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Carbohydrate Research 338 (2003) 385–388
CARBOHYDRATE RESEARCH
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Note

The structure of the O-specific polysaccharide chain of the *Shewanella algae* BrY lipopolysaccharide

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Received 12 December 2001; accepted 26 October 2002

Abstract

An acidic O-specific polysaccharide was obtained by mild acid degradation of the *Shewanella algae* strain BrY lipopolysaccharide and was found to contain L-rhamnose, 2-acetamido-4-[D-3-hydroxybutyramido)]-2,4,6-trideoxy-D-glucose (D-BacNAc4NHbu), and 2-amino-2,6-dideoxy-L-galactose, *N*-acylated by the 4-carboxyl group of L-malic acid (L-malyl-(4→2)-α-L-FucN) in the ratio 2:1:1. ^{1}H and ^{13}C NMR spectroscopy was applied to the intact polysaccharide, and the following structure of the repeating unit was established:

-3)-α-D-BacNAc4NHbu-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→2)-L-malyl-(4→2)-α-L-FucN-(1-

The repeating unit includes linkage via the residue of malic acid, reported here for the first time as a component of bacterial polysaccharides.

Keywords: Lipopolysaccharide; O-Chain; *Shewanella*, *S. algae*

1. Introduction

Shewanella algae BrY is a Gram-negative bacterium that was isolated from subsurface sediments.[1] It can attach to amorphous iron oxides and, in so doing, utilizes the Fe(II)/Fe(III) couple as a terminal electron acceptor during dissimilatory iron reduction.[2] Like all Gram-negative bacteria it has lipopolysaccharide (LPS) as a major macromolecule on the external face of the outer membrane. The polysaccharide part of the LPS may be responsible for adhesion of this microorganism to the mineral surface. *S. algae* BrY is an opportunistic pathogen which has been implicated in certain infections,[3] and thus O-side chains of the LPS may be essential for virulence. Here we present the results of structural analysis of the O-specific polysaccharide chain of *S. algae* BrY LPS.

2. Experimental

2.1. NMR spectroscopy

^{1}H and ^{13}C NMR spectra were recorded using a Varian Inova 500 spectrometer in D_2O solutions at 60 °C with acetone standard (2.225 ppm for ^{1}H and 31.5 ppm for ^{13}C) using standard pulse sequences COSY, TOCSY (mixing time 120 ms), NOESY (mixing time 200 ms), HSQC, gHMBC (optimized for a 5 Hz coupling constant), and HSQC–TOCSY. Spectra were assigned with the help of the Pronto program.[4]

2.2. Monosaccharide identification

The polysaccharide (1 mg) was hydrolysed (0.5 ml of 3 M TFA, 100 °C, 2 h), the solution evaporated to dry-

Abbreviations: LPS, lipopolysaccharide; FucN, 2-amino-2,6-dideoxygalactose; BacN, 2,4-diamino-2,4,6-trideoxyglucose; BacNAc4NHbu, 2-acetamido-4-[D-3-hydroxybutyramido)]-2,4,6-trideoxy-D-glucose.

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0008-6215/03/$ - see front matter
PII: S0008-6215(02)00469-X

ness under a stream of nitrogen, the residue dissolved in water (0.5 mL), reduced with $NaBH_4$ (~ 5 mg, 30 min), treated with AcOH (0.5 mL), dried, and MeOH (1 mL) added. The mixture was dried twice, and the residue acetylated with Ac_2O (0.5 mL, 100 °C, 30 min), dried, and analysed by GLC on a DB-17 capillary column (25 m × 0.25 mm) with a flame ionization detector (HP 5890 instrument) in a temperature gradient of 180–240° at 2 °C/min, or on a Varian Saturn 2000 instrument equipped with an ion-trap mass spectral detector. For absolute configuration determination, a sample of the polysaccharide (1 mg) was treated with (*R*)-2-butanol (0.5 mL) and acetyl chloride (0.05 mL) for 3 h at 100 °C, dried, acetylated as already described, and analysed by GLC in a temperature gradient of 120–240 °C at 2°/min. Retention times were compared with those of standard samples prepared from L-rhamnose, *N*-acetyl-L-fucosamine, malic acid, and 3-hydroxybutyric acid with (*R*)- and (*S*)-2-butanol.[5]

