Identification of sn-Glycero-1-phosphate and Phosphoethanolamine Residues Linked to the Membrane-derived Oligosaccharides of Escherichia coli*

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A previous report from this laboratory (van Golde, L. M. G., Schulman, H., and Kennedy, E. P. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 1368-1372) described the discovery in Escherichia coli of a novel class of oligosaccharides, containing glucose as the sole sugar, substituted with glycerophosphate units derived from membrane phospholipids, and with succinic acid in O-ester linkage. These membrane-derived oligosaccharides, comprising about 0.5 to 1.0% of the dry weight of E. coli, represent a family of closely related oligosaccharides that may be subfractionated on anion exchange resins. The present paper describes studies of the oligosaccharide A-1 described by van Golde et al. in the previous report. The glycerophosphate linked to the oligosaccharide in phosphodiester bond is the sn-glycero-1-P enantiomer. This finding strongly supports the previous conclusion that the oligosaccharides are the acceptors of the polar head groups of membrane phospholipids, since the unesterified glycerophosphate of phosphatidylglycerol is an sn-glycero-1-P residue, otherwise rare in nature. The glycerophosphate residues in the membrane-derived oligosaccharide are not substituted in the sn-2 or sn-3 positions, since they are readily oxidized by periodate under mild conditions. Alkaline hydrolysis liberates glycerophosphate, and only negligible amounts of free glycerol, consistent with the view that the glycerophosphate residues are linked to glucose units through position 6, unfavorable for the formation of glucose cyclic phosphate intermediates that would eliminate free glycerol.

Oligosaccharide A-1 (but not Fraction A-2) contains phosphoethanolamine residues equivalent to 30 to 40% of the total phosphorus. The phosphoethanolamine residues are linked to position 6 of glucose units, as proved by the isolation of glucose 6-phosphate as a product of partial acid hydrolysis.

Isotope tracer experiments by Kanfer and Kennedy in 1963 (1) revealed that 32P is continuously lost from phosphatidylglycerol during logarithmic growth of Escherichia coli. In 1973, van Golde et al. (2) discovered that the polar head groups of phosphatidylglycerol are continuously transferred to a novel class of oligosaccharides found in the water-soluble fraction of E. coli. These oligosaccharides contain glucose as the sole sugar. The average chain length corresponds to about 9 glucose units. It will be shown in this report that the glycerophosphate residues linked to the membrane-derived oligosaccharides are of the sn-1 configuration in the stereospecific numbering system of Hirschmann (3). It will also be shown that some species of oligosaccharides contain phosphoethanolamine in phosphodiester linkage to position 6 of glucose units. The presence of succinyl-O-ester linkages in these oligosaccharides has already been reported (2).

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‡Work on the structure of the oligosaccharide moiety of these substances will be described in another publication.

The membrane-derived oligosaccharides constitute a closely related family of compounds. Considerable variation in type and amount of substitution has been revealed by anion exchange chromatography (2). The studies reported here will be concerned almost entirely with the fraction designated A-1 by van Golde et al. (2).

MATERIALS AND METHODS

Preparation of Membrane-derived Oligosaccharides—Frozen cells of Escherichia coli K12 harvested in the late logarithmic phase of growth on rich medium were obtained from the Grain Processing Corp., Muscatine, la. The membrane-derived oligosaccharide fraction was prepared by a large scale adaptation of the procedures of van Golde et al. (2), involving extraction with 50% ethanol, concentration of the extract in a rotary evaporator, and fractionation of the concentrated extract on Bio-Gel P6. The membrane-derived oligosaccharides, detected by the anthrone method (4) emerge in a peak of intermediate molecular weight (2). The fractionations were carried out at room temperature and propanol (7%, v/v) was included in all buffers to prevent bacterial growth. The membrane-derived oligosaccharides were next fractionated successively on columns of DEAE-cellulose and of Dowex 1 acetate, essentially by adaptations of the procedure of van Golde et al. (2).

