Phospholipid Composition and Membrane Function in Phosphatidylserine Decarboxylase Mutants of Escherichia coli*

(Received for publication, October 11, 1977)

Edward Hawrot‡ and Eugene P. Kennedy

From the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115

Our understanding of membrane biogenesis and function has been greatly enriched by studies of Escherichia coli mutants defective in membrane lipid synthesis (for recent reviews, see Refs. 1 and 2). The isolation of fatty acid auxotrophs has permitted extensive manipulation of the membrane fatty acids (3, 4). These auxotrophs have been widely used to examine the relationship between the physical properties of membrane lipids and membrane-localized physiological processes (2). A similar study of genetically produced alterations in the polar groups of E. coli membrane phospholipids is just now beginning (5-8). Mutants are now available with blocks in the synthesis of a given enzyme is temperature-sensitive. Based on the kinetics of heat inactivation of phosphatidylserine decarboxylase activity in extracts, intact nongrowing cells, and in growing cells, it appears that the enzyme newly synthesized at 42°C is more thermostable in vivo than enzyme molecules previously inserted into the membrane at the lower temperature. Thus, the older, stable enzymatic activity must be diluted during growth before physiological effects are observed.

Temperature-sensitive conditional lethal mutants in phosphatidylserine decarboxylase (psd) accumulate large amounts of phosphatidylserine under nonpermissive conditions (42°C) prior to cell death. In addition, the ratio of cardiolipin to phosphatidylylglycerol is increased. At an intermediate temperature (37°C), high levels of phosphatidylserine can be maintained with little effect on cell growth or viability. Under these conditions, both the rate of induction and the function of the laclose transport system are normal. At 42°C addition of Mg²⁺ or Ca²⁺ to mutant cultures produces a partial phenotypic suppression. Growth is prolonged and the filaments normally present at 42°C do not form.

Upon transfer to the nonpermissive temperature, there is a considerable lag before accumulation of phosphatidylserine begins and the growth rate is affected. Based on the kinetics of heat inactivation of phosphatidylserine decarboxylase activity in extracts, intact nongrowing cells, and in growing cells, it appears that the enzyme newly synthesized at 42°C is more thermostable in vivo than enzyme molecules previously inserted into the membrane at the lower temperature. Thus, the older, stable enzymatic activity must be diluted during growth before physiological effects are observed.

In this study, we examine in detail the alteration in phospholipid composition produced by the psd defect. In addition, the effects of phosphatidylserine accumulation on growth, viability, and cell morphology have been studied. A partial characterization of the thermostability of one of the psd mutants (psd2) suggests an unusual form of enzyme temperature sensitivity. Although the enzyme is indeed thermostable, the mutant phenotype is similar to that seen in mutants in which the synthesis of a given enzyme is temperature-sensitive. Finally, the effects of phosphatidylserine accumulation on the induction of a membrane transport protein (lac gene product) and on the function of several transport systems are described.

EXPERIMENTAL PROCEDURES

Materials—Culture media were obtained from Difco, Detroit. L-[³H]Proline, L-¹⁴C]glutamine, and ³²P, were supplied by New England Nuclear. Boston. Methyl-a-D-[¹⁴C]glucose (a-methyl glucoside) was obtained from Amersham/Searle, Chicago. β-D-[³H]Galactosyl-1-thio-D-β-galactoside (thiogalactoside) was prepared by the oxidation of unlabeled thiogalactoside with galactose oxidase, followed by reduction with tritiated borohydride, according to the general procedures described by Kennedy et al. (11). Phosphatidylserine was prepared enzymatically as previously described (10). Triton X-100 (octylphenoxypolyethoxyethanol) was a product of Rohm and Haas, Philadelphia.

Media and Strains—Bacterial cultures were grown in a low phosphate medium, Medium 56-LP (12) containing 0.3 mM phosphate when efficient labeling of cells with ³²P, was required. For other experiments, cells were grown in L broth (13). Carbon cource and supplements were added as indicated.

