Carbohydrate Research 388 (2014) 73–80

Contents lists available at ScienceDirect

Carbohydrate Research

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

# Structural investigation of the antagonist LPS from the cyanobacterium *Oscillatoria planktothrix* FP1

Sara Carillo, Giuseppina Pieretti, Emiliano Bedini, Michelangelo Parrilli, Rosa Lanzetta, Maria Michela Corsaro *

*Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario di Monte S.Angelo, Via Cintia 4, 80126 Napoli, Italy*

ARTICLE INFO

*Article history:*
Received 11 July 2013
Received in revised form 18 September 2013
Accepted 14 October 2013
Available online 23 October 2013

*Keywords:*
Cyanobacteria
*Oscillatoria planktothrix*
Lipopolysaccharide
Lipid A
Structural determination
Rhamnan

ABSTRACT

Cyanobacteria are aquatic and photosynthetic microorganisms, which contribute up to 30% of the yearly oxygen production on the earth. They have the distinction of being the oldest known fossils, more than 3.5 billion years old, and are one of the largest and most important groups of bacteria on earth. Cyanobacteria are an emerging source of potentially pharmacologically active products and, among these, there are the lipopolysaccharides. Despite their significant and well documented activity, very little is known about the cyanobacteria lipopolysaccharides (LPS) structure. The aim of this work is to investigate the structure of the highly TLR4-antagonist lipopolysaccharide from the cyanobacterium *Oscillatoria planktothrix* FP1.

The LPS was purified and analysed by means of chemical analysis and $^{1}H$ and $^{13}C$ NMR spectroscopy. The LPS was then degraded by Smith degradation, HF and acetic acid hydrolyses. All the obtained products were investigated in detail by chemical analysis, NMR spectroscopy and by mass spectrometry. The LPS consists of a high molecular mass and very complex molecule lacking Kdo and heptose residues, where the polysaccharide chain is mainly constituted by a backbone of 3-substituted α-L-rhamnose units. The core region is rich in galacturonic acid and mannose residues.

Moreover a glycolipid portion, similar to Gram-negative lipid A, was identified. This was built up of a non phosphorylated (1′→6) linked glucosamine disaccharide, acylated with 3-hydroxylated fatty acids. In particular 3-hydroxypentadecanoic and 3-hydroxyesadecanoic acids were found, together with esadecanoic and tetradecanoic ones. Finally the presence of a galacturonic acid residue at 6-position of the distal glucosamine in place of the Kdo residue is suggested.



## 1. Introduction

Cyanobacteria arguably constitute the most important group of organisms ever found on our planet. The most interesting peculiarity of these microorganisms is that they have virtually not changed evolutionarily over the last 2 billion years, providing a unique opportunity to understand the paleobiological features of the ancient biosphere and to retrieve the geochemical and biogeochemical processes that occurred in the Precambrian.[1,2]

Cyanobacteria, also called blue-green algae, are very important members of the global ecosystem, and contribute up to 30% of the yearly oxygen production on the hearth. In order to produce molecular oxygen these photosynthetic microorganisms, as well as plants and green algae, use two photosystems (PSI and PSII), which contain pigmented proteins that give the characteristic colour to the bloom. Cyanobacteria can survive in almost every habitat such as from oceans to fresh water, soil to bare rocks, deserts to ice shelves, hot springs to Arctic and Antarctic lakes as well as in the form of endosymbionts in plants, lichens and several protists.[3]

Cyanobacteria are a source of novel bioactive natural compounds that may be exploited biotechnologically for the benefit of human beings. Several cyanobacterial secondary metabolites have been shown to have significant pharmaceutical potential ranging from antimicrobial, anticancer, antiviral to enzyme inhibiting activities important for biomedical research. A number of metabolites from cyanobacteria may be employed ecologically as allelochemicals. Commercial development and application of these compounds as biocides (such as algaecides, herbicides and insecticides) have been predicted to be more beneficial in comparison to synthetic biocides from an environmental point of view.

In contrast many cyanobacterial metabolites are reported to be extremely toxic for animals and human. Many cyanotoxins are neurotoxins, affect the liver and other organs, and are potent tumour promoters.[4–6] Cyanobacterial LPS are also reported to have

* Corresponding author. Tel.: +39 081 674 149; fax: +39 081 674 143.
*E-mail address:* corsaro@unina.it (M.M. Corsaro).

http://dx.doi.org/10.1016/j.carres.2013.10.008
0008-6215/

a range of pathological effects in humans, from gastro-intestinal illness, cutaneous signs and symptoms, allergy, respiratory disease, headache and fever.[7–10]

Recently, an LPS fraction derived from the cyanobacterium *Oscillatoria planktothrix* FP1 was reported to act as a selective TLR4–MD-2 receptor antagonist.[11] This molecule is also a potent antagonist of *Neisseria meningitidis* lipopolysaccharide, inhibiting cytokine production in an in vitro model of septicemia[12] and augmenting the anti-inflammatory response when combined with benzylpenicillin.[13] In addition the *O. planktothrix* LPS fraction, acting as antagonist for the TLR4, has allowed the elucidation of TLR4/MD2 mediated pathologies, such as epilepsy seizures,[14] meconium aspiration syndrome[15] and neuroinflammation.[16]

The lipopolysaccharides derived from cyanobacteria and from Gram-negative bacteria differ in their chemical characteristics. In particular only few authors reported for cyanobacteria LPS the presence of a low amount of Kdo, found ubiquitously in Gram-negative bacterial LPS. In addition, the finding of heptose, usually present in Gram-negative LPS, has never been described for this kind of cell wall molecules.[17–19]

Despite the high potential application in the pharmaceutical field very few papers describe the difference between the Gram-negative bacteria and cyanobacteria lipopolysaccharide structure. Up to now only the LPS structure from the marine cyanobacterium *Synechococcus* has been elucidated.[20] In this paper we report the purification and chemical characterisation of the highly TLR4-antagonist LPS from *O. plankthotrix* FP1.

