# Bacterial membranes and lipid packing theory

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**Abstract** Recent physical studies on the lipids of biological membranes have emphasized the potential instability of the lamellar phase of mixtures of lipids containing unsaturated species of phosphatidylethanolamine, plasmenylethanolamine, or monoglycosyldiacylglycerols, all of which are important constituents of the membranes of different groups of prokaryotes. The polar lipid compositions of bacteria are examined in terms of lipid packing theory. This survey reveals that gram-negative species with high proportions of unsaturated fatty acids (>65%), often have phosphatidylcholine (PC), in addition to the more common phosphatidylethanolamine (PE), phosphatidylglycerol, and cardiolipin. Physical studies have shown that PC is capable of inducing the bilayer phase when added to unsaturated PE. Many bacteria that are rich in unsaturated fatty acids and contain PC, have intracytoplasmic membrane systems (ICM), and the potential role of bilayer instability in the formation of ICM is discussed. Two groups of bacteria that are either natural fatty acid auxotrophs or utilize exogenous fatty acids when endogenous synthesis is inhibited, *Acholeplasma laidlawii* and the butyric acid-producing clostridia, are capable of adjusting their lipid class compositions according to the degree of unsaturation of their lipid aliphatic chains. Lipid class composition is also affected by growth temperature in both groups of organisms, and by incorporation of cholesterol in *A. laidlawii*. As the content of *cis*-unsaturated fatty acids or temperature is increased, lipids that form an unstable lamellar phase at physiological temperatures are replaced with lipids that have larger effective polar head groups, and can therefore form more stable bilayers.—**Goldfine, H.** Bacterial membranes and lipid packing theory. *J. Lipid Res.* 1984. **25:** 1501–1507.

**Supplementary key words** phospholipids • gram-negative bacteria • gram-positive bacteria • intracytoplasmic membranes • glucosyldiacylglycerols

## INTRODUCTION

A central problem of membrane biochemistry is the need for complex mixtures of lipid classes, each of which consists of a multitude of molecular species. It is clear from reconstitution studies, that stable bilayers can be formed from a single lipid class, for example PC. In addition to stable bilayers, however, biological membranes require mixtures of lipids that are fluid, in order to function. Specific lipids may also be required for their charge contributions, and others for their interactions with membrane proteins.

That polar lipids can self-assemble into aggregates other than bilayers has been known for over two decades (for reviews see 1, 2). The structures and sizes of these aggregates are determined by intermolecular forces and thermodynamic laws. In addition to these considerations, a set of relatively simple rules, arising from the shapes of lipid molecules, has been used to predict the structures of lipid aggregates formed in an aqueous environment. Israelachvili, Marčelja, and Horn (3) have expressed the geometric packing properties of polar lipids in terms of a dimensionless 'critical packing parameter', $v/a_o l_c$, characteristic of each lipid. In this expression, $v$ is the volume of the hydrocarbon chains(s), $l_c$ is the maximum length of the chains, and $a_o$ is the optimal surface area per amphiphile, which is determined by the volume of the head group, its hydration, charge, and hydrogen bonding capabilities. The volume of the chains is dependent on their thermal motion. The possible values of the critical packing parameter and the predicted aggregates are listed in **Table 1.**

In this article, the packing properties of the major polar lipids of bacteria will be examined and their ability to form stable bilayers will be assessed. This discussion will be limited to mesophilic organisms, which grow optimally at 25° to 45°C. It will become apparent that certain combinations of aliphatic chains and polar head groups may lead to bilayer instability, and the potential importance of this condition for membrane elaboration will be discussed. Recent work on the ability of certain species to prevent the accumulation of lipids that destabilize bilayers, will also be reviewed.

## GRAM-NEGATIVE BACTERIA

### Enteric bacteria

Gram-negative bacteria are surrounded by two membranes. The outer membrane is outside the rigid cell

Abbreviations: DPG, diphosphatidylglycerol (cardiolipin); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PME, phosphatidyl-N-methylethanolamine; PDME, phosphatidyl-N,N-dimethylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; MGDG, monoglucosyldiacylglycerol; DGDG, diglucosyldiacylglycerol; ICM, intracytoplasmic membranes; cyc, cyclopropane.