Bacterial strains used in this study were all derivatives of E. coli K-12 and are listed in Table I. In general, all experiments compared temperature-sensitive psd mutants with isogenic wild type (psd+) transductant strains. The turbidity of bacterial cultures was measured with a Coleman Jr. spectrophotometer at 550 nm.

Analysis of Phospholipids—Phospholipids were extracted from bacterial cultures uniformly labeled with ³²P, by means of the Ames modification (14) of the Bligh and Dyer procedure (15). The phospholipid classes were then separated by thin layer chromatography as described previously (6). Medical x-ray film (Kodak N54T and RP/R-54) was used for radioautography of the chromatograms.

Transport Assays—The release of o-nitrophenol from o-nitrophenyl-β-D-galactoside was used as one measure of lactose transport function since transport of this β-galactoside across the membrane is the rate-limiting step in the release of o-nitrophenol via the action of intracellular β-galactosidase (16, 17). As an index of the nonspecific leakiness of membranes, hydrolysis of o-nitrophenyl-β-D-galactoside was measured in cells incubated in the presence of excess thiogalactoside, a substrate that inhibits the transport of o-nitrophenyl-β-D-galactoside mediated by the lac permease.

Portions (0.5 ml) of the cultures were taken directly from the growth flasks and added to 1.5 ml of medium containing chloramphenicol (100 µg/ml) and o-nitrophenyl-β-D-galactoside (2 mM). In control experiments, thiogalactoside (10 mM) was included in the assay mix. After a 10-min incubation at 37°C, 3.5 ml of 1 N K₂CO₃...
that phosphatidylserine decarboxylase in this strain is rate-limiting for phosphatidylethanolamine synthesis even at this temperature. The phenotypic expression of the psd4 mutation at 30°C is apparently quite sensitive to the genetic background. Different levels of phosphatidylserine accumulation at 30°C, varying from 2 to 20 mol % of total lipid, have been observed in different strains into which the psd4 mutation has been transduced.

In addition to the accumulation of phosphatidylserine by the psd mutants, there is also an increase in the ratio of cardiolipin to phosphatidylglycerol as compared to revertant and wild type strains. For strains EH150 psd2 and EH160 psd3 grown at 42°C, this ratio is increased by a factor of 2 or 3 over and above the normal 2-fold increase seen upon transfer of control cultures from 30°C to 42°C (Table II and see Fig. 4). Strain EH170 psd4, with a higher level of phosphatidylserine accumulation at 42°C, shows a correspondingly greater increase (6- to 7-fold) in the ratio of cardiolipin to phosphatidylglycerol in a number of experiments.

### Table I

<table>
<thead>
<tr>
<th>Strains of Escherichia coli K-12</th>
<th>Relevant characteristics</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES4</td>
<td>purA</td>
<td>CGSC*</td>
</tr>
<tr>
<td>EH150</td>
<td>psd3 derivative (temperature-sensitive phosphatidylserine decarboxylase) of ES4</td>
<td>Ref. 8</td>
</tr>
<tr>
<td>EH150-RI</td>
<td>Spontaneous revertant of EH150</td>
<td>See text that grows normally at 42°C</td>
</tr>
<tr>
<td>EH160</td>
<td>psd4 derivative (temperature-sensitive phosphatidylserine decarboxylase) of ES4</td>
<td>Ref. 8</td>
</tr>
<tr>
<td>EH170</td>
<td>psd4 derivative (temperature-sensitive phosphatidylserine decarboxylase) of ES4</td>
<td>Ref. 8</td>
</tr>
<tr>
<td>EH170-RA3</td>
<td>Spontaneous revertant of EH170</td>
<td>See text that grows normally at 42°C</td>
</tr>
<tr>
<td>M2508</td>
<td>Hfr, metB, metA, rel</td>
<td>CGSC</td>
</tr>
<tr>
<td>EH250</td>
<td>melA', psd2 transductant of M2508</td>
<td>Ref. 8</td>
</tr>
<tr>
<td>EH251</td>
<td>melA', psd4 transductant of M2508</td>
<td>Ref. 8</td>
</tr>
<tr>
<td>W3110</td>
<td>Prototroph</td>
<td>D. Franekel</td>
</tr>
<tr>
<td>EH450</td>
<td>psd2 transductant of W3110</td>
<td>Ref. 8</td>
</tr>
<tr>
<td>EH451, EH471</td>
<td>psd4 transductant of W3110</td>
<td>Ref. 8</td>
</tr>
<tr>
<td>EH470</td>
<td>psd4 transductant of W3110</td>
<td>Ref. 8</td>
</tr>
</tbody>
</table>