Fractions containing oligosaccharide A-1 were pooled, concentrated to a small volume, and precipitated by the addition of 4 volumes of ethanol, followed by 2 volumes of ether. The precipitate was recovered.
Membrane-derived Oligosaccharides of Escherichia coli

by centrifugation, taken up in a minimum volume of water, and similarly reprecipitated. The material was finally dried in a desiccator under vacuum or P₂O₅.

The A₁ fraction so obtained contained (per mg) 4.1 μmol of glucose determined by the anthrone method, 0.67 μmol of total phosphorus (P), and 0.67 μmol of succinyl ester. Succinyl esters were determined by a variation of the method of Lee (9).

Reduction with Sodium Borohydride—A sample containing about 8.3 mg of oligosaccharide Fraction A-1 dissolved in 0.1 ml of 5 M sodium carbonate was treated with 0.1 ml of a fresh 0.5 M solution of NaBH₄, and the mixture was held at 25°C for 2 hours to complete the reduction. To destroy excess borohydride, 0.3 ml of 1 M H₂O₂ was added. When the evolution of hydrogen had ceased, the solution was taken to dryness at room temperature in a stream of air and repeatedly taken up in methanol and dried. The product was then taken up in 1 ml of water and purified by chromatography on Sephadex G-25 with 7% propanol as eluent.

Determination of sn-Glycerol-3-phosphate by Dehydrogenase Method—sn-Glycerol-3-phosphate was determined in a variation of the procedure of Bublitz and Kennedy (7) with glyceraldehyde dehydrogenase, an enzyme specific for the sn-glycerol-3-phosphate enantiomer. A glycine/hydrazine buffer was made by mixing 10 ml of 0.2 M glycine containing 0.05 M KCl with 0.68 ml of hydrazine hydrate (95%; Eastman Kodak). Best results appeared to be obtained if this buffer was prepared on the day it was to be used. The pH should be 9.7 upon 25-fold dilution of the buffer with distilled water. The test system contained the following components in a final volume of 1.0 ml: 0.4 ml of the hydrazine/glycine buffer, 5 mM 2-mercaptoethanol, 0.5 mM DPN, and 0.05 ml of a solution of crystalline dehydrogenase (Boehringer, an enzyme specific for the sn-glycero-3-phosphate enantiomer. The reaction was stopped by the addition of 0.5 ml of 1 N acetic acid. The precipitate was removed by centrifugation, and the sample was treated with 0.5 ml of 0.5 N trichloroacetic acid at room temperature for 10 min in a final volume of 0.3 ml. The method depends upon the preferential partition of N-dinitrophenyl phosphoethanolamine into the aqueous phase. Phosphoethanolamine and other substances that form dinitrophenyl derivatives preferentially soluble in chloroform do not interfere, but the method does not distinguish between phosphoethanolamine and other amines whose dinitrophenyl derivatives are preferentially soluble in water.

Determination of Glycerol by the Glycerokinase Method—The test system contained the following components in the indicated final concentrations: 0.1 M Tris chloride buffer of pH 7.6, 0.01 M MgCl₂, and 2 mM TPN. Neutralized samples to be tested, or standards containing 50 or 100 nmol of authentic glucose-6-P, were mixed with the above components, and the volume of each incubation was adjusted to 0.9 ml with water. The optical density at 340 nm was read and recorded. The reaction was then started by the addition of 0.1 to 0.2 unit of purified glucose-6-P dehydrogenase (Boehringer Mannheim) dissolved in 0.19 ml of 0.05 M Tris buffer of pH 7.0 containing 1% bovine serum albumin. After 30 min at 37°C, the increment of optical density at 340 nm was read, and compared with that of appropriate controls and standards.

RESULTS

Oligosaccharide Fraction A-1 Contains No Phosphomonoester when Tested with Alkaline Phosphatase—Samples of oligosaccharide Fraction A-1 were incubated with alkaline phosphatase (as described under “Materials and Methods”) under the conditions in which glycerophosphate is quantitatively hydrolyzed. No orthophosphate was produced. However, when the sample of oligosaccharide or the reduced product obtained by treatment with borohydride was hydrolyzed in 0.5 N NaOH at 100°C in polypropylene tubes, under conditions expected to break phosphodiester bonds, there was a progressive appearance of phosphomonoester until a maximum of 60 to 70% of the total phosphorus of the sample was liberated as phosphomonoester. Further hydrolysis did not increase this value significantly.

