Carbohydrate Research 357 (2012) 75–82

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Structure of the lipopolysaccharide isolated from the novel species *Uruburuella suis*

Alba Silipo [a,*], Luisa Sturiale [b], Cristina De Castro [a,†], Rosa Lanzetta [a,†], Michelangelo Parrilli [a,†], Domenico Garozzo [b], Antonio Molinaro [a,†]

[a] Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte S. Angelo, Via Cintia 4, 80126 Napoli, Italy
[b] Istituto di Chimica e Tecnologia dei Polimeri—ICTP, CNR, Via P. Gaifami 18, 95126 Catania, Italy

ARTICLE INFO

Article history:
Received 28 March 2012
Received in revised form 10 May 2012
Accepted 13 May 2012
Available online 22 May 2012

Keywords:
Uruburuella suis
Neisseriaceae
Lipooligosaccharide
NMR spectroscopy
MS spectrometry

ABSTRACT

Uruburuella suis is a novel species isolated from lungs and heart of pigs with pneumonia and pericarditis. Phenotypic and phylogenetic evidences showed that it represented a hitherto unknown subline within the family *Neisseriaceae*. In the present work we defined the whole structure of the LPS isolated from *Uruburuella suis*. The structural determination, which was achieved by chemical, spectroscopic and spectrometric approaches, indicates a novel rough type lipopolysaccharide rich in negatively charged groups in the lipid A-inner core region. The elucidation of the structural features of the LPS from *Uruburuella suis* is a first step toward the comprehension of the characteristics of the cell envelope in such new and interesting microorganisms.

1. Introduction

Lipopolysaccharides[1,2] are heat-stable complex amphiphilic macromolecules indispensable for the growth and the survival of Gram-negative bacteria, for the correct assembly of the external membrane and the right positioning of porins. The very low fluidity of the highly ordered structure of the LPS monolayer contributes to form a defensive barrier which helps the bacteria to resist antimicrobial compounds and environmental stresses. LPS are also called endotoxins because they are cell-bound and, once released, can play a key role in the pathogenesis of Gram-negative infections in both plant and animal hosts,[3] in which they trigger the activation of both the innate and adaptative immune systems.

Lipopolysaccharides are composed of three distinct domains: a hydrophilic polysaccharide, the O-specific chain, covalently linked to an oligosaccharide named core which in turn is connected to the glycolipid portion, the lipid A. In the core oligosaccharide, inner and outer regions are usually distinguished: the inner core, proximal to the lipid A, consists of peculiar monose residues like heptoses and an acidic sugar termed Kdo (3-deoxy-D-*manno*-oct-2-ulosonic acid) which is a marker of LPS together with 3-OH fatty acids. The inner core region can be decorated with negatively charged substituents, often present in not stoichiometric amounts, like phosphate (P), pyrophosphate (PP), pyrophosphorylethanolamine (PPEtN), phospho-arabinosamine (PAra4N), and uronic acids (as Gal*p*A). The outer core region is more variable and is usually composed by hexoses. Bacteria can also biosynthesize LPS without O-specific chain and in this case LPS is defined of R-type (rough type) or lipooligosaccharide (LOS) and confers a rough appearance to the colony morphology. The lipid A[4,5] is a vital component of the microbial metabolism and has crucial functions of protection and defense, acting as a strong stimulator of the innate immune system. Lipid A is the less variable portion of LPS and possesses a rather conservative structure usually consisting of a β-(1→6) disaccharide backbone usually consisting of glucosamine, phosphorylated at positions 1 and 4′ and acylated with 3-hydroxy fatty acids at positions 2 and 3 of both GlcNs via amide and ester linkage. These acyl chains, defined primarily because they are directly linked to the sugar backbone, are further acylated to their hydroxy groups by secondary acyl moieties. Kdo, or derivative of this sugar, is linked to the vicinal glucosamine of lipid A backbone at the primary function at position 6′.

