# *THE ROLE OF CELL-WALL LIPID IN THE BIOSYNTHESIS OF BACTERIAL LIPOPOLYSACCHARIDE**

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In gram-negative enteric bacteria, the cell envelope is composed largely of protein glycopeptide, lipopolysaccharide, and in addition, substantial amounts of lipid.[1] The lipopolysaccharide contains the somatic O-antigens as well as the structures responsible for endotoxin activity,[2] and is distinct from the lipid fractions obtained by usual lipid extraction procedures. Until now, no biologic activity has been established for these lipid fractions, and their relation to the other components of the intact cell envelope has been unknown.

In the present communication we present evidence that a specific interaction occurs between the lipopolysaccharide and the phospholipid component of the cell envelope, and that this interaction is essential for the enzymatic reactions leading to the synthesis of the polysaccharide portion of the lipopolysaccharide.

Biosynthesis of the lipopolysaccharide has been studied in mutant strains which are unable to synthesize specific nucleotide sugars and which, therefore, produce incomplete lipopolysaccharides.[3, 4] In *Salmonella typhimurium*, these studies have revealed the existence of several soluble enzymes which catalyze the sequential transfer of specific sugars into the growing lipopolysaccharides.[5] Two of these reactions can be described as follows:

$$\text{Glucose-deficient lipopolysaccharide} + \text{UDP-glucose} \rightarrow \text{Glucosyl-lipopolysaccharide} (+\text{UDP}) \quad (1)$$

$$\text{Glucosyl-lipopolysaccharide} + \text{UDP-galactose} \rightarrow \text{Galactosyl-glucosyl-lipopolysaccharide} (+\text{UDP}). \quad (2)$$

In reaction (1), the incorporation of glucose into the lipopolysaccharide is catalyzed by the enzyme UDP-glucose lipopolysaccharide glucosyl transferase, and the acceptor is obtained from a mutant deficient in UDP-glucose. In reaction (2), the incorporation of galactose is catalyzed by the enzyme UDP-galactose lipopolysaccharide galactosyl transferase, and the acceptor is derived from a mutant deficient in UDP-galactose.

The major portion of these enzyme activities is found in the particulate cell envelope fraction, which also contains the acceptor lipopolysaccharide, but significant enzyme activity is also present in the soluble fraction. The soluble enzymes show an absolute requirement for the addition of the appropriate acceptor. In previous studies we have used the crude cell envelope fractions, heated to destroy their endogenous enzyme activity, as acceptors. It has been demonstrated that the monosaccharide units are transferred into the deficient lipopolysaccharides,[5] but the lipopolysaccharides themselves, separated from the other components of the cell envelope by phenol extraction and purified by $Mg^{++}$ precipitation and dialysis, do not act as acceptors in the transferase reactions. This unexpected finding suggested a requirement for an additional component of the cell envelope.

We have now found that extraction of the cell envelope with lipid solvents completely destroys its capacity to accept sugars in the transferase reactions. This treatment removes most of the fatty acid and phospholipid, but does not remove the lipopolysaccharide itself. We have found that recombining the lipid extract with the residual cell envelope material completely restores the acceptor activity. It has also been possible, for the first time, to convert the purified lipopolysaccharide into a completely active acceptor by recombining it with the lipid extract. The activity of the lipid fraction is recovered in the phospholipid component, and similar fractions from various species of bacteria are equally active.

*Materials and Methods.*—(1) *Mutant strains of S. typhimurium:* Strain SL-797,[6] which is unable to synthesize UDP-glucose because of a deficiency in phosphoglucose isomerase, was the source of the glucose-deficient cell envelope fraction and lipopolysaccharide. Strain G-30,[4] which lacks UDP-galactose-4-epimerase, was the source of galactose-deficient cell envelope and lipopolysaccharide. Strain EI-1,[5] which is deficient in both enzymes, was the source of transferase enzymes. Cells were grown and disrupted by sonication, as previously described.[5] The 105,000 $\times$ *g* supernatant fraction from cells in exponential growth phase was the source of enzyme. The 1200–12,000 $\times$ *g* sediment from cells grown to stationary phase was suspended in 0.01 *M* Tris buffer, pH 7.8, and heated in a boiling water bath for 10 min to inactivate the endogenous transferase enzymes. This suspension was used as the cell envelope fraction. Lipopolysaccharide was purified from this fraction as previously described.[4]

