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STRUCTURE OF THE O-ANTIGEN OF *Pseudomonas syringae* PV. *phaseolicola* STRAIN NPS 3121

STANLEY F. OSMAN*, WILLIAM F. FETT, AND KEVIN B. HICKS
U. S. Department of Agriculture, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118 (U.S.A.)

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ABSTRACT

The structure of the O-antigen portion of the lipopolysaccharide isolated from the bean pathogen *Pseudomonas syringae* pv. *phaseolicola* strain NPS 3121 has been determined by chemical and n.m.r.-spectral analysis. The polysaccharide consists of a repeating unit of alternating 2- and 3-*O*-sustituted α-D-rhamnopyranosyl residues with every second 3-*O*-substituted D-rhamnose also substituted at O-4 with a terminal α-D-fucofuranosyl group.

INTRODUCTION

The ability of a particular plant cultivar to respond to microbial invasion of one race of a particular bacterial species, thereby preventing infection while being susceptible to another race of the same bacterium, suggests host recognition to be an important factor in this form of highly specific disease-resistance[1]. A high degree of specificity also exists between symbiotic rhizobia and their legume hosts[2]. In the latter interactions, such bacterial cell-surface polysaccharides as extracellular polysaccharides (EPS) and lipopolysaccharides (LPS) are considered to be important in recognition and in subsequent phenomena leading to successful nodule development[2]. It has also been proposed that bacterial cell-surface polysaccharides are involved in recognition phenomena between phytopathogenic bacteria and plants[3]; however, very little information is available on the complete structure of these polysaccharides. In conjunction with studies in this laboratory on plant disease-resistance, we have initiated an investigation on the structure of the LPS of strains representing different physiologic races of the bean pathogen *Pseudomonas syringae* pv. *phaseolicola*. If race-specific differences do exist, such differences may be important for recognition phenomena *in planta*. We now present the structure of the O-antigen portion of the LPS isolated from a strain of *Pseudomonas syringae* pv. *phaseolicola*. The whole LPS of a number of strains of *P. s.* pv. *phaseolicola* has recently been showed to contain rhamnose, fucose, and glucose[4].

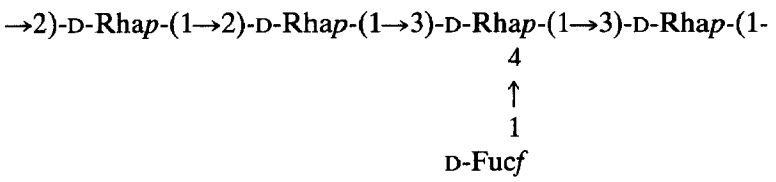
*To whom correspondence should be addressed.

RESULTS AND DISCUSSION

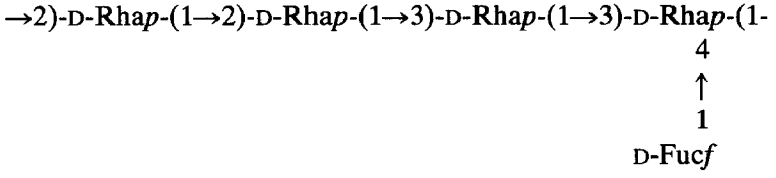
The O-antigen-containing column-fraction, $[\alpha]_D^{23}$ +88°, was shown to consist of D-rhamnose and D-fucose in the ratio of 4:1, along with traces of glucose, uronic acids, and hexosamines. Permethylation analysis (see Table I, column A) indicated that the polymer contained 2-, 3-, and 3,4-substituted and unsubstituted 6-deoxyhexoses (standards were not available to distinguish a substituted rhamnose from the correspondingly substituted fucose by gas–liquid chromatography) in the ratios of 2:1:1:1. The substituted sugars were all pyranosidic, whereas the unsubstituted sugar was in the furanoside form.

The polymer isolated after mild hydrolysis with acid contained rhamnose as the only sugar, and permethylation analysis (see Table I, column B) indicated equal amounts of the 2- and 3-substituted pyranosides. From these results, it may be concluded that the mild acid treatment had removed a D-fucofuranosyl group from O-4 of the 3,4-disubstituted rhamnose, yielding a linear polysaccharide composed of 2- and 3-substituted D-rhamnopyranosyl residues.