3. Results

The lipopolysaccharide and its polysaccharide O-chain were isolated from the cells of *S. algae* by conventional methods, including phenol–water extraction and mild acid hydrolysis.[6] Monosaccharide analysis (GLC of alditol acetates) of the polysaccharide revealed the presence of rhamnose and fucosamine. NMR data (Table 1, Fig. 1) showed that polysaccharide has a regular structure with a repeating unit consisting of four monosaccharides. The repeating unit included also an acetamido group and the residues of 3-hydroxybutyric acid (**Hbu**) and malic acid (**M**). The identities of the monosaccharides and other components were established on the basis of ^1H and ^{13}C NMR chemical shifts and $^3J_{H,H}$ coupling constants, which for monosaccharide residues were in agreement with values expected for the respective pyranosides. Thus, two residues of α-rhamnopyranose (**C** and **D**), one residue of α-fucosamine (**A**), and one residue of 2,4-diamino-2,4,6-trideoxy-α-glucopyranose (bacillosamine, **B**) were identified. The α anomeric configuration of the rhamnose residues was confirmed by the high-field position of their C-5 signals at 70.1 ppm (compared to ~ 73 ppm expected for the β anomers) and the absence of an intraglycosidic NOE between H-1 and H-3, H-5. Determination of the monosaccharide sequence was achieved on the basis of NOE and HMBC data. The observed NOE correlations between protons **A**1 and **B**3, **B**1 and **C**3, **C**1 and **D**2, **D**1, and **M**2, **D**1 and **C**5, and HMBC correlations (Table 1, Fig. 1) corresponded to the structure presented. HMBC correlations between C-1 of acyl substituents and the protons at the acylation position were used for the identification of N-acylation (Fig. 1). Thus, correlation of H-4 of the BacN residue with C-1 of 3-hydroxybutyrate, and of H-2 of BacN with C-1 of an acetyl group, indicated the acylation pattern of BacN. Proton A2 correlated with one of the carboxyl groups of malic acid. HMBC data allowed to identification of the signals of both carbonyl carbons of malic acid, but did not reliably differentiated between them because both carbonyl carbons gave similar intensity correlations with the H-2 and H-3 protons of the malic acid

Table 1
NMR data for the *S. algae* BrY polysaccharide

Unit, residue	1	2	3	4	5	6	Interresidual NOE from H-1	Interresidual HMBC from H-1
A, α-L-FucN	5.12	4.01	3.72	3.73	3.90	1.21	**B**3	**B**3
	97.5	50.6	68.1	72.0	68.0	16.5		
B, α-D-BacN	4.85	4.13	3.96	3.75	4.14	1.16	**C**3	**C**3
	95.5	55.2	73.2	56.3	67.8	17.7		
C, α-L-Rha	4.94	4.13	3.71	3.51	3.67	1.24	**D**2	**D**2
	102.9	67.7	76.9	71.4	70.1	17.6		
D, α-L-Rha	4.90	3.97	3.85	3.35	3.57	1.22	**M**3, **C**5	**M**2
	98.1	79.1	70.7	73.1	70.1	17.6		
Hbu, 3-hydroxy-butyrate		2.33	4.16	1.21				
		2.40						
	175.6	46.2	65.8	23.4				
M, malate		4.60	2.63					
			2.77					
	176.1	72.6	40.0	172.7				
Ac		1.96						
	174.6	23.1						

