Transient Methylation of Dolichyl Oligosaccharides Is an Obligatory Step in Halobacterial Sulfated Glycoprotein Biosynthesis*  

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Biosynthesis of sulfated saccharides that are linked to asparagine residues in the cell surface glycoprotein of Halobacterium halobium via a glucose residue involves sulfated dolichyl-monophosphoryl oligosaccharide intermediates (Lechner, J., Wieland, F., and Sumper, M. (1985) J. Biol. Chem. 260, 860–866). During isolation and characterization of these lipid oligosaccharides we detected a group of related compounds containing additional unidentified sugar residues. Here we report that: 1) the unknown sugar residues were 3-O-methylglucose, linked peripherally to the lipid-saccharide intermediates; 2) the 3-O-methylglucose residues in the oligosaccharides occur only at the lipid-linked level but are absent at the protein-linked level; 3) cell surface glycoprotein biosynthesis in Halobacteria in vivo is drastically depressed when S-adenosylmethionine-dependent methylation is inhibited, indicating that methylation is an obligatory step during glycoprotein synthesis.  

We propose a mechanism for the transport of lipid oligosaccharides through the cell membrane, involving an intermediate stage in which the saccharide moieties are transiently modified with 3-O-methylglucose.

EXPERIMENTAL PROCEDURES

General Procedures  

Quantitative as well as qualitative sugar analyses, either by colorimetric assays or by gas liquid chromatography (GLC), were as described in Ref. 1. Hydrolysis of uronic acid-containing saccharides was more complete, and losses of material by destruction were smaller if the samples were first methanolysed (0.5 N HCl in methanol for 20 h at 80 °C), and the resulting methylglycosides subsequently treated with 2 N HCl for 2 h at 80 °C.

Procedures for mild hydrolysis of dolichylphosphate oligosaccharides, desulfation, reduction of sugars with sodium borohydride and sodium [3H]borohydride, as well as permethylation of carbohydrates according to Refs. 3 and 4 and 3H reduction of uronic acids according to Ref. 6 are also described in Ref. 1. Combined GLC-mass spectroscopy was performed using a Hewlett-Packard 5955B system equipped with a Durabond 1701 capillary column (30 m, ICI, Frankfurt), with an entrance voltage of 70 kV. The temperature of the interface was 280 °C, and that at the injection port, 220 °C. For analyses of partially methylated perylitol acetates, chromatography was started at 140 °C, with a subsequent linear increase of 2 °C/min. Pentfluoropropionyl methylglycosides were chromatographed starting at 120 °C with a linear increase of 2 °C/min.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis in gels containing 12% (w/v) acrylamide and 2.5% (w/v) N,N'-methylenebisacrylamide was performed according to Ref. 6. Fluorography of the gels was performed according to Ref. 7.

Special Procedures  

3SO4- -Pulse-Chase Labeling—For 3SO4- pulse-chase experiments, Halobacterium halobium RIMl, grown to early stationary phase in sulfate-free synthetic medium (8), was resuspended at 20 times the original cell concentration in sulfate-free basal salts (8), and incubated with 3SO4- (carrier free, 100 μCi/ml, Amersham Buchler, Braunschweig) as described (9). For the chase, the cells were harvested by centrifugation (3 min, 10,000 rpm, 20 °C) and resuspended in complete basal salts (containing 60 mM SO4 and 0.5% (w/v) alanine). Conditions used in the inhibition experiments with adenosine and homocysteine (added in the form of its thioldactone (Sigma) and performed essentially as described in Ref. 10) are outlined in the legend to Fig. 3. Aliquots were taken at intervals, and, after centrifugation, the cells were lysed by the addition of 50 mM Tris-HCl buffer, pH 7.5, containing 10 μg of DNase (Boehringer Mannheim) per ml. 

[35S]Methionine labeling (25 μCi/0.4-ml cell suspension, 1200 Ci/mmol, Amersham Corp.) was performed as a control under the conditions used for 3SO4- labeling.

