Poly(arabitol phosphate) teichoic acid in the cell wall of *Agromyces cerinus* subsp. *cerinus* VKM Ac-1340^T

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Abstract On the basis of NMR studies and analysis of the products of acid and alkaline hydrolyses the following structures were established for the repeating units of poly(arabitol phosphate) teichoic acid: α-6-deoxy-L-Tal*p*-(1 → 3)-β-D-Glc*p*NAc-(1 → 2)-α-L-Rha*p*-(1 → 4(2)-D-Arabitol-PO_4 and β-D-Glc*p*NAc-(1 → 2)-α-L-Rha*p*-(1 → 4(2)-D-arabitol-PO_4. The molar ratio of these units is about 1.2:1.0, respectively. Poly(arabitol phosphate) teichoic acid is here reported in bacterial cell walls for the first time.

Key words: Cell wall; Poly(arabitol phosphate) teichoic acid; 6-Deoxytalose; *Agromyces*

1. Introduction

Teichoic acids of gram-positive bacteria display a wide structural diversity [1,2]. Polymers of phosphoric esters of glycerol, ribitol, mannitol and erythritol have been found [3,4].

As has been previously shown, within the genus *Agromyces* (the order *Actinomycetales*) the structure of cell wall teichoic acids is characterized by a high variability. Teichoic acid from *Agromyces cerinus* subsp. *nitratus* is represented by a poly-(ribofuranosylribitol phosphate) [5] and that from *Agromyces fucosus* subsp. *hippuratus* is a 1,5-poly(ribitol phosphate) in which each ribitol residue carries a tetrasaccharide substituent [6]. In this communication, we report the established structure of the repeating units of the teichoic acid from *Agromyces cerinus* subsp. *cerinus* VKM Ac-1340^T.

2. Materials and methods

2.1. Strain, growth conditions and preparation of cell walls

Agromyces cerinus subsp. *cerinus* VKM Ac-1340^T was grown as described previously [6]. To prepare cell wall, the crude mycelium was disrupted with an ultrasonic disintegrator and then incubated at 60°C for 1 h to inactive autolytic enzymes. Cell walls were separated by differential centrifugation and purified by digestion with trypsin, boiling in 2% SDS, treatment with trichloroacetic acid (10%, 4°C, 48 h and 5%, 90°C, 15 min) and 0.1 N NaOH, 3 h, 100°C [7]. The resulting preparation was lyophilized and subjected to chemical analysis.

2.2. Analytical procedures, paper chromatography and electrophoresis

Acid and alkaline hydrolyses, treatment with aqueous HF, and analytical methods were detailed in [3]. Paper chromatography and electrophoresis were carried out on Filtrak FN-13 paper (Germany). The following solvent systems were used in descending chromatography: (1) pyridine/benzene/butan-1-ol/water (3:1:5:3, v/v) to separate glycosides, arabitol and monosaccharides; (2) pyridine/ethyl acetate/acetic acid/water (5:5:1:3, v/v) to separate amino sugars. Phosphoric esters were separated by paper electrophoresis using pyridine-acetate buffer, pH = 5.6. Phosphorus esters were detected with the Isherwood reagent; alditol, monosaccharides, and glycosides with 5% $AgNO_3$ in aqueous NH_3, glucosamine with ninhydrin. Alkaline phosphomonoesterase (EC 3.1.3.1.) was purchased from Sigma (USA).

2.3. Optical rotation determination

Optical rotation was obtained on Jasco DJP-360 instrument (Japan) in water at 20°C.

2.4. NMR spectroscopy

NMR spectra were recorded with a Bruker AM-3000 spectrometer for solutions in D_2O at 40°C with acetone (δ_{1H} 2.225 p.p.m. and δ_{13C} 31.45 p.p.m.) as the internal standard. Homonuclear $^1H/^1H$ COSY spectrum and heteronuclear HMQC spectrum [8] were obtained using a standard Bruker program from the software package for the Aspect 3000 computer.

3. Results and dicussion

The cell wall of *Agromyces cerinus* subsp. *cerinus* VKM Ac-1340^T contains 2.2% of phosphate that was present in the acid hydrolysate in the form of phosphoric esters of an alditol.