(2) *Analytic and preparative techniques:* UDP-glucose-$C^{14}$ and UDP-galactose-$C^{14}$ were prepared as previously described.[5] Dry weights were determined after drying in a vacuum oven at 60° for 18 hr. Thin-layer chromatography was performed on silica gel G (Brinkmann), using chloroform-methanol-water (65:25:4) and visualizing the spots with iodine vapor and with 2,7-dichlorofluoroscein. Other analytic techniques were as previously described.[4, 5]

*Results.*—*Requirement for lipid fraction in the UDP-galactose lipopolysaccharide galactosyl transferase reaction:* The crude cell envelope fraction prepared from sonic extracts of the galactose-deficient mutant was an effective acceptor for galactose transfer (Table 1), but became completely inactive following extraction with lipid solvents. The lipid extracts contained no lipopolysaccharide, and were also in-

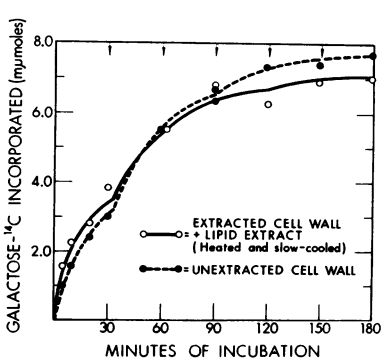


FIG. 1.—Effect of lipid extract on the course of the UDP-galactose transferase reaction. In this figure, cell wall refers to cell envelope. The preparation of the intact cell envelope fraction, extracted cell envelope fraction, and lipid extract, the amounts used, and the conditions of heating and slow-cooling were described in the legend to Table 1. The reactions were carried out as described in Table 1, each point representing a separate assay tube. Mixtures of the extracted cell envelope and lipid extract were heated and slow-cooled as described in Table 1. Controls without acceptor were run in parallel and the values subtracted from the experimental values. In tubes with lipid extract alone, or with extracted cell envelope fraction plus 0.05 ml of ethanol, the incorporation of galactose-$C^{14}$ was equal to that in the control tubes. Additional enzyme (0.6 mg) and UDP-galactose-$C^{14}$ (20 mμmoles) were added to each tube and to each control tube at 30-min intervals between 30 and 150 min of incubation, as indicated by the arrows.

TABLE 1

THE EFFECT OF LIPID EXTRACTION OF THE INTACT CELL ENVELOPE ON THE UDP-GALACTOSE LIPOPOLYSACCHARIDE GALACTOSYL TRANSFERASE REACTION

| Acceptor | Galactose-$C^{14}$ Incorporation ($m\mu$moles/10 min) Expt. A | Expt. B |
|---|---|---|
| None (enzyme alone) | 0.07 | 0.04 |
| Unextracted cell envelope | 1.04 | 0.60 |
| Extracted cell envelope | 0.09 | 0.08 |
| Lipid extract | 0.08 | 0.08 |
| Extracted cell envelope + lipid extract | 0.45 | 0.33 |
| Extracted cell envelope + lipid extract (heated and slow-cooled) | 0.97 | 0.63 |

For assay of UDP-galactose lipopolysaccharide galactosyl transferase, the assay mixture contained 80 mM Tris buffer, pH 8.5, 8 mM $MgCl_2$, 0.08 mM UDP-galactose-$C^{14}$, 0.6 mg of enzyme protein, and acceptor, in a total volume of 0.25 ml. Sufficient ethanol was added so that the solvent concentration was equal in all tubes. At these levels, the solvent had no effect on the rate of the transferase reactions. Samples were incubated for 10 min at 37°, the reaction mixtures were precipitated by the addition of 5% trichloroacetic acid, and radioactivity was determined as previously described.[5] This time period gives an accurate measure of the initial rate of the reaction. Controls without acceptor were included in each series of assays.