Uruburuella suis has been isolated from lungs and heart of pigs with pneumonia and pericarditis.[6] Phenotypic and phylogenetic evidences showed that the new unidentified strain represented a hitherto unknown subline within the family *Neisseriaceae*, a family of Gram-negative, parasitic bacteria including several important human pathogens. The understanding of the molecular mechanisms involved in the inflammatory process requires the knowledge of the structure of the bacteria derived inflammatory

* Corresponding author. Fax: +39 (0)81 674393.
E-mail address: silipo@unina.it (A. Silipo).
† Fax: +39 (0)81 674393.

0008-6215/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carres.2012.05.010

molecules, and among these LPS plays a key role. No structural data are so far available for LPS structure for such a new species. Moreover, the LPS structural characterization is the first step to the design of antimicrobial compounds and therapeutic strategies. These considerations, together with the intrinsic interest derived from its nature of clinical isolate belonging to a new species and genus within the *Neisseriaceae* family, prompted us to start the isolation of the pure LPS molecules to determine the whole structure.

2. Results

2.1. Isolation and compositional analysis of LPS isolated from *Uruburuella suis*

LPS fraction was extracted from and analyzed by SDS electrophoresis. The isolated and purified LPS was found to have the typical run to the bottom of the gel as R-type LPS (LOS).

The compositional analysis of the isolated LOS revealed the presence of L-Rha, D-GlcN, D-Glc, L-*glycero*-D-*manno*-heptose (L,D-Hep), 3-deoxy-D-*manno*-oct-2-ulopyranosonic acid (D-Kdo). Methylation analysis revealed the presence of terminal Rha, 2,3-substituted Rha, 3,4-substituted Hep, 2,3,7-substituted Hep, terminal Hep, 4-substituted Glc, terminal Glc, terminal GlcN, 6-substituted GlcN, 4,5-substituted Kdo, terminal Kdo. Furthermore, at high retention time a heptose disaccharide Hep-(1→7)-Hep was found (Fig. 1).

Fatty acids analysis revealed the presence of (*R*)-3-hydroxytetradecanoic (C14:0(3-OH)) in amide linkage and (*R*)-3-hydroxydodecanoic (C12:0(3-OH)) acid and dodecanoic acid (C12:0) in ester linkage.

In order to achieve the primary structure of the core portion of *Uruburuella suis*, LOS was fully de-acylated to obtain an oligosaccharide fraction **OS** that, by gel-permeation chromatography, was further purified. The compositional analysis of the isolated **OS** confirmed the presence of the sugar residues found in the intact LOS fraction.

2.2. Structural characterization of OS product

The ^{1}H NMR spectrum of **OS** is shown in Figure 2. A combination of homo- and heteronuclear 2D NMR experiments (DQF-COSY, TOCSY, ROESY, ^{1}H–31P, ^{1}H–13C HSQC and ^{1}H–13C HMBC) was executed in order to assign all the spin systems of **OS** and the monosaccharide sequence. In the anomeric region of the ^{1}H NMR spectrum (Fig. 2) 11 anomeric signals were identified (**A–M**, Table 1, Fig. 2a); furthermore, signals resonating at 1.80/2.04 and 1.69/2.09 ppm were identified as the H-3 methylene protons of two Kdo residues, $\mathbf{K_1}$ and $\mathbf{K_2}$. The anomeric configuration of each monosaccharide unit was assigned on the basis of the $^3J_{H1,H2}$ coupling constants obtained by the DQF-COSY and the *intra*-residual NOE contacts observable in the ROESY spectrum, whereas the values of the vicinal $^3J_{H,H}$ coupling constants allowed the identification of the relative configuration of each residue. The proton resonances of all spin systems were obtained by DQF-COSY and TOCSY spectra and were used to assign the carbon resonances in the HSQC spectrum.