On paper chromatography the alditol was indistinguishable from authentic arabitol. After acid hydrolysis of the cell wall the alditol was accumulated by preparative paper chromatography and identified as arabitol based on ^1H and ^{13}C NMR spectra (Table 1). Positive optical rotation of 0.4% arabitol solution is indicative of D-configuration ($[\alpha]_D^{20°}$ +1.5° in saturated solution of $Na_2B_4O_7$.)

Based on chromatographic and electrophoretic analysis, acid hydrolysis (2 N HCl, 3 h, 100°C) of the cell wall yielded arabitol, its mono- and diphosphates, rhamnose, 6-deoxytalose and glucosamine as the major products. Alkaline hydrolysis (1

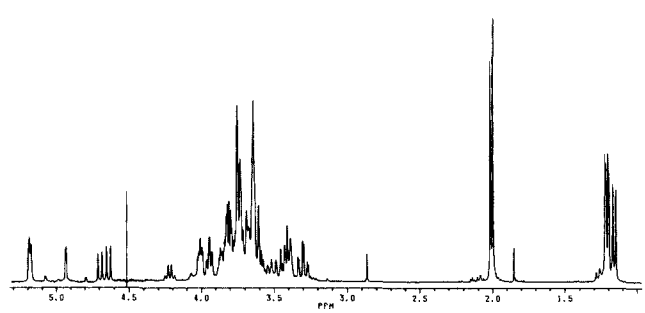

Fig. 1. 300 MHz ^1H NMR spectrum of the glycosides.

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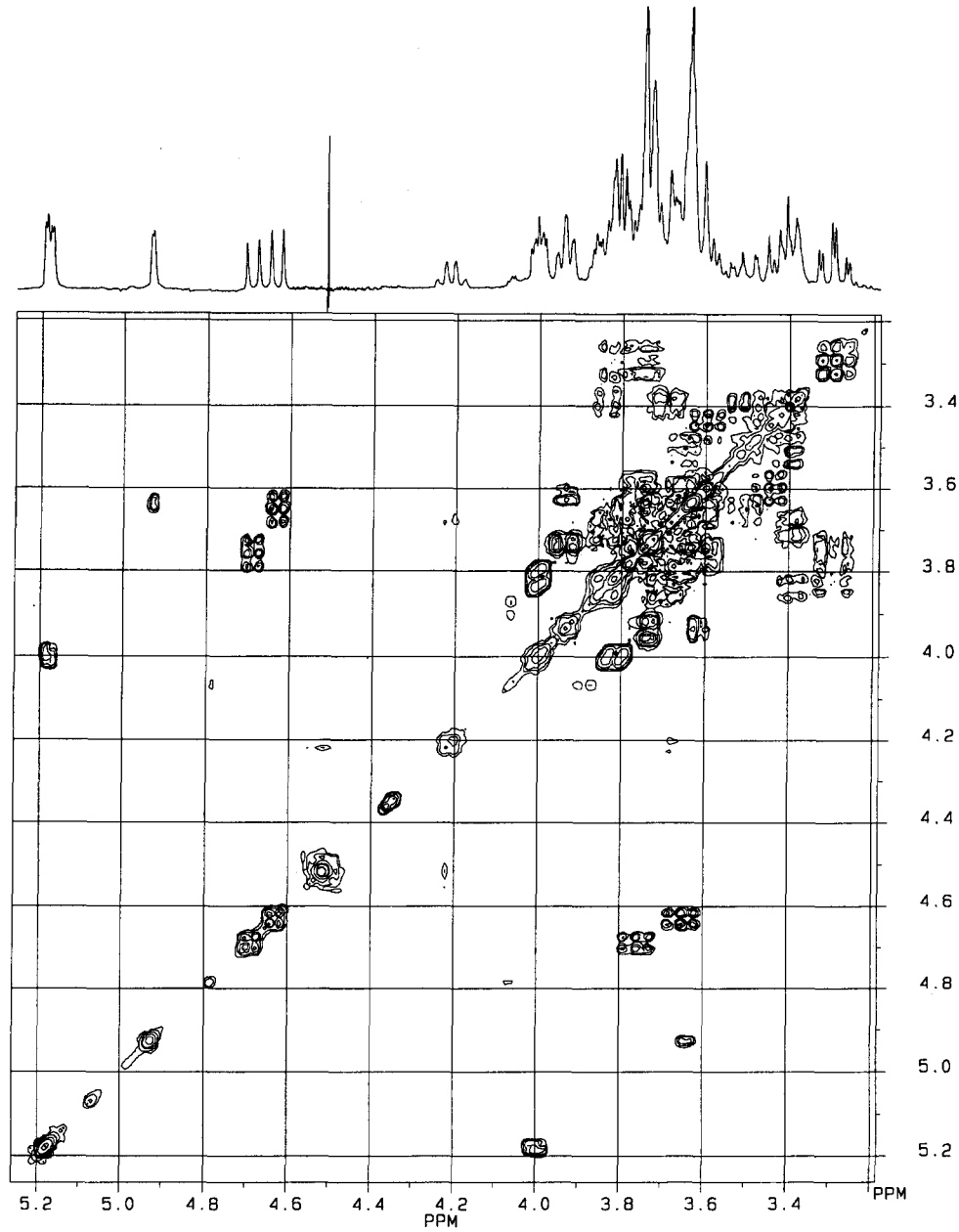

