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Structure of the O-specific polysaccharide from the marine bacterium *Rheinheimera japonica* KMM 9513^T, containing N-glycosidic bond between monosaccharides

Maxim S. Kokoulin [a,*], Anatoly I. Kalinovsky [a], Svetlana V. Tomshich [a], Lyudmila A. Romanenko [a], Valery V. Mikhailov [a,b], Nadezhda A. Komandrova [a]

[a] G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, 100 Let Vladivostoku Prosp., 159, 690022 Vladivostok, Russia

[b] Far Eastern Federal University, Suhanova St., 8, 690950 Vladivostok, Russia

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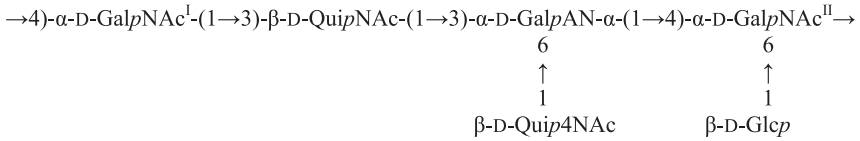
ABSTRACT

The O-specific polysaccharide was isolated from the lipopolysaccharide of type strain *Rheinheimera japonica* KMM 9513^T and studied by sugar analysis, Smith degradation, and two-dimensional ^{1}H and ^{13}C NMR spectroscopy including ^{1}H,^{1}H-TOCSY, ^{1}H,^{1}H-COSY, ^{1}H,^{1}H-ROESY, ^{1}H,^{13}C-HSQC, ^{1}H,^{13}C-HMBC, ^{1}H,^{13}C-H2BC and ^{1}H,^{13}C-HSQC-TOCSY experiments. The new structure of the O-specific polysaccharide of *R. japonica* KMM 9513^T containing N-glycosidic bond was established:



1. Introduction

Gram-negative bacteria are an important component of marine ecosystems where they occupy diverse habitats including deep-sea and hydrothermal vents, sea ice as well as open and coastal water areas. The latter attract attention because such ecosystems are known to be of ecological significance, providing favorable conditions for marine biota nursery, biocontrol and bioremediation activities. Sea coastal environments differ in physical parameters (such as sharp changes in temperature and salinity, agitation, terrestrial influx and radiation) from open or deep seawater, implying the existence of indigenous bacteria capable of adaptation to peculiar coastal conditions.

The defining feature of Gram-negative bacteria is the presence of an outer membrane, which comprises the outermost surface of the cell envelope and is in regular contact with the surrounding environment. Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria, therefore, it is plausible that many of the functional changes induced by the harsh habitats can target LPS structure. LPS is unique to Gram-negative bacteria as it plays a key role as an elicitor of innate immune responses, ranging from localized inflammation to disseminated sepsis.[1] Recent studies have shown that some LPS of marine Gram-negative bacteria have a weak toxicity compared to other Gram-negative bacteria and can be considered as potential active substances in the development of new drugs to prevent septic shock.[2,3]

LPS can be classified as smooth (S-form) or rough (R-form) depending on their structural characteristics. Both types contain lipid A, covalently linked to an oligosaccharidic region core. Only in the S-form LPS, the core region is substituted by an O-specific polysaccharide (OPS) portion. The chemical structure of the carbohydrate moiety of LPSs of marine Gram-negative bacteria is highly diverse and includes an ever-extending number of rare and unusual monosaccharides and non-carbohydrate substituents.[4] At present, considerable attention is being given to the elucidation of the chemical structure of LPSs of Gram-negative bacteria from marine environment.

LPS structures of bacteria genus *Rheinheimera* have not been studied intensively. Recently, we have established the structure of the OPS of *R. pacifica* KMM 1406^T isolated from a seawater sample

* Corresponding author. G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, 100 Let Vladivostoku Prosp., 159, 690022 Vladivostok, Russia. Tel.: +74232 310719; fax: +7 4232 314050.
E-mail address: maxchem@mail.ru (M.S. Kokoulin).

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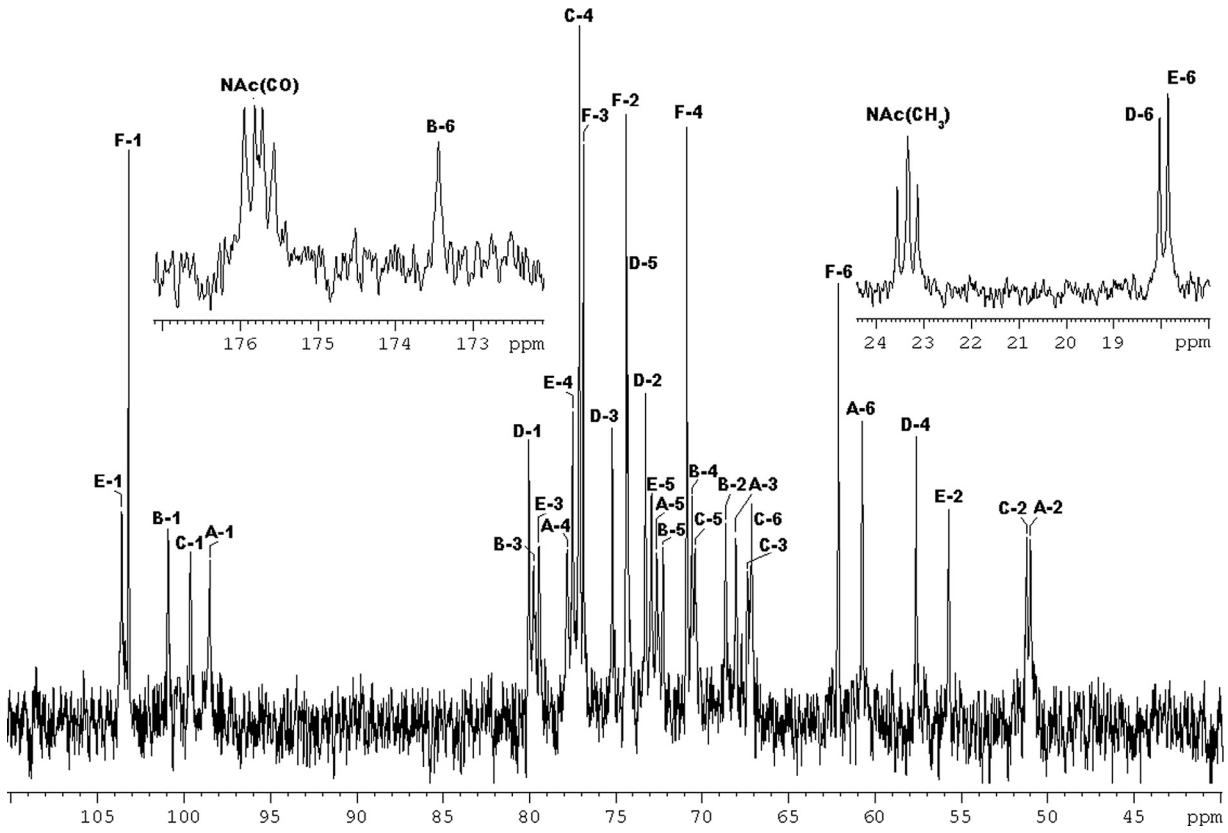

