A Receptor for a *Proteus vulgaris* Bacteriocin

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**SUMMARY**

Lipopolsaccharide was isolated from a *Proteus vulgaris* strain susceptible to the killing action of *P. vulgaris* bacteriocin 45, from two resistant mutants and from a wild *P. vulgaris* strain, none of which adsorb the bacteriocin. The carbohydrate composition of the lipopolysaccharide of the sensitive organism differs from that of the resistant strains. Neutralization tests and electron microscopy showed that this phage-tail-like bacteriocin only adsorbed to the cell wall or the lipopolysaccharide fraction of the sensitive *P. vulgaris* strain. Adsorption was accompanied by triggering of the bacteriocin particle.

**INTRODUCTION**

Bacteriophage-like structures with a bacteriocin action (Reeves, 1965) have been isolated from different bacterial species (Coetzee *et al.* 1968; Lang, McDonald & Gardner, 1968; Jayawardene & Farkas-Himsley, 1968). Coetzee *et al.* (1968) showed that the killing activity of phage-tail-like particles from strains of *Proteus vulgaris* was associated with adsorption to susceptible bacteria. Electron microscopic observation of the adsorption of bacteriophages to isolated cell walls (Weidel, 1951; Hotchin, Dawson & Elford, 1952) demonstrated the presence of specific phage receptors on bacterial cell surfaces. Phage receptor activity has been attributed to lipopolysaccharide (Jesaitis & Goebel, 1952, 1953; Lindberg, 1967), lipoprotein (Weidel, Koch & Lohss, 1954; Zickler, 1967), polysaccharide (Taylor, 1966) and lipoglycoprotein (Weidel & Kellenberger, 1955) fractions of the cell walls of hosts. More recently the cytoplasmic membrane has been implicated in the adsorption of a *Streptococcus lactis* phage (Oram & Reiter, 1968) and the lethal adsorption of colicins (Šmarda & Taube-neck, 1968). The adsorption mechanism of phage-tail-like bacteriocins is unknown and this paper reports the isolation of a lipopolysaccharide receptor for *Proteus vulgaris* bacteriocin 45.

**METHODS**

*Bacterial strains.* The origin of the bacteriocin-producing *Proteus vulgaris* strain 45 and the *P. vulgaris* indicator strain 10 were described by Coetzee *et al.* (1968). In addition two resistant mutants derived from strain 10 (nos 9, 37) and a wild resistant *P. vulgaris* strain 7 were examined. The resistant mutants were isolated by flooding with sterile bacteriocin plates which had been inoculated with indicator strain 10. Colonies which grew after incubation at 37° for 2 days were picked and tested for
ability to adsorb the bacteriocin (Coetzee et al. 1968). *P. vulgaris* strain 7 was also examined for bacteriocin adsorption.

**Media.** The media were Difco brain-heart infusion broth and SS agar.

**Bacteriocin techniques.** Bacteriocin from *Proteus vulgaris* strain 45 was produced in fluid medium, purified, concentrated and assayed as previously described (Coetzee et al. 1968). It was suspended in 0.1 M-ammonium acetate, pH 7.2.

**Electron microscopy.** Samples suspended in 0.1 M-ammonium acetate, pH 7.2, were negatively stained with neutral potassium phosphotungststate (Brenner & Horne, 1959). These were mounted on carbon support films by a spreading technique (Bradley, 1962) and examined in a Phillips EM 200 electron microscope.

**Preparation of cell wall fraction.** Frozen packed cells were disrupted in a Raytheon 10 kyc./sec. sonic oscillator, partially purified by differential centrifugation (Osborn, 1966) and freeze-dried.

**Isolation of lipopolysaccharide.** Acetone-dried cells were extracted with 45% (w/v) phenol at 68 to 70°C and lipopolysaccharide (LPS) purified according to Westphal & Jann (1964). A 3% (w/v) of LPS in 0.1 M-tris + HCl buffer, pH 7.8, was treated with 1 mg./ml. lysozyme (British Drug Houses Ltd) and 50 µg./ml. ribonuclease (BDH) at 37°C for 12 hr. Final purification was accomplished by three cycles of alternate low-(10,000 g for 15 min.) and high-speed (100,000 g for 3 hr) centrifugation (Lüderitz et al. 1965). The pellet was dissolved in a small volume of water and freeze-dried.

**Hydrolysis of lipopolysaccharide.** Hexoses and uronic acids were liberated with N-H₂SO₄ at 100°C for 4 hr. The hydrolysate was neutralized with barium hydroxide, centrifuged and the supernatant lyophilised. Amino sugars were released with 4 N-HCl at 100°C for 10 hr and the acid removed under vacuum in the presence of NaOH.

**Chromatography of carbohydrates.** Two-dimensional chromatograms on Whatman no. 54 paper were developed for 16 hr by the descending technique. The solvents used were: first dimension, formic acid + ethyl methyl ketone + 2-methylpropan-2-ol + water (3 + 6 + 8 + 3, by vol.); second dimension, butan-1-ol + pyridine + 0.1 M-HCl (6 + 4 + 3, by vol.). Uronic acids and sugars were detected by spraying the dried chromatograms with 3% (w/v) p-anisidine hydrochloride in moist butan-1-ol or with silver nitrate + sodium hydroxide spray (Trevelyan, Procter & Harrison, 1950). Amino sugars were detected with ninhydrin or with Morgan-Elson reagent (Partridge, 1948).

**Estimation of heptose and 2-keto-3-deoxyoctonic acid.** The presence of heptose was demonstrated according to Osborn (1963) and 2-keto-3-deoxyoctonate by the method of Weissbach & Hurwitz (1959) as modified by Osborn (1963).