Fig. 2. Downfield part of $^1H/^1H$ COSY spectrum of the glycosides.

N NaOH, 3 h, 100°C) of the cell wall produced a mixture of isomers monophosphate of glycosylarabitol. Treatment of the latter with alkaline phosphomonoesterase resulted in a complete loss of the phosphoryl groups and formation of glycosides. These data could be considered as indicative of the presence of a poly(arabitol phosphate) chain.

However, consecutive treatment with trichloroacetic acid (10%, 48 h, 4°C; 5%, 15 min, 90°C) and alkali (0.1 N NaOH, 1 h, 100°C) failed to extract the polymer or its degradation products from the cell wall.

In order to release glycosylarabitols, the dephosphorylated repeating units of the teichoic acid, the cell wall was treated with 40% hydrogen fluoride (72 h, 20°C) [9] and the resulting glycosides were accumulated by preparative paper chromatography and subjected to ^1H and ^{13}C NMR spectroscopy.

The ^1H NMR spectrum of the dephosphorylated product contained five signals in the anomeric proton resonance region. Two of them were of a slightly higher integral intensity than the

Table 1
NMR-data for D-arabitol isolated from *Agromyces cerinus* subsp. *cerinus* cell wall.

Chemical shift of ^1H (δ, p.p.m.)		Coupling constant (J, Hz)		Chemical shift of ^{13}C (δ, p.p.m.)	
H-1,1′	3.71	$1/2(J_{1,2} + J_{1',2})$	7.1	C-1	64.3
H-2	3.97	$J_{2,3}$	2.0	C-2	71.5
H-3	3.61	$J_{3,4}$	7.9	C-3	71.7
H-4	3.79	$J_{4,5}$	2.8	C-4	72.3
H-5	3.87	$J_{5,5'}$	11.1	C-5	64.1
H-5′	3.68	$J_{5',4}$	5.9		

