Origin and Fate of the Lysophosphatidylethanolamine in a Chain-forming Mutant (*envC*) of *Escherichia coli*

By G. P. F. MICHEL* AND J. STARKA

Laboratoire de Physiologie Microbienne, Groupement Scientifique de Luminy, 13288 Marseille Cedex 9, France

(Received 18 October 1983; revised 28 November 1983)

The role of phospholipid metabolism in the functioning of the bacterial envelope was investigated in the chain-forming *Escherichia coli envC*. Lysophosphatidylethanolamine (LPE) which accumulated in this strain during growth was indentified as the product of phosphatidylethanolamine (PE) hydrolysis by a phospholipase A₁, i.e. 2-acylLPE. Isotopically labelled LPE transferred into intact mutant and parent cells by liposome/bacteria interaction was rapidly reacylated to PE. However, in *envC* the final PE/LPE ratio was lower than that in the parent, thus showing that the fate of LPE is modified. Crude cell extracts degraded LPE to a lesser extent in *envC* than in the parent but were unable to promote reacylation activity under our experimental conditions. In both strains, the lysophospholipase activity was neither calcium-dependent nor inhibited by the SH-group inhibitors *p*HMB or *p*CMPS, and hydrolysed 1-acylLPE as well as 2-acylLPE. These results indicate the existence of a deacylation–reacylation cycle in *E. coli* and show that this cycle is perturbed in *envC* cells, especially at the lysophospholipase step.

INTRODUCTION

Lysophospholipids produced by phospholipase A present in the outer membrane of *Escherichia coli* are rapidly reacylated under normal conditions and do not accumulate in bacterial membranes (Homma *et al.*, 1981; Proulx & Van Deenen, 1966; Vos *et al.*, 1978). However, this is not the case in the PM61 (*envC*) strain, which exhibits lysophosphatidylethanolamine (LPE) accumulation during growth (Michel, 1979), in addition to antibiotic hypersensitivity and septation deficiency.

pldA mutants of *E. coli*, which are defective in phospholipase A, grow almost normally. It was proposed, therefore, that this enzyme is not essential, at least in laboratory conditions (Ohki *et al.*, 1972; Raetz, 1978). However, Hardaway & Buller (1979) showed that the breakdown of the permeability barrier of *E. coli* towards actinomycin D is related to activation of phospholipase A.

The micellar organization of lysophospholipids can create unstable zones in the envelope structure (Cullis & De Kruijff, 1979; Luzzati *et al.*, 1968). Under certain conditions, lysophospholipids are bactericidal (Duckworth *et al.*, 1974), haemolytic (Bierbaum *et al.*, 1979) and inhibit enzyme activity (Mookerjee, 1979). Conversely, as suggested by Gerritsen *et al.* (1979), lysophospholipids can correct defective interaction between some proteins and some phospholipids. This idea emerged from the observation that introduction of conical lipids (Cullis & De Kruijff, 1978, 1979), like unsaturated phosphatidylethanolamine (PE) or unsaturated lysophosphatidylcholine, into liposomes consisting of phosphatidylcholine and glycophorin established a permeability barrier to dysprosium chloride (Gerritsen *et al.*, 1979).

Abbreviations: LPE, lysophosphatidylethanolamine; PE, phosphatidylethanolamine; *p*HMB, *p*-hydroxy-mercuribenzoate; *p*CMPS, *p*-chloromercuriphenylsulphonate.

Consequently, it is possible that LPE accumulation in PM61 may have a physiological significance and could be related in some manner to envelope perturbation.

This suggestion is supported by an earlier observation that there is a relation between LPE accumulation and the increase in chain length of strain PM61. Conversion of this mutant from the chain form to individual rods could be induced by addition of sugars to increase the osmolarity of the growth medium, or by decreasing the growth temperature. This partial phenotypic correction was accompanied by a decrease in cellular LPE content, although it was not lowered to the level of the parent strain (G. P. F. Michel, unpublished results).

Because little is known about the physiological role of phospholipid metabolism in envelope function, there is no doubt that the *envC* mutant, which is characterized by changes in envelope properties and phospholipid metabolism, is a useful tool for the study of the involvement of membrane-phospholipids in cellular events, such as septation and permeability. By taking this approach, we tried as a first step to elucidate the origin of the perturbation of phospholipid metabolism in *envC* which leads to LPE accumulation.