Residues **A** and **B** were both identified as composing the disaccharide backbone of the lipid A. The *gluco* configuration was indicated by high $^3J_{H,H}$ ring proton values (all around 8–10 Hz). In details, residue **A** was plainly identified as the α-GlcN of lipid A skeleton probed by the correlation of H-2 with a nitrogen bearing carbon signal at 55.2 ppm, testifying its nature of amino-sugar whose C-6 carbon signal, downfield shifted at 69.5 ppm, was proof of glycosyl substitution at this position (Table 1). The high-field shift of proton resonances of H-2 was indicative of the absence of acylation at these positions. The chemical shifts values of H-1/C-1, the low $^3J_{H1,H2}$ coupling constant and the *intra*-residual NOE contact of H-1 with H-2 were all in agreement with α-anomeric configuration of **A**. Analogously, spin system **B** was identified as GlcN; the HSQC spectrum showed the correlation of H-2 with nitrogen bearing carbon signals; the high-field shift of proton resonances of H-2 was indicative of the absence of acylation. The chemical shifts of H-1/C-1, the $^3J_{H1,H2}$ coupling constant (8.0 Hz)

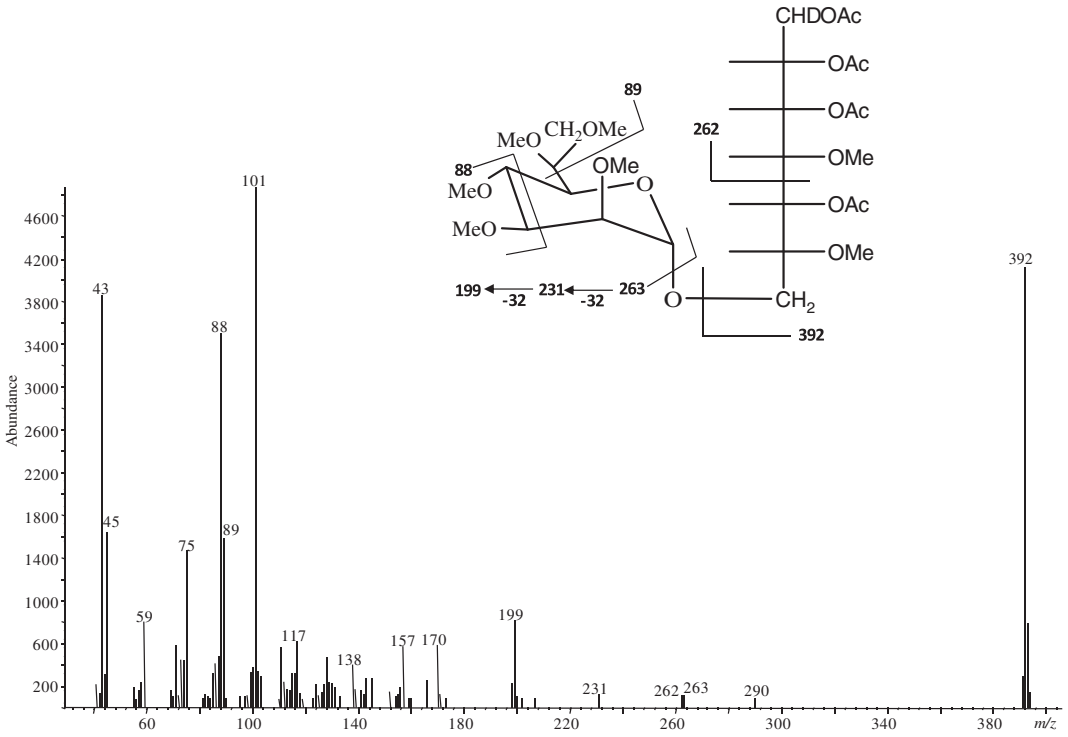

Figure 1. Electron impact mass spectrum of the terminal Hep-(1→7)-Hep disaccharide isolated via methylation analysis; the structure and the main fragments are shown in the inset.