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Effect of Membrane-Associated f1 Bacteriophage Coat Protein upon the Activity of *Escherichia coli* Phosphatidylserine Synthetase

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The effects of insertion of the major coat protein of f1 bacteriophage into *Escherichia coli* membranes were investigated under conditions allowing in vivo analysis of phosphatidylserine synthesis. An *E. coli* strain possessing a temperature-sensitive phosphatidylserine decarboxylase was utilized under conditions in which the decarboxylase activity was reduced but nonlethal. The presence of the coat protein in the host membranes inhibits the activity of the phosphatidylserine synthetase and perhaps affects the activity of the phosphatidylserine decarboxylase.

When most nonpermissive *Escherichia coli* hosts are infected with f1, fd, or M13 bacteriophage possessing an amber mutation in any gene except gene 2, cell division and most macromolecular synthesis cease within one uninfected generation time (9, 19). Infection by mutants for gene 1, 3, 4, 5, 6, or 7 alters the host phospholipid metabolism such that the relative level of cardiolipin increases and that of phosphatidylethanolamine decreases (13, 19). The relative level of phosphatidylethanolamine decreases due to the failure to accumulate phosphatidylethanolamine when the cellular levels of phosphatidylglycerol and cardiolipin are increasing (4). This decreased accumulation of phosphatidylethanolamine correlates with a reduction in phosphatidylethanolamine synthesis; the increased cardiolipin content results from increased cardiolipin synthesis and decreased cardiolipin turnover (4). The alterations have been attributed to the presence of "free" coat protein within the host membranes, since they do not occur after infection with an amber mutant for the major coat protein gene (4, 19). (Free coat protein is defined as membrane-associated coat protein which, because of the mutant used, is not involved in the formation of phage.)

It was suggested (4) that the effect upon phosphatidylethanolamine synthesis might result from an altered activity of cytidine 5'-diphosphate–diglyceride:L-serine phosphatidyltransferase (phosphatidylserine synthetase), the first enzyme of the branch of the phospholipid biosynthetic pathway leading to phosphatidylethanolamine. However, direct in vivo analysis of phosphatidylserine synthesis was not possible, since phosphatidylserine was rapidly decarboxylated to form phosphatidylethanolamine. In this paper, we employ an *E. coli psd-4* mutant possessing a temperature-sensitive phosphatidylserine decarboxylase (7, 8). At intermediate temperatures, the decarboxylase activity is only partially reduced. It was therefore possible to measure in vivo phosphatidylserine synthesis and to correlate alterations in this activity with the presence of coat protein in host membranes.

MATERIALS AND METHODS

Materials. Carrier-free [^{32}P]orthophosphoric acid was purchased from New England Nuclear Corp. Thin-layer chromatography was performed on MN Polygram Sil G 22 plastic sheets from Brinkmann Instruments Inc. Kodak No-Screen medical X-ray film was used for autoradiography. All chemicals were of reagent grade.

Bacteria, bacteriophage, and media. *E. coli* strain EH470, obtained from Edward Hawrot, Harvard Medical School, Boston, Mass., is a *psd-4* transductant of strain W3110 (8) possessing a thermolabile phosphatidylserine decarboxylase. At elevated temperatures, such mutants accumulate substantial quantities of phosphatidylserine (7). To utilize this strain in these experiments, an F′ episome was introduced by mating with strain 200 PS (Thi$^-$ Lac y$^-$ Str^s T6^s Amp^s/F$^+$ Lac$^+$) obtained from Philip Harriman, University of Missouri, Kansas City. A culture of ampicillin-sensitive 200 PS was grown at 34°C to approximately 2 × 10^7 bacteria per ml. A portion of the F$^-$, ampicillin-resistant EH470 grown to approximately 2 × 10^8 bacteria per ml was added, such that the ratio of males to females was 2:1. After a 4-h mating, ampicillin was added to a final concentration of 12 μg/ml. The culture was diluted with buffer containing 12 μg of ampicillin per ml and plated on an agar plate containing 0.5 mg of ampicillin. Ampicillin-resistant colonies which grew at 34°C were tested for temperature-sensitive growth at 42°C, for plaque formation by male-specific f1 and

f2 bacteriophage, and for "killing" in liquid culture by amber mutants of f1 bacteriophage. One isolate with exhibited ampicillin resistance, temperature-sensitive growth, and sensitivity to male-specific phage was termed EH470-B2 and was utilized in the subsequent experiments. Bacteriophage and media were as described previously (4).