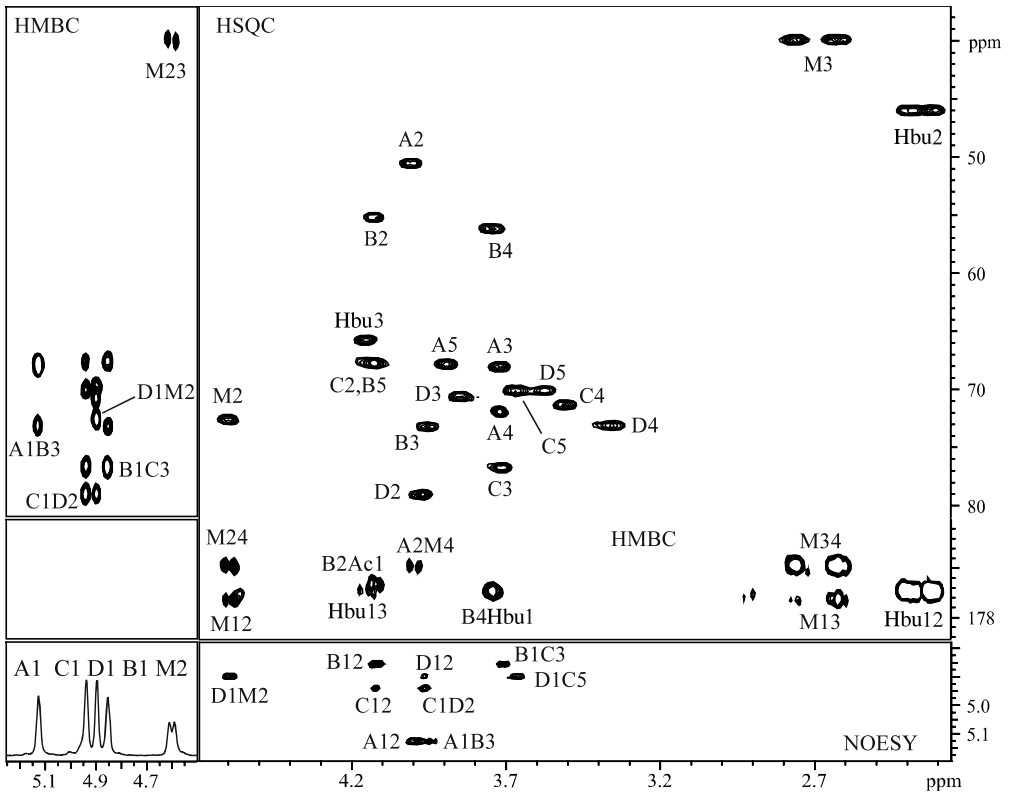
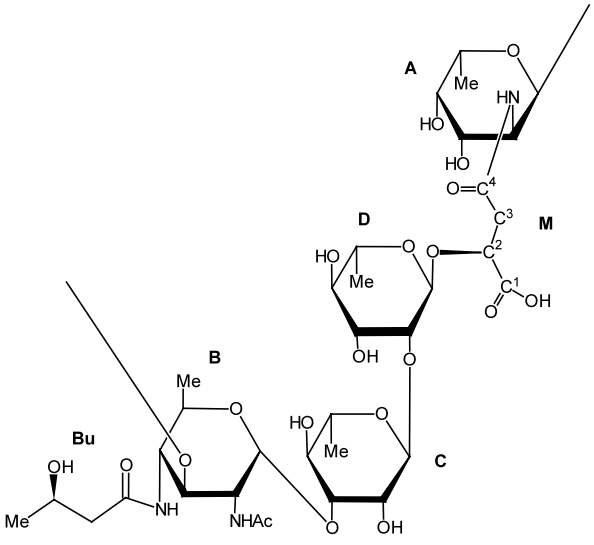


Fig. 1. Fragments of HSQC, HMBC, and NOESY spectra of the O-specific polysaccharide from *S. alga* BrY.

residue (Fig. 1). To solve this problem, a NOESY spectrum in 10% D_2O was recorded, wherein the NH-2 proton of fucosamine gave an NOE correlation with H-3 of malic acid, but not with its H-2. Thus it was concluded that FucN is acylated with the 4-carboxyl group of malic acid.

The absolute configuration of all components except bacillosamine was determined by GLC of the acetates of 2-butyl glycosides or esters. It was found that 3-hydroxybutyrate has the D configuration, malic acid the -L configuration, and rhamnose and fucosamine both have the L configurations. The absolute configuration of D-bacillosamine was determined by the analysis of ^{13}C NMR glycosylation effects as described.[7] The position of C-1 of BacN at 95.5 ppm and of Rha **C** C-3 at 76.9 ppm is expected for α-BacN-(1→3)-α-Rha with different absolute configurations of components. In the case of identical absolute configurations C-1 of BacN would be at ~100 ppm, and C-3 of Rha at ~81 ppm. Since Rha **C** has the L configuration, BacN has the D configuration. Additional proof of the identical absolute configuration of both rhamnose residues was the strong NOE between protons **D**1 and **C**5. This is characteristic for an α-(1→2)-linked disaccharide of two manno-sugars having the same absolute configuration, as can be seen from molecular modelling. On the basis of experimental data, the following structure of the O-specific polysaccharide *S. algae* BrY is proposed:



The structures of the polysaccharide O-chains from three other strains of *Shewanella* have been described previously;[8–10] they show no resemblance with each other. The core structure of the rough-type LPS from *S. putrefaciens* CN32 have been also described.[11] The structure, analysed in current work, includes the residue of malic acid, found for the first time in a bacterial polysaccharide.

Acknowledgements

This work was supported by grants from the Canadian Bacterial Diseases Network—National Centre of Excellence and the USA-NABIR-DOE program.

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