For labeling with 1-[methyl-3H]methionine (90 Ci/mmol, Amersham Corp.) cells were grown in sulfate-free synthetic medium as described (8). At early stationary phase the cells were collected and resuspended in an equal volume of sulfate-free basal salts containing 0.24% (w/v) of alanine and shaken for 4 h at 37 °C. Thereafter, the bacteria were harvested and resuspended at 20 times the original cell concentration in sulfate-free basal salts (conditions of inhibition are given in the legend to Fig. 5) and stirred under illumination at 37 °C. After 2 h, the cells were centrifuged and resuspended in an equal volume of sulfate-containing basal salts (400 μl) with 150 μCi of [3H] CH3-methionine. Incubation was again for 2 h at 37 °C with stirring under illumination. Subsequently the fraction of sulfated dolichyl oligosaccharides was isolated as described in Ref. 1 for 3SO4- labeled lipid oligosaccharides. After addition of unlabeled 3-O-methylglucose as a carrier the samples were hydrolyzed (4 N trifluoroacetic acid) for 4 h at 100 °C, dried, and passed through a small column containing 1 ml of anion exchange resin (AG 1-X8, acetate form, Bio-Rad) and 1 ml of cation exchange resin (AG 50W-X8 H+) in water. The effluents were concentrated and chromatographed on silica 60 thin layer plates (Merck, Darmstadt) in acetonitrile/water, 80/20. This chromatog-
subsequently chromatographed on a Bio-Gel P-2 (-400 mesh) column. The material was repeated once. Unlabeled 3-O-methylglucose was visualized by spraying with orcinol reagent. 3H-labeled material was detected by scintillation counting in 10 fractions were collected, and radioactivity was determined by scintillation partitioning between chloroform and water, the oligosaccharides were combined in three separate pools: SPI, SPII, and SPIII. The material contained in each pool was submitted to mild hydrolysis to split the bond between dolichylphosphate and the saccharide moiety and then reduced with sodium [3H]borohydride. After mild methanolysis to remove the sulfate residues (12), the samples were subjected to permeation chromatography on Bio-Gel P-2. As shown in Fig. 1, each of the three pools SPI, SPII, and SPIII was further separated to yield 3-5 peaks. The material in each of these peaks (lettered A through J in Fig. 1) was hydrolyzed to yield monosaccharides, reduced, and the hexoses then analyzed by GLC as their peracetylated, and reanalyzed. As a result, a decrease in the amount of methylhexose and an increase in Glc was observed. In addition, the 3-O-methylhexose co-migrated with authentic 3-O-

Fig. 1. Gel filtration of the oligosaccharide moieties of SPI, -II, and -III on Bio-Gel P-2 after high performance liquid chromatography (1). After mild hydrolysis (10 mM HCl, 100 °C, 5 min) of the material in each of the pools, SPI, -II, and -III, and partitioning between chloroform and water, the oligosaccharides (aqueous phase) were reduced with sodium [3H]borohydride and subsequently chromatographed on a Bio-Gel P-2 (-400 mesh) column (180 × 0.8 cm) in pyridine/acetate buffer, 0.1 M, pH 5.5. 1.16-ml fractions were collected, and radioactivity was determined by scintillation counting in 10 µl of each fraction. ——, 3H radioactivity.

To quantitate the yields during isolation of the sulfated lipid oligosaccharides, 35S-labeled membranes were added to the samples.

Demethylation of methylated hexoses with BBBr was carried out according to Ref. 11.

Transfer to Exogenous Hexapeptide—Cells for these experiments were grown in a medium composed of 1 volume of sulfate-depleted complex medium (in which MgSO4 is substituted by MgCl2) and 2 volumes of sulfate-free synthetic medium (8) by shaking at 150 rpm and 37 °C. Three- or four-day-old cultures with an A at 578 nm of 0.8–1.5 were used in the experiments. (An A of 1 corresponds to a cellular protein concentration of 0.5 mg/ml.) More detail is given in the legend to Fig. 6.

3-O-Methylglucose Occurs in Dolichylmonophosphate Oligosaccharide Intermediates—Isolation of sulfated dolichylphosphate oligosaccharides is described in Ref. 1. After high performance liquid chromatography the fractions collected were combined in three separate pools: SPI, SPII, and SPIII. The material contained in each pool was submitted to mild hydrolysis to split the bond between dolichylphosphate and the saccharide moiety and then reduced with sodium [3H]borohydride. After mild methanolysis to remove the sulfate residues (12), the samples were subjected to permeation chromatography on Bio-Gel P2. As shown in Fig. 1, each of the three pools SPI, SPII, and SPIII was further separated to yield 3-5 peaks. The material in each of these peaks (lettered A through J in Fig. 1) was hydrolyzed to yield monosaccharides, reduced, and the hexoses then analyzed by GLC as their peracetate derivatives. Some of the peaks (black in Fig. 1) showed, besides Glc, an unknown component, which on combined GLC chromatography-mass spectroscopic analysis was found to be a 3-O-methylhexose (Fig. 2, A–C). To determine the nature of this 3-O-methylhexose, the sample was demethylated by treatment with BBBr according to Ref. 11 and reanalyzed. As a result, a decrease in the amount of methylhexose and an increase in Glc was observed. In addition, the 3-O-methylhexose co-migrated with authentic 3-O-