* E. coli Genetic Stock Center, Yale University, New Haven, Conn.

was added and the cells were removed by centrifugation. The formation of α-nitrophenol was measured in a Gilford spectrophotometer at 420 nm.

The rates of induction of lactose transport and β-galactosidase activity were determined as described by Nunn and Cronan (17). After a 17-min induction period with thiospropyl-β-galactoside, portions (0.5 ml) of the cultures were transferred to 0.5 ml of medium containing chloramphenicol and α-nitrophenyl-β-D-galactoside as described above. Controls with added 10 mm thiodigalactoside were also included. For assay of β-galactosidase activity, cells were first disrupted by addition of sodium dodecyl sulfate and CHCl3 (18). After incubation with substrate for 17 min at 37°C, 1.5 ml of 1 M K2CO3 was added and the extent of α-nitrophenyl-β-D-galactoside hydrolysis was determined as described above.

In addition, the function of the lactose system, as well as that of three other transport systems, was measured by the uptake and accumulation of radioactive substrates. Cells (about 1010) suspended in 2 ml, were incubated at 37°C for 5 min before the substrate was added in a volume of 0.3 ml. Samples (0.5 ml) were withdrawn, filtered on 95-mm nitrocellulose filters (0.45 μm, Millipore Corp.), and quickly washed twice with 5 ml of Medium 56-5P at 25°C. Controls to determine the level of nonspecific uptake were carried out by the addition of high levels of unlabeled substrate to the system. Radioactivity trapped on the filters was determined by liquid scintillation counting.

**Other Procedures** Phosphatidylserine decarboxylase activity was assayed as previously described (6, 19). Extracts were prepared by sonic irradiation with either an MSE-100 ultrasonic disintegrator or a Branson Sonifier. Protein was determined by the method of Lowry et al. (20).

### RESULTS

**Alternations in Phospholipid Composition in psd Mutant Strains**—As previously reported (6), the most dramatic change in the phospholipid composition of psd mutant strains is the accumulation of high levels of phosphatidylserine at nonpermissive temperatures. As indicated in Table II, after 4 h growth at 42°C, all three temperature-sensitive mutants (psd2, psd3, and psd4) accumulate phosphatidylserine to levels at least 50-fold higher than normal when compared to revertant strains. The accumulation of phosphatidylserine, in general, corresponds to the decrease of phosphatidylethanolamine as expected from the pathway of biosynthesis proposed for this organism (10). The psd4 strain, EH170, accumulates an intermediate level of phosphatidylserine at 30°C suggesting...
Phosphatidylserine Decarboxylase Mutants of E. coli

As reported previously (22), cell division is also affected in psd mutant cells under conditions of phosphatidylserine accumulation such as in the experiment of Fig. 1. Chains of unseparated mutant cells form upon growth at 42°C (Fig. 2). Separations in these filaments are often irregularly spaced along the length of the chain. The chain formation of mutant psd cultures may explain a small part, but certainly not all, of the observed decrease in viable cell count seen in Fig. 1B where the viability fell by a factor of 10^4 after 21 h. In some cases, clumping of cells into aggregates also occurs. This is especially true of the mutant psd transductants of strain W3110. The reason for this aggregation has not been explored further.

Time Course of Effects on Phospholipid Synthesis—Since the temperature-sensitive psd mutant, EH250, continues to grow in liquid culture for a considerable length of time at 42°C (Fig. 1), it was of obvious importance to determine the factors governing the relatively long delay in lethal effect. It would be especially useful to know the time at which significant levels of phosphatidylserine first appear in mutant cells after a shift to 42°C, since the physiological effects of a defect in phosphatidylserine decarboxylase would not be expected to appear prior to this accumulation and might possibly appear only some time later.