The heated cell envelope suspension from strain G-30 was centrifuged, and 1.4 gm (wet weight) of the pellet was extracted by stirring with 80 ml of absolute ethanol at room temperature for 60 min. The mixture was centrifuged at 12,000 × $g$ for 20 min, and the ethanol solution was evaporated to 7 ml under reduced pressure [final concentration 4 mg (dry weight) per ml]; this is referred to as the lipid extract. The sediment was mixed with 80 ml of absolute ethanol, recentrifuged, and the final extracted sediment was suspended in 7 ml of 0.01 *M* Tris buffer, pH 7.8, 0.001 *M* EDTA. This was sonicated for 15 sec with a Branson "Sonifier" to yield a uniform milky suspension; this was the extracted cell envelope fraction. An equal amount of the unextracted cell envelope was suspended in 7 ml of the same buffer, and sonicated in the same manner; this was the unextracted cell envelope fraction. Aliquots of 0.05 ml of the fractions were included as acceptors in the standard assay as indicated. This quantity of cell envelope fraction (containing 0.06 $\mu$moles of lipopolysaccharide heptose) gave maximal reaction rates. In the last experiment, all components except enzyme (105,000 $g$ supernatant fraction from strain E1-1) and UDP-galactose-$C^{14}$ (1000 cpm per m$\mu$mole) were mixed and heated in covered tubes in a 60° water bath for 30 min. The water bath was allowed to cool to 25° over a 2-hr period, the tubes were chilled, enzyme and UDP-galactose-$C^{14}$ were added, and the assays were carried out as usual. Expts. A and B represent experiments with different enzyme preparations.

active as acceptors. When both the lipid extract and the extracted cell envelope were included in the reaction mixture, significant reconstitution of acceptor activity was seen. However, this return of acceptor activity was variable, and ranged from 10 to 55 per cent of the original activity of the intact cell envelope. It was found that 100 per cent of the original acceptor activity was restored when the lipid extract and suspensions of the extracted cell envelope were mixed, heated at 60° for 30 min, and gradually cooled to room temperature. During heating and slow-cooling the presence of both the lipid extract and extracted cell envelope fraction were necessary; heating and slow-cooling of both components separately prior to mixing did not cause any significant increase of acceptor activity. The reconstituted cell envelope was also similar to the unextracted cell envelope in its capacity to accept galactose when maximal incorporation was achieved by repeated additions of enzyme and UDP-galactose-$C^{14}$ (Fig. 1). In these experiments, total incorporation of galactose-$C^{14}$ into the reconstituted cell envelope was 90–95 per cent of the incorporation obtained with the intact cell envelope. Absolute ethanol was usually employed for lipid extraction because of greater ease in handling the extracts, but similar results were obtained by extraction with chloroform-methanol (3:1, v/v) or ethanol-diethyl ether (3:1, v/v).

*Purified lipopolysaccharide as acceptor in the UDP-galactose lipopolysaccharide galactosyl transferase system:* As previously reported,[5] the purified lipopolysaccharide was inactive as an acceptor in the galactosyl transferase reaction. However, high rates of incorporation of galactose were observed if lipopolysaccharide and lipid extract were first mixed, heated, and slow-cooled as described above, and then assayed for acceptor activity (Table 2). Negligible acceptor activity was seen if lipopolysaccharide and lipid extract were merely mixed in the assay tube;

TABLE 2

THE EFFECT OF LIPID EXTRACT ON LIPOPOLYSACCHARIDE IN THE UDP-GALACTOSE LIPOPOLYSACCHARIDE GALACTOSYL TRANSFERASE REACTION

| Acceptor | Galactose-$C^{14}$ incorporated[a] (mμmoles/10 min) |
|---|---|
| None (enzyme alone) | 0.07 |
| Unextracted cell envelope | 0.93 |
| LPS[b, d] | 0.05 |
| Lipid extract[c, d] | 0.08 |
| LPS + lipid extract | 0.09 |
| LPS + lipid extract[d] (heated and slow-cooled) | 0.94 |

[a] Assays were performed as described in the legend to Table 1.

