THE CHANGES IN CELL SIZE AND PHOSPHOLIPID COMPOSITION DURING GROWTH OF A CHAIN-FORMING *envC* MUTANT OF *ESCHERICHIA COLI*

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1. Introduction

The relative proportions of phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL) in the envelope of *Eschericha coli* vary with the growth phase [1–4] and can be modified by many physical and chemical factors [4–10]. The changes in phospholipid (PL) composition almost invariably involve an increase in CL and a decrease in PG [1–10]. In some cases, eg., in cells infected with phage [11], or treated with phenethyl alcohol [7] or complement [12], accumulation of lysophosphatidylethanolamine (LPE) accompanied CL increase. The nonconditional *envC* mutant of *E. coli* [13] which is characterized by chain morphology and alterations of the outer membrane has an unusually low PG/CL ratio [14]. Incubation of this mutant in the presence of hypertonic concentrations of sucrose restores the division process as well as the PG/CL ratio to normal [15,16].

In this study I have examined the relation between PL composition and size of *envC* chains at different phases of growth and compared the changes in PL composition in normal and outer membrane-altered bacteria. It was found that during growth *envC* chains become shorter and accumulate a relatively large amount of LPE. However, despite this the ratio between zwitterionic and anionic PL diminishes but with a transient stabilization at the end of exponential phase.

2. Materials and methods

2.1. *Bacteria and growth*

The characteristics and origin of *E. coli* P678 (*envC*$^+$) and PM61 (*envC*$^-$) have been described [13,14]. Bacteria were grown at 37°C on a gyratory shaker in complete medium containing per liter: 5 g tryptose (Difco); 5 g yeast extract (Difco); 8 g NaCl. A_{450} was measured with a Spectronic 20 spectrophotometer. The cell size distributions were determined with a Coulter counter (model Z2, Coultronics, Margency) equipped with a 50 μm aperture probe. Since the growth rates of *envC*$^+$ and *envC*$^-$ strains are different [13], cell samples were removed at five comparable stages of growth (fig.1A, P1–P5).

2.2. *Phospholipid extraction and analysis*

^{32}P-labelled PL were prepared as in [16]. They were separated on pre-coated thin-layer chromatographic silica gel plates (Merck) by one-dimensional chromatography using solvent system I (chloroform–methanol–acetic acid, 65 : 25 : 8) or by bi-dimensional chromatography (first dimension, solvent sytem II chloroform–methanol–ammonia–water (65 : 25 : 4 : 1; second dimension, solvent system I) and identified by co-chromatography with authentic standards.

3. Results

Particle numeration, size distribution and PL composition of *envC* and its parent P678 were examined at different stages of growth (P1–P5) shown in fig.1A. During growth of *envC* the particle volume distribution which was initially characterized by a preponderance of long cells changed progressively to reflect a shortening of the longer chains (fig.1B, table 1). Figure 2 shows the patterns of change in the

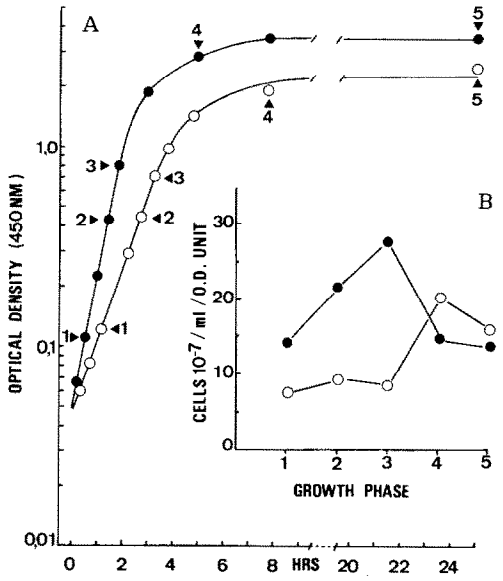

Fig.1. (A) Growth of *envC*$^+$ (•) and *envC*$^-$ (○) strains. (▾) indicates when samples are taken. (1) Beginning of the exponential phase (P1). (2) Middle of the exponential phase (P2). (3) End of the exponential phase (P3). (4) Early stationary phase (P4). (5) Stationary phase (P5). (B) Particle number evolution in *envC*$^+$ (•) and *envC*$^-$ (○) during growth.