Fig. 2. GLC-mass spectroscopy of the material of peak B in Fig. 1. The sample was hydrolyzed to monosaccharides, reduced, peracetylated, and submitted to GLC-mass spectroscopy as described under "Experimental Procedures." A, total ion profile; B, mass spectrum of peak 1 after reduction of the hydrolyzed sample with sodium [3H]borohydride; C, mass spectrum of peak 1 after reduction with sodium [3H]borohydride.

**RESULTS**

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Transient Methylation of Dolichyl Oligosaccharides

Table I

<table>
<thead>
<tr>
<th>Partially methylated alditol acetates obtained after permethylation of the material in peaks B, C, and E in Fig. 1</th>
<th>Compound originating from</th>
</tr>
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<tr>
<td>1,5-Di-O-acetyl-2,3,4,6-tetra-O-methylhexitol</td>
<td>3-O-Methyl-Glc₁-R</td>
</tr>
<tr>
<td>1,4,5,6-D₄-Tetra-O-acetyl-2,3-di-O-methyl-hexitol</td>
<td>R₄GlcUA₄-R</td>
</tr>
<tr>
<td>4-O-Acetyl-1,2,3,5,6-penta-O-methylhexitol</td>
<td>R₅Glc-ol</td>
</tr>
</tbody>
</table>

Fig. 3. Inhibition of in vivo glycoprotein biosynthesis by incubation with adenosine/homocysteine: ³⁵S labeling of Halobacterium halobium. Cell suspensions were incubated with the amounts of radioactivity as described under “Experimental Procedures” for 4 h at 37°C with illumination and gentle stirring. Thereafter, cells were harvested by centrifugation, lysed by the addition of 50 mM Tris/HCl buffer, pH 7.5, containing 10 μg of DNase (Boehringer Mannheim)/ml, and aliquots were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subsequent fluorography as described under “Experimental Procedures.” Lane a, ³⁵SO₄⁻ labeling; lane b, ³⁵SO⁻ labeling in the presence of 10 mM adenosine and 10 mM homocysteine; lane c, [³⁵S]methionine labeling; lane d, [³⁵S]methionine labeling in the presence of 10 mM adenosine and 10 mM homocysteine.

Fig. 4. A, ³⁵SO⁻ pulse-chase labeling of Halobacteria. Lane a, pulse labeling for 30 min; lanes b–d, chase with 80 mM MgSO₄ for 1, 2, and 3 h, respectively; lane e, same as in a but in the presence of 10 mM adenosine and 10 mM L-homocysteine; lanes f, g, and h, chase with 80 mM MgSO₄ for 1, 2, and 3 h in the presence of 10 mM adenosine and 10 mM homocysteine, respectively. Aliquots were taken at the times indicated, and cell lysates were submitted to electrophoresis. Details of incubation and sodium dodecyl sulfate-gel electrophoresis as well as fluorography are described under “Experimental Procedures.” B, quantitative evaluation of the pulse-chase experiment. Using the fluorogram as a guide, bands corresponding to the SPs (arrow in A) were cut from the gel and their radioactivity determined.

Methylglucose in GLC on a capillary column, both as its peralditol acetate as well as its pentafluoropropionyl derivative. Thus, the unknown compound is shown to be 3-O-methylglucose.

