the permeability of colicin-treated cells.

*Lysis of colicin-treated cells by sodium dodecylsulfate*

Strain 17 became more sensitive to sodium dodecylsulfate after treatment with colicin K (Fig. 3), in spite of the absence of lysophosphatidylethanolamine. Strain S15 was equally sensitive with or without colicin treatment (Fig. 3); the untreated cells were lysed at lower concentrations of sodium dodecylsulfate than are required to lyse strain 17. Ohki et al. [11] had selected strain S15 for increased sensitivity, since their procedure for detecting phospholipase A in colonies depended on lysis of colonies by sodium dodecylsulfate. Apparently neither the increased lysophosphatidylethanolamine nor any other alteration of membrane structure in colicin-treated cells of S15 can further increase their sensitivity.

## DISCUSSION

Phospholipase A activity plays no role in the action of colicin K. The colicin itself cannot have any phospholipase A activity, since it fails to bring about any hydrolysis of lysophosphatidylethanolamine in mutants lacking the major, detergent-resistant phospholipase A. Lysophosphatidylethanolamine cannot be responsible for killing, for colicin-promoted permeability to divalent cations, or for increased sensitivity to sodium dodecylsulfate, since the mutants are killed, become permeable, and become more sensitive to the detergent. The amount of lysophosphatidylethanolamine in the colicin-treated mutants never exceeded the amount in control cells of the wild type. It is unlikely, therefore, that a critical level attained within a few minutes in the wild type cells could account for the abnormal properties of the membrane.

The cellular localization of the phospholipase A may be responsible for the irrelevance of its action to ion permeability. The phospholipase and most of the lysophosphatidylethanolamine have been found in the outer membrane [19, 20]. If the lysophosphatidylethanolamine of colicin-treated cells is localized in the outer membrane, it may have little effect on permeability to small molecules, for which the outer membrane is thought not to be a barrier to diffusion.

Lysophosphatidylethanolamine in the outer membrane might increase its permeability to detergents, thus accounting for the greater sensitivity of colicin-treated cells to lysis by sodium dodecylsulfate. However, we found treatment of strain 17 with colicin K to increase sensitivity to sodium dodecylsulfate. Structural changes other than the formation of lysophosphatidylethanolamine must sensitize the colicin-treated cells.

Cramer and Keenan [9] have shown that colicin E1 increases lysophosphatidylethanolamine only 25 min after perturbing the fluorescence of *N*-phenyl-1-naphthylamine in the membrane. They concluded that lysophosphatidylethanolamine accumulates only as a secondary consequence of damage to the cells and plays no direct role in the action of colicin E1. Similarly, our results show that formation of lysophosphatidylethanolamine plays no direct role in the action of colicin K.

ACKNOWLEDGMENTS

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