TABLE 1. Critical packing parameters and lipid aggregates (2, 3)

| Critical Packing Parameter | Critical Packing Shape | Structures Formed | Lipid |
|---|---|---|---|
| $v/a_o l_c$ | | | |
| <1/3 | cone ($a_o$ large relative to $v$) | Spherical micelles | Lysophospholipids, detergents |
| 1/3–1/2 | truncated cone | Globular or cylindrical micelles | Non-ionic lipids, lyso-PC |
| 1/2–~1 | cylindrical | Bilayer | PC, PS, PG diglycosyldiglycerides, saturated PE, sphingomyelin |
| >1 | inverted, truncated cone ($a_o$ small relative to $v$) | Inverted micelles, hexagonal ($H_{II}$) | PE with mostly unsaturated chains, plasmenylethanolamine, monoglycosyldiglycerides, DPG + $Ca^{2+}$ |

wall and contains proteins, lipopolysaccharide, and phospholipids. The cytoplasmic and outer membranes do not have identical phospholipid compositions. In *Salmonella typhimurium* the phospholipids of the outer membrane are PE, PG, and DPG in the ratio 81:17:2. In the cytoplasmic membrane the ratio is 60:33:7. Similar results have been reported for the separated membranes of *Escherichia coli* K-12, *Pseudomonas* BAL-31, and *Proteus mirabilis* (for references see 4). In *E. coli* and in some of the other bacteria studied (4), the inner membrane has 10 to 20% more unsaturated acyl chains than the outer membrane. Therefore, the composition of the total lipids of these bacteria does not exactly reflect the lipids of the cytoplasmic membrane, and this affects their phase behavior. In *E. coli*, for example, the gel to liquid crystalline phase transition temperature of the cytoplasmic membrane is lower than that of the outer membrane (5). The organization of the outer membrane is different from that of the cytoplasmic membrane (6), and it will not be discussed.

Of the three major lipids of enteric bacterial cell membranes, both PG and DPG form stable bilayers, but the addition of equimolar concentrations of $Ca^{2+}$ to DPG results in the formation of an hexagonal ($H_{II}$) phase (Table 1). Only PE has a tendency to form the $H_{II}$ phase in most ionic environments. The phase behavior of PE isolated from *E. coli* has been studied (7, 8). A sample rich in cyclopropane fatty acids underwent a reversible bilayer to $H_{II}$ transition at 55 to 60°C ($T_{BH}$) (8). The total lipids of *E. coli* K-12 dispersed in 25 mM Tris-HCl were found to be almost completely lamellar by $^{31}P$ NMR. However, when 100 mM NaCl was added, to approximate intracellular conditions, lipid dispersions gave strong signals characteristic of isotropic motion, which increased in intensity as temperature was increased above 31°C. Destabilization of PE was thought to involve salt interactions with anionic phospholipids (9). $^{31}P$ NMR studies on the cytoplasmic membrane also indicated some lipid polar group isotropic motion.

**Photosynthetic bacteria**

The lipids of many of the purple nonsulfur bacteria are characteristically rich in monounsaturated fatty acids, especially *cis*-vaccenic acid (*cis*-11-18:1) (10). In *Rhodopseudomonas sphaeroides, R. capsulata,* and *R. palustris,* over 75% of the acyl chains of the phospholipids of the whole cells and of the intracytoplasmic membranes are *cis*-9-16:1 and *cis*-11-18:1 (10). In addition to PG and PE, all three species have from 20 to 25% PC, which is capable of stabilizing the bilayer arrangement of unsaturated PE. For example, the addition of 30 mol% PC to soya bean PE induces the bilayer phase (2). These bacteria also contain up to 25% of total lipid by weight of ornithine lipids and lipids containing other nitrogenous bases, which do not contain phosphorus. The ornithine lipids have two acyl chains, one of which is an amide, and the effects of these lipids on the phase behavior of phospholipids are not known.[1]

Many of the purple sulfur bacteria, the Chromatiaceae, have the same phospholipids as enteric bacteria: PE, PG, and DPG, but no PC. They have a higher proportion of PG and small amounts of glycosyldiglycerides (10, 11). The fatty acids of these organisms are somewhat more saturated than in the purple nonsulfur family, and the higher content of PG and the presence of glycolipids may suffice to produce stable bilayers, but this needs to be verified by experiment. *Ectothiorhodospira,* a subgroup of Chromatiaceae, which have 65 to 76% unsaturated

[1] Two subgroups of the purple nonsulfur bacteria have been characterized with respect to polar lipids (11). Subgroup 1 has little PE, no PC, PG, or DPG, and major amounts of ornithine lipids. This group includes species classified as *Rhodopseudomonas, Rhodomicrobium,* and *Rhodospirillum.* Subgroup 2a has PG, DPG, PE, and an ornithine lipid. This group includes *Rhodospirillum tenue* and *R. rubrum,* which has from 60 to 80% unsaturated fatty acids. The presence of relatively large amounts of neutral lipids in many of these organisms should also be noted. It is not clear whether they are in membranes or in separate vesicles.

plus cyclopropane fatty acids, have PC, PG, and DPG, with relatively little PE (10–12).