The synthesis of phosphatidylethanolamine and phosphatidylserine was followed in EH250 psd2 and EH251 psd+ grown as described in the experiment of Fig. 1 but labeled with ^32P, from the time of shift to 42°C. As indicated in Fig. 3A, the earliest accumulation of phosphatidylserine occurred only after 3 h of growth at 42°C. Correspondingly, incorporation of ^32P into phosphatidylethanolamine was normal over the initial 3 h. With further incubation at 42°C, synthesis of phosphatidylethanolamine was curtailed (Fig. 3B), whereas phosphatidylserine continued to accumulate. After 3 h at 42°C, the total synthesis of phospholipid per ml of culture was markedly reduced in rate (Fig. 3C). Both phospholipid (Fig. 3C) and protein (data not shown) synthesis were affected somewhat prior to the effect on culture turbidity (Fig. 1A).

The apparent delayed response of the turbidity of the culture grow at a normal rate for approximately three generations (Fig. 1). The number of generations of growth observed after shift to 42°C varied with the medium. In general, richer media allowed longer growth at 42°C. As Fig. 1A indicates, after about 5 h of growth at 42°C, growth declined in rate and eventually came to a complete halt. Macromolecular synthesis in mutant cells at 42°C, as measured by net synthesis of DNA, RNA, protein, and phospholipid follows the same general time course as the increase in culture turbidity (data not shown). Upon continued incubation of cultures at 42°C, a sharp drop in viability occurs (Fig. 1B).

**Fig. 1.** Growth and viability of mutant strain EH250 psd2 at 42°C. Mutant, EH250 psd2, and isogenic control, EH251 psd+, cultures were grown at 30°C in Medium 56-LP containing 1% glycerol and supplemented with methionine. At a cell density of 2.5 x 10^7/ml, the cultures were transferred to an incubator at 42°C. Panel A, bacterial growth at 42°C was measured as the increase in turbidity at 650 nm. Panel B, viable cell count was determined after serial dilution in Medium E (21) by plating diluted samples on plates of supplemented Medium E. Colony formers were scored after incubation at 30°C for 36 to 48 h. The relative viability was normalized by dividing the viable count by the culture turbidity (A). A, EH251 psd+; ▲, EH250 psd2.

**Fig. 2.** Light microscopy of mutant EH170 psd4 cells at 42°C. A culture of strain EH170 psd4 was grown at 42°C in L broth into stationary phase. A sample was air-dried, heat-fixed, and stained by the Gram procedure. The length of a single bacterium is about 2 μm.
Fig. 3. Synthesis of phosphatidylserine and phosphatidylethanolamine in strains EH250 psd2 and EH251 psd+ at 42°C. Bacterial cultures were grown at 30°C in Medium 56-I.P in parallel to those described in Fig. 1. At the time of shift to 42°C, $^{32}$P was added at a final specific activity of 11 Ci/mol. At the appropriate times, samples (0.8 ml) of the cultures were removed and the phospholipids were extracted. One-fourth of this extract was used for the analysis of phospholipids by thin layer chromatography. Panels A and B, the amount of $^{32}$P incorporated into phosphatidylserine and phosphatidylethanolamine was determined as described in Table II. Significant accumulation of phosphatidylserine at the first time point could have been easily detected with the analytical procedures that were used. Panel C, synthesis of total phospholipid. ▲, EH251 psd+; ●, EH250 psd2.

Fig. 4. Incorporation of $^{32}$P into the individual phospholipid components upon transfer of EH250 psd2 cells to 42°C. The bacterial phospholipids were labeled with $^{32}$P, extracted, and analyzed as described in Fig. 3. The radioactivity in each phospholipid is presented as the percentage of the $^{32}$P incorporated into total phospholipid at each given time point. The values for cardiolipin have been corrected for its content of 2 mol of phosphate/mol. PG, phosphatidylglycerol; CL, cardiolipin; PS, phosphatidylserine; PE, phosphatidylethanolamine. ●, EH251 psd+; ▲, EH250 psd2.