In a previous report, we showed that labelled PE transferred by means of liposomes into intact cells is hydrolysed to LPE more rapidly in PM61 than in its parent, P678; this result was interpreted to be a consequence of phospholipase A activation (Michel & Starka, 1979). The present paper describes results showing that the fate of LPE is modified in PM61, and suggests that three different alterations of phospholipid metabolism could be involved in 2-acylLPE accumulation in this strain.

METHODS

Bacterial strains, media and culture conditions. The origin and characteristics of *E. coli* P678 *envC*⁺ and PM61 *envC* have been described previously (Rodolakis *et al.*, 1974). S15 *pldA*⁺ and S17 *pldA* (phospholipase A deficient) were described by Abe *et al.* (1974) and Ohki *et al.* (1972). Bacteria were grown at 37 °C in a gyratory shaker on complete medium containing (l⁻¹): 5 g tryptose (Difco), 5 g yeast extract (Difco) and 8 g NaCl. Optical density was measured at 450 nm.

Extraction and analysis of phospholipids. Phospholipids were extracted with chloroform/methanol from rapidly chilled cells and separated by thin-layer chromatography as described previously (Starka & Moravova, 1970). The portions of silica gel containing individual [2-³H]glycerol-labelled phospholipids were scraped off and transferred to scintillation vials containing 0.4 ml water and 5 ml Ready-Solv MP (Beckman) in order to minimize quenching due to silica gel. Each vial was then placed in a water-bath sonicator for 5 min before the radioactivity was estimated. Total phospholipid phosphorus was determined as described by Ames & Dubin (1960).

Fatty acid analysis. Fatty acid methyl esters prepared with boron trifluoride in methanol were analysed by gas-liquid chromatography on a BDS column 2 m long (Interchim, France) using a Varian 1440 series chromatograph equipped with a flame-ionization detector. The temperatures of the injector, detector and column were respectively 235 °C, 235 °C and 185 °C. Identification of fatty acids was made by comparison with authentic standards. Cyclopropane fatty acids were identified after hydrogenation in acetic acid (Kaneshiro & Marr, 1961).

Phospholipid isolation. Total lipid extracts were separated from neutral lipids by silicic acid column chromatography. Neutral lipids were eluted with chloroform and adsorbed phospholipids were eluted with methanol. Individual phospholipids were separated and purified by thin-layer chromatography on preparative plates (Merck 5745) developed successively in chloroform/methanol/ammonia/water (70:30:4:2, by vol.), chloroform/methanol/water (65:25:4, by vol.) and chloroform/methanol/acetic acid (65:25:9, by vol.). Silica gel bands containing the phospholipids were scraped off, dispersed by sonication in a water-bath and the phospholipids eluted sequentially with chloroform containing increasing proportions of methanol, and finally with methanol alone. At each elution step, silica gel was recovered by centrifugation (1500 g, 10 min, 20 °C) and the supernatant fluid containing the phospholipids was evaporated to dryness.

Hydrolysis by phospholipase A₂. Labelled LPE or PE extracted from PM61 grown on complete medium containing 2 µCi [³²P]phosphate ml⁻¹ (74 kBq ml⁻¹) was incubated at 37 °C for 12 h with phospholipase A₂ (*Naja naja*, Sigma). The incubation mixture contained 70 nmol phospholipid in 100 µl methanol and 1 ml HEPES buffer, pH 8.0. The mixture was dispersed by sonication for 30 s in a water-bath sonicator and 10 units of phospholipase A₂ and 5 mM-CaCl₂ (final concentration) were added. The reaction was stopped by successive additions of 2 ml methanol, 2 ml chloroform and 0.8 ml distilled water. The upper phase was removed and shaken with 2 ml chloroform for a second lipid extraction. The two chloroform phases were mixed, evaporated to dryness, dissolved in a small volume of chloroform and chromatographed on thin-layer silica gel plates (Merck 5721)

using chloroform/methanol/acetic acid (65:25:9, by vol.) as solvent. The phospholipids were detected by autoradiography.