Phospholipid analyses. Phospholipids were extracted and quantitated as described previously (4), except that the chromatography solvent chloroform-methanol-glacial acetic acid (65:25:8) of Ames (1) was utilized for fractionation. Determinations of phospholipid composition, synthesis, and turnover were as described previously (4), except that the bacteria were grown for two generations at 34°C and then for two generations at 38°C before the experiment. All experiments were performed with bacteria growing at 38°C with aeration.

RESULTS

We previously showed (4) that the presence of the f1 coat protein in wild-type *E. coli* K-12 membranes results in a decrease in phosphatidylethanolamine synthesis. It was not possible to test directly whether the synthesis of phosphatidylserine was affected, since phosphatidylserine was rapidly decarboxylated to phosphatidylethanolamine in these strains. However, when EH470-B2 is grown at 38°C, phosphatidylserine accounts for approximately 30% of the phospholipid of the bacterium (Fig. 1). In addition, the presence of free coat protein in this strain at 38°C results in an accumulation of cardiolipin (Fig. 1A) in the same manner as occurs in wild-type *E. coli* infected with an appropriate f1 amber mutant phage (4). These results suggested that this strain could be utilized to examine the effect of the coat protein on phosphatidylserine synthesis.

To observe this effect, EH470-B2 was infected with f1 R16, which contains an amber mutation in gene 5. These bacteria can synthesize coat protein, but no phage DNA and, consequently, no phage-type particles. In this case, the bacterial membrane contains only free coat protein. As a control, the bacteria were infected with M13 8H1, which contains an amber mutation in the coat protein gene. These bacteria can synthesize all other detectable phage-specific proteins and DNA species (6, 17) but no coat protein. Figure 2 shows phospholipid synthesis in EH470-B2 at 38°C after infection by these two amber mutants. The pattern of phosphatidylserine synthesis in f1 R16-infected EH470-B2 (Fig. 2A) resembled that of phosphatidylethanolamine synthesis observed previously in f1 R12-infected bacteria (4), with levels decreasing to approximately 20% of the uninfected rate. At later times after M13 8H1 infection, phosphatidylserine synthesis also declined to approximately 60% of the uninfected rate (Fig. 2B), but this decrease probably reflects the general decline in metabolism in amber mutant-infected bacteria (4, 9, 19). Comparison of the phosphatidylserine synthesis in both infected bacteria shows that the presence of the coat protein produced a threefold further reduction in phosphatidylserine synthesis immediately after infection. Therefore, the coat protein-induced decline in phosphatidylethanolamine synthesis observed in wild-type *E. coli* may be caused by a decrease in phosphatidylserine synthesis.

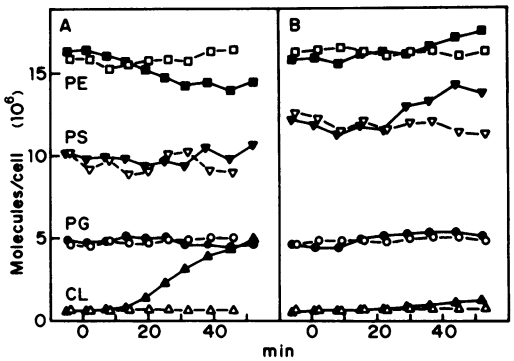

FIG. 1. *Molecular phospholipid composition of (A) f1 R16- and (B) M13 8H1-infected bacteria. ^{32}P-labeled cultures of EH470-B2 were divided in half and, at a density of 1.5 × 10^8 cells per ml, one-half was infected (0 min) with the appropriate amber mutant at a multiplicity of infection of 200; the other half remained uninfected. At the indicated times, portions were withdrawn, and the phospholipids were extracted and quantitated as described in the text. Symbols: ▲, △, cardiolipin (CL); ■, □, phosphatidylethanolamine (PE); ●, ○, phosphatidylglycerol (PG); ▼, ▽, phosphatidylserine (PS). Solid lines and closed symbols represent the amber mutant-infected cultures. Dashed lines and open symbols represent the parallel uninfected cultures.*

However, phosphatidylethanolamine synthesis in f1 R16-infected EH470-B2 also declined (Fig. 2A) such that the level was sixfold lower than in M13 8H1-infected bacteria (Fig. 2B). It is difficult to determine whether this effect is a result of the mutant decarboxylase, since the exact nature of the temperature-sensitive lesion is unknown. If the mutant enzyme has a higher apparent K_m for phosphatidylserine at this temperature, then the decrease of phosphatidylserine synthesis caused by the presence of the coat protein (Fig. 2A) might be expected to affect the formation of phosphatidylethanolamine. However, the total phosphatidylserine content of uninfected and f1 R16-infected EH470-B2 are nearly identical and quite high during the time period of the experiment (Fig. 1A). Under these conditions, for the concentration of phosphati-