Isolation of α- and β-Glycerophosphates as Products of Alkaline Hydrolysis—A sample of Fraction A-1 containing 11.8 μmol of total phosphorus was hydrolyzed in 0.5 ml of 0.5 N NaOH at 100°C in a stopped polypropylene tube. The sample was cooled, diluted to 4.0 ml, and neutralized by treatment with 75 mg of CG 50 cation exchange resin, H⁺ form. The resin was removed by centrifugation, washed twice with water, and the washings were combined with the supernatant.

A portion of the alkaline hydrolysate containing 11 μmol of total phosphorus was chromatographed on Dowex 1 acetate under conditions known to give a clean and reproducible separation of α- and β-glycerophosphates, as described in the
legend to Fig. 1. The α-glycerophosphate peak contained a total of 3.15 μmol of phosphomonoester, and the β-glycerophosphate peak contained 3.84 μmol, emerging in the exact positions predicted from chromatography of authentic samples.

Paper Chromatography—The identity and purity of the glycerophosphate preparations was tested by ascending paper chromatography on Whatman No. 43 paper in Solvent System I containing 60 parts of acetone (v/v) and 40 parts of 0.2 M ammonium acetate/acetic acid buffer of pH 4.7. Each of the isolated preparations gave a single phosphorus-containing spot with an Rf identical with an authentic standard (0.61 for α-glycerophosphate and 0.63 for β-glycerophosphate).

Identification of sn-Glycerol-1-P—The α-glycerophosphate isolated after alkaline hydrolysis of oligosaccharide A-1 in the experiment of Fig. 1 could be either sn-glycerol-3-P (by far the most abundant form of α-glycerophosphate in nature) or its enantiomer, sn-glycerol-1-P. The following procedures offered unequivocal evidence that it is in fact sn-glycerol-1-P.

Liberation of Orthophosphate and of Glycerol by Alkaline Phosphatase—A portion of the isolated α-glycerophosphate was tested with alkaline phosphatase, and the liberated phosphate and glycerol were determined on the same sample (“Materials and Methods”). They were equivalent (Table I), strongly supporting the chromatographic identifications of glycerophosphate described above.

Periodate Lability—Treatment of a sample with a microadaptation of the method of Burmaster (8) (“Materials and Methods”) quantitatively released orthophosphate in good agreement with the amount found by the alkaline phosphatase method (Table I). Since β-glycerol-P is resistant to periodate, this offers strong evidence that the isolated material is indeed one of the enantiomers of α-glycerol-P.

Assay with sn-Glycerol-3-P Dehydrogenase—The dehydrogenase from rabbit muscle is completely specific for the sn-3 isomer. When assayed by the dehydrogenase method (“Materials and Methods”) the isolated α-glycerophosphate contained less than 3% of sn-3 isomer (Table I). To make sure that the negative finding was not the result of some technical problem, the sample of isolated glycerol-P was mixed with a known amount of authentic sn-glycerol-3-P and reassayed. The increment in optical density at 340 nm was exactly that predicted for the added authentic compound.

Acid Isomerization of sn-Glycerol-1-P to Form sn-Glycerol-3-P—If the isolated α-glycerophosphate is sn-glycerol-1-P, the migration of phosphate during treatment with 1 N HCl should result in an equilibrium mixture containing about 9% of sn-glycerol-2-P with the remainder an equal mixture of sn-1 and sn-3 isomers, as shown by the work of Baer and Kates (10). A sample of the isolated glycerophosphate was treated with 1 N HCl at 100° for 1 hour, cooled, neutralized, and analyzed. Acid isomerization led to the production of 50% of sn-glycerol-3-P, in satisfactory agreement with the expected amount (45.5%).