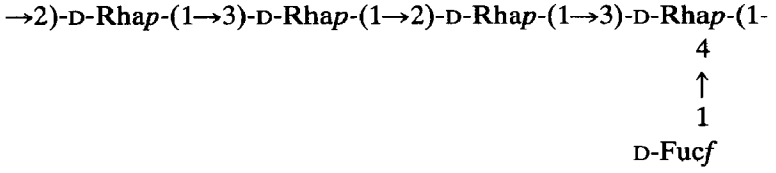
From these data, three sugar sequences are possible for the O-antigen, namely,



1a



1b



1c

TABLE I

METHYLATION ANALYSIS OF *P. s.* PV. *phaseolicola* STRAIN NPS 3121 AND DEGRADATION PRODUCTS

Position of alkoxyl groups in 6-deoxyhexose	*RRT*[a]	*Relative amount*[b]			
		A	*B*	*C*	*D*
3,4 diMe	1.20	2	1		
2,4 diMe	1.21	1	1		
2 Me	1.33	1			
2,3,5 triMe	**1.00**	1			
2,3,4 triMe	0.95			1	
2,4 diMe, 3 Et	1.08				1
2 Me, 3,4 diEt	1.21				1

[a]Retention time of the alkylated alditol acetate relative to that of 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-D-fucitol (13.59 min) on a 20-m OV-101 column programmed under the following conditions: 100–120° at 2°/min; 120–140 at 4°/min; and 140–225° at 6°/min. [b]Key: A, strain 3121 O-antigen; B, strain 3121 O-antigen hydrolyzed with 0.1M H_2SO_4 for 3 h at 75°; C, strain 3121 O-antigen treated with $NaIO_4$, procedure 1; and D, strain 3121 treated with $NaIO_4$, procedure 2.

The correct sequence was determined by Smith degradation and n.m.r.-spectral analysis. Two protocols were used in the degradation analysis. In the first procedure the sample was oxidized, the product reduced with $NaBH_4$, the product hydrolyzed under mild conditions (50% HOAc), the material permethylated, and then the products completely hydrolyzed, reduced, and acetylated. In the second procedure the oxidized sample was permethylated before, and perethylated after, hydrolysis with 50% HOAc. The products derived from these reactions (see Table I, columns C and D) were those expected from an alternating 2- and 3-substituted rhamnan main-chain (**1c**).

^{13}C-n.m.r. data for the polysaccharide are summarized in Table II. The observed shifts are in good agreement with reported values. Correlations for shifts of C-1 and C-6 of the fucofuranoside were made with those of methyl α-D-fucofuranoside prepared in our laboratory. Both the ^{1}H (see Experimental section) and the ^{13}C-n.m.r. (Including ^{13}C–^{1}H coupling constants) data are consistent with all the sugars' having the α configuration[5,6]. There was no evidence for the presence of acetyl or other substituents in the n.m.r. spectra.

The O-antigen structure of *P. s.* pv. *phaseolicola* strain NPS 3121 is similar to that of other phytopathogenic pseudomonads, in that rhamnose is a major component of the backbone structure[8–12]. The presence of fucose as the furanoside is rare in bacterial LPS, and, to the best of our knowledge, has been reported in only one other species[13]. The O-antigen of *P. s.* pv. *syringae* is reported to contain rhamnose and fucose[14], and it may be similar to the O-antigen of *P. s.* pv. *phaseolicola*; however, the complete characterization of the former has not yet been reported. A recent report indicated that different pathovars of *P. syringae* may share a common O-antigen structure[11]. There appears to be no structural differences in the O-antigen fractions of LPS isolated from three races of *P. s.* pv. *glycinea*[12], although the complete O-antigen structure has not been determined.

TABLE II

^{13}C-N.M.R. SHIFT DATA FOR 3121 O-ANTIGEN (A), AND PRODUCT DERIVED FROM ITS MILD HYDROLYSIS (B)

Substituted sugar	*Carbon atom*	*Shift (p.p.m.)*		*Lit.*[7]
		A	*B*	
→2)-α-D-Rha*p*-(1→	C-1	101.7	101.8	101.6
	C-2	79.0	79.0	78.8
	CH_3	17.4[a]	17.5[b]	17.5
→3)-α-D-Rha*p*-(1→	C-1	102.8	102.9	102.6
	C-3	78.4	78.1	78.6
	CH_3	17.7[a]	17.4[b]	17.5
↓ 3				
-α-D-Rha*p*-(1→	C-1	103.5[c]		
4	C-3	77.7		
↑	CH_3	18.5[d]		
-α-D-Fuc*f*-(1→	C-1	102.5[c]		102.7[e]
	CH_3	18.2[d]		

[a–d]Assignments with same lettered superscripts may have to be interchanged. [e]Shift for methyl α-D-fucofuranoside (see Experimental section).

