**Introduction**

Lipopolysaccharides (LPSs, endotoxins) are complex lipid-linked carbohydrates which are found in the outer membranes of Gram-negative bacteria (Fig. 1 and 2). These negatively charged molecules are usually composed of a polymeric carbohydrate (O-antigen), a short oligosaccharide (R-core) and a fatty-acylated region (lipid A). Through its action on macrophages, LPS can trigger responses that are protective or injurious to the host.

In order to get more insight into the mechanisms of blood purification and toxic action, FTIR ATR spectroscopy was used to investigate in situ the interactions of LPS from Pseudomonas aeruginosa (serotype 10) with a hydrophilic and a hydrophobic surface, with a positively charged crosslinked polymer and a positively charged bilayer.

**Materials and Methods**

In each FTIR ATR experiment, an aqueous buffer solution of LPS from Pseudomonas aeruginosa (serotype 10) (Sigma, L-7018) was pumped into a flowthrough cuvette and contacted with the surface of interest. Furthermore, a chopper behind the cuvette split the IR-beam and allowed one to measure sample and reference spectra quasi-simultaneously (single beam sample reference (SSBR) technique).

First, the interaction of LPS with a plasma-cleaned, hydrophilic germanium (Ge) surface was investigated. A trapezoidal Ge plate was always used as internal reference element and represented the carrier for all other surfaces. Second, with the help of a film balance, a monolayer consisting of dipalmitoyl phosphatidic acid (DPPA) (Fig. 2) was transferred to the Ge plate creating a hydrophilic surface (Fig. 3, step I). Third, the interaction of LPS was supported positively charged lipid bilayer was monitored. The bilayer consisting of DPPA as inner and a 1:1:mixture of dimyristoyl phosphatidylcholine (POPC) and Hexadecylpyridinium (HDPyr) as outer leaflet was prepared using the Langmuir Blodgett (LB) Vesicle Method (schematically shown in Fig. 3, step II-III).

Finally, a positively charged crosslinked polymer consisting of γ-amino propyl triethoxysilane (ATS) was prepared by polymerization of ATES on Ge (Fig. 4). This method of surface activation gives few adjacent monolayers of silane across the carrier surface.

**Results and Discussion**

Whereas the hydrophilic LPS exhibits only weak adsorption to the Ge plate and the DPPA monolayer, it binds strongly to the ATS polymer (Fig. 7). However, in each case considerable and similar amounts of adsorbed amide I and II peaks are found. This can be explained by contamination of the LPS with protein which is able to bind quite unspecifically to any surface. After washing with buffer, only the hydrophilic Ge and, to a greater extent, the positively charged ATS-polymer are able to retain some LPS molecules which possess phosphate and carboxyl groups for electrostatic interaction. The main effect of the endotoxin on DPPA is the solubilization of DPPA molecules. Moreover, LPS causes the adsorption of POPC vesicles on the ATS polymer exhibiting a strong detergent-like effect (data not shown).

Furthermore, the endotoxin fraction removes predominantly the positively charged HDPyr from the DPPA/(POPC:HDPyr) bilayer. With the help of the SSBR-technique, this effect can be seen even at the lowest concentration of LPS (1 µg/ml) (Fig. 5). After reaching our highest concentration (1000 µg/ml), almost no amount of the positively charged HDPyr is found in the membrane that is now consisting of pure POPC. Fig. 6 shows that the spectra of the remaining bilayer resemble closely the spectra of pure POPC. The absorption of the CO ester peak at 1738 cm⁻¹ is significantly reduced while the absorption of the COH ester peak at 1715 cm⁻¹ is almost the same (data not shown).

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