Budapest University of Technology and Economics
Department of Physical Chemistry

Edit Urbán

The effects of *Salmonella minnesota* R595 lipopolisaccharide on model membranes

Ph.D. theses

Supervisor: Attila Bóta Ph.D.D. (associate professor)
BUTE Department of Physical Chemistry

Consultant: Dr. Béla Kocsis Ph.D. (associate professor)
University of Pécs, Institute of Medical Microbiology and Immunology

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1. Motivations

Lipopolysaccharides (LPS) are major lipid components of the outer membrane of Gram-negative bacteria, located only in their outer leaflets. Its hydrophobic lipid-A segment is responsible for a variety of biological functions. The LPS may be released from the bacteria into the surrounding medium (e.g. blood circulation) and their aggregates have been shown to have relevant dose-dependant biological effects in the human host organism. At their low concentration the LPS molecules may have beneficial effect on the mechanisms of the complex living systems, but the presence of LPS in high concentrations causes pathophysiological alterations and may lead to sepsis, septic shock, and consequently to multi-organ failure. The LPS can modify the immune response either by binding to specific membrane receptors or by interacting with the host cell membrane in a non-specific way. These toxic effects are supposed to originate from their intercalation into the phospholipid bilayers of membranes.

In my work the effects of *Salmonella minnesota* R595 LPS on the phase behaviour of human and bacterial model membranes (liposomes) consisting of different phospholipids were investigated (the main features of liposomes are shown in

the Figure). This kind of LPS was used because of its reproducible preparation and its well characterised liotropic properties.

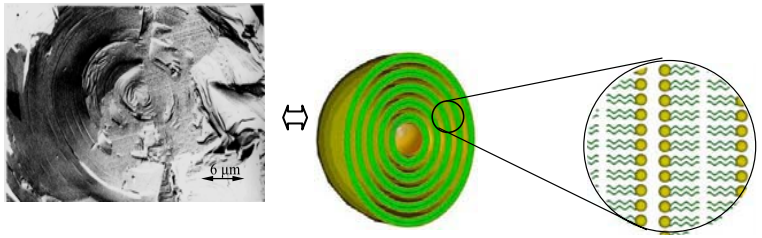

Figure: The electron micrograph of a liposome and its idealised structure

The biological membranes are able to control their phospholipid content to achieve the appropriate ratio of bilayer lipids related to the non-bilayer ones. This feature plays important role in the membrane physiology whereas the local accumulation of non-bilayer lipids forms metastable structures in the membrane which are responsible for specific transport mechanisms and fusion processes.

The goal of the work was to reveal the effect of a well characterised LPS on human and bacterial model membrane systems containing of different biological relevant lipids.

The fully hydrated dipalmitoylphosphatidylcholine (DPPC) vesicles as a model system for human cytoplasmic cell membranes and the fully hydrated mixtures of 80 mol % dipalmitoylphosphatidylethanolamine (DPPE) and 20 mol % other lipid component (the bilayer dipalmitoylphosphatidylglycerol (DPPG), the non-bilayer dipalmitoylphosphatidycacid (DPPA), the non-bilayer dipalmitoylglycerol (DPG)) as bacterial model membranes were investigated. In these systems the direct damaging effects on the layer structure and the formation of other liotropic structures induced by the presence of LPS were studied from physico-chemical point of view.

2. Applied methods

The concentration dependent effects of LPS on the model membranes were investigated by different methods.

Differential scanning calorimetry (DSC) was used for the elucidation of the thermotropic behaviour (the phase transition temperature, change in enthalpy).

The small angle X-ray scattering (SAXS) was applied to determinate the parameters of the layer structure and to identify the 2- and 3-dimensional formation. The simultaneous detection of the parameter of subcells with the changes occurred in the colloidal range was executed by using a small- and wide-angle X-ray scattering (WAXS) apparatus.

The morphology of the samples was studied by using freeze-fracture technique combined by electron microscopy giving possibilities for the direct visualisation of the appearance of the different structural formations, typically in the size range between 0.1 and 10 μm.

3. Ph.D. theses

1. In the presence of the *Salmonella minnesota* R595 lipopolisaccharide (LPS) the well ordered multilamellar arrangement of the fully hydrated dipalmitoylphosphatidylcholine (DPPC) vesicles was strongly distorted and the phase transition between the gel and rippled gel (i.e. the pretransition) was abolished up to the molar ratio of 1/10 LPS/DPPC while the main transition was not significantly affected. At the equimolar mixture of LPS and DPPC, a complex structure, the coexistence of the LPS induced cubic and the LPS preferred lamellar structures, was formed. At this high LPS concentration the thermotropic behaviours of the system was significantly affected, the change in enthalpy between the states formed at 26 and 45 °C, was drastically decreased. Therefore this phase transition exhibits a weak first order character and the structures formed at the two characteristic temperature domains (i.e. above the phase transition and below it) became to be similar.