Fate of exogenous LPE integrated into cells. Bacteria harvested from the exponential phase of growth were suspended in 2 ml 10 mM-HEPES buffer (pH 7.5) and incubated for 60 min with 2 ml liposomes in the presence of 20 mM-CaCl₂ (final concentration). Liposomes were prepared with unlabelled total phospholipids extracted from PM61 and [³H]LPE in a ratio 1:2.5 (based on phosphorus determination). The liposome-cell interaction was interrupted by addition of 20 mM-EDTA and the bacteria were separated from non-integrated liposomes by centrifugation through 10% (w/v) Ficoll in 10 mM-HEPES (pH 7.5), as described by Jones & Osborn (1977). These cells were washed successively with prewarmed 1 mM-MgCl₂ in 10 mM-HEPES (pH 7.5) and with complete medium before being suspended in 150 ml of prewarmed complete medium and grown at 37 °C in a gyratory shaker. Samples were withdrawn at intervals; phospholipids were extracted directly from the cells, chromatographed and the radioactivity of individual phospholipids measured as described above.

Lysophospholipase assays. Bacteria in the exponential growth phase were harvested (7000 g, 5 min, 4 °C), washed with cold 10 mM-HEPES containing 1% (w/v) NaCl (pH 7.5) and suspended in 10 mM-HEPES (pH 7.5). The cells were sonicated in ice with a Sonifier B-12 (Branson Sonic Power Co., Soest, The Netherlands) for five periods of 30 s at 45 W. Unbroken cells were removed by centrifugation (1500 g, 10 min, 4 °C) and the supernatant (crude extract) was used as a source of enzyme. The incubation mixture consisted of 0.2 ml LPE dispersion prepared by sonication in a water-bath sonicator (30 s) in either 50 mM-Tris/maleate buffer (pH 6.0) for 2-acylLPE, or in 10 mM-HEPES buffer (pH 7.5) for 1-acylLPE, and 0.5 ml crude extract. The reaction was carried out at 37 °C for 60 min and stopped by phospholipid extraction. The radioactivity of individual phospholipids recovered on silica gel plates after thin-layer chromatography was determined.

SH-group inhibitor treatment. Cells harvested from the exponential growth phase were suspended in 10 ml 10 mM-HEPES buffer (pH 7.5) containing either 1 mM-*p*-hydroxymercuribenzoate (Fluka, Buchs, Switzerland) or 1 mM-*p*-chloromercuriphenylsulphonate (Sigma) and incubated at 37 °C for 30 min.

RESULTS

Identification of the LPE from PM61

Analysis of the molecular species of LPE extracted and isolated from early stationary phase PM61 cells showed an enrichment in unsaturated and cyclopropane fatty acids as compared to the PE and 1-acylLPE obtained after phospholipase A₂ treatment of PE (Table 1). Considering the position specificity of fatty acids in the phospholipid molecules, i.e. saturated fatty acids in position 1 and unsaturated and cyclopropane fatty acids in position 2 (Raetz, 1978), such a result suggests the presence of a 2-acylLPE in PM61. This was confirmed by showing that LPE isolated from ³²P-labelled PM61 cells was hydrolysed by the phospholipase A₂ of *Naja naja* (Fig. 1) indicating the presence of a 2-acylLPE, produced in PM61 by a phospholipase A₁.

Table 1. Fatty acid composition of lysophosphatidylethanolamine isolated from PM61 (*envC*) and of 1-acyllysophosphatidylethanolamine obtained from phosphatidylethanolamine after phospholipase A₂ treatment

The results are mean values from two experiments. tr, trace.

Fatty acid	Percentage of total fatty acid* in:		
	LPE	PE + A ₂ *	PE
14:0	3.0	4.2	5.4
16:0	19.0	83.5	53.9
16:1	40.0	4.0	27.0
C ₁₇ Δ	28.0	5.2	9.3
18:0	tr	0.5	tr
18:1	10.0	2.5	4.4
Unsaturated (U)	50.0	6.5	31.4
Saturated (S)	22.0	88.2	59.3
Cyclopropane (Cy)	28.0	5.2	9.3
(Cy + U)/S	3.5	0.1	0.7

* PE hydrolysed by phospholipase A₂.