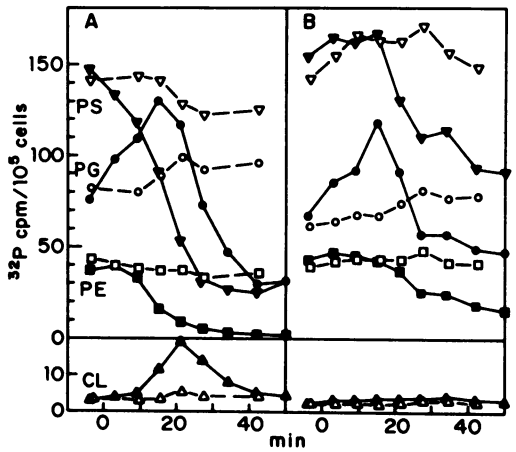

FIG. 2. *Phospholipid synthesis in (A) f1 R16- and (B) M13 8H1-infected bacteria. Cultures of EH470-B2 were divided and infected as described for Fig. 1. At intervals, portions were withdrawn and pulsed with $^{32}P_i$ for 4 min, and the rates of phospholipid synthesis were determined as described in the text. Results are presented as ^{32}P-labeled counts per minute incorporated into the phospholipid species per 10^5 cells in the sample and plotted at the midpoint of the pulse. Symbols are as in Fig. 1. Solid lines and closed symbols represent the amber mutant-infected cultures. Dashed lines and open symbols represent the parallel uninfected cultures.*

dylserine to have any effect on the activity of the mutant decarboxylase, it must be postulated that it is a special class of phosphatidylserine, perhaps only the newly synthesized phosphatidylserine molecules, which can be decarboxylated. This would mean that previously labeled phosphatidylserine could not be converted to phosphatidylethanolamine in EH 470-B2 at 38°C. When this was tested in the turnover experiment shown in Fig. 3, the decrease in phosphatidylserine was accompanied by an equal increase in phosphatidylethanolamine in uninfected EH 470-B2 at 38°C, strongly suggesting that preexisting phosphatidylserine can be used as a substrate for the decarboxylase under these conditions. These data, together with the fact that there is less phosphatidylethanolamine synthesized in f1 R16-infected EH470-B2 under the same conditions, suggests that the presence of the coat protein at least has an indirect effect on the decarboxylase activity.

The failure to accumulate both phosphatidylserine and phosphatidylethanolamine in f1 R16-infected EH470-B2 (Fig. 1A) compared with levels in M13 8H1-infected bacteria (Fig. 1B) is consistent with this conclusion. The decreased turnover of phosphatidylserine into phosphatidylethanolamine after f1 R16 infection (Fig. 3A), compared with that after M13 8H1 infection (Fig. 3B), also indicates a reduced decarboxylase activity. The cellular content (Fig. 1), synthesis (Fig. 2), and turnover (Fig. 3) of phosphatidylglycerol and cardiolipin for both f1 R16 and M13 8H1 infections are consistent with previous findings (4). Phosphatidylglycerol turnover in f1 R16-infected bacteria in the presence of phospholipid synthesis (Fig. 3A) differed from that observed in the absence of phospholipid synthesis (4) only because an increased turnover from 20 to 30 min postinfection reflected the burst of cardiolipin synthesis (Fig. 2A). Otherwise, phosphatidylglycerol turnover, as determined previously (4), was unaffected by the presence of the coat protein.

DISCUSSION

The presence of the f1 bacteriophage major coat protein in the *E. coli* membrane has been shown to increase the synthesis and decrease the turnover of cardiolipin and also to decrease the synthesis of phosphatidylethanolamine (4). In this paper, the latter effect is shown to result from a reduction of both phosphotidylserine synthetase and decarboxylase activities.

