Lipopolysaccharide from a Gram-Negative Marine Bacterium

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A lipopolysaccharide molecule was isolated from a marine bacterium. The molecule seems to be composed of lipid A and the hexoses, glucose and galactose.

The O antigen (lipopolysaccharide) of gram-negative bacteria has been under investigation by many researchers. The physical (8, 9, 14) and chemical (7–9, 17) structure of the molecule and the immunological properties (9, 10) have been investigated. These studies have been almost entirely limited to terrestrial organisms. Marine bacteria in ultrathin section show the same physical structure in their cell wall, indicating a lipopolysaccharide (LPS) molecule similar to the terrestrial microorganisms (6). O'Leary and MacLeod (Bacteriol. Proc., p. 57, 1969) described the isolation of a lipopolysaccharide from a marine pseudomonad by using a modification of the Westphal phenol-water technique. They found that the molecule could be isolated by the addition of growth medium salts to the phenol-water procedure. Upon further analyses, the LPS was found to be dependent upon cations for its structural integrity (15). Vibrio marinus and V. parachamolyticus, which are considered marine bacteria, have been found to contain a lipopolysaccharide molecule (C. F. Denke and R. R. Colwell, Bacteriol. Proc., p. 44, 1971). The LPS from the vibrios does not seem to be structurally dependent on cations, as is that from the marine pseudomonad (personal communication, C. F. Denke).

The question arose as to how consistent was the marine form of the LPS. To investigate the occurrence of marine lipopolysaccharides, a number of marine microorganisms were isolated from sand and seawater collected from Narragansett Bay, R.I. Upon initial screening, one organism was chosen for analysis. The organism was isolated from sand at the low-tide watersand interface and grown on a seawater medium containing 5.0 g of nutrient broth and 8.0 g of yeast extract per 1 liter of filter-sterilized seawater. Cultural and biochemical characteristics indicated that the organism was a nonmotile pseudomonad, closely related to Pseudomonas iridescens as described by Stanier (3). The organism did not grow in medium without sodium chloride. Nutritional studies (11, 12) indicated that the organism preferred 1.0 M NaCl, 0.026 M MgCl₂, and 0.01 M KCl for growth. The organism was routinely cultivated at room temperature with constant shaking in a medium containing 5.0 g of nutrient broth, 8.0 g of yeast extract, and the complete salts solution (1.0 M NaCl, 0.026 M MgCl₂, and 0.01 M KCl) per liter of distilled water. The organism was grown for 24 h, harvested by centrifugation, washed in complete salt solution, and extracted by the phenol-salts method (15). Purification of the material was accomplished by centrifugation at 105,000 × g, treatment with deoxyribo nuclease and ribonuclease, and precipitation with cold acetone.

Initial quantitative analysis of the purified material showed 5.7% neutral carbohydrate (1) and 1.5% amino sugar (4) (Table 1). These values are rather low for an LPS molecule. Paper chromatography (18) after hydrolysis in 2 N HCl for 2 h indicated the presence of glucose, galactose, and glucosamine. The neutral sugars were detected as described by Block et al. (2). No heptose or 2-keto-3-deoxy-octonate could be detected. This indicated that the preparation did not contain the backbone portion of the LPS molecule. The method of Leive et al. (9) for the isolation of free lipid and the lipid A was applied to the sample. The free lipid was estimated to be 19.4%. The material isolated as lipid A was hydrolyzed in 4 N HCl for 4 h and chromatographically (13) checked for the presence of amino sugars. Aniline hydrogen phthalate, ninhydrin, and the Elson Morgan sprays (2, 16) revealed only 2-amino-glucose. Phosphate was also detected but not quantitated (5).

The material isolated from this marine pseudomonad as lipid A showed many of the characteristics of other lipid A molecules: it contained glucosamine, and phosphate; its solubility in lipid solvents such as chloroform indicated the
TABLE 1. Partial composition of the lipopolysaccharide isolated from a marine pseudomonad

<table>
<thead>
<tr>
<th>Composition</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total neutral sugar</td>
<td>5.7</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.3</td>
</tr>
<tr>
<td>Total protein</td>
<td>1.6</td>
</tr>
<tr>
<td>Free lipid</td>
<td>19.4</td>
</tr>
<tr>
<td>Total 2-amino sugar</td>
<td>1.5</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>—</td>
</tr>
<tr>
<td>Inorganic phosphate</td>
<td>—</td>
</tr>
</tbody>
</table>

*Glucose and galactose were estimated with glucostat (special) and galactostat reagents (Worthington Biochemical Corp.).

Identified but not quantitated from the lipid A portion.

**presence of fatty acids; and the two hexoses present, glucose and galactose, are also commonly found in the polysaccharide portion of the LPS molecule. Absent from our preparation was the heptose monosaccharide common to the polysaccharide core of most LPS molecules. The absence of the core could indicate that this pseudomonad may only produce an incomplete LPS, as is the case in some of the Salmonella (10). Another possibility is that the proper concentration of cations was not present during the extraction to hold the core portion intact. The organism does, however, possess a type of lipopolysaccharide molecule.

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**LITERATURE CITED**