Structure of 3-O-Methylglucose-containing Oligosaccharides—The structures of saccharides F and G (Fig. 1) are described in Ref. 1. The saccharides containing 3-O-methylglucose (peaks B, C, and E in Fig. 1) were submitted to structural analyses. They contained GlcUA, Glc, and 3-O-methylglucose in the ratio 3:2:1 (B, E) and 2:1:1 (C). Permethylation according to Ref. 3, described in more detail in Ref. 1, revealed that the following structural elements occur in each of the samples (cf. Table I): a peripherally bound hexapyanose, (1→4)-linked hexuronic acids, and a 4-linked hexitol. The 3-O-methylglucose residue in each of the saccharides must be assigned to the nonreducing terminus, since each of the saccharides is linked via Glc to the dolichylphosphate residue (1). Thus, the peripherally bound hexapyanose must result from the 3-O-methylglucose residue. Therefore, the structures are as follows: peaks B and E, 3-O-methyl-Glc(1→4)GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)Glc-ol; and peak C, 3-O-methyl-Glc(1→4)GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)Glc-ol.

No 3-O-Methylglucose Is Found in Halobacterial Proteins—The main acceptor proteins for sulfated saccharides are the cell surface glycoprotein and two low molecular weight proteins (M₉ = 26,000 and 36,000). All of these glycoproteins are located on the surface of the cell. Therefore, we analyzed both, purified cell surface glycoprotein and a total envelope preparation (13, 14), which had been delipidated according to Ref. 1, by GLC-mass spectroscopy for their content of 3-O-methylglucose. No 3-O-methylglucose could be detected in these samples (limit of detection less than 3% of their Glc content). From this lack of 3-O-methylglucose in the acceptor proteins, we must conclude that this residue represents a...
Transient modification of the lipid oligosaccharides.

**In Vitro Glycoproteins Biosynthesis Is Inhibited by Methyl-ation Inhibitors**—The intermediate occurrence of 3-O-methylglucose suggested a processing of the sulfated oligosaccharides. Therefore, we investigated the inhibition of methylation on the biosynthesis of the sulfated glycoproteins. According to Ref. 10, a combination of adenosine and homocysteine (which is converted in vivo to S-adenosylhomocysteine) by product inhibition suppresses S-adenosylmethionine-dependent methylations. We used this inhibition in an in vivo 35SO₄²⁻-labeling experiment to detect a possible effect on the biosynthesis of sulfated glycoproteins. The result is shown in Fig. 3. Clearly, biosynthesis of sulfated glycoproteins is depressed (lane b), although general protein biosynthesis remains unaffected, as shown by incorporation of [³⁵S]methionine in control incubations (lanes c and d). Remarkably, biosynthesis of the sulfated dolichylmonophosphate oligosaccharides (arrow in Fig. 3) is not influenced and takes place just as in the control incubation. This inhibition of glycoprotein synthesis implies that methylation is an obligatory step in the biosynthesis of saccharides preceding transfer of the saccharide to the protein. In accordance with this conclusion is our observation that under inhibitor conditions newly synthesized sulfated dolichyl oligosaccharides remain stable in a subsequent chase experiment (Fig. 4, A and B, lanes e–h). In the absence of inhibitor, almost all of the radioactive precursors are transferred to protein during the chase period (Fig. 4, A and B, lanes a–d).

Adenosine Combined with Homocysteine Inhibits the Formation of 3-O-Methylglucose-containing Lipid Oligosaccharides—The combination of adenosine and homocysteine indeed affects the formation of methylated dolichyl oligosaccharides, as is demonstrated by the following experiment. Halobacteria were labeled with [methyl-²H]methionine in vivo in the presence or absence of adenosine and homocysteine. Subsequently the sulfated dolichylmonophosphate saccharides were isolated according to Ref. 1. After hydrolysis to mono-saccharides their [methyl-²H]-3-O-methylglucose contents were analyzed by radioscanning after thin layer chromatography are described under “Experimental Procedures.” Radioscanning traces of the thin layer chromatograms are shown. A, labeling in the presence of 10 mM adenosine and 10 mM L-homocysteine, which were present during the whole period of illumination (cf. “Experimental Procedures”). B, control without adenosine and homocysteine. The dotted line shows integration of the ³H radioactivity.