Heat Inactivation of Mutant Phosphatidylserine Decarboxylase—In order to gain further information on the lag in physiological effect observed in cells of mutant psd2 upon shift to 42°C (Fig. 1), it was necessary to determine the extent of inactivation of phosphatidylserine decarboxylase in vivo at nonpermissive temperature. Mutant psd2 cells, in the absence of growth, are not killed to any greater extent than wild type cells even after a 22-h exposure to a temperature of 42°C (data not shown). This is in marked contrast to the killing observed during growth as seen in Fig. 1.

Cells of mutant psd2 were incubated at 42°C for various periods in the presence of chloramphenicol, added to prevent growth. When phosphatidylserine decarboxylase was then assayed in extracts of these cells, it was found that the activity had decreased only at a very slow rate (Fig. 5). The half-life of inactivation of the enzyme in intact cells was on the order of 12 h. This rate is much too slow to account for the physiological effects observed in vivo. Furthermore, the mutant psd2 decarboxylase bound to the membrane in cell-free extracts is resistant to heat inactivation, like the enzyme from the originally isolated "silent" mutant psd1 (6). Although the mutant psd2 phosphatidylserine decarboxylase is present in membrane-bound form, the rate of inactivation is no more sensitive to heat inactivation than wild type enzyme as long as no detergent is added (Fig. 6). However, when the heat treatment is carried out in the presence of 0.1% Triton X-100, the mutant psd2 activity is rapidly inactivated with a half-life on the order of 25 s while the half-life for inactivation of wild type enzyme is about 80 min. The detergent effect in extracts, together with the results from enzyme inactivation in whole cells, suggest that the mutant psd2 phosphatidylserine decarboxylase is resistant to heat inactivation when membrane-bound. Once extracted from the membrane, however, it is markedly thermolabile.

Mutant (psd2) levels of activity are similar whether or not extracts are previously incubated in Triton at 28°C (data not shown). This is true for the glycerol-containing assay system used in the heat-inactivation studies. Therefore, the psd2 mutation does not produce Triton sensitivity per se (i.e., at all temperatures).

The physiological lag in accumulation of phosphatidylserine can now be explained by the following hypothesis. It would appear that mutant psd2 phosphatidylserine decarboxylase synthesized at the permissive temperature in vivo is incorpo-
Phosphatidylserine Decarboxylase Mutants of E. coli

Fig. 5 (left). Heat inactivation of phosphatidylserine decarboxylase in whole cells of EH450 psd2 and EH451 psd+. Bacterial cultures were grown at 30°C to a cell density of 5 × 10^8/ml. Cells were harvested and resuspended at 10^7/ml in Medium 56, containing chloramphenicol (115 μg/ml). The cell suspension was placed in an incubator at 43°C and at the appropriate times samples (4 ml) were removed and the cells disrupted by sonic irradiation. Phosphatidylserine decarboxylase activity was assayed at 28°C in the presence of 20% glycerol (6, 8). The specific activities of the unheated samples were: 27.1 nmol of CO_2 released/min/mg of protein for EH451 psd- and 10.7 for EH450 psd2. ●, strain EH451 psd-; ▲, strain EH450 psd2.