Fig. 1. Hydrolysis of lysophosphatidylethanolamine and phosphatidylethanolamine by phospholipase A_2 . [^{32}P]LPE and [^{32}P]PE isolated from PM61 cells were incubated with phospholipase A_2 as indicated in Methods. After incubation, the phospholipids were extracted, separated by thin-layer chromatography and detected by autoradiography. 1, Untreated LPE; 2, hydrolysed LPE; 3, untreated PE; 4, hydrolysed PE.

Effect of SH-group inhibitors on LPE accumulation

Large quantities of LPE for analytical use were isolated by incubating bacteria in HEPES buffer containing an SH-group inhibitor, *p*HMB or *p*CMPS, at 37 °C. In the presence of *p*HMB, we found that LPE accumulation in P678 and PM61 attained, respectively, 8.6% and 12.4% of the total phospholipid content (Table 2). Similar results, not shown here, were obtained with *p*CMPS. Moreover, the LPE obtained after *p*HMB treatment was a 2-acylLPE, as indicated by its susceptibility to phospholipase A_2 and its high proportion of unsaturated fatty acids (data not shown). The content of unsaturated fatty acids in phosphatidylglycerol, diphosphatidylglycerol, and particularly phosphatidylethanolamine is higher in untreated PM61 than in P678. These differences are not due to the incubation of cells in the HEPES buffer, since phospholipids directly extracted from PM61 are also more unsaturated than those of P678 (data not shown). It should be noted that the treatment of bacteria with SH-group inhibitors could also be a useful test for the detection of phospholipase A-deficient strains. Indeed, the mutant S17 (*pldA*), unlike its parent strain S15, when incubated in the presence of *p*HMB produced no detectable LPE (unpublished result).

Fate of the liposomal LPE integrated into cells

Exogenous LPE was integrated into cells via liposome-cell interaction. The increase of the [3H]PE:[3H]LPE ratio in the cells showed that liposomal LPE was converted to PE by a reacylation process (Table 3). Although this ratio was higher in P678 than in PM61, it is not clear what kind of process is altered in the mutant, since reacylation and lysophospholipase activities take place simultaneously in intact cells of both strains.

LPE degradation by P678 and PM61 cell extracts

Incubation of LPE with crude cell extracts did not induce conversion of LPE to PE (i.e. reacylation) under our experimental conditions. Deacylation was assessed by hydrolysis of LPE: 2-acylLPE was hydrolysed faster in P678 than in PM61. This hydrolysis was slightly stimulated

Table 2. Effect of *p*-hydroxymercuribenzoate on the phospholipid composition of P678 and PM61

Lysophosphatidylserine (LPS), lysophosphatidylethanolamine (LPE), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) were extracted from untreated cells (Expt 1), from cells incubated for 30 min in 10 mM-HEPES pH 7.5 at 0 °C (Expt 2) or at 37 °C (Expt 3), and from cells incubated as in Expt 3 but with 1 mM-*p*-hydroxymercuribenzoate (Expt 4). In each case the results represent mean values from three experiments. tr, trace; –, not detectable.

Strain	Expt	Percentage of total [³² P]phospholipids					
		LPS	LPE	PS	PE	PG	DPG
P678	1	–	–	–	74.4	14.5	11.1
	2	–	tr	–	74.3	14.8	10.9
	3	–	1.9	–	72.3	8.7	17.1
	4	tr	8.6	–	65.8	13.3	12.3
PM61	1	–	1.7	tr	72.7	11.0	14.6
	2	–	2.1	tr	72.8	10.0	15.1
	3	tr	5.5	tr	71.4	4.8	18.3
	4	–	12.4	tr	64.1	8.3	15.2

Table 3. Fate of liposomal lysophosphatidylethanolamine integrated into P678 and PM61 cells

Cells were grown and exposed to liposomes containing [³H]LPE as described in Methods. Samples were withdrawn immediately after resuspension in growth medium, and after 60 min and 12 h of incubation at 37 °C. Cellular phospholipids were extracted and analysed by thin-layer chromatography. The results are mean values from two experiments for P678 and three for PM61; the values in parentheses represent the PE/LPE ratios.