Identification of sn-Glycerol-2-P—The identity of the isolated β-glycerophosphate was confirmed by a series of analyses almost identical with those described above for the α isomer, as shown by the dehydrogenase assay (Table II). Acid isomerization converted the isolated β compound to the sn-3 isomer in 48% yield, in satisfactory agreement with the anticipated value.

Alkaline Hydrolysis of Oligosaccharide A-1 Does Not Yield Free Glycerol—In the experiments described above, the yield of glycerophosphate accounted very nearly quantitatively for the phosphomonoester released. Only traces of orthophosphate were found. This suggested that when the glycerol-P-saccharide

![Graph](http://www.jbc.org/)

**Fig. 1.** Separation and isolation of α- and β-glycerophosphate fractions. A portion (4 ml) of an alkaline hydrolysat of oligosaccharide A-1 was prepared and neutralized as described in the text, and containing 11 μmol of total phosphorus, was applied to a column (1.2 x 41 cm) of Dowex 1 acetate (8% cross-linked). All solvents contained 7% (v/v) propanol, and eluted with a linear gradient, beginning with 0.25 M potassium acetate adjusted to pH 6.4 with acetic acid, and ending with 0.50 M potassium acetate of the same pH, in a total volume of 600 ml. This program had previously been shown to separate α- and β-glycerophosphate cleanly and reproducibly. Fractions of 5 ml each were collected at the rate of four per hour. Samples of each fraction in the region in which glycerophosphate should be eluted were assayed for their content of phosphomonoester with the alkaline phosphatase method (“Materials and Methods”).

**Table I**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Result (μmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate released by alkaline phosphatase</td>
<td>1.80</td>
</tr>
<tr>
<td>Glycerol released by alkaline phosphatase (glycerokinase assay)</td>
<td>1.80</td>
</tr>
<tr>
<td>α-Glycerophosphate (periodate assay)</td>
<td>1.90</td>
</tr>
<tr>
<td>sn-Glycerol-3-P (dehydrogenase assay)</td>
<td>0.04</td>
</tr>
<tr>
<td>sn-Glycerol-3-P after acid isomerization (dehydrogenase assay)</td>
<td>0.90</td>
</tr>
</tbody>
</table>
diester bonds were broken during alkaline hydrolysis, only glycerol, and no free glycerol, was released. To test this more directly, a sample of oligosaccharide A-1 containing a total of 180 nmol of phosphorus, of which 126 nmol were glycerol, was hydrolyzed in 0.5 N NaOH for 80 min at 100°. A standard containing 100 nmol of free glycerol was treated in identical fashion. The samples were cooled, neutralized, and assayed for glycerol by the sensitive and specific glycerokinase method with 3P-labeled ATP ("Materials and Methods"). Only 5 nmol of glycerol were found in the hydrolysate of A-1.

Glycero-1-P Residues Attached to Oligosaccharide Fraction A-1 Are Not Further Substituted—If the glycerol-1-P residues linked by phosphodiester bonds to the oligosaccharide are further substituted on positions 2 or 3 of the glycerol, they should be resistant to treatment with periodate under mild conditions. To test this, a sample of Fraction A-1 containing 150 nmol of total phosphorus was treated with 0.033 M periodate in 0.033 M acetic acid for 15 min at room temperature in a total volume of 0.15 ml. The reaction was terminated by the addition of 0.1 ml of 0.3 M sodium bisulfite. In a control experiment, the bisulfite was added prior to the periodate to prevent oxidation. Each sample was then treated with 0.25 ml of 1 N NaOH and hydrolyzed at 100° for 80 min. The samples were cooled, neutralized with a stoichiometric amount of CG 50 H+ cation exchange resin, and made up to a total volume of 1.0 ml. Samples of the hydrolysate were next treated with alkaline phosphatase to liberate glycerol from glycerophosphate, and the liberated glycerol was assayed with glycerokinase, and 3P-labeled ATP ("Materials and Methods"). The control sample yielded 90 nmol of glycerol, whereas the sample oxidized with periodate yielded only 5 nmol of glycerol. Clearly the glycerophosphate residues in oligosaccharide A-1 are readily attacked by periodate and thus cannot be substituted either in the sn-2 or sn-3 positions.