### Other gram-negative bacteria

Inspection of the fatty acid compositions of other gram-negative bacteria reveals additional groups of organisms that have high contents of unsaturated fatty acids, which I have somewhat arbitrarily set at 65% or more of the total. As is the case with the photosynthetic bacteria, many of these organisms have polar lipids such as PDME or PC, which would be expected to enhance the stability of bilayers containing unsaturated PE. The methylotrophs, organisms that have an obligate or facultative requirement for one-carbon organic compounds as a source of carbon, represent a well-characterized group with respect to fatty acid and lipid compositions (**Table 2**). Of the four organisms that are rich in unsaturated fatty acids, three have substantial amounts of PDME plus PC. Only *M. methanolica,* which has 70% unsaturated fatty acids, has no N-methylated products of PE. Goldberg and Jensen (13) have also analyzed the phospholipids of a group of methanol-utilizing strains of pseudomonads. Those that had 75% or more unsaturated fatty acids had substantial amounts of PC, while one strain with less unsaturated fatty acids had none. Many species of *Thiobacillus* have PME, but no PDME or PC. One species that has about 35% PC in addition to 25% PME is *T. novellus,* which has from 62 to 92% 18:1 plus 19:cyc, depending on the growth medium.

Other bacteria that are rich in unsaturated fatty acids and have PC include various strains of the stalked hyphomicrobia, which also have about 35% PDME, and *Paracoccus denitrificans,* which has, in addition, an ornithine lipid. *Azotobacter agilis,* which has 57% unsaturated fatty acids, has small amounts of PME and PC, but *A. vinelandii,* which has 68% unsaturated fatty acids, has mainly PE (Table 2).

Some gram-negative animal and plant pathogens and symbionts are rich in unsaturated fatty acids and have PC in addition to the typical phospholipids of gram-negative bacteria. These include *Brucella abortus* and *Brucella melitensis,* both with over 30% PC. By contrast *Bordetella pertussis,* which has about 45% unsaturated fatty acids, has no PC but does have 20% PS (Table 2). Interestingly, *Legionella* strains, which are unusual for gram-negative bacteria in having predominantly *iso-* and *anteiso*-methyl branched fatty acids (26), have 9 to 15% PME, 1 to 2% PDME, and 29 to 36% PC (27). Among the bacteria that infect plants are *Rhizobium,* which produce nodules on the roots of leguminous plants, and *Agrobacterium,* which produces gall hypertrophies. All species of both genera examined have PC in addition to PE and polyglycerol phosphatides (4, 28). In the free-living rhizobia, PS, PI, PME, or PDME may also be

TABLE 2. Polar lipids and unsaturated fatty acid content of some gram-negative bacteria

| | PE | PME | PDME | PC | PG | DPG | Other | Ref. | UFA[a] | Ref. | ICM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | % | | |
| Methylotrophs | | | | | | | | | | | |
| *Methylomonas methanolica* | 77[b] | 0 | 0 | 0 | 17 | 6.3 | | 13 | 70 | 13 | + |
| *Methylosinas trichosporium* | 0 | 21 | 49 | 11 | 13 | <1 | 5 | 14 | 90 | 14 | + |
| *Methylococcus capsulatus* | 74 | 0 | 0 | 8 | 13 | 5 | | 14 | 35 | 14 | + |
| *Methylobacterium organophilum* | 25–57[c] | | 24–31 | 15–44 | | | PS, 1–9 | 15 | | | + |
| OBT strain | 0 | 31 | 42 | 7 | 19 | <1 | | 14 | 92 | 14 | +? |
| La Paz strain | 0 | 52 | 2 | 30 | 11 | <1 | 4 | 14 | 91 | 14 | +? |
| Other | | | | | | | | | | | |
| *Thiobacillus novellus* | 25 | 7 | | 35 | 27 | 6 | | 16 | 62–92 | 17 | − |
| *Hyphomicrobium vulgarae* | 23 | 0 | 36 | 29 | 10 | — | | 5 | 92 | 18 | + |
| *Paracoccus denitrificans* Strain N3 | 19 | 0 | 0 | 32 | 34 | 15 | orn. lipid | 19 | ~75 | 20 | − |
| *Azotobacter agilis* | 64 | 5 | | 2 | 13 | 23 | | 21 | 58 | 22 | + |
| *Azotobacter vinelandii* | >85 | | | | ←7→ | | [e] | 23 | 68 | 23 | + |
| *Brucella abortus*[d] Strain Scherle II | ←28→ | | | 35 | 23 | 7.2 | 7.9[f] | 24 | 86[g] | 25 | Unk. |
| *Brucella melitensis*[d] | ←33→ | | | 38 | 9.4 | 20 | | 24 | 77 | 25 | Unk. |
| *Bordetella pertussis*[d] | 46 | | | | trace | 24 | 20 (PS) | 24 | ~45 | 24 | − |