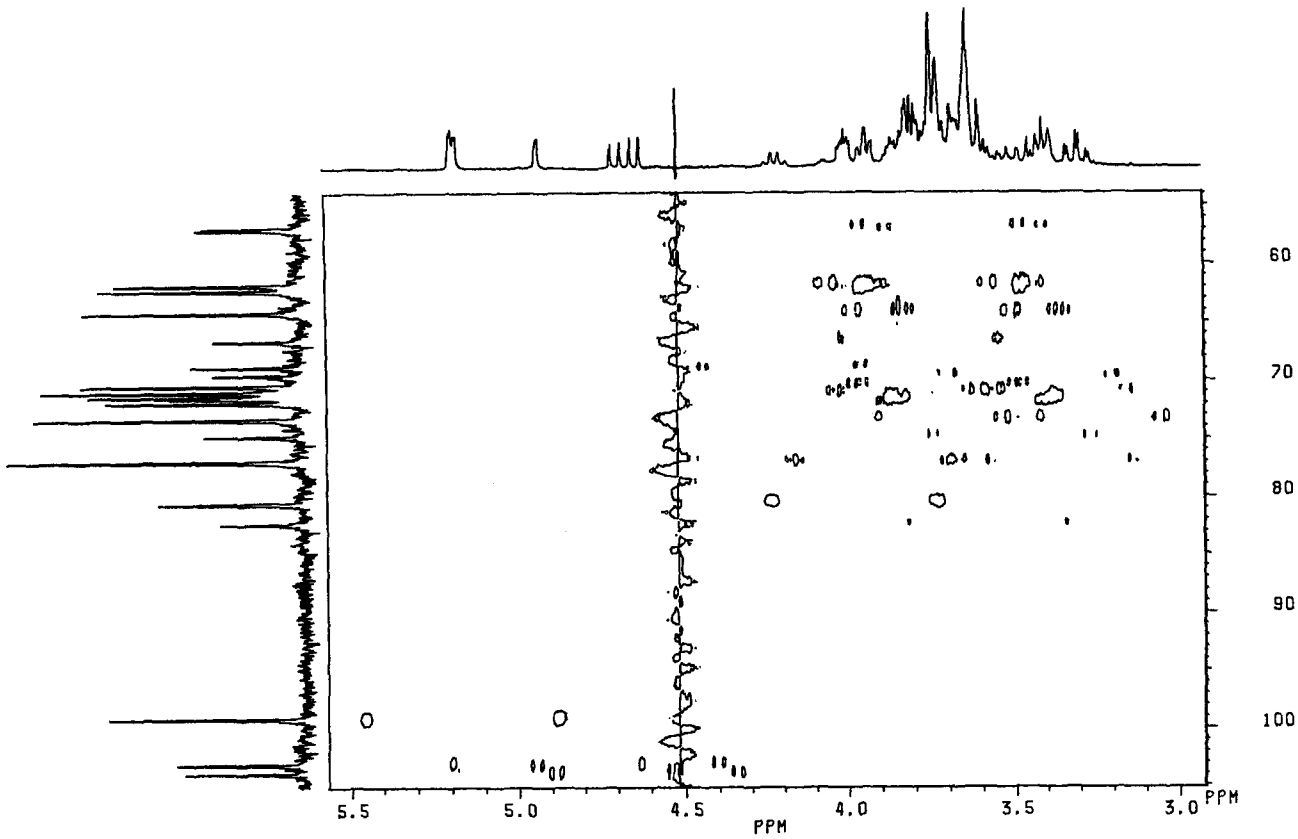

Fig. 3. Downfield part of 300 MHz HMQC spectrum of the glycosides.

Table 2
NMR-data for the glycoside I (G I) and the glycoside II (G II) of the teichoic acid from *Agromyces cerinus* subsp. *cerinus* cell wall

Residue		Chemical shift of ^1H (δ, p.p.m.)		Coupling constant (J, Hz)			Chemical shift of ^{13}C (δ, p.p.m.)	
		GI	GII				GI	GII
→2)-Arabitol	H-1	3.74	3.74	$1/2(J_{1,2} + J_{1',2})$	5.5	C-1	62.2	62.2
	H-1′	3.74	3.74			C-2	76.95	76.95
	H-2	3.93	3.93	$J_{2,3}$	1.7	C-3	71.2	71.2
	H-3	3.62	3.62			C-4	71.85	71.85
	H-4	3.65	3.65			C-5	64.15	64.15
	H-5	3.76	3.76					
	H-5′	3.63	3.63					
→2)-α-L-Rha*p*-(1→	H-1	5.19	5.18	$J_{1,2}$	1.9	C-1	99.1	99.1
	H-2	4.00	4.02	$J_{2,3}$	3.2	C-2	80.6	80.5
	H-3	3.82	3.83	$J_{3,4}$	9.6	C-3	70.95	70.95
	H-4	3.29	3.30	$J_{4,5}$	9.6	C-4	73.3	73.3
	H-5	3.75	3.75	$J_{5,6}$	6.1	C-5	70.4	70.4
	H-6	1.22	1.21			C-6	17.8	17.8
→3)-β-D-Glc*p*NAc-(1→	H-1	4.63	4.70	$J_{1,2}$	8.1	C-1	103.9 (7.5)[a]	103.15 (7.0)[a]
	H-2	3.66[b]	3.77[b]	$J_{2,3}$	9.7	C-2	57.0[b]	56.8 (−0.2)[a]
	H-3	3.52	3.60	$J_{3,4}$	9.7	C-3	74.8	82.35
	H-4	3.41	3.45	$J_{4,5}$	9.7	C-4	70.5	69.5 (−1.7)[a]
	H-5	3.40	3.43			C-5	76.95	76.95
	H-6	3.86	3.86			C-6	61.8	61.8
	H-6′	3.65	3.65					
6d-α-L-Tal*p*-(1→	H-1		4.93	$J_{1,2}$	1.7	C-1		103.1
	H-2		3.64	$J_{2,3}$	3.5	C-2		71.3
	H-3		3.76	$J_{3,4}$	3.5	C-3		66.6
	H-4		3.67	$J_{4,5}$	1.2	C-4		73.3
	H-5		4.22	$J_{5,6}$	6.3	C-5		68.8
	H-6		1.16			C-6		16.6