![FIG. 5. Quantification of [methyl-³H]-3-O-methylglucose after in vivo labeling with L-[methyl-³H]methionine. Methyl-³H labeling, isolation of sulfated lipid oligosaccharides, hydrolysis, and thin layer chromatography are described under “Experimental Procedures.” Radioscanning traces of the thin layer chromatograms are described under “Experimental Procedures.” Radioscanning traces of the thin layer chromatograms are shown. A, labeling in the presence of 10 mM adenosine and 10 mM L-homocysteine, which were present during the whole period of illumination (cf. “Experimental Procedures”). B, control without adenosine and homocysteine. The dotted line shows integration of the ³H radioactivity.](image-url)

![FIG. 6. Glycosylation of exogenously added acceptor peptide Tyr-Asn-Leu-Thr-Ser-Val (18). A, Halobacterium halobium cells, grown as described under “Experimental Procedures,” were suspended in sulfate-free basal salts containing 0.5% (w/v) Ala and 6 mg of bovine serum albumin/ml to give an A of 10 (corresponding to a cellular protein concentration of 5 mg/ml (17)) at 578 nm. Four µl of ethidium bromide (100 µCi) of carrier-free sodium [³⁵S]sulfate (Amersham Corp.) were added to each of two 1-ml portions of suspension. After illumination for 10 min at 37 °C with gentle stirring, 25 µl (125 µg) of the hexapeptide was added to the sample and 25 µl of water to the control. After 2 h of illumination at 37 °C and gentle stirring, another 125 µg of hexapeptide was added to the sample (25 µl of water to the control). This was repeated once more after one further hour, and the incubation was terminated after a total of 5 h. Cells were removed by centrifugation (10,000 X g, 6 min), and the supernatants were chromatographed on Bio-Gel P-2 (200–400 mesh, 50 X 1 cm, with pyridinium acetate, 0.1 M, pH 5.5 as eluant). 1.2-ml fractions were collected. •—•, control; ○—○, sample with hexapeptide. B, Halobacteria (as above) were suspended in sulfate-free basal salts containing 0.5% Ala (control) or in sulfate-free basal salts containing 0.5% Ala and, in addition, 10 mM adenosine and 10 mM L-homocysteinethiolactone (Sigma) (sample), both to give a final A of 10 at 578 nm. After incubation for 1 h at 37 °C under illumination and gentle stirring, to both suspensions (1 ml each) was added 4 µl (40 µg) of aqueous ethidium bromide solution, 5 µg of bovine serum albumin, 10 µl (90 µCi) of [³⁵SO₄] (carrier free, Amersham Corp.), and 20 µl (100 µCi) of an aqueous solution of acceptor hexapeptide. After 2 and 3 h of illumination, additional 100-µg portions of hexapeptide solution were added. After a total of 5.5 h of incubation at 37 °C under illumination and gentle stirring (4.5 h in the presence of [³⁵SO₄] and hexapeptide), cells were removed and the supernatants chromatographed on Bio-Gel P-2. •—•, control; ○—○, sample in the presence of 10 mM adenosine and 10 mM homocysteine.](image-url)
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**TABLE II**
Structure of some oligosaccharides occurring linked to dolichyl phosphate after mild acid hydrolysis, desulfation, and reduction with sodium borohydride

<table>
<thead>
<tr>
<th>Material from peak in Fig. 1</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>3-O-CH₂Glc(1→4)GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)Glc-ol</td>
</tr>
<tr>
<td>C</td>
<td>3-O-CH₂Glc(1→4)GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)Glc-ol</td>
</tr>
<tr>
<td>D</td>
<td>GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)Glc-ol</td>
</tr>
<tr>
<td>E</td>
<td>3-O-CH₂Glc(1→4)GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)Glc-ol</td>
</tr>
<tr>
<td>F</td>
<td>Glc(1→4)GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)Glc-ol</td>
</tr>
<tr>
<td>G</td>
<td>GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)Glc-ol</td>
</tr>
<tr>
<td>H</td>
<td>Glc(1→4)GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)Glc-ol</td>
</tr>
<tr>
<td>I</td>
<td>Glc(1→4)GlcUA(1→4)GlcUA(1→4)GlcUA(1→4)Glc-ol</td>
</tr>
</tbody>
</table>

**Discussion**

The result is shown in Fig. 5; the content of 3-O-methylglucose is strongly decreased by the inhibitors.