Strain	[³ H]Phospholipid	Radiolabelled PE and LPE [nmol (mg cell protein) ⁻¹] at:		
		0	60 min	12 h
P678	PE	12.1	4.4	1.35
	LPE	1.0	0.4	0.11
		(12.0)	(11.0)	(12.0)
PM61	PE	22.0	14.1	2.25
	LPE	9.6	5.3	0.45
		(2.3)	(2.7)	(5.0)

Table 4. Hydrolysis of lysophosphatidylethanolamines by cell homogenates of P678 and PM61

Supernatants of sonicated cells were incubated either with 2-acylLPE or with 1-acylLPE (40 nmol) at 37 °C for 60 min. Where indicated, the reaction mixture contained 10 mM-CaCl₂. Undigested substrate was separated by thin-layer chromatography and its radioactivity determined. Results are mean values from two experiments with duplicate samples.

Strain	Percentage hydrolysis (mg protein) ⁻¹ h ⁻¹					
	2-acylLPE				1-acylLPE	
	–Ca ²⁺	+Ca ²⁺	–pHMB	+pHMB*	–Ca ²⁺	+Ca ²⁺
P678	12.4	14.0	9.2	9.2	9.0	10.2
PM61	6.7	8.0	6.0	7.4	4.5	5.0

* Cells preincubated in 1 mM-*p*-hydroxymercuribenzoate (pHMB) for 10 min at 37 °C were sonicated and the supernatants incubated with 16 nmol substrate in presence of pHMB.

by calcium, although this cation was not absolutely required (Table 4). Furthermore, LPE hydrolysis was not inhibited by pHMB and it was not specific for the 2-acylLPE, since 1-acylLPE prepared from PE after phospholipase A₂ treatment was hydrolysed as well as 2-acylLPE. However, 1-acylLPE was hydrolysed less efficiently by PM61 than by P678 extracts.

These observations would mean that either only one enzyme participates in the hydrolysis of 1- and 2-acylLPE, or if two distinct enzymes are involved, both activities are modified in PM61.

DISCUSSION

Lysophospholipids, and especially LPE, do not accumulate in more than trace amounts in growing cells of *E. coli* because they are quickly utilized as substrates for enzyme reactions involving reacylase and lysophospholipase activities (Doi *et al.*, 1972; Doi & Nojima, 1975; Homma *et al.*, 1981). Together with phospholipase A these enzyme activities constitute a deacylation–reacylation cycle, which is shown schematically in Fig. 2. Phospholipase A₁ causes degradation of PE to LPE (step 1) which can be either hydrolysed to glycerylphosphorylethanolamine (GPE) and fatty acid (FA) by a lysophospholipase (step 2) or converted to PE by a reacylase activity (step 3). It should be pointed out that the reacylation process leading to the neosynthesis of PE from LPE could be achieved either by addition of a free fatty acid or by transfer of a fatty acid from a phospholipid or even a lysophospholipid (transacylation) to LPE. Perturbation of the cycle at any step could induce a modification of the phospholipid composition and, as in PM61, accumulation of LPE.

For this reason, studies on the functioning of the deacylation–reacylation cycle are important for the understanding of the role of phospholipid metabolism in cellular physiology. One can imagine that this cycle is involved in the rearrangement of cellular components following perturbation, and in the control of membrane growth.

In a previous paper (Michel & Starka, 1979) we showed that PE was converted to LPE more rapidly in PM61 than in its parent P678. This led us to suggest that LPE accumulation is the consequence of an abnormal phospholipase A₁ activation in PM61. However, LPE accumulation could also involve other enzyme systems using LPE as substrate, i.e. reacylase and lysophospholipase. Indeed, if one considers the deacylation–reacylation cycle (Fig. 2), LPE accumulation might result from phospholipase A₁ activation (step 1), lysophospholipase deficiency (step 2) or reacylase deficiency (step 3).