Evidence for Phosphoethanolamine Residues in Oligosaccharide A-1—A sample of oligosaccharide A-1 was reduced with borohydride ("Materials and Methods"), hydrolyzed in 0.5 N NaOH for 80 min at 100°, and purified by chromatography on Sephadex G-25. The recovered material contained 40% of its original phosphorus. To make sure that this finding was not the result of incomplete hydrolysis, the isolated material was subjected to a second hydrolysis and purification. Based on the ratio of total phosphorus to sugar, determined by the anthrone method, the rehydrolyzed material retained 38% of its original total phosphorus.

The reduced hydrolyzed oligosaccharide fraction was tested for phosphoethanolamine with the fluorodinitrobenzene method ("Materials and Methods"). A sample containing 0.34 μmol of alkali-resistant phosphate was found to contain the equivalent of 0.32 μmol of phosphoethanolamine, consistent with the view that the alkali-resistant phosphate represents a diester of phosphoethanolamine, which if linked to position 6 of a glucose unit would be strongly resistant to hydrolysis in alkal.

Quantitative Analysis for Ethanolamine on Automated Amino Acid Analyzer—Since the fluorodinitrobenzene method is not specific for ethanolamine derivatives, the ethanolamine content of oligosaccharide A-1 was determined by chromatography on the automated amino acid analyzer. Two samples of oligosaccharide A-1 that contained 17 and 34 nmol, respectively, of apparent phosphoethanolamine by the fluorodinitrobenzene method were hydrolyzed in redistilled 6 N HCl at 110° for 18 hours in sealed tubes. Standards of authentic phosphoethanolamine were similarly treated, since the recovery of ethanolamine from phosphoethanolamine is not quantitative. All samples were analyzed on the amino acid analyzer. The samples of hydrolyzed oligosaccharide contained 17.7 and 33.5 nmol of ethanolamine in good agreement with the values for phosphoethanolamine by the fluorodinitrobenzene method, and with values for alkali-resistant phosphodiester.

Isolation of Phosphoethanolamine—If ethanolamine is attached through a phosphodiester bond to position 6 of glucose in the membrane-derived oligosaccharide (as the above evidence strongly indicates) treatment of the oligosaccharide with periodate under acid conditions would be expected to attack the glucose residues, with the formation of ethanolamine phosphoglycolalddehyde, which upon subsequent hydrolysis should yield phosphoethanolamine. Since the yield of phosphoethanolamine could not be expected to be quantitative, in the experiment described here, a small amount of phospho[1,2-14C]ethanolamine was added to the sample of oligosaccharide, and carried through the entire procedure to allow an estimate of the yield.

A sample of oligosaccharide A-1 containing a total of 7 μmol of phosphorus was mixed with 0.22 μmol of phospho[1,2-14C]ethanolamine (104,000 cpm/μmol) in a total volume of 0.20 ml, to which was added 3.0 ml of 0.1 M sodium periodate and 0.1 ml of 10 N sulfuric acid. The solution was heated at 100° for 1 hour. The solution was cooled, and neutralized with aqueous barium hydroxide (about 0.7 ml). The precipitate of barium sulfate was removed by centrifugation, and washed twice with 1 ml of water. The combined supernatant and washes (5.4 ml) contained 85% of the added radioactivity.

The solution was then chromatographed on Dowex 1 formate (Fig. 2). A single peak of radioactivity was recovered exactly in the position expected for authentic phosphoethanolamine. The peak fractions were combined and dried in a rotary evaporator, and taken up in 1.0 ml of water. Samples were analyzed for phosphoethanolamine by the fluorodinitrobenzene procedure and for radioactivity. The specific activity of the recovered phosphoethanolamine was 13,000 cpm/μmol, corresponding to a release of 1.54 μmol of unlabeled phosphoethanolamine from the oligosaccharide. The value expected from direct analysis of the oligosaccharide for phosphoethanolamine residues was 2.4 μmol. However, any phosphoethanolamine residues that are destroyed on the oligosaccharide prior to release will not contribute to the observed isotope dilution, so the value of 1.54 μmol is a minimum figure.