**Adsorption of bacteriocin to cell wall and cell wall fractions.** Cell walls, LPS and polysaccharide fractions (50 µg./ml.) suspended in 0.1 M-ammonium acetate, pH 7.2, were mixed with equal volumes of purified bacteriocin (titre 1/1000) similarly suspended. The mixtures were incubated at 37°C for 30 min. and assayed for bacteriocin activity. Samples of those containing LPS were examined in the electron microscope.

**RESULTS**

The resistant mutants (nos 9, 37) of *Proteus vulgaris* strain 10 and the wild strain 7 did not adsorb bacteriocin 45. Electron micrographs of purified LPS of all strains examined appeared as filaments and condensed particles with no contaminating cell
All preparations in ammonium acetate and potassium phosphotungstate.
A. Purified lipopolysaccharide extracted from *Proteus vulgaris* strain 10.
B. Phage tail-like structures of bacteriocin 45 adsorbed to lipopolysaccharide from *Proteus vulgaris* strain 10. Some of the particles have contracted sheaths. Note the predominantly rounded form of the lipopolysaccharides.

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*(Facing p. 34)*
All preparations in ammonium acetate and potassium phosphotungstate.
Mixture of bacteriocin 45 and lipopolysaccharide from resistant *Proteus vulgaris* strain 37. The bacteriocin particles do not appear to be adsorbed.

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wall (Pl. 1 A). Bacteriocin (titre 1/1000) was completely neutralized when incubated with cell wall or purified LPS from the P. vulgaris strain 10. No inactivation of bacteriocin occurred with similar fractions from resistant strains even when the mixtures contained 5 mg. material/ml. Polysaccharide isolates from all four strains did not inactivate bacteriocin. Further evidence that the bacteriocin receptor site was associated with the LPS fraction of the cell wall was found on electron microscopy. In mixtures which contained LPS from the sensitive strain 10 (Pl. 1 B) most of the phage-tail-like particles were arranged with their ‘base-plate’ ends towards the LPS molecule. Many of the particles were triggered and the projecting cores appeared to be adsorbed. This adsorption is often accompanied by a loss of the filamentous nature of the LPS. It then assumes a rounded structure (Pl. 1 B). No specific orientation or contraction of bacteriocin particles suggestive of adsorption was seen in mixtures which contained LPS from resistant strains and the LPS morphology remains unchanged (Pl. 2).

Table 1. Constituent sugars of lipopolysaccharide of Proteus vulgaris strains

Lipopolysaccharide was isolated from Proteus vulgaris strain 10 susceptible to the killing action of P. vulgaris bacteriocin 45, from two resistant mutants (nos 9, 37) and from a wild resistant strain no. 7. Presence of a sugar is indicated by +.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>7</th>
<th>9</th>
<th>37</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heptose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2-keto-3-deoxyoctonate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glucoronic acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Galactoronic acid</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>-</td>
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</tr>
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</table>

The constituent sugars of LPS hydrolysates of each strain are shown in Table 1. The resistant mutants 9, 37 differed from the sensitive Proteus vulgaris strain 10 in that they contained no glucoronic acid. Strain 37 also lacked galactosamine. The wild P. vulgaris strain 7 resistant to bacteriocin 45 differed from the resistant mutants in that while also lacking uronic acids it contained galactose and rhamnose.

DISCUSSION

The possibility always exists that the LPS contains unaltered cell wall. This is unlikely because the purified LPS was treated with lysozyme and was free of cell wall material as shown by electron microscopy. Colicins (Smarda & Taubeneck, 1968) and some phages (Oram & Reiter, 1968) adsorb to the cytoplasmic membrane. The phenol extraction procedure for LPS employed here excludes the cytoplasmic membrane as the adsorption site for bacteriocin 45. The LPS fraction, in contrast to the polysaccharide from the sensitive strain, neutralized and adsorbed bacteriocin 45. This bacteriocin is thought to be a defective phage (Coetzee et al. 1968) and electron micrographs of its adsorption to LPS show similarities to the adsorption of Felix o-1 phage (Lindberg, 1967). The altered appearance of LPS from strain 10 after adsorption of bacteriocin is unexplained but it could be due to a change in the forces which
determine its filamentous nature and may provide proof of the 'changed surface hypothesis' of Weidel & Kellenberger (1955).

The LPS from the sensitive \textit{Proteus vulgaris} strain 10 has a similar sugar composition to that found by Kotelko, Lüderitz & Westphal (1965) for the LPS fraction of \textit{P. mirabilis}. Mutant 9 lacks glucoronic acid and mutant 37 in addition also lacks galactosamine. It is not known whether mutant 37 is a double mutant. Although Kotelko \textit{et al.} (1965) consider the presence of uronic acids possibly due to contamination by acidic polysaccharides we would like to attribute the receptor specificity to glucoronic acid. In support of this is the fact that the LPS from the wild \textit{P. vulgaris} strain 7 also lacks glucoronic acid although the issue is complicated by the presence of two additional sugars in the LPS of this strain. Goebel & Jesaitis (1952) noticed a radical difference in the carbohydrate composition of LPS prepared from a phage T3,4,7-resistant mutant of \textit{Shigella sonnei} and Lindberg (1967) found that the adsorption of Felix 0–1 phage by LPS from \textit{Salmonella minnesota} is dependent on the presence of a terminal N-acetyl-D-glucosamine unit. Once the sequence and structure of the \textit{P. vulgaris} LPS is known the role of glucoronic acid in the specificity of the receptor site for bacteriocin 45 may be clarified.

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\textbf{REFERENCES}


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