It has been shown that in *E. coli*, LPE degradation can be achieved either by the lysophospholipase activity of the detergent-resistant phospholipase A which is calcium-dependent (Nishijima *et al.*, 1977) or by a specific lysophospholipase showing no dependence on calcium (Doi & Nojima, 1975). We found that LPE hydrolysis by crude cell extracts of the two strains studied is neither calcium dependent nor inhibited by SH-group inhibitors and acts as well on 1-acylLPE as on 2-acylLPE. Therefore, it seems likely that the enzyme activity detected in our experiments is probably a specific lysophospholipase rather than a lysophospholipase activity of the detergent-resistant phospholipase A. Furthermore, it cannot be excluded that in *E. coli*, a single enzyme is responsible for the hydrolysis of the 1- and 2-acyl ester bonds in lysophospholipid molecules. This possibility is also supported by the results of Doi & Nojima (1975).

Although we found diminished lysophospholipase activity in PM61, it is still not clear if LPE

Fig. 2. Deacylation–reacylation cycle in *E. coli*. PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; FA, fatty acid; GPE, glycerylphosphorylethanolamine. (1) Phospholipase, (2) lysophospholipase, (3) reacylase.

accumulation is the result of phospholipase A₁ activation and/or lysophospholipase and reacylase deficiencies. It would be necessary to block two of the three steps of the deacylation–reacylation cycle to test each of the possibilities, at least for steps (1) and (3). Nevertheless, our results show conclusively that the fate of LPE is modified in *envC* since the [³H]PE:[³H]LPE ratio found in growing bacteria having integrated exogenous [³H]LPE via liposome–cell interaction is lower in the mutant than in the parent.

Further studies will be required to check if the apparent deficiency of the deacylation–reacylation cycle in PM61 is directly involved in envelope alterations of this mutant, or if a regulatory control system triggered by envelope alterations acts on the enzyme activities involved in this cycle, in order to provide for some proteins a lipid environment more favourable to their functions. According to the hypothesis of Gerritsen *et al.* (1979), the presence of unsaturated lysophospholipid and the increase of unsaturated PE in PM61 could be indicative of the second possibility.

We are currently pursuing other studies in order to clarify the relationship of *envC* and LPE accumulation. Transduction of the mutant gene *envC* out of PM61 by P1 phage grown on CBK 286 *cysE::Tn5* led to disappearance of chains and decrease of LPE. These preliminary results suggest that *envC* could be involved both in cellular division and in the functioning of the deacylation–reacylation cycle.

We are grateful to B. Entressangles and D. Karibian for helpful advice and discussions.