Several possibilities have been suggested (4) to explain how the coat protein might affect the synthetase activity. (i) The coat protein might enter or exist in the membrane in an environment similar to that of phosphatidylserine synthetase, and the enzyme either cannot associate

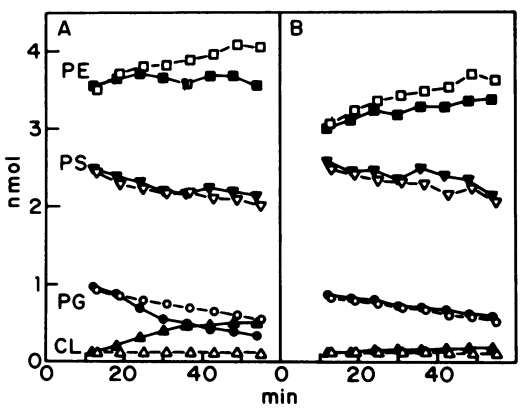

FIG. 3. *Phospholipid turnover in (A) f1 R16- and (B) M13 8H1-infected bacteria. ^{32}P-labeled cultures of EH470-B2 were divided and infected as described in Fig. 1. Five minutes after infection, the bacteria were filtered, washed, and resuspended in media lacking ^{32}P. At the times indicated, portions were withdrawn, and the label remaining in the phospholipid species was quantitated as described in the text. Symbols are as in Fig. 1. Solid lines and closed symbols represent the amber mutant-infected cultures. Dashed lines and open symbols represent the parallel uninfected cultures.*

with the membrane or is displaced as the coat protein enters. This alternative must be considered in view of the tenuous nature of any membrane association this enzyme might exhibit (12). Whereas most phospholipid biosynthetic enzymes are found associated with the cytoplasmic membrane (2, 19), this enzyme has been found as a ribosomal associated form or loosely associated with membranes (11, 15–17). (ii) The presence of the coat protein in the membrane in close proximity to the site of action of the synthetase inhibits its activity. This would include any complex site of action involving ribosomes, synthetase, and membrane. (iii) Phosphatidylserine synthetase activity may have specific phospholipid requirements which are disturbed in the modified phospholipid composition created by coat protein insertion (4, 13, 19). This alternative is significant, since cardiolipin activates phosphatidylserine synthetase in vitro and lowers its K_m for L-serine (10). Previous data have suggested that, after the coat protein has been inserted into the membrane, it can form a tight association with cardiolipin (4). Since the amount of cardiolipin is low during the time of logarithmic growth of this experiment, any sequestering of cardiolipin around the coat protein might decrease the cardiolipin level in the environment of phosphatidylserine synthetase and thus reduce the activity of the enzyme.

Activity of the phosphatidylserine decarboxylase might be inhibited by mechanisms analogous to (ii) and (iii) proposed above for the synthetase. However, the delayed response to the decarboxylase activity (Fig. 2A) indicates that it is less sensitive (to either the coat protein itself or to the altered phospholipid environment) than is the synthetase. It is doubtful that mechanism i is a viable alternative in this case, since the decarboxylase is tightly associated with the cytoplasmic membrane (2, 5, 18).

Thus, insertion of the filamentous phage major coat protein into *E. coli* membranes affects three phospholipid biosynthetic activities: phosphatidylserine synthetase, phosphatidylserine decarboxylase, and cardiolipin synthetase (4; Fig. 2). Although these alterations are attributable to the presence of the coat protein in the membranes, the possibility that some of them are secondary responses cannot be excluded. For example, the relative cardiolipin content in *E. coli* can increase when there is a defect in phosphatidylserine synthetase (14). The results presented previously (4) and in this paper indicated that coat protein in the membranes decreased phosphatidylserine synthetase activity and caused an accumulation of cardiolipin. The data do not exclude the possibility that some of the increased cardiolipin content arises indirectly through the inhibition of phosphatidylserine synthetase activity. It was previously suggested that there is a direct interaction in the membrane between cardiolipin and the coat protein, perhaps due to the negative charges on the cardiolipin molecule, and that this interaction might be responsible for the decrease in cardiolipin turnover (4). In mutant bacteria defective in cardiolipin, any requirement of coat protein for negatively charged lipids appears to be replaced by phosphatidylglycerol (G. Pluschke, Z. Hirota, and P. Overath, J. Biol. Chem., in press). However, when cardiolipin is present as in EH470-B2, the cardiolipin-coat protein interaction cannot be replaced by a coat protein-phosphatidylserine interaction, since the cardiolipin accumulation and the decrease in turnover still occur when coat protein is present in membranes containing 30% phosphatidylserine.

It is hoped that the study of alterations produced by the insertion of a foreign protein into *E. coli* membranes might clarify interactions among the various phospholipid biosynthetic activities. Eventually such information could facilitate elucidation of the mechanisms by which the *E. coli* phospholipid metabolism is regulated. Furthermore, analysis of the requirements for incorporation of the coat protein into host membranes (3, 4) might prove to be applicable to *E. coli* membrane biogenesis in general.

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