[a] Unsaturated fatty acid (includes cyclopropane fatty acids where applicable).
[b] Percent of lipid P unless otherwise indicated.
[c] Cells grown under various conditions.
[d] Percent by weight.
[e] Unidentified.
[f] Including 5.3% PS.
[g] PC fatty acids.

present (4, 28, 29). *Agrobacterium tumefaciens* (84%) (22) and *Rhizobium japonicum* strains grown on defined media (74–88%) (30), are rich in monosaturated and cyclopropane fatty acids. Other strains infective on *Lotus pedunculatus*, contain large amounts of branched saturated and unsaturated fatty acids in addition to normal unsaturated and cyclopropane chains. It should be noted that the free-living forms of these bacteria also contain large amounts of free fatty acids and other neutral lipids (31). Thus, a recurrent pattern among gram-negative bacteria is the occurrence of PC, sometimes with PDME, in organisms that are rich in unsaturated acyl chains. At over 65% of these moieties, one would expect a sharp increase in polar lipid molecules with two unsaturated chains, and pure dioleoyl PE does not form stable bilayers above 10°C (7) without the addition of bilayer-stabilizing lipids. As the number of methyl groups added to PE increases, $a_o$ will increase; the molecule will be more cylindrical in shape and should be more effective in stabilizing the lamellar phase.

Rohmer, Bouvier-Nave, and Ourisson (32) have reported the presence of 0.1 to 2 mg per g dry wt of hopanoids in a wide variety of prokaryotes. These sterol-like triterpenes are postulated to reinforce bilayers by virtue of their rigid structures. It is interesting to note that they have been found in two of the groups listed above that are rich in unsaturated fatty acids and contain PC, the purple nonsulfur bacteria and the methylotrophs, including *Hyphomicrobium*. They were not found in the purple sulfur bacteria, *A. tumefaciens* and *P. denitrificans* among others.

### Non-bilayer lipids and intracytoplasmic membranes

Many of the photosynthetic and chemolithotrophic bacteria that are rich in unsaturated fatty acids and contain PC also have intracytoplasmic membranes (ICM) (Table 2). A correlation between PC and ICM in prokaryotes was noted in 1966, and we suggested that PC may play "an important role . . . in the elaboration of cytoplasmic membranes" (33) by virtue of the "unique charge or size (or both) of the polar head group" (34). In the intervening years this hypothesis has been supported by additional studies on bacterial lipids and membranes, but there are bacteria, like the Rhizobiacea, that have PC but no ICM, and the reverse (4).

Is it now possible to construct lipid-packing models for the elaboration of ICM in bacteria? Considerable work has been done on the ICM of phototrophic bacteria, but space limitations do not permit extensive review of this literature (35, 36). A dominant model for formation of ICM in these bacteria involves the insertion of proteins and pigments into preexisting cytoplasmic membrane along with extension by invagination, presumably as a result of lipid synthesis and accretion in excess of that needed for cell elongation (35, 36). As noted above, when PE is unsaturated it tends to assume the $H_{II}$ phase and the presence of PC stabilizes the bilayer phase. Thus, in bacteria with mostly unsaturated PE and limited quantities of PC and other bilayer-stabilizing lipids, local conditions may occur that permit formation of non-bilayer structures.[2]

Cullis and deKruijff (2) have presented a detailed model for the role of such non-bilayer domains in the process of blebbing-off observed in erythrocytes upon ATP depletion. A combination of lipids in the erythrocyte inner monolayer consisting of 49% PE and 25% PS along with smaller amounts of PC and sphingomyelin may be induced to form non-lamellar structures by increased cytosolic $Ca^{2+}$, which blocks the ability of PS to stabilize unsaturated PE bilayers. This in turn reduces the surface area of the inner monolayer and produces blebbing. In bacteria with the combination of unsaturated PE and limiting PC, decreasing the content of bilayer-stabilizing lipid in the cytoplasmic membrane could similarly lead to the formation of non-lamellar structures. If this were to take place in the outer leaflet of the cytoplasmic membrane it would, according to their model, lead to invagination, which is the opposite of the blebbing off process.

