Feasibility, via a new interface, of mass spectrometry of lipopolysaccharides separated in polyacrylamide gels

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REPORT

ABSTRACT

For the first time, it was demonstrated the feasibility of analyzing by mass spectrometry (MS) Gram-negative bacteria lipopolysaccharides (LPS) electrophoretically separated with very high resolution in polyacrylamide gels (slab-PAGE). Particularly, the direct impact of this result in four aspects of LPS analysis is demonstrated: spectrometric analysis of the microisolated species, sample preparation for MS, bidimensional separation and heterogeneity of LPS, and the structural characterization of lipopolysaccharides of medical (for example, LPS from strain RM.118 [Rd-] of Haemophilus influenzae) and biological interest (for example, LPS from strain HMK of Vibrio fischeri). To build an interface between high-resolution slab-PAGE separation and MS analysis, it was necessary to develop a new method, with unique characteristics, that made possible the microisolation of LPS species: high-efficiency recovery, high precision, no modification of the microisolated material and general applicability (applicable to LPS of different nature and sources). In this way, the bases for the fine structural analysis of the highly complex nature, in molecular species, of bacterial LPS are laid. The experiments showing these results were published in five papers in the leading journals of analytical biochemistry.

Introduction

Lipopolysaccharides (LPS) are a major component of the outer membrane of Gram-negative bacteria. From the medical point of view, the study of these biomolecules is very important due mainly to: (i) LPS play a key mediating role in infectious processes, sepsis and septic shock induced by Gram-negative bacteria in humans, that is associated with a high rate of morbility and mortality, (ii) LPS are primary targets of the immune response against Gram-negative bacteria in humans and they are important antigens for developing vaccines against those pathogens; and (iii) because of their high immunostimulating activity, LPS and their synthetic derivatives are powerful adjuvants being developed for vaccination of humans.

An essential problem faced in LPS research at present, in studies of its structure and of its biological function, is the high heterogeneity of these macromolecules and the lack of methods that allow the complete separation of their individual molecular species for subsequent analysis and use. In this research, a group of new methods that enabled the microisolation of intact LPS molecular species and their subsequent structural characterization by mass spectrometry were found.

Results

The main contribution of this research is that, for the first time, it is achieved the structural analysis, by mass spectrometry of LPS species that have been separated with very high resolution, by polyacrylamide gel electrophoresis (slab-PAGE) (see a typical result in figure 1). It laid the foundations for the fine structural analysis of the highly complex nature, in molecular species, of bacterial LPS.

This result particularly had a direct impact on four directions related to LPS analysis.

Spectrometric analysis

It was demonstrated that, by using electrospray ionization mass spectrometry (ESI-MS), it is possible to estimate the relative abundance of the molecular species contained in each LPS fraction separated by slab-PAGE, and the sequence of their dephosphorylated and permethylated oligosaccharides. [1].

It was found that the direct analysis by matrix-assisted laser desorption/ionization-based mass spectrometry (MALDI-TOF-MS) allows determining the relative abundance of the molecular species contained in each LPS fraction separated by slab-PAGE. Moreover, the precise molecular mass of the *O*-desacylated LPS or that of their intact oligosaccharides, and the presence or absence in them of different phosphorylation states of lipid A can be determined [2].

Sample preparation for mass spectrometry

It was demonstrated that it is possible to improve the sensitivity of LPS analyses by ESI-MS and MALDI-TOF-MS, by using the microisolated LPS species. The mass spectra of the microisolated species are much simpler, have a higher intensity and a better signal-to-noise ratio [1, 2].

LPS separation and heterogeneity

The separation in a second dimension by mass spectrometry of the microisolated species conclusively proved that LPS fractions separated by slab-PAGE are not homogeneous. The fractions are not really comprised of one, but several different molecular species [1, 2].

Analysis of LPS of interest

The higher sensitivity of the analysis allowed detecting and identifying, by ESI-MS, minor LPS molecular species from Haemophilus influenzae strain