Fig. 6 (center). Heat inactivation of phosphatidylserine decarboxylase in sonicates of EH450 psd2 and EH451 psd+. Bacterial cultures grown at 30°C were harvested and the cells resuspended at a density of 10^9/ml. Extracts were prepared by sonic disruption. The irreversible heat inactivation of phosphatidylserine decarboxylase was determined by incubating these preparations (about 1.5 mg of protein/ml) rated into the membrane and thus remains nearly completely functional during subsequent incubation at elevated temperatures. On the other hand, the decarboxylase synthesized during incubation at 42°C is presumably rapidly inactivated at some point prior to effective insertion into the membrane. In this way, the overall phenotype should resemble that seen with mutants containing temperature-sensitive defects in enzyme synthesis (25). The appearance of physiological effects is thus dependent on dilution through growth of the stable enzyme to a point where the enzyme function becomes rate-limiting. This hypothesis was tested by determining the rate of decline of phosphatidylserine decarboxylase specific activity (activity/mg of cell protein) as measured in extracts at the low temperature, in growing cells after transfer to the nonpermissive temperature. It was observed that the decline of specific activity was much more rapid in growing cells than in nongrowing cells (compare Fig. 7 and Fig. 5). Since the membrane-bound mutant decarboxylase is fairly resistant to heat treatment of whole cells (Fig. 5), it would appear that the rapid decline of specific activity in growing cells (Fig. 7) is primarily due to a differential heat sensitivity of the newly synthesized mutant phosphatidylserine decarboxylase or to some other effect of growth.

Function of Transport Systems—In order to ascertain the effect of accumulated phosphatidylserine upon membrane function, the activities of several transport systems were examined. For these studies, strain EH470 psd4 was grown at 37°C, a temperature intermediate between the permissive and nonpermissive conditions. Whereas at 30°C, strain EH470 psd4 contains phosphatidylserine at a level of about 2% of the total phospholipid, at 37°C phosphatidylserine is accumulated at 43°C for the appropriate time intervals and with the indicated additions. Samples were then quickly placed in an ice bath and the initial rate of phosphatidylserine decarboxylase activity was assayed at 28°C in the presence of 20% glycerol. The specific activities for the unheated samples were: 21.4 nmol of CO_2 released/min/mg of protein for EH451 psd- and 9.0 for EH450 psd2. ●, EH451 psd-; ○, EH450 psd2; ▲, EH451 psd- + 0.1% Triton X-100; - - - - , EH450 psd2 + 0.1% Triton X-100.

Fig. 7 (right). Specific activity of phosphatidylserine decarboxylase in EH450 psd2 and EH451 psd+ cells grown at 43°C. Bacterial cultures grown at 30°C were shifted to 43°C at a cell density of about 5 × 10^8/ml. At the times indicated, samples of the growing cultures were removed and the cells were disrupted by sonic irradiation after harvesting by centrifugation. Initial rates of phosphatidylserine decarboxylase activity were measured at 28°C in the presence of a protective level of glycerol. The initial specific activity of the EH450 psd2 extract was approximately 20% of that in the wild type extract. ●, EH451 psd-; ▲, EH450 psd2.
grown in 10 4/L h of growth. At the same time, the mutant tidylserine amounted to 31% of the cellular phospholipid after stabilize the enzyme grown in minimal medium in the presence or absence of 10 Mg' or Ca2+ to the growth medium produces a partial phe-

Table IV, the rate of induction of transport function in cells, after normalization to the amount of induced P-galact-

cumulation of intermediate levels of phosphatidylserine has been previously discussed dependence of phenotypic expression of the psd4 mutation on genetic background, suggest that the psd4 mutant decarboxylase is highly sensitive to the membrane environment.

On the other hand, phenotypic suppression may result from a stabilization of the phosphatidylserine-containing membrane. Strain EH450 psd2, when transferred to 42°C in rich medium containing 0.8 mM Mg++, ceases growth after about five doublings. At this point, the phosphatidylserine content of the mutant culture was 48 mol %, MgSO4 was added at a final concentration of 20 mm to a parallel culture upon transfer to 42°C and growth continued for several generations more than in the untreated culture. The final level of phosphatidylserine attained in the Mg+-treated culture was increased to 76 mol %, while phosphatidylethanolamine was reduced to only 5% of the total phospholipid. The addition of Mg++ under these conditions brings about the greatest accumulation of phosphatidylserine so far observed. It is as yet unclear whether the different modes of phenotypic suppression are due to differences in sensitivity of the two mutants psd2 and psd4 or rather due to differences in the effects of Ca++ versus Mg++.

In addition to their effect on increasing growth yield, both Mg++ and Ca++ also serve to suppress the filamentation that is coincident with phosphatidylserine accumulation (Fig. 2).