[a]Glycosylation effect. [b]CH_3CON at 2.01 and 2.02 p.p.m. (^1H) and 23.45 (CH_3), 175 and 176.0 p.p.m. (CO, ^{13}C).

other three (Fig. 1). This was suggestive of a 5.5:4.5 mixture of two glycosides with two and three sugar residues, respectively. The spectrum was assigned using 2D COSY (Fig. 2), relayed coherence transfer COSY, and 1D double resonance in difference mode [10]. The coupling constants [11] and the chemical shifts [12] indicated that both disaccharide and trisaccharide glycosides contained α-rhamnopyranose and β-2-acetamido-2-deoxyglucopyranose residues and arabitol as an aglycon. The additional residue in the trisaccharide glycoside proved to be 6-deoxytalopyranose (Table 2).

2D NOE spectrum in rotating frames (ROESY [13,14]) contained *inter alia* inter-residue peaks for H-1 of talopyranose and H-3 of 2-acetamido-2-deoxyglucopyranose, for H-1 of 2-acetamido-2-deoxyglucopyranose and H-2 of rhamnopyranose, for H-1 of rhamnopyranose and H-2 of arabitol residues.

The heteronuclear $^{1}H/^{13}C$ 2D spectrum (HMQC [8]) (Fig. 3), allowed us to assign the signals in the ^{13}C NMR and calculate the glycosylation effects. The glycosylation effect for 2-acetamido-2-deoxy-β-glucopyranose (glycoside I) was 7.5 p.p.m. that was characteristic of different absolute configuration of 2-acetamido-2-deoxy-β-glucopyranose and α-rhamnopyranose. The small β-glycosylation effect for C-2 (−0.2 p.p.m.) and the significant negative effect for C-4 (−1.5 p.p.m.) of 2-acetamido-2-deoxy-β-glucopyranose also suggested opposite absolute configuration of 2-acetamido-2-deoxy-β-glucopyranose and 6-deoxytalopyranose in the trisaccharide (glycoside II) [15]. Absolute configuration of α-rhamnopyranose isolated from hydrolysates of the glycosides was determined after its conversion into 2-octyl-glycoside by comparing the obtained derivatives to the standard samples of (*S*+)- and (*R*−)-2-octyl-rhamnopyranoside using gas-liquid chromatography [16]. It was inferred to have L-configuration.

Taking into account L-configuration of rhamnopyranose, 2-acetamido-2-deoxy-β-gluco-pyranose should have D-configuration, whereas 6-deoxytalopyranose L-configuration.

To test whether elimination of 6-deoxytalose occurs during HF-treatment, the glycosylarabitol monophosphates from the alkaline hydrolysate of the cell wall were accumulated by preparative paper electrophoresis and subjected to ^{1}H NMR spectroscopy. The spectrum showed that the mixture of phosphoric esters contained diglycosylarabitol and triglycosylarabitol phosphates in the molar ratio identical to that found for glycosides (see above).

Experiments aimed at establishing the type of phosphodiester linkages between the repeating units in the polymer are in progress.

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