[b] LPS refers to galactose-deficient lipopolysaccharide purified from strain G-30 by the phenol extraction and $Mg^{++}$ precipitation procedure.[4] The amount added, equivalent to 0.1 μmoles of heptose, resulted in maximal reaction rates.

[c] Lipid extract was prepared as described in Table 1; 0.05 ml was added where indicated.

[d] All components were mixed, heated, and slow-cooled (see Table 1) before addition of UDP-galactose-$C^{14}$ (1000 cpm per mμmole).

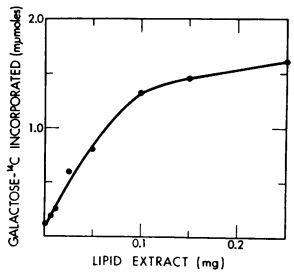


FIG. 2.—Effect of concentration of lipid extract on rate of UDP-galactose lipopolysaccharide transferase reaction. Lipid extract and galactose-deficient lipopolysaccharide (0.1 μmoles of heptose) were heated and slow-cooled prior to addition of UDP-galactose-$C^{14}$ (1500 cpm per mμmole) and enzyme (see Table 1). Galactose-$C^{14}$ incorporated is expressed as mμmoles in 10 min.

heating and slow-cooling of the lipid-lipopolysaccharide mixture were essential for the lipopolysaccharide to act as an active acceptor. The heating and slow-cooling procedure seemed optimal, although appreciable acceptor activity was also seen with shorter periods of heating, or with more rapid cooling. Higher temperatures and extended heating periods were not extensively studied. Heating to 100° for 20 min did not reduce the acceptor activity if lipopolysaccharide and lipid extract had been previously heated to 60° and slow-cooled.

The effect of varying concentrations of the lipid extract on the rate of the transferase reaction is shown in Figure 2. The rate was proportional to the concentration of lipid from 0.1 to 0.35 mg per ml, and a maximal rate was seen with concentrations above 0.75 mg per ml (dry weights).

The reaction with UDP-galactose-$C^{14}$ was specific for lipopolysaccharides derived from galactose-deficient cells. The lipopolysaccharide from strain SL-797 (glucose-deficient) lacks the sites of attachment for gaactose,[7] and was inactive as an acceptor for galactose, even when heated and slow-cooled with the lipid extract. The lipopolysaccharide extracted from wild-type *S. typhimurium*, which already contains the full complement of sugars, also did not serve as acceptor.

*Requirement for lipid fraction in the UDP-glucose lipopolysaccharide glucosyl transferase reaction:* The requirement for the lipid fraction of the cell envelope is not unique for the galactosyl transferase system. Similar experiments have revealed an identical requirement in the glucosyl transferase system [reaction (1)]. Extraction with lipid solvents of the cell envelope fraction from the glucose-deficient mutant caused complete loss of acceptor activity; essentially no glucose was transferred into the extracted cell envelope (Table 3). Significant acceptor activity returned when the lipid extract and extracted cell envelope fraction were recombined under the same conditions of heating and slow-cooling that were optimal for the galactosyl transferase system. In this experiment, 35 per cent of the original acceptor activity of the intact cell envelope was restored.

The purified lipopolysaccharide from glucose-deficient cells was also transformed

TABLE 3

EFFECT OF THE LIPID EXTRACT ON THE UDP-GLUCOSE LIPOPOLYSACCHARIDE GLUCOSYL TRANSFERASE SYSTEM

| Additions | Cpm | Glucose-$C^{14}$ incorporated ($m\mu$moles/10 min) |
|---|---|---|
| *Experiment A* | | |
| None (enzyme alone) | 44 | 0.02 |
| Unextracted cell envelope | 504 | 0.25 |
| Extracted cell envelope | 18 | 0.01 |
| Lipid extract | 25 | 0.01 |
| Extracted cell envelope and lipid extract | 174 | 0.09 |
| *Experiment B* | | |
| None (enzyme alone) | 39 | 0.02 |
| Unextracted cell envelope | 728 | 0.37 |
| Glucose-deficient LPS | 89 | 0.04 |
| Glucose-deficient LPS and lipid extract | 400 | 0.20 |

The assay mixture contained 80 mM Tris buffer, pH 7.8, 20 mM $MgCl_2$, 0.08 mM UDP-glucose-$C^{14}$ (2000 cpm per $m\mu$mole), 0.12 mg of enzyme protein, and acceptor, in a total volume of 0.25 ml. The assay procedure was as described in the legend to Table 1.

In expt. A, 62 mg (wet weight) of the cell envelope fraction prepared from strain SL-797 (glucose-deficient) was extracted with 2.5 ml of absolute ethanol at room temperature for 60 min. The supernatant solution obtained by centrifugation at 12,000 $\times$ *g* for 20 min was evaporated to 0.25 ml under reduced pressure; this was the lipid extract. The sediment was washed once with 2 ml of 0.01 *M* Tris buffer, pH 7.8, and then was suspended in 0.25 ml of the same buffer and used as the extracted cell envelope. An equal amount of the original cell envelope fraction was suspended in 0.25 ml of the same buffer and was the unextracted cell envelope. Aliquots of 0.03 ml of each fraction were included when indicated. This amount of cell envelope contained 0.05 $\mu$moles of lipopolysaccharide heptose, and gave maximal reaction rates. All components were heated and slow-cooled as described in Table 1, before the addition of enzyme and UDP-glucose-$C^{14}$.

In expt. B, the lipid extract was prepared as described in Table 1, and 0.05 ml was added to the indicated tubes. All tubes were heated and slow-cooled as described above. The glucose-deficient lipopolysaccharide contained 0.07 $\mu$moles of heptose.

into an active acceptor if it was first mixed with the lipid extract, heated, and slow-cooled (Table 3).

*Characterization of the radioactive products:* With both enzyme systems it was established that the reactions involved addition of the glycosyl residue to a lipopolysaccharide acceptor (Table 4). Essentially all of the radioactivity incorporated was recovered in the purified lipopolysaccharides. In paper electrophoresis of the polysaccharide components, the radioactivity corresponded to the major anionic fractions of the mutant lipopolysaccharides. Galactose and glucose were the only radioactive compounds detected after complete hydrolysis of the products of the galactosyl transferase and glucosyl transferase reactions, respectively. The radioactive products were, therefore, identical to those found when the unextracted cell envelope fraction was used as acceptor in the transferase reactions.[5]

*Purification and characterization of the active lipid factor:* Chloroform-methanol extracts of the cell envelope were subjected to repeated silicic acid column chromatography. The bulk (80–90%) of the original activity of the lipid extract was recovered in the fractions which contained the phospholipid components. The two most active fractions, 2 and 3, were equal in activity (Table 5), and each showed a single component in thin-layer chromatography. The $R_f$ of fraction 2 was identical to authentic phosphatidyl ethanolamine. Fraction 3 appears to be lysophosphatidyl ethanolamine, but has not yet been positively identified.

Identification of the active component as phosphatidyl ethanolamine was supported by studies with purified lipid preparations. Phosphatidyl ethanolamines isolated from several gram-negative organisms by Dr. J. Law of Harvard University

TABLE 4

PURIFICATION OF RADIOACTIVE PRODUCTS[a]

| Stage of purification | Galactose-C$^{14}$ [b] A[d] Cpm | % | B[e] Cpm | % | Glucose-C$^{14}$ [c] C[d] Cpm | % | D[e] Cpm | % |
|---|---|---|---|---|---|---|---|---|
| Trichloroacetic acid precipitate | 28,120 | 100 | 47,500 | 100 | 14,000 | 100 | 7,500 | 100 |
| Crude lipopolysaccharide (after phenol extraction) | 26,300 | 94 | 41,900 | 88 | 15,900 | 110 | 8,000 | 106 |
| Purified lipopolysaccharide (after $Mg^{++}$ precipitation and dialysis) | 26,000 | 93 | 40,700 | 86 | 15,100 | 108 | 7,200 | 96 |
| Soluble polysaccharide (after hydrolysis at pH 3.4) | 26,300 | 94 | 39,400 | 83 | 14,400 | 103 | 6,200 | 83 |
| Electrophoresis of soluble polysaccharide (cpm in anionic peaks)[f] | 100% | — | 100% | — | 100% | — | 90% | — |

[a] Products were prepared by increasing the reaction mixtures described in Tables 1–3 by 5- or 10-fold. Lipid extract and acceptor were mixed under conditions of heating and slow-cooling, prior to addition of enzyme and nucleotide sugar. Maximal loading was achieved by repeated additions of enzyme and nucleotide sugar (see Fig. 1). After incubation at 37° for a total of 180 min, the reactions were terminated by the addition of trichloroacetic acid (final concentration 5%), and the precipitates were washed twice with 5 vol of 5% trichloroacetic acid. Lipopolysaccharide was purified, soluble polysaccharide was obtained, and electrophoresis was performed, as previously described.[4, 5]

[b] The donor was UDP-galactose-C$^{14}$ (2000 cpm per mμmole).

[c] The donor was UDP-glucose-C$^{14}$ (2000 cpm per mμmole).

[d] The acceptors were the extracted cell envelope fractions of strain G-30 (expt. A), and strain SL-797 (expt. C).

[e] The acceptors were the purified lipopolysaccharides isolated from strain G-30 (expt. B), and from strain SL-797 (expt. D).

[f] The anionic electrophoretic peaks corresponded to the major polysaccharide components of the nonradioactive lipopolysaccharides.[5]

were found to be fully as active as the phospholipid fraction isolated from *S. typhimurium* (Table 5). Of great interest is the relatively low activity of the three synthetic phosphatidyl ethanolamines tested, which contained decanoic, hexanoic, and palmitic acids as their fatty acid components. None of these compounds, which contain saturated fatty acids of varying chain length, showed activity approaching that of the bacterial phosphatides. This indicates a specific role for the fatty acid components of the bacterial phosphatidyl ethanolamine preparations. The activity of other phospholipids, and the specific role of individual fatty acids is under investigation.

*Discussion.*—The results demonstrate a requirement for the lipid fraction of the cell envelope in the biosynthesis of the endotoxin lipopolysaccharide. Not only does the lipid fraction restore the acceptor activity of the extracted cell envelope, but it also transforms the purified lipopolysaccharide into an active acceptor for the enzymatic incorporation of sugars.

It is clear that the lipid affects the lipopolysaccharide component of the transferase systems, rather than the enzyme. This is shown by the requirement that the lipid fraction and acceptor lipopolysaccharide must be mixed under specific conditions of heating and slow-cooling *prior* to the addition of transferase enzyme and nucleotide sugar. The reactions can be schematically represented for the galactosyl transferase reaction as follows:

$$\text{Lipopolysaccharide} + \text{Lipid} \xrightarrow[\text{slow-cool}]{\text{heat,}} \text{Lipopolysaccharide:Lipid complex}$$

$$\text{Lipopolysaccharide:Lipid complex} + \text{UDP-galactose} \xrightarrow[\text{transferase}]{\text{galactosyl}} \text{Galactosyl lipopolysaccharide:Lipid complex.}$$

The lipid may act by providing an essential site for enzyme binding or by altering

TABLE 5

EFFECT OF PURIFIED LIPIDS ON THE UDP-GALACTOSE LIPOPOLYSACCHARIDE GALACTOSYL TRANSFERASE SYSTEM

| Lipid | Units[a] per mg | Units[a] per μmole P |
|---|---|---|
| Purified column fractions, *S. typhimurium*[b] | | |
| Fraction 1 (fatty acids) | 0.8 | — |
| Fraction 2 (phosphatidyl ethanolamine) | 5.5 | 6.7 |
| Fraction 3 | 5.4 | 6.3 |
| Authentic phosphatidyl ethanolamine[c] | | |
| *E. coli* | — | 5.5 |
| *A. agilis* | — | 5.5 |
| Dihexanoyl (synthetic) | — | 0.13 |
| Didecanoyl (synthetic) | — | 0.97 |
| Dipalmitoyl (synthetic) | — | 0.35 |
| Crude bacterial lipid extracts[d] | | |
| *S. typhimurium* | 4.8 | — |
| *S. paratyphi A* | 4.1 | — |
| *Shigella flexneri* | 5.0 | — |
| Other lipids[e] | | |
| Phosphatidyl serine | — | 2.2 |
| Phosphatidyl choline (dipalmitoyl) | — | 0.19 |
| Oleic acid | 0.4 | — |
| Triolein | 0.0 | — |