REFERENCES

- ABE, M., OKAMOTO, N., DOI, O. & NOJIMA, S. (1974). Genetic mapping of the locus for detergent-resistant phospholipase A (*pldA*) in *E. coli* K12. *Journal of Bacteriology* **119**, 543–546.
- AMES, B. & DUBIN, D. T. (1960). Role of polyamines in the neutralisation of bacteriophage DNA. *Journal of Biological Chemistry* **235**, 769–775.
- BIERBAUM, T. J., BOUMA, S. R. & HUESTIS, W. J. (1979). A mechanism of erythrocytes lysis by lysophosphatidylcholine. *Biochimica et biophysica acta* **555**, 102–110.
- CULLIS, P. R. & DE KRUIFF, B. (1978). Polymorphic phase behaviour of lipid mixtures as detected by ³¹P-NMR. Evidence that cholesterol may destabilize bilayer structure in membrane systems containing phosphatidylethanolamine. *Biochimica et biophysica acta* **551**, 248–259.
- CULLIS, P. R. & DE KRUIFF, B. (1979). Lipid polymorphism and the functional roles of lipids in biological membranes. *Biochimica et biophysica acta* **559**, 399–420.
- DOI, O. & NOJIMA, S. (1975). Lysophospholipase of *E. coli*. *Journal of Biological Chemistry* **250**, 5208–5214.
- DOI, O. & NOJIMA, S. (1976). Nature of *E. coli* mutants deficient in detergent-resistant and/or detergent-sensitive phospholipase A. *Journal of Biochemistry* **80**, 1247–1248.
- DOI, O., OHKI, M. & NOJIMA, S. (1972). Two kinds of phospholipase A and lysophospholipase in *Escherichia coli*. *Biochimica et biophysica acta* **260**, 244–258.
- DUCKWORTH, D. H., BEVERS, E., VERKLEIJ, A. J., OP DEN KAMP, J. A. F. & VAN DEENEN, L. L. M. (1974). Action of phospholipase A₂ and phospholipase C on *E. coli*. *Archives of Biochemistry and Biophysics* **165**, 379–387.
- GERRITSEN, W. J., VAN ZOELN, E. J. J., VERKLEIJ, A. J., DE KRUIFF, B. & VAN DEENEN, L. L. M. (1979). A ¹³C-NMR method for determination of the transbilayer distribution of phosphatidylcholine in large unilamellar, protein free and protein containing vesicles. *Biochimica et biophysica acta* **551**, 248–259.
- HARDAWAY, K. L. & BULLER, C. S. (1979). Effect of EDTA on phospholipid and outer membrane function in *E. coli*. *Journal of Bacteriology* **137**, 62–68.
- HOMMA, H., NISHIJIMA, M., KOBAYASHI, T., OKUYAMA, H. & NOJIMA, S. (1981). Incorporation and metabolism of 2-acyllysophospholipid by *E. coli* K12. *Biochimica et biophysica acta* **663**, 1–13.
- JONES, N. C. & OSBORN, M. J. (1977). Interaction of *S. typhimurium* with phospholipid vesicles. Incorporation of exogenous lipids into intact cells. *Journal of Biological Chemistry* **252**, 7398–7404.
- KANESHIRO, T. & MARR, A. G. (1961). *cis*-9,10-Methylenehexadecanoic acid from the phospholipids of *E. coli*. *Journal of Biological Chemistry* **236**, 2615–2619.
- LUZZATI, V., GULIK KRZYWICKI, T. & TARDIEU, A. (1968). Polymorphism of lecithins. *Nature, London* **218**, 1031–1034.
- MICHEL, G. P. F. (1979). The changes in cell size and phospholipid composition during growth of a chain-forming *envC* mutant of *E. coli*. *FEBS Letters* **100**, 258–260.
- MICHEL, G. P. F. & STARKA, J. (1979). Phospholipase A activity with integrated phospholipid vesicles in intact cells of an envelope mutant of *E. coli*. *FEBS Letters* **108**, 261–265.
- MOOKERJEE, S. (1979). Effect of lysolecithin in solubilizing membrane-bound galactosyltransferases. *Canadian Journal of Biochemistry* **57**, 66–71.
- NISHIJIMA, M., NAKAIKE, S., TAMORI, Y. & NOJIMA, S. (1977). Detergent-resistant phospholipase A of *E. coli* K12. Purification and properties. *European Journal of Biochemistry* **73**, 115–124.
- OHKI, M., DOI, O. & NOJIMA, S. (1972). Mutants of *E.*

- coli* K12 deficient for detergent-resistant phospholipase A. *Journal of Bacteriology* **110**, 864–869.
- PROULX, P. R. & VAN DEENEN, L. L. M. (1966). Acylation of lysophospholipids by *E. coli*. *Biochimica et biophysica acta* **125**, 591–593.
- RAETZ, C. R. H. (1978). Enzymology, genetics and regulation of membrane phospholipid synthesis in *E. coli*. *Microbiological Reviews* **42**, 614–659.
- RODOLAKIS, A., CASSE, F. & STARKA, J. (1974). Morphological mutant of *E. coli* K12. *Molecular and General Genetics* **130**, 177–181.
- SCANDELLA, C. J. & KORNBERG, A. (1971). A membrane-bound phospholipase purified from *E. coli*. *Biochemistry* **10**, 4447–4456.
- STARKA, J. & MORAVOVA, J. (1970). Phospholipids and cellular division of *Escherichia coli*. *Journal of General Microbiology* **60**, 251–257.
- TAMORI, Y., NISHIJIMA, M. & NOJIMA, S. (1979). Properties of purified detergent-resistant phospholipase A of *E. coli*. Inactivation and protection with detergents and phospholipids. *Journal of Biochemistry* **86**, 1129–1138.
- VOS, M. M., OP DEN KAMP, J. A. F., BEQUERDITE QUAGLIATA, S. & ELSBACH, P. (1978). Acylation of monoacylglycerophosphoethanolamine in the inner and the outer membranes of the envelope of an *E. coli* K12 strain and its phospholipase A-deficient mutant. *Biochimica et biophysica acta* **508**, 165–173.