**TABLE III**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Culture turbidity</th>
<th>Transport of o-nitrophenyl-β-D-galactoside</th>
<th>Control with added thiogalactoside</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mmol/min/mg protein</td>
<td></td>
</tr>
<tr>
<td>EH471 psdA</td>
<td>0.078</td>
<td>603</td>
<td>179</td>
</tr>
<tr>
<td>EH470 psd4</td>
<td>0.070</td>
<td>651</td>
<td>109</td>
</tr>
</tbody>
</table>

**TABLE IV**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Culture turbidity</th>
<th>Transport of o-nitrophenyl-β-D-galactoside</th>
<th>β-Galactosidase activity</th>
<th>Transport of β-galactosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mmol/min/mg protein</td>
<td>β-Galactosidase activity</td>
<td>Transport of β-galactosidase</td>
</tr>
<tr>
<td>EH451 psdA</td>
<td>0.050</td>
<td>222</td>
<td>900</td>
<td>0.23</td>
</tr>
<tr>
<td>EH470 psd4</td>
<td>0.042</td>
<td>172</td>
<td>723</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Phenotypic Suppression—The addition of cations such as Mg++ or Ca++ to the growth medium produces a partial phenotypic suppression of the temperature sensitivity of the psd mutants. In the case of the psd4 mutation, Ca++ appears to stabilize the enzyme in vivo. This is suggested by the results of an experiment where psd4 cells, after transfer to 37°C, were grown in minimal medium in the presence or absence of 10 mm CaCl2. In the control cells, grown without Ca++, phosphatidylserine amounted to 31% of the cellular phospholipid after 4 h of growth. At the same time, the mutant psd4 cells grown in 10 mm CaCl2 had a phosphatidylserine content of only 6%. Moreover, the growth rate was unaffected by this level of Ca++. The fact that Ca++ reduces the level of accumulated phosphatidylserine in psd4 cells suggests that a higher level of decarboxylase activity is present in these cells as compared to the controls. It is likely, therefore, that Ca++ acts in this case by protecting the mutant psd4 decarboxylase from heat inactivation. These findings, together with the previously discussed dependence of phenotypic expression of the psd4 mutation on genetic background, suggest that the psd4 mutant decarboxylase is highly sensitive to the membrane environment.
Few filaments are seen in mutant psd cultures grown at nonpermissive temperatures in the presence of Mg\(^{2+}\) or Ca\(^{2+}\) despite the continued accumulation of phosphatidylserine.

**DISCUSSION**

The cytoplasmic membrane of *E. coli* is the site of a large number of processes and systems essential to the life of the cell. These include the highly organized electron transport system, the energy transduction and conservation systems associated with it, a large number of specific transport systems, and numerous biosynthetic processes. *A priori*, it might therefore have been anticipated that the lipid composition of the membrane must be rather precisely and specifically regulated if the cell is to continue to live and grow. The present studies, however, reveal the rather surprising fact that the lipid composition may vary quite widely without preventing continued growth.

In the mutants studied, phosphatidylserine replaced phosphatidylethanolamine during growth at the nonpermissive temperatures. Ordinarily, phosphatidylserine is present only in traces in *E. coli* since it is decarboxylated almost as rapidly as it is formed. Phosphatidylserine is strikingly different from phosphatidylethanolamine, in that it possesses an additional net negative charge at neutral pH. One of the mutants (psd4) can be grown at 37°C for indefinite periods. Under these conditions, phosphatidylserine constitutes about 13 to 26% of the total phospholipid. Obviously, the presence of these levels of phosphatidylserine is not incompatible with the vital functions of the membrane. More strikingly, a psd2 mutant growing in medium containing 20 mM MgSO\(_4\) did not cease growth until the content of phosphatidylserine reached 76 mol %, while the content of phosphatidylethanolamine had been reduced to only 5%.

The sequestration of accumulated phosphatidylserine in some structure of the cell other than the cytoplasmic membrane might offer an explanation for the resistance of membrane function to its presence. However, the accumulated phosphatidylserine, like the phosphatidylethanolamine that it replaces, is almost equally distributed between the inner, cytoplasmic membrane, and the outer membrane.