There are conflicting reports on the asymmetry of PE in chromatophores isolated by French press disruption of *R. sphaeroides*. These closed vesicles have the same orientation as the membrane invaginations seen in intact cells, i.e., their outer, cytoplasmic surface is topologically equal to the inner, cytoplasmic surface of the cell membrane (37). The outer monolayer of these chromatophores has been reported to have 35% (37) and 78% (38) of chromatophore PE. This uncertainty, and the lack of knowledge of the localization of PC and ornithine lipid, do not permit a detailed lipid packing model of membrane invagination at this time. Further, even if the sidedness of cell lipids were known, local domains of lipids and the influences of proteins may be more important than the overall composition. At this time one can only say that there may be a role for non-bilayer lipids in ICM formation, but considerably more work is needed before a dynamic picture can be proposed.

[2] Oelze and Drews have noted that among the phototrophic bacteria, all those producing up to 60% monounsaturated fatty acids do not produce ICM, and all species with 80–90% monounsaturated fatty acids do. They attribute this to the higher degree of fluidity. (Oelze, J., and G. Drews. 1981. *In* Organization of Prokaryotic Cell Membranes. Vol. II. B. K. Ghosh, editor. CRC Press, Boca Raton, FL. 131–195).

## GRAM-POSITIVE BACTERIA

Studies on lipid assembly in gram-positive species are facilitated by the presence of a single cytoplasmic membrane.[3] In many species the ease of preparation of protoplasts by enzymic digestion of the cell wall permits direct study of phospholipid asymmetry (39). Among the gram-positive bacteria, the genus *Bacillus* is closest to the gram-negative bacteria in polar lipid composition. PE is 20 to 45% of total phospholipid, with PG and DPG the bulk of the rest (4). About 90% of the PE is in the outer monolayer of the cell membrane of *B. amyloliquefaciens,* but in other species it is less asymmetrically distributed (39). In *B. megaterium* MK 10, about 40% of the PG was found to be in the outer monolayer (39). In addition to these lipids, some *Bacillus* species have varying amounts of diglycosyldiglycerides and O-aminoacyl-PG (4). The major lipid acyl chains in these organisms are *iso-* and *anteiso*-methyl branched. Among these polar lipids, PG, diglycosyldiglycerides and DPG, if intracellular $Ca^{2+}$ is not high, should form stable bilayers. In addition, branched acyl chains should decrease the tendency of PE to form the hexagonal phase since they have smaller cross-sectional areas than *cis*-monosaturated fatty acids (40). Less is known about the polar lipids of the anaerobic, spore-forming clostridia. One well-studied group, the butyric acid-producing clostridia, is discussed below.

In the gram-positive cocci and the lactobacilli the major polar lipids are PG and DPG. These may be accompanied by O-aminoacyl PG, especially when the pH of the medium decreases, diglycosyldiglycerides, and PI (4, 29). The PG/DPG ratio is variable; therefore, any tendency towards bilayer instability will be influenced both by this ratio and the intracellular $Ca^{2+}$ concentration. As in *Bacillus* species, *iso-* and *anteiso*-methyl branched fatty acids are abundant in the gram-positive cocci (29). The phase behavior of the O-aminoacyl PG lipids is not known, but the increase in bulk of the polar head group should decrease the critical packing parameter, $v/a_o l_c$, thus further decreasing the tendency to form the hexagonal phase. A further complication in the gram-positive cocci is the presence of *sn*-glycerol-1-P derivatives of diglycosyldiglycerides in which the glycerol-1-P may be acylated. These lipids can serve as membrane anchors for teichoic acids, polymers of glycerol-P or ribitol-P.[4]

[3] The mesosomes, which are considered by some investigators to be artifacts of electron microscopy, will not be considered here.

[4] The lipids of the *Actinomycetes* are considerably more complex (4), and since little is known about the phase behavior of many of their lipids, they do not lend themselves to this type of analysis.