Fig. 1. ^{13}C NMR spectra of the OPS of *R. japonica* KMM 9513^{T}. Arabic numerals refer to the carbons in the sugar residues denoted as described in Table 1. NAc stands for the N-acetyl group.

collected at a depth of 5000 m in the northwest area of the Pacific Ocean.[5] The polysaccharide contains both D and L isomers of 2-acetamido-2-deoxy-galacturonic acid. Strain *R. japonica* KMM 9513^{T} was isolated from a sediment sample collected offshore of the Sea of Japan.[6] The main aim of current study was to establish the structure of OPS of *R. japonica* KMM 9513^{T}.

2. Result and discussion

2.1. Isolation and component analysis of OPS

Wet cells of *R. japonica* KMM 9513^{T} were treated with aqueous saline to remove the minor capsular material, which was not investigated further. The LPS was isolated from dried bacterial cells by the phenol–water procedure.[7] The SDS–PAGE analysis showed that LPS was of smooth type, according to the presence of high molecular weight species in the upper part of the gel (data not shown).

The OPS was obtained by mild acid degradation of the LPS followed by gel chromatography of the carbohydrate moiety. Sugar analysis of the OPS by GC and GC–MS of the acetylated methyl glycosides after full methanolysis of the polysaccharide revealed the presence of 2-amino-2,6-dideoxy-glucose (QuiN), 4-amino-4,6-deoxy-glucose (Qui4N), glucose (Glc), galacturonic acid (GalA) and 2-amino-2-deoxy-galactose (GalN) (the full identification of monosaccharides were completed by NMR spectroscopy, see below).

The D configuration of all sugar residues was determined by GC of the acetylated (S)-2-octyl glycosides[8] using appropriate standards. The D configuration of QuiN, GalA and GalN was also confirmed by analysis of the ^{13}C NMR chemical shifts in the polysaccharide using known regularities in glycosylation effects.[9]

2.2. NMR analysis of OPS

The ^{13}C NMR spectrum of the OPS (Fig. 1) showed, *inter alia*, five signals in anomeric region at δ_C 98.5–103.6 ppm and one more anomeric signal at δ_C 80.1 ppm (see below), four nitrogen-bearing carbons of amino sugars at δ_C 51.0–57.6 ppm, one carboxyl carbon of uronic acid at δ_C 173.4 ppm, two non-substituted and one substituted hydroxymethylene groups at δ_C 60.7, 62.1 and 67.1 ppm, respectively, two methyl groups of 6-deoxy sugars at δ_C 17.9 and 18.0 ppm, four N-acetyl groups at δ_C 23.1–23.6 (CH_3) and 175.6–176.0 ppm (CO). The absence from ^{13}C NMR spectrum of any signals for sugar ring carbons in the region δ_C 82–88 ppm (characteristic for C-4 of furanosides) demonstrated the pyranose form of all sugar residues.

The ^{1}H NMR spectrum of the OPS contained, *inter alia*, nine signals in the low-field region at δ_H 4.46–5.45 ppm (including six signals of anomeric protons), two methyl groups of 6-deoxy sugars at δ_H 1.18 and 1.32 ppm and four methyl groups of N-acetyl groups at δ_H 2.04–2.07 ppm in the high-field region.

The ^{1}H and ^{13}C NMR spectra of the OPS were assigned by using two-dimensional homonuclear ^{1}H,^{1}H-COSY, ^{1}H, ^{1}H-TOCSY and heteronuclear ^{1}H, ^{13}C-HSQC, ^{1}H, ^{13}C-H2BC, ^{1}H, ^{13}C-HSQC-TOCSY and ^{1}H,^{13}C-HMBC experiments (Table 1).

The ^{1}H, ^{1}H-COSY and ^{1}H, ^{1}H-TOCSY spectra (Table 1) revealed isolated proton spin systems for six sugar residues. Two spin systems with *galacto* configuration were identified by H-1/H-2 up to H-4 and H-6/H-5 correlations (units **A** and **C**). One more spin system consisting of five protons belonged to pyranosidic hexuronic acid with *galacto* configuration (unit **B**, followed from the absence of protons at C-6). Two spin systems belonged to pyranosidic 6-deoxy sugars