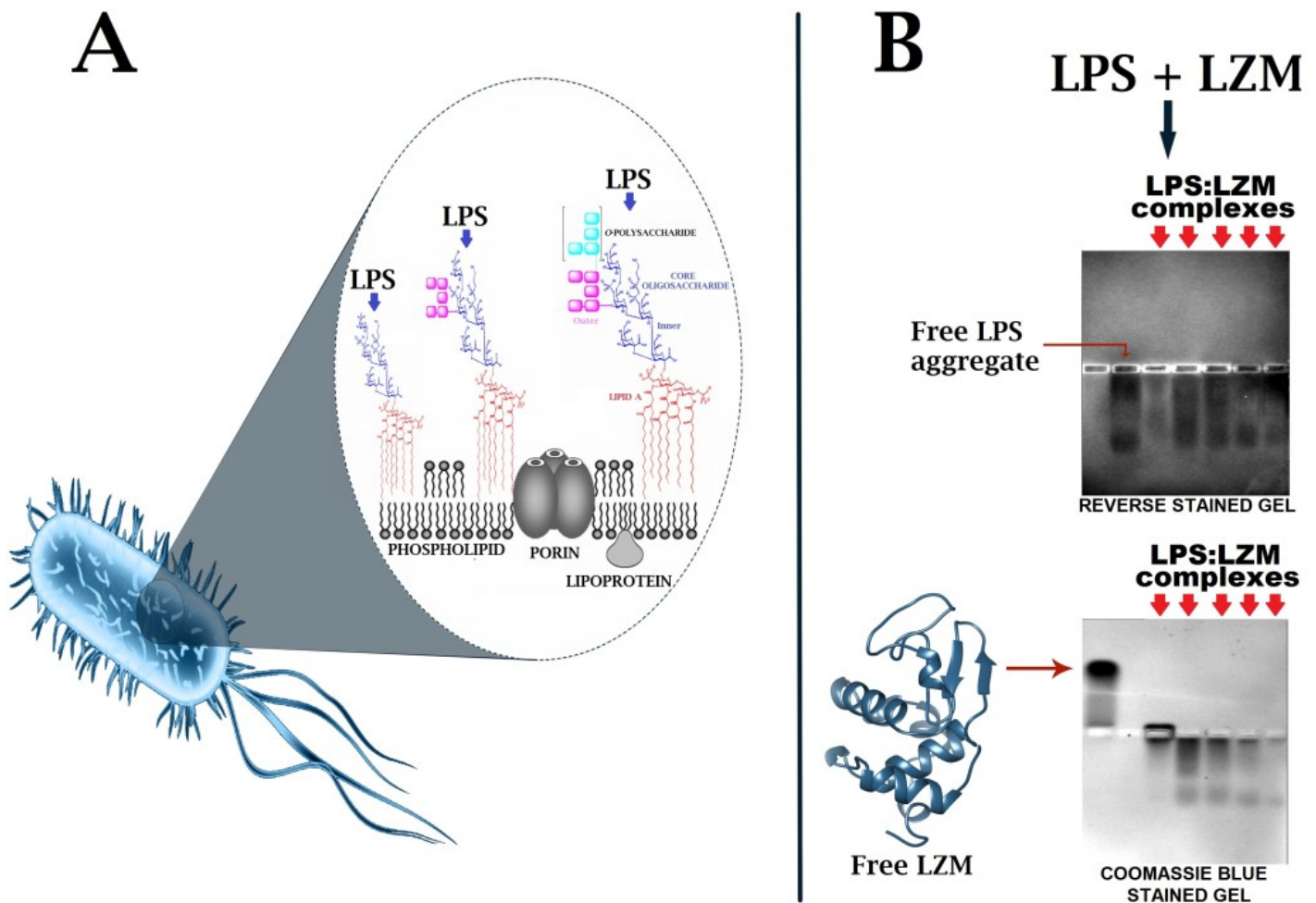


Molecular giants detected in agarose gels

Lipopolysaccharides (LPS), which are the major biological macromolecules of the surface from Gram-negative bacteria (fig. A), are of huge interest in medicine. Large amounts of LPS in the bloodstream cause diverse pathophysiological effects, causing thousands of deaths annually worldwide. Ironically, LPS are essential for the survival of Gram-negative bacteria by playing a vital role in a variety of recognition processes and by providing a permeation barrier for harmful substances.



Interaction of proteins with LPS. There is interest in finding proteins or peptides that protect from sepsis by strongly binding LPS, breaking its aggregates, and suppressing LPS-induced pro-inflammatory responses. In line with this, varieties of cationic proteins and peptides (e.g., lysozyme [LZM]) which form complexes with lipopolysaccharide have been discovered. We found that LZM-LPS complexes can be detected using native agarose gel electrophoresis (NAGE) (fig. B). Specifically, our results validate that lysozyme can disrupt LPS aggregates and that concentration of either LPS or LZM has a significant effect on the amount of LPS disaggregated and the amount

of LZM-LPS complexes formed. In a wider scope, the results of this study indicate that three different complementary criteria may be considered to infer a strong interaction and stable binding of cationic proteins/peptides to LPS. These criteria are: (i) any change in NAGE-separated LPS pattern (e.g., increased electrophoretic mobility), (ii) shift in electrophoretic mobility of cationic proteins/peptides, and (iii) specific Coomassie blue staining of charged protein/peptide-LPS complexes.

Identification of LPS aggregates using a novel two-dimensional LPS separation technique. LPS aggregates are separated in the first dimension by preparative NAGE. Next, LPS aggregates are visualized by means of inducing a clear, transparent pattern, contrasted against an opaque background (e.g., gel in the upper part of fig. B). The agarose gel (1D-NAGE) is cut into a few segments, processed for LPS passive elution, and the resulting samples are electrophoresed in a second-dimension analytical sodium dodecyl sulfate (SDS)-polyacrylamide gel (2D-PAGE). The LPS molecules fall into bands of discreet sizes, which are then stained with silver to make them visible to imaging techniques and reveal the composition of each region of the agarose gel. As previously shown, a wealth of information can be obtained using slab-PAGE alone or in combination with other characterization techniques (e.g., mass spectrometry, bioassays). This includes purity of LPSs, glycoform-specific composition, molecular weight of components and primary (e.g., oligosaccharide) sequences, minor structural changes due to mutations in genes responsible for LPS biosynthesis, and cell-based biological activities.

On the micropurification of LPS aggregates. This is important to allow the experimenter to get more information out of the molecular species under investigation. Although the diffusion of LPS aggregates out of the gel matrix is extremely difficult due to the amphipathic nature and little solubility of LPS in water, the amounts of eluted and recovered LPS is sufficient to allow determination and quantitation of the biological activities of LPS aggregates. In fact, concentrations of LPS ranging from a few nanograms per milliliter to a few micrograms per milliliter are often used to evaluate LPS-induced cell responses. It is worth noting that purification based on NAGE allows the effective removal of proteins, nucleic acids, polysaccharides, lipids, or small ionic compounds, which are common contaminants in LPS preparations.

We believe the present methodology may become a useful tool offering the opportunity of (i) screening a large number of proteins or host defense peptides from different natural sources and (ii) getting insight into the interaction between lipopolysaccharide-binding proteins/peptides and LPS aggregates. In addition, screened proteins or peptides may have interest as candidates for further biological evaluations and development into therapeutically valuable anti-sepsis or anti-infective agents.

Publication

[Lipopolysaccharide aggregates in native agarose gels detected by reversible negative staining with imidazole and zinc salts.](#)

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