## 2. Results

### 2.1. Isolation and purification of *O. planktothrix* FP1 LPS

The cells of the freshwater cyanobacterium *Oscillatoria planktothrix* FP1 were extracted as reported previously.[21]

The $^1$H NMR of the crude extract (Fig. 1) showed the presence of several signals widely distributed in the entire proton spectrum, indicating the presence of different kind of molecules in the sample. The most intense signals fell into the range $\delta$ 5.5–4.5 and 4.3–3.0, suggesting that the main component was polysaccharide material. Moreover the signal at $\delta$ 4.9 was particularly intense, thus suggesting the presence of a homopolysaccharide. In particular, the presence of a signal at $\delta$ 1.3 suggested that the main monosaccharide unit was a 6-deoxy sugar.

The glycosyl investigation of the crude extract was performed by GC–MS analysis of both acetylated methyl glycosides and acetylated alditols. Both methods gave the same results, indicating the presence of L-rhamnose, D-xylose, D-glucose, D-galactose, D-mannose, D-galacturonic acid and D-glucosamine. In addition, neither heptose nor Kdo were revealed in these analyses. To check if these residues were not revealed because they were phosphorylated, a hydrolysis with 48% aqueous HF was performed to remove the potentially present phosphates.[22] Again no traces of heptose and Kdo were found.

The fatty acids composition demonstrated the presence of 3-hydroxylated fatty acids. In particular, 3-hydroxypentadecanoic and 3-hydroxyesadecanoic acids were found, together with esadecanoic and tetradecanoic ones. The 8% DOC–PAGE of the sample showed only two bands (Fig. 2, lane 1), which indicated two different molecular mass LPS species. When a more concentrated sample was loaded on the gel, the typical ladder pattern of LPS appeared (Fig. 2, lane 2). In addition, the low polyacrylamide percent necessary to perform this experiment indicated a high molecular mass for the LPS of *O. planktothrix*.

Many attempts to purify the LPS fraction were performed. More specifically, several gel filtration media with different exclusion sizes of molecular masses were used, even in presence of detergents and metal ion chelators, but none of them gave satisfying results. In addition, a preparative SDS gel electrophoresis was

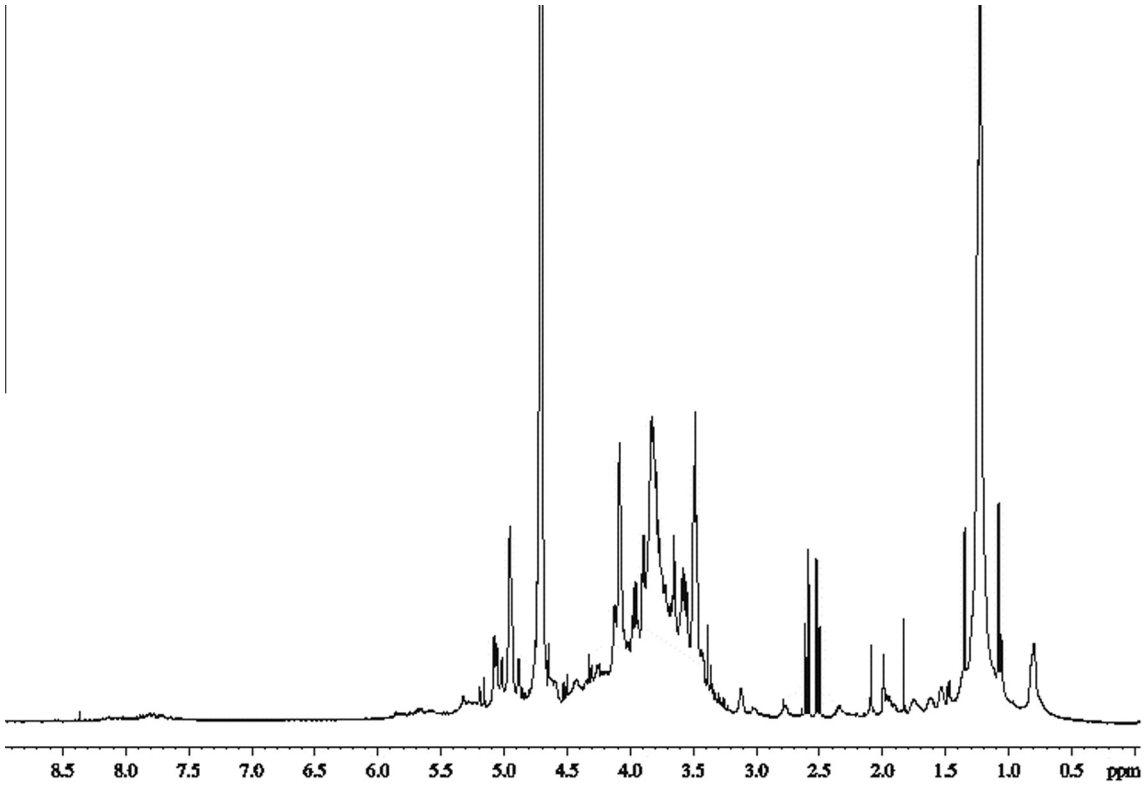


**Figure 1.** $^1$H NMR spectrum of the crude extract from *O. planktothrix* FP1 cyanobacterium. The spectrum was recorded in $D_2O$ at 298 K at 600 MHz.