The conclusion that the functions of the membrane of *E. coli* are surprisingly resistant to variations in the composition of membrane lipid is borne out by studies of the lac transport system, and certain other transport systems. Neither the induction nor the function of the lac system is affected by the presence of 19 to 24 mol % of phosphatidylserine in the membrane lipids.

Systems for the uptake of α-methyl glucoside, L-proline, and L-glutamine were somewhat reduced in activity in cells which had accumulated phosphatidylserine, but it is not easy to determine whether this reduction is a direct or an indirect consequence of the presence of phosphatidylserine in the membrane. The experiments cited above in which growth continued at relatively high levels of phosphatidylserine support the argument that transport systems in general, although perhaps somewhat reduced in activity, nevertheless function at a rate permitting continued growth.

In the available mutants, the accumulation of phosphatidylserine begins only after a considerable lag. This observation is consistent with the proposal that phosphatidylserine decarboxylase activity is in relative excess in cells as compared to phosphatidylserine synthetase activity; a conclusion that is supported by the fact that phosphatidylserine is present only in traces during the growth of wild type cells. Thus, most of the phosphatidylserine decarboxylase activity would have to be lost before any effect on phosphatidylethanolamine synthesis would be observed.

Phosphatidylserine decarboxylase appears to be greatly stabilized against thermal inactivation when it is localized in the membrane, whether in intact, resting cells (Fig. 5) or in membrane-containing cell-free extracts (Fig. 6). In contrast, there is a rapid drop of specific activity (activity/mg of cell protein) in growing cells at 43°C (Fig. 7). This finding argues that it is the newly synthesized enzyme which is acutely thermolabile. If all of the newly synthesized decarboxylase were immediately inactivated, the time needed for the specific activity of the enzyme to fall to one-half of its original value (85 min in the experiment of Fig. 7) would be equal to the doubling time for the culture, which was in fact about 60 min. This finding suggests that some of the newly synthesized decarboxylase escapes inactivation and is inserted into the membrane.

Under nonpermissive conditions, the sum of phosphatidylserine and phosphatidylethanolamine remains constant in the psd mutants. Thus, the accumulation of phosphatidylserine does not affect the relative rate of synthesis in the phosphatidylglycerol-cardiolipin branch of the biosynthetic pathway for the lipids of *E. coli* (10). The increase in the ratio of cardiolipin to phosphatidylglycerol, typical of the psd mutants at 42°C, may be a result of a generalized response to growth-limiting conditions as has been previously observed (24).

The specific site of the lethal action of phosphatidylserine in the psd mutants remains unknown. In general, during phosphatidylserine accumulation, the mutant cell membrane appears to remain functionally intact and growth slows gradually before finally stopping. The amount of phosphatidylserine accumulated at the time of growth cessation is somewhat variable but is in the range of 35 to 50 mol %. The fact that higher levels of phosphatidylserine accumulation are obtained in Mg\(^{2+}\)-treated cultures suggests that Mg\(^{2+}\) ions may be shielding the negatively charged phosphatidylserine and thus permitting further synthesis and incorporation of this anionic phospholipid. Under these conditions of increased phosphatidylserine accumulation, growth of mutant cultures is, in fact, prolonged. The partial phenotypic suppression of the growth effect by cations suggests that ultimately it is not the lack of a critical amount of phosphatidylethanolamine that is lethal to the mutant cell, but instead the accumulation of negatively charged phosphatidylserine is itself responsible for the toxic effects.

**REFERENCES**

13. Lennox, E. S. (1955) *Virology* 1, 190–206

E. Hawrot and E. P. Kennedy, manuscript in preparation.
Phosphatidylserine Decarboxylase Mutants of E. coli

Phospholipid composition and membrane function in phosphatidylserine decarboxylase mutants of Escherichia coli.
E Hawrot and E P Kennedy


Access the most updated version of this article at http://www.jbc.org/content/253/22/8213

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/253/22/8213.full.html#ref-list-1