Lipopolysaccharide as Receptor for Rhizobium Phage 1P

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INTRODUCTION

There are only a few papers concerning the sites of phage adsorption in *Rhizobium*. Barnett & Vincent (1970) suggested that the receptor site for phages 7, 7cr, and 8 was associated with the somatic antigen of *Rhizobium*. Atkins & Hayes (1972) found alterations in lipopolysaccharide (LPS) and lipoprotein (LP) of *Rhizobium trifolii* mutants that adsorbed phage poorly.

To elucidate the attachment site of phage 1P to *Rhizobium*, we studied cell walls and their LPS preparations as phage receptors.

METHODS

Bacterial and phage strains. *Rhizobium trifolii* strains 24SM, HR, 15, 24AR and XSM were used in these experiments. Mutants HR and 15, resistant to phage 1P, were derived from 24SM. The former was obtained by u.v. treatment, whereas the latter was a spontaneous mutant isolated by selection with phage 1P. Phage 1P propagated on *R. trifolii* 24SM was employed in assays of receptor activity of cell walls and LPS. It does not require divalent ions for its attachment to bacteria (Staniewski, 1968).

Media. Bacteria were grown in medium 4, containing (g/l glass-distilled water): K_2HPO_4 , 3.6; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 0.5; ferric ammonium citrate, 0.05; Casamino acids, 3.0; glucose, 10.0; pH 7.6.

Preparation of cell walls, LPS and polysaccharides (PS). *Rhizobium trifolii* cell walls were prepared by a slight modification of the procedure of Roberson & Schwab (1960). LPS was prepared according to Westphal & Jann (1965). LPS of strain 24SM, instead of being centrifuged, was fractionated on a Sepharose 2B column and eluted with 0.01 M- NH_4HCO_3 (Romanowska, 1970). PS was prepared according to Berst *et al.* (1969).

Deacetylation of cell walls and LPS by NaOH and NH_4OH . Cell walls and LPS (5 mg/ml) were treated with 0.1 M-NaOH or 2.5 M- NH_4OH at 37 °C for 1 h. The mixture was chilled, neutralized with 0.1 M-HCl and centrifuged. The pellet was washed three times and lyophilized.

LPS oxidation with $NaIO_4$. LPS was oxidized according to a modification of the procedure of Foster, Davies & Crumpton (1958). One ml of LPS (10 mg/ml) was mixed with 0.5 ml of 2% (w/v) $NaIO_4$ and incubated at 20 °C for 12 min. Excess $NaIO_4$ was decomposed by ethylene glycol. After 20 min, 0.5 ml of 1% (w/v) $NaBH_4$ was added. Excess $NaBH_4$ was then decomposed by acetic acid (0.2 ml) and dialysed overnight against distilled water.

Inactivation of phage 1P. Cell walls of LPS, at a concentration of 1 to 50 µg/ml in tris-HCl buffer pH 7.4, were mixed with an equal volume of phage 1P containing 6×10^3 plaque forming units (p.f.u.). The mixture was incubated at 37 °C without shaking. At intervals, samples were removed and tested for phage survival using *R. trifolii* 24SM as the indicator culture.

Table 1. *Composition of sugars in LPS preparations*

Sugar	Retention times	Content of sugars (mol %)			
		24SM	XSM	HR	15
Rhamnose	0.18	—	8.0	—	—
Fucose	0.195	0.8	—	—	—
Ribose	0.27	—	—	3.0	0.3
Arabinose	0.3	—	0.5	—	Trace
Mannose	0.75	14.3	2.2	35.0	16.2
Galactose	0.86	6.0	2.6	33.5	12.4
Glucose	1.00	1.5	86.2	7.0	4.8
X ₁	1.27	2.8	—	—	—
X ₂	1.47	3.1	—	—	2.6
X ₃	1.77	Trace	—	—	1.5
Heptose I	1.97	10.0	—	—	5.0
Heptose II	2.32	62.3	—	22.0	57.2

Retention times are expressed relative to that of glucose. Results are expressed as percentages of the total sugars. Heptose I, D-glycero-D-mannoheptose; heptose II, L-glycero-D-mannoheptose; compounds X₁, X₂ and X₃ were unidentified.

RESULTS AND DISCUSSION

Preliminary studies showed that both cell walls and LPS from several phage-sensitive strains effectively inactivated phage IP, while those from the two phage-resistant mutants inactivated the phage only slightly. Thus, approximately 0.1 µg LPS from strain 24SM inactivated 50 % of the p.f.u. within 1 h, while LPS from the two mutants inactivated less than 10 % during the same period.

The effect of various modifications of cell walls and LPS on their ability to inactivate phage was determined. Deacetylation of cell walls and LPS caused a loss of phage-inactivating ability. Untreated cell walls or LPS from strain 24SM inactivated 93 to 96 % of p.f.u. within 30 min, while cell walls and LPS deacetylated by treatment with 2.5 M-NH₄OH or 0.1 M-NaOH inactivated only 9 to 16 % and 1 to 8 % of p.f.u., respectively. The need for acetyl groups for effective phage adsorption has been noted in other species. For example, the capacity of *Staphylococcus aureus* cells walls to inactivate phage 52A was lost by removing O-acetyl groups (Shaw & Chatterjee, 1971). Adsorption of Vi phage onto bacterial surfaces results in the formation of a complex phage-bound Vi-polysaccharide deacetylase with Vi polysaccharide, leading to deacetylation. Phage-treated Vi polysaccharide regains receptor activity after acetylation (Taylor, 1966).

Oxidation of LPS also caused a significant decrease in phage inactivation. LPS from strain 24SM, oxidized with 2 % (w/v) NaIO₄ and then reduced with 1 % (w/v) NaBH₄, inactivated only 5 % of the phage.

Intact LPS was required for phage inactivation. Polysaccharide obtained from the LPS of strain 24SM after hydrolysis with acetic acid at 100 °C failed to inactivate phage IP. The function of the lipid may be to stabilize the configuration of the attachment site (Lindberg, 1973).

The molar ratios of neutral sugars in the LPS isolated from strains 24SM, XSM, HR and 15 were determined (Table 1). A high content of L-glycero-D-mannoheptose (more than 50 % of the molar ratio) was characteristic of the neutral sugars of LPS preparations from strains 24SM and 15 but was missing from strain XSM, a strain whose LPS inactivates phage IP poorly. This suggested that heptoses might form part of the phage receptor, and was consistent with the effects of oxidation on phage inactivation since this treatment degrades L-glycero-D-

mannoheptose (Foster *et al.* 1958). However, the LPS of the phage-resistant mutant, strain 15, contained a large amount of L-glycero-D-mannoheptose but failed to inactivate phage 1P.

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