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Interaction of four-antennary oligoglycines and lipopolysaccharides in aqueous media

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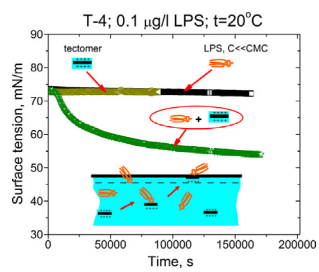
HIGHLIGHTS

- Significant decrease in surface tension values of mixed LPS/T-4 systems is recorded.
- LPS interact with hydrophilic T-4 tectomers and form amphiphilic complexes.
- Foam film study validates formation of amphiphilic LPS/tectomer complexes.
- The study promotes T-4 for registration and efficient LPS capture in aqueous media.

GRAPHICAL ABSTRACT



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ABSTRACT

Aqueous solutions containing both four-tailed oligoglycine $[Gly_7{-}NHCH_2{-}]_4C$ and lipopolysaccharides (Ra-LPS from *Escherichia coli* EH100) are studied in the present work. The experimental procedure comprises examination of the properties of interfacial layers at the solution/air boundary and the drainage characteristics of microscopic foam films. Significant decrease of the dynamic and equilibrium surface tension values is established upon raising the content of the oligoglycine in the mixture. This effect is related to the plausible formation of amphiphilic LPS/tectomer complexes. The drainage properties of the films go in line with the notion for the possible onset of amphiphilic entities in the solutions. The reported data may be regarded as experimental evidences for the existence of surface active LPS/tectomer complexes in the investigated systems. The results suggest that aqueous solutions of four-antennary oligoglycines could be used for registration of trace quantities and efficient capture of LPS in aqueous media.

1. Introduction

The lipopolysaccharides (LPS) are a major component of the outer surface layer of the cell walls of Gram-negative bacteria like human pathogens *Escherichia coli* (*E. coli*). They determine the surface properties of these bacteria at a molecular level and are therefore, very important for the interaction with host cells or other interfaces [1,2]. Besides, it is well-known that LPS play a key role in the bacterial resistance to hydrophobic antibiotics [3] and antimicrobial peptides [4]. They are also called bacterial endotoxins, because can cause fever and a wide range of pathology in humans [1,5].

LPS molecules possess hydrophobic portions composed of hydrocarbon chains (Lipid A) and hydrophilic regions including polysaccharides, phosphate and other functional groups [1]. The polysaccharide section is composed of a core oligosaccharide and an outer portion called O-specific chain of identical repeating

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oligosaccharide units. The presence of both hydrophilic and hydrophobic portions determines the amphiphilic nature of the LPS molecules and in aqueous solutions they can self-aggregate into various supramolecular structures with different shapes and sizes [6]. The formation of micelles (above the critical micelle concentration CMC), or LPS layer on hydrophobic surfaces (at concentrations lower than CMC), and the onset of premicelle aggregates (at the so-called apparent critical micelle concentration CMC_a), have been reported in Ref. [7].

Bacteria themselves can build incomplete LPS, lacking the O-specific chain [8]. Certain mutations which make the bacteria unable to add complete polysaccharide chains to Lipid A cause the display of a series of characteristics known as "rough" phenotypes. Depending on the length of the polysaccharide chain, Ra to Rd mutants are distinguished. Thus Rd LPS ("deep rough" mutants) contains only an inner polysaccharide core, whereas Ra LPS ("rough" mutants) contains a longer polysaccharide portion [9]. Compared to