Further identification of the recovered phosphoethanolamine was made by paper chromatography on Whatman No. 43
(ascending) in Solvent System II containing 75 parts ethanol (v/v) and 25 parts of 0.5 M formic acid/1.0 M pyridine. The recovered material showed only a single ninhydrin-reactive spot (RF = 0.29) indistinguishable from authentic phosphoethanolamine. The dinitrophenyl derivative was prepared using the procedure for quantitative estimation of phosphoethanolamine ("Materials and Methods"). The aqueous phase after the chloroform extraction step was concentrated to a small volume and similarly chromatographed. It yielded a principal yellow spot of RF = 0.58 containing 77% of the radioactivity on the paper strip, and a faint spot of RF = 0.76 containing 8% of the radioactivity. Authentic phosphoethanolamine similarly yielded two spots of identical RF. The more rapidly migrating substance is evidently a minor by-product of the treatment of phosphoethanolamine with fluordinitrobenzene under the conditions of the assay.

The recovered phosphoethanolamine contained no detectable orthophosphate. It contained 1.1 μmol of phosphomonoester by the alkaline phosphatase method per mol of phosphoethanolamine determined by the fluordinitrobenzene method.

**Phosphoethanolamine Is Attached to Position 6 of Glucose Residues**—A sample of oligosaccharide A-1 was reduced with borohydride and treated with alkali to eliminate glycerophosphate. It contained 1.3 μmol of phosphoethanolamine, accounting quantitatively for the total phosphorus of the sample. The sample was hydrolyzed in 0.40 ml of 1 N HCl for 2 hours at 100°. It was neutralized, and found to contain 0.91 μmol of glucose-6-P by the dehydrogenase method ("Materials and Methods") representing a 70% yield of the phosphorus bound to phosphoethanolamine.

To confirm the identification of glucose-6-P, a portion of the hydrolysate was mixed with a tracer amount of 14C-labeled glucose-6-P prepared by the phosphorylation of [U-14C]glucose with hexokinase. The sample, containing 639 nmol of glucose-6-P, 96% of which represented the product of hydrolysis of phosphoethanolamine residues, was chromatographed on Dowex 1 formate as described in Fig. 3. A sharp peak of radioactivity (representing the tracer glucose-6-P) was accompanied by a total of 619 nmol of glucose-6-P as determined by the dehydrogenase method, representing a quantitative recovery of glucose-6-P.

In further confirmation of the identity of the recovered material, samples of the peak fractions recovered from the chromatogram of Fig. 3 were pooled, after removal of salt as described, and chromatographed on Whatman No. 43 paper in System II (ascending). A single phosphorus-containing spot was found with RF = 0.25, indistinguishable from authentic glucose-6-P standards.

**DISCUSSION**

The alkaline hydrolysis of phosphodiester derivatives of glycerol, in a reaction in which negligible amounts of free glycerol are produced, undoubtedly involves the formation of glycerol-1(3),2-cyclic phosphate as intermediate. Such cyclic phosphates are unstable in alkali and are cleaved to give a mixture of the phosphomonoesters glycerol-1(3)-P and glycerol-2-P. Once formed, the phosphomonoesters do not isomerize in alkali (10).