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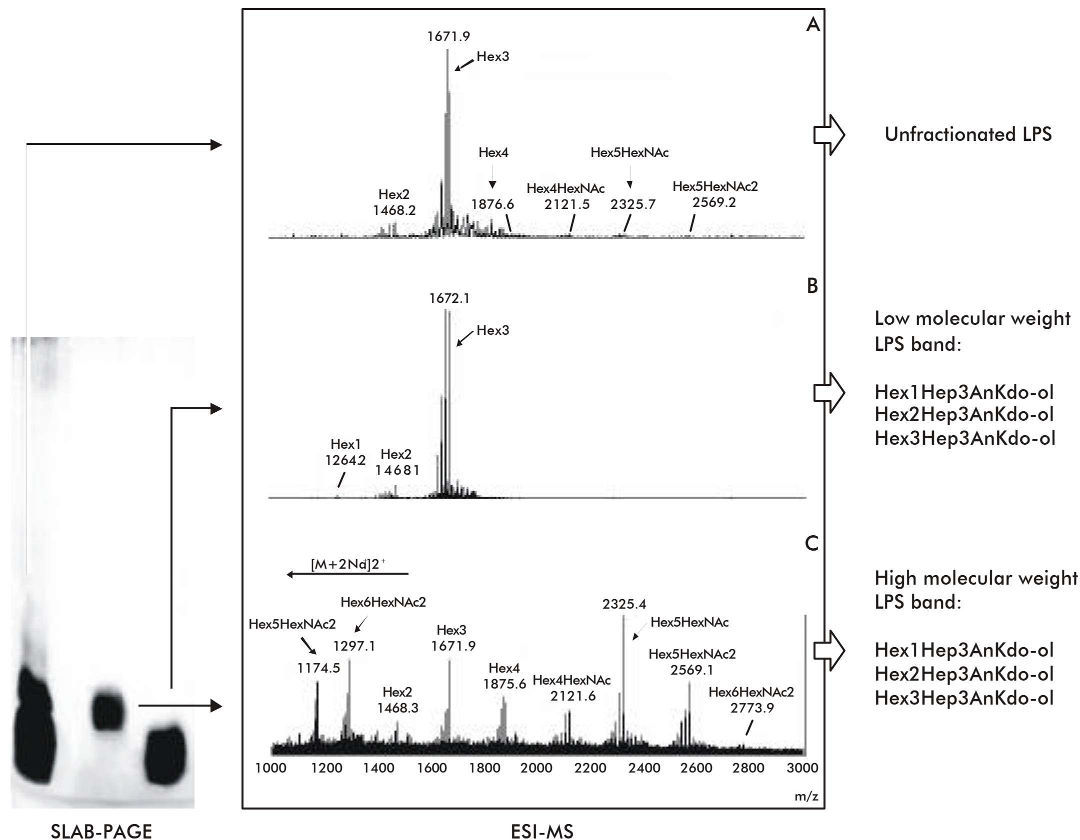

Figure 1. Analysis by ESI-MS of the lipooligosaccharides from H. influenzae strain Rd separated by slab-PAGE (1). Observe in the right column of the figure, the molecular species contained in each slab-PAGE separated band.

Rd, which were either isomeric or of a higher molecular weight, which had never been described previously in the literature [1].

For the first time, the high-resolution separation, detection and precise determination of the molecular mass of LPS molecular species from Vibrio fischeri strain HMK is performed. The *O*-desacylayted LPS mass spectrum showed that this biomolecule consists of 6 molecular species. The species with the highest molecular weight, with the higher relative abundance, showed an m/z of 3767.1 and 3890.1, while the species with the lowest molecular weight had an m/z 2522.5, 2645.4, 2725.7, and 2848.7. Those LPS species differed not only in the molecular mass of their oligosaccharides, but also in the phosphorylation state of their lipid A (biphosphorylated or biphosphorylated with a phosphoethanolamine substitute) [2].

To achieve an interface between slab-PAGE high-resolution separation and mass spectrometry analysis, it was necessary to find and use a method that allows the microisolation of LPS species. In this subject, the contributions are as follows:

For the first time, single species of LPS were recovered from polyacrylamide gels. The characteristics of the new recovery method were unique: high efficiency, precision and general applicability (applicable to LPS of different nature and sources) [3, 4].

Through biochemical assays (determination of Limulus amebocyte lysate activity, induction of tumoral necrosis factor-alpha and generation of anti-LPS polyclonal antibodies) [3-5] and mass spectrometry [1, 2], it was demonstrated that the microisolated LPS species are functional and structurally intact.

Perspectives

The availability of the new methodology established in this work for the isolation and characterization, for the first time, of individual species of LPS will be very useful for understanding the structure and the biological activity of complex LPS mixtures. Moreover, it will allow the study, at the molecular level, of the interaction of LPS with animals and humans.

This knowledge can offer some new useful strategies for (i) the diagnosis, treatment and prevention of Gram-negative bacterial infections and septic shock, (ii) the rational design of vaccines that use LPS as antigen and (iii) the rational design of new vaccine formulations that use LPS as an adjuvant.

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