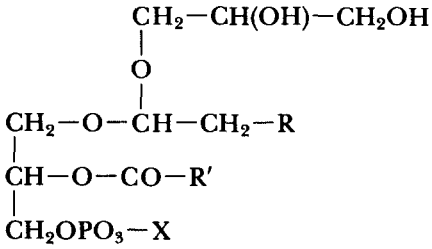
## CELLULAR CONTROL OF BILAYER STABILITY

While most bacteria do not use exogenous fatty acids in preference to those synthesized endogenously (41), certain species are fatty acid auxotrophs, and others can become fatty acid-dependent if a cofactor for fatty acid synthesis is limiting. This poses a problem for controlling bilayer stability, especially if the variety of fatty acids the cells can utilize is extensive, as is the case for *Acholeplasma laidlawii* and the butyric acid-producing clostridia.

The A strain of *A. laidlawii* responds to increasing growth temperatures or increased incorporation of unsaturated acyl chains or cholesterol by increasing the ratio of diglucosyldiglyceride (DGDG) to monoglucosyldiglyceride (MGDG), two of its major polar lipids. The other major lipids are PG, DPG, and glycerophosphate derivatives of the glycolipids. MGDG prefers the hexagonal or cubic phase at physiological temperatures, especially when enriched with unsaturated fatty acids; whereas DGDG by itself forms a stable lamellar phase and can stabilize the lamellar phase of MDGD (42, 43). The phase behavior of these lipids follows the rules set forth in Table 1, since $a_o$ of DGDD is larger than that of MGDG. Cholesterol is viewed as having a large hydrocarbon volume relative to surface area and increases the tendency of unsaturated MGDG/DGDG mixtures to form non-lamellar phases (43). Addition of cholesterol to MGDG/DGDG mixtures with nearly equal amounts of 16:0 and 18:1 chains did not disrupt the lamellar phase. These results parallel those obtained with mixtures of unsaturated soya PE plus PC (44). Addition of cholesterol to PE/PC mixtures in which PC contained unsaturated chains destabilized the bilayer phase, whereas addition to soya PE/dipalmitoyl PC stabilized the bilayer configuration. Wieslander et al. (42, 43) have postulated that the changes in lipid composition seen in *A. laidlawii* represent a cellular response to domains of non-lamellar lipid. As temperature, acyl chain unsaturation, or cholesterol content is increased, according to this view, the bilayer will be destabilized, and this tendency is counteracted by the conversion of MGDG to DGDG.

A second example of regulation of lipid class composition in response to growth temperature and the degree of lipid unsaturation has been described in the butyric acid-producing clostridia. The major lipids in these cells include either PE (*C. butyricum*) or PME (*C. beijerinckii*), both of which are approximately three-fourths plasmalogen, and PG plus DPG, which are approximately one-third plasmalogen (45). These organ-

isms also have a unique glycerol acetal of their major nitrogen-containing plasmalogens (I).



(I)

In both species, as the degree of unsaturation of the membrane lipids is increased, the ratio of glycerol acetal lipid to plasmalogen increases (46, 47). The effect of temperature has only been studied in *C. beijerinckii* ATCC 6015 (formerly *C. butyricum*) (48), in which the ratio of glycerol acetal lipid to total PE plus PME decreases as the growth temperature is lowered. However, this response is complex because the plasmalogen form of PG also replaces the plasmalogen forms of PE plus PME at lower growth temperatures (49). Thus, as in *A. laidlawii*, increasing lipid unsaturation or growth temperature leads to an increase in the proportions of polar lipids with larger effective head group areas, and these replace lipids that do not form a stable lamellar phase when their aliphatic chains are largely unsaturated (42, 43, 50). In both species the lipids involved have been localized in the outer leaflet of the cell membrane (39, 51), although the experiments on *A. laidlawii* have been questioned (52). Recent $^{31}P$ NMR experiments in this laboratory have shown that the oleate-enriched glycerol acetal of PE plasmalogen (I) forms a stable bilayer at temperatures up to 60°C and can stabilize the bilayer phase of egg PE, in a manner similar to PC (2) (H. Goldfine and N. C. Johnston, unpublished).

These two well-regulated systems represent metabolic responses of bacterial cells to the potential dangers of bilayer instability. Bacteria that cannot mount such a response, as is the case in *E. coli*, normally do not utilize exogenous fatty acids promiscuously (41) and, as described above, their lipid composition appears to be suitable for the fatty acids normally synthesized by the cell. The regulation of the lipid class composition of *E. coli* and other bacteria is currently under intensive study, especially with respect to genetic control mechanisms (53). Future studies need to be directed toward the contribution of membrane phase state in the control of the synthesis of bacterial and other membrane lipids.

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