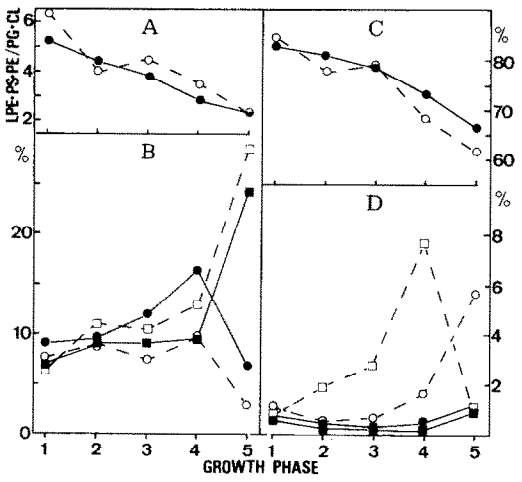

Fig.2. Changes in the PL composition of *envC*$^+$ and *envC*$^-$ during growth. (A) zwitterionic PL/anionic PL: *envC*$^+$ (•—•), *envC*$^-$ (○- - -○). (B) PG (anionic PL): *envC*$^+$ (•——•); *envC*$^-$ (○- - -○). CL (anionic PL): *envC*$^+$ (▪—▪); *envC*$^-$ (□- - -□). (C) PE (zwitterionic PL): *envC*$^+$ (•—•); *envC*$^-$ (○- - -○). (D) PS (zwitterionic PL): *envC*$^+$ (•—•); *envC*$^-$ (○- - -○). LPE (zwitterionic PL): *envC*$^+$ (▪—▪), *envC*$^-$ (□- - -□). For B–D the results are expressed in % of the total [^{32}P]PL recovered on thin-layer chromatographic silica gel plates.

contents of the major PLs during growth. The patterns are similar in the two strains although the relative contents of the individual PLs in *envC* differ from those of the parent. Quantitative measurements showed that PG increased until P4 and decreased in P5 as CL increased (fig.2B).

However, the PG/CL ratio was always lower in *envC*. The PE content decreased in both strains (fig.2C) but the *envC* chains displayed a transient stabilization at P3 which coincides with the slow down in the chain shortening process (fig.1B). Moreover, *envC* cells contained significant amounts of an unknown phosphoglyceride with R_F 0.093 in solvent system I throughout growth whereas the parent contained only traces of it (<1%). This compound was ninhydrin-positive, co-chromatographed with an LPE standard, and was characterized as LPE using mono- and bi-dimensional thin-layer chromatography (see section 2.2). In solvent system I, the R_F values were CL, 0.54; PG, 0.43; PE, 0.29; phosphatidylserine (PS), 0.164. Lysophosphatidylserine standard obtained by digestion of PS with phospholipase A_2 had R_F 0.037 in the same system.

LPE accumulated in *envC* until P4 when it accounted for ~8% of the total PL, and then dropped to 1% in P5 (fig.2D). PS, on the other hand, rose from 1.7–5.7% in this period. Figure 2A shows that with age the polyglycerophosphatides increase at the expense of the zwitterionic PLs despite the transient

Table 1
Size distribution of *envC*$^+$ and *envC*$^-$ during growth

	Growth phase				
	1	2	3	4	5
envC$^+$					
I	94.2	97.4	99.4	93.2	96.9
II	5.8	2.6	0.6	6.8	3.1
envC$^-$					
I	26.5	36.9	44.8	96.1	96.9
II	70.5	62.2	55.2	3.9	3.1

The results are expressed in % of the total number of particles: I, vol. 0.4–1.6 nl; II, vol. 1.6–8 nl

and reproducible stabilization observed in P3 in *envC* cells.

We reported [16] that *envC* chains incubated in the presence of 0.4 M sucrose in rich medium divided into normal rods and adjusted their PG/CL ratio to that of the parent. However, these conditions did not reduce the LPE content to the level found in $envC^+$ cells (results not shown).

4. Conclusions

The results presented here show that the envelope alterations determined by *envC* mutation and expressed phenotypically as aberrant morphology and division, antibiotic- and detergent hypersensitivity, release of periplasmic enzymes, and modified outer membrane composition [15–17], are paralleled by alterations of PL metabolism during growth. The finding of a relatively high proportion of LPE lends serious support to the idea that the alteration of *envC* membranes is due to the detergent action of this lysophospholipid. Moreover, the decrease in chain length in early stationary phase is concomitant with the increase in LPE. It appears likely that separation of daughter cells in *envC* chains is also prompted by the detergent action of LPE on the outer membrane in the septum division zone. On the other hand, the formation of shorter fragments of chains in later phases of growth could also be the result of lysis of individual cells in the chain. Indeed, it is possible that LPE activates murein hydrolases and thus sets off the autolytic process. This should not be surprising since it is well known that detergents like Triton X-100 solubilize and activate membrane-bound murein hydrolases.

PE has been shown [18] to be more easily deacylated by autochthonous phospholipase A in a strain of *E. coli* releasing various constituents of the outer membrane (PL, lipopolysaccharide and phospholipase A) into the medium. They suggested that phospholipase A activity is triggered by factors affecting the structure of the cell envelope. In the same way, growing *envC* release PL and lipopolysaccharide material (unpublished observations). Furthermore, it was shown that PE and other PL of intact *envC* cells are accessible to the action of phospholipase C added to the medium and thus indicating a serious disorganization of outer membrane [19]. Because of these considerations it seems likely that LPE accumulation is the result of phospholipase activation in the outer membrane. This question is currently under investigation.

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