2. In the presence of LPS in the fully hydrated dipalmitoylphosphatidylethanolamine (DPPE)-dipalmitoylphosphatidylglycerol (DPPG)/water system at 1/4 DPPE/DPPG molar ratio, a phase separation was induced. This phenomenon was observed up to of 1/10 LPS/DPPE-DPPG molar ratio. During the phase separation two different - in DPPE enriched and depleted - domains was formed and in these domains cubic and lamellar structures were present. Increasing the temperature, firstly the chain melting of the lamellar phase, than the destruction of the cubic phase occurred.

3. At the equimolar ratio of LPS related to lipids (LPS/DPPE–DPPG) the cubic phase is typical for the system. The formation of cubic structures can be explained by the effect of the molecular geometry of the constituent; the self organisation of the amphiphilics is governed by non-bilayer properties of the conical shaped LPS dominantly, and the effect of the bilayer lipids (DPPE, DPPG) becomes insignificant. Although, the concomitant phenomenon of the formation of the cubic structures is the competition between the bilayer and non-bilayer arrangement, therefore the form of this cubic phase is not specified and its space-group can not be observed.

4. In the fully hydrated DPPE-DPPA system (1/4 DPPE/DPPA mol/mol)) the cubic phase was induced by the presence of LPS in the temperature range corresponding to the gel phase of the pure lipid/water system. At the 1/10 LPS/lipid molar ratio the local presence of the cubic phase was observed while at higher LPS concentration (1/1 LPS/lipid molar ratio) the cubic phase is well determined, it belongs to the Q^{224} space group. The pregnant cubic formation can be explained by the non-bilayer properties of the LPS and the DPPA.

5. The fully hydrated DPPE/DPG (8/2 mol/mol) system shows special phase behaviour; the typical multilamellar arrangement of the gel phase was transformed during the chain melting into the mixture of the inverted hexagonal and the cubic structures. This special feature can be explained by the change in the molecular geometry of the lipids from the cylindrical into the conical shape.

6. By the low presence of LPS (1/100 LPS/DPPE-DPG molar ratio) in the fully hydrated DPPE-DPG/water system a higher positional correlation in the layer structure was induced in the gel phase. The structural behaviour was affected only at higher LPS concentration: at 1/10 LPS/lipid molar ratio of the

distortion of the lamellae and at the equimolar mixture of LPS and lipids a cubic structure appears. Above the chain melting the ratio of the cubic structure related to the hexagonal one was changed and at the equimolar ratio of LPS and lipids a complex and at the same time amorphous state was formed.

7. Above the chain melting the bacterial model membranes containing DPPE form amorphous states which do not depend on the other lipid species (DPPG, DPPA, DPG). Their similar morphologies originate from the character of the dominant LPS. In the complex structures destructed lamellar and cubic phases coexist. Moreover, these complex structures are amorphous which is a consequence of the higher mobility of the molecules in this temperature domain.

4. Publications

1. Urbán E., Bóta A., Kocsis B.: Effect of *Salmonella minnesota* R595 LPS on the dipalmitoylphosphatidyl-ethanolamine(DPPE)-dipalmitoylglycerol (DPG)-water model membrane system, Chem. Phys. Lipids (in press,2006).
2. Urbán E., Bóta A., Kocsis B.: Non-bilayer formation in the DPPE-DPPG vesicle system induced by deep rough mutant of *Salmonella minnesota* R595 lipopolysaccharide, J. of Colloids & Surfaces B 48 (2006) 106-111.
3. Urbán E., Bóta A., Kocsis B., Lohner K.: Distortion of the lamellar arrangement of phospholipids by deep rough mutant lipopolysaccharide from *Salmonella minnesota* (R595), J. Therm. Anal. Cal. 82 (2005) 463-469.
4. Urbán E., Bóta A., Klumpp E., Csiszár Á.: Vesicle system for mimicking of effects of toxic 2,4-dichlrorophenol on cell-membranes, J. of Colloids & Surfaces A: Physicochem. Eng. Aspects 230 (2004) 201-206.

5. Urbán E., Bóta A.: DPPE (dipalmitoil-foszfatidil-etanolamin)/víz alapú liposzómák előállítása és szerkezetének tanulmányozása, Olaj Szappan Kozmetika 52. évf. (2003) I.: 6-10.