EXPERIMENAL

General methods. — Evaporations were conducted under a stream of N_2 at ambient temperature. An OV-101 capillary column (20 m) was used for g.l.c. (Hewlett–Packard 5880A) and g.l.c.–m.s. (Hewlett–Packard 5995B) analysis. Sugars were identified by g.l.c.–m.s. of their aldononitrile derivatives. Standard procedures were used for uronic acid[15] and hexosamine[16] determinations. Dimsyl sodium was used for permethylation and ethylations, as previously described[17]. Alkylated alditol acetates were unambiguously identified by g.l.c.–m.s. The optical rotation of the sugars was determined for the rhamnose and fucose isolated by h.p.l.c. (Aminex HPX-87C column, H_2O eluant) from an acid hydrolyzate of the polymer; rhamnose $[\alpha]_D^{23}$ −8.8°; fucose $[\alpha]_D^{23}$ +71.4°.

N.m.r. analysis. — Spectra were recorded with a JEOL CX-400 spectrometer. The parameters for ^{13}C spectra (100 MHz) were: pulse delay, 5 s; completely decoupled mode; probe T, 23°; ref., 1,4-dioxane (67.4 p.p.m.) Typically, 10,000 scans were accumulated. Proton spectra were recorded at 400 MHz for samples dissolved in D_2O, with an acquisition time of 2 s and a pulse delay of 2 s. The probe was maintained at 50° and, typically, 500 scans were accumulated. Me_4Si was the reference standard. The signals for the anomeric protons of strain 3121 O-antigen were centered at 5.0 (2 H), 5.1 (1 H), 5.2 (1 H), and 5.3 (1 H). The heteronuclear coupling-constants for the anomeric carbon atoms, obtained from the ^{13}C–^{1}H coupled spectrum were 184, 180, 169, and 184 Hz.

Preparation of O-antigen. — Strain NPS 3121 was obtained from Dr. Peter Lindgren (Dept. of Plant Pathology, Univ. of California, Berkeley, CA). The bacterium was grown to early stationary phase at room temperature in a semi-synthetic, liquid medium[18] containing 1% of D-glucose as the carbon source and with potassium phosphate substituted for yeast extract. LPS was extracted from the cells with hot, aq. phenol[19], and purified by ultracentrifugation (100,000*g*; three times). After hydrolysis in 1% acetic acid for 2 h at 100°, the liberated lipid A was removed by centrifugation and $CHCl_3$ extraction. The supernatant fluid was lyophilized, and the polysaccharide was fractionated on a column of Sephadex G-50 eluted with pyridine acetate buffer, pH 4.25.

Partial hydrolysis of polysaccharide. — The polysaccharide (4 mg) was hydrolyzed for 3 h at 75° with 0.1M H_2SO_4 and, after neutralization of the acid with $BaCO_3$ and centrifugation, the supernatant liquor was evaporated to dryness and the residue mixed with EtOH. Both the EtOH-soluble and -insoluble materials were subjected to sugar and permethylation analysis.

Periodate oxidation. Oxidations were conducted under standard conditions using 0.2M $NaIO_4$ in 0.1M acetate buffer (pH 3.9). Conditions for the modified Smith degradation (procedure 2) were identical to those described by Lindberg *et al.*[20].

Preparation of methyl α-D-fucofuranoside. — A mixture of the methyl glycosides of fucose was prepared by the Fischer reaction[21]. The methyl pyranosides (major components) were separated from the methyl furanosides by preparative h.p.l.c. on a column of HPX-42A (Ag^+) with H_2O as the mobile phase. The anomeric furanosides were separated by preparative h.p.l.c. on a Dynamax C-18 prep column (H_2O, mobile phase). The isomers were characterized by their relative retention-times[21] in g.l.c.

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