**Inhibition of Methylation**

**Transfer to the Acceptor Protein of the Sulfated Oligosaccharides**—Does the above described inhibition of in vivo sulfated glycoprotein biosynthesis reflect that methylation of the dolichyl oligosaccharide sulfate is necessary for transfer to the protein of the sugar moiety? As we had obtained evidence for the occurrence of typical Asn-X-Thr(Ser)-acceptor sequences (1), we used the hexapeptide Tyr-Asn-Leu-Thr-Thr-Val as an artificial exogenous acceptor. The profiles are shown in Fig. 6A. Clearly, the presence of exogenous acceptor leads to the appearance of a peak a few fractions after the excluded volume. The radioactive material of this peak on high voltage thin layer electrophoresis behaved very similar to Pronase-derived sulfated glycopeptides from the cell surface glycoprotein (not shown here). Thus, a transfer system of the sulfated oligosaccharides to an exogenous acceptor was available, and we investigated the influence of methylation inhibitors on this transfer. To this end, cells were incubated with or without adenosine/homocysteine each in the presence of 35SO₄⁻. Thereafter, cells were removed by centrifugation and the supernatants chromatographed on a Bio-Gel P-2 column. The radioactivity profiles are shown in Fig. 6B. The hexapeptide-dependent radioactive peak is depressed drastically in the presence of the methylation inhibitors. Hence, the observed inhibition of sulfated glycoprotein synthesis by adenosine/homocysteine does indeed appear to be a function of inhibition of methylation of the sulfated dolichyl oligosaccharide intermediates rather than some sort of unspecific (and unknown) effect on glycoprotein core biosynthesis. Moreover, this methylation seems to be involved in the passage through the membrane of the sulfated dolichyl oligosaccharides and/or the transfer of the completed saccharide to protein.

**DISCUSSION**

The above data show that a methylated hexose, namely 3-O-methylglucose, is peripherally linked to dolichylphosphate oligosaccharide intermediates in Halobacteria. This compound is no constituent of a product, i.e. glycoprotein. Therefore, a lipid oligosaccharide containing 3-O-methylglucose must be regarded as an intermediate form.

We do not yet know whether 3-O-methylglucose is introduced from a corresponding activated sugar compound or is generated by direct methylation of a peripheral glucose in the lipid oligosaccharide. The structures presented in Table II, however, favor a mechanism involving direct transfer (or removal) of methyl groups. We found only terminal saccharide sequences with Glc-GlcUA-R and 3-O-methyl-Glc-GlcUA-R but no structure with 3-O-methyl-Glc-GlcUA-R.

Suppression of methylation of the lipid intermediates results in a concomitant arrest of glycoprotein biosynthesis. What might be the role of this methylation?

Essentially two possible functions are conceivable. Methylation of a glucose residue in the 3'-position would clearly increase the hydrophobicity of the sugar, a change resulting in a molecule with one face devoid of hydrophilic groups. Alternatively, the methylated glucose might serve as a specific recognition marker. Thus, this methylation (for both reasons) could represent an obligatory step for translocation of the lipid oligosaccharides through the membrane. In accordance with this idea is the fact that 3-O-methylglucose-free lipid oligosaccharides accumulate in the presence of ethidium bromide, which inhibits protein synthesis, i.e. lack of oligosaccharide acceptor, as can be seen in Fig. 3B in Ref. 1. In this experiment inhibition of protein synthesis led to a shift of 35SO₄⁻ SP radioactivity; SPI and II decreased, whereas SPIII accumulated drastically. As we know now, SPIII is free of 3-O-methylglucose-containing lipid oligosaccharides (cf. Fig. 1).

In addition this idea is supported by our finding that an exogenous acceptor-hexapeptide is glycosylated at a much lower extent if methylation of the sulfated dolichyl oligosaccharides is inhibited.

3-O-Methylglucose occurs as a structural component of bacterial polysaccharides (15), and, most recently, 3-O-methylmannose has been described to be a structural constituent of N-linked oligosaccharides in glycoproteins from *Mucor rouxii* (16), but no methylated hexose has been described so far as occurring in lipid-saccharide intermediates during glycoprotein biosynthesis.

In many respects archaeabacteria show similarities with eucaryotic organisms, rather than with eubacteria, e.g. in their protein synthesis machinery or in the presence of cell surface glycoproteins. In addition, we have shown that *Halobacteria* use a dolichyl residue rather than a bacterial undecaprenyl residue during biosynthesis of oligosaccharide intermediates (1). In view of our data, we are interested, therefore, in the possibility that the observed modification with methylated
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Sugars might play a role in the biosynthesis of eucaryotic acidic glycoproteins as well.

Acknowledgments—We wish to thank R. Heitzer and S. Stammler for their expert technical assistance, Prof. Tanner and Dr. Lehle for a generous gift of Tyr-Asn-Leu-Thr-Ser-Val hexapeptide, and Dr. P. Orlan for reading the manuscript.

REFERENCES