The glycerophosphates obtained on alkaline hydrolysis of the phosphodiester linkage in the membrane-derived oligosaccharide A-1 consists of an approximately equal mixture of pure sn-glycero-1-P and sn-glycero-2-P. The glycerophosphate linked to the native molecule must therefore be entirely the sn-glycero-1-P enantiomer. Diesters containing glycero-3-P would yield approximately equal amounts of glycero-2-P and

![Fig. 2. Isolation of phosphoethanolamine by chromatography on Dowex. The hydrolysate (5.4 ml) after removal of barium sulfate as described in the text was passed over a column (0.8 x 13 cm) of Dowex 1 formate (2% cross-linked). The column had been washed with 10 ml of 0.1 M ammonium formate just prior to use. After application of the sample, the column was eluted with a linear gradient (water to 0.04 M formic acid) in a total of 100 ml. Samples of each fraction were counted to locate the tracer phospho[14C]ethanolamine.](http://www.jbc.org/content/252/10/4212/F2)

![Fig. 3. Isolation of glucose-6-P by chromatography on Dowex. The sample of hydrolysate, prepared as described in the text, containing 639 nmol of glucose-6-P in 3 ml of 0.09 M HCl was passed over a column (0.8 x 20.5 cm) of Dowex 1 formate (8% cross-linked). The column was washed with 10 ml of water and eluted with a linear gradient in a total volume of 200 ml, beginning with water and ending with a buffer containing 0.5 M formic acid and 0.5 M ammonium formate. Fractions (4.2 ml) were collected at the rate of two per hour. The tracer glucose-6-P was located by counting a sample of each fraction in a scintillation counter. Individual fractions in the region of the peak of radioactivity were separately freed of ammonium ions by passage over a short column of Dowex 50 H+ cation exchanger, and each fraction was then taken to dryness under a jet of air. The fractions were redissolved in 0.50 ml of water. The content of glucose-6-P in each fraction was then determined by the dehydrogenase method ("Materials and Methods"), and the radioactivity was also redetermined. From these data the concentration of glucose-6-P in the fractions originally collected was plotted in the figure above (right-hand scale).](http://www.jbc.org/content/252/10/4212/F3)
glycerol-3-P, and no glycerol-1-P. A diester of sn-glycerol-2-P would yield a mixture of all three phosphates.

In our previous study (2) it was concluded that the glycerol-P units attached to the membrane-derived oligosaccharides of *Escherichia coli* must be derived from phosphatidylglycerol, or from dihydrophosphatidylglycerol, or from both. Phosphatidylglycerol is sn-1,2-diacylglycerol-3-phospho-1'-glycerol. Its polar head group is thus an sn glycerol 1 P residue. The finding of this unusual form of glycerophosphate in phosphodiester linkage to the membrane-derived oligosaccharides strongly supports the conclusion that it is derived from the polar head group of phosphatidylglycerol, whether directly (as seems most probable) or via cardiolipin.

Most Gram-positive organisms contain a cell wall teichoic acid which is a derivative of polyglycerophosphate or polyribitolphosphate, and in addition contain a membrane-localized lipoteichoic acid which functions as carrier for the biosynthesis of cell wall polymers (11–13). In *Streptococcus faecalis* and in *Streptococcus lactis* and in *Escherichia coli* does not contain phosphoethanolamine residues in diester linkage, with the strong presumption that these phosphoethanolamine residues are derived from the polar headgroups of phospholipids, other potential sources have not been excluded. Further experiments are needed to resolve this question.

When oligosaccharide A-1 was reduced with borohydride, and treated with alkali to eliminate glycerophosphate, phosphoethanolamine residues were quantitatively retained. Upon further acid hydrolysis, these yielded glucose-6-P in 70% yield, offering unequivocal evidence that most, if not all, of the phosphoethanolamine residues are attached to position 6 of sugar moieties in the oligosaccharide. Since the possibility of microheterogeneity cannot be ruled out, the possibility remains that small amounts of phosphoethanolamine are attached at other positions as well. Glycerophosphate residues are probably also attached to position 6, as is suggested by the failure to eliminate free glycerol upon treatment with alkali, but further work is needed to verify this tentative conclusion, and to elucidate other features of the structure of this oligosaccharide.

### REFERENCES

Identification of sn-glycero-1-phosphate and phosphoethanolamine residues linked to the membrane-derived Oligosaccharides of Escherichia coli.
E P Kennedy, M K Rumley, H Schulman and L M Van Golde


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