[a] The assay mixture was as described in the legend to Table 1, except that each tube contained galactose-deficient lipopolysaccharide (0.1 μmoles of heptose). The lipids were dissolved in methanol (4 mg per ml, dry weight), and 0.05-ml aliquots were added. The reaction mixture was heated and slow-cooled (see Table 1) prior to the addition of enzyme and UDP-galactose-$C^{14}$ (2000 cpm per mμmole). Tubes were incubated at 37° for 10 min, trichloroacetic acid was added, and radioactivity was determined as usual. Units are mμmoles of galactose-$C^{14}$ incorporated in 10 min. Total phosphate was determined on aliquots of the phospholipid solutions,[8] and the results of the assays were expressed per μmole phosphate; with other lipids, dry weights were determined prior to dissolving the individual lipids, and these measurements are less accurate than the phosphate determinations.

[b] *S. typhimurium* column fractions were obtained by silicic acid column chromatography of a chloroform-methanol extract of the cell envelope fraction of strain G-30.[9] Fatty acids were eluted with diethyl ether-petroleum ether (1:9, v/v) and chloroform; phospholipids were eluted with increasing concentrations of methanol in chloroform (from 1:9 to 1:1, v/v), and were rechromatographed in an identical manner to obtain the purified fractions.

[c] The purified phosphatidyl ethanolamines from *E. coli* and *Azotobacter agilis*, and the synthetic dihexanoyl and didecanoyl phosphatidyl ethanolamines (prepared by the method of Maurukas, Kairys, and Holland[10]), were generous gifts from Dr. John Law.

[d] Crude bacterial lipid extracts were prepared from cell envelope preparations as described in the legend to Table 1.

[e] The other lipids were purchased from the Sigma Chemical Co.; the phosphatidyl serine was a crude preparation, prepared from bovine brain.

the physical state of the lipopolysaccharide. The latter mechanism could involve the formation of a micellar structure, the production of an altered conformation of the lipopolysaccharide, or a change in the state of aggregation or dispersion of the lipopolysaccharide. Further study is required to clarify the nature of the lipopolysaccharide-lipid interaction.

The precise location of the lipid fraction in the cell envelope has not been established. In the present study it has not been possible to distinguish cell-wall lipid from the lipid of the cytoplasmic membrane.

In the present communication, the lipid fraction is defined as the material which can be extracted from the intact cell envelope by treatment with lipid solvents. This includes "Lipid B" of Westphal,[2] but not the lipid portion of the lipopolysaccharide (Westphal's "Lipid A"), which is not extracted under these conditions. The studies of Kaneshiro and Marr[11] and of Law[12] have shown that the lipid fractions of gram-negative bacteria usually consist largely of phosphatidyl ethanolamine, with smaller or trace amounts of phosphatidyl serine, free fatty acids, esterified fatty acids, and coenzyme Q.

We have shown that the activity of the lipid extract in the enzymatic glycosyl transferase reactions resides in the phosphatidyl ethanolamine of the cell envelope. It is of particular interest that synthetic preparations containing saturated fatty

acids, hexanoic (C-6), decanoic (C-10), and palmitic (C-16) acids, showed negligible or only slight activity as compared with the phosphatidyl ethanolamines derived from gram-negative bacteria. The bacterial compounds characteristically have high proportions of either unsaturated fatty acids,[11] or of unusual fatty acids containing cyclopropane rings,[11-13] and it is possible that the fatty acid components are responsible for the specificity of the bacterial phospholipids in the lipid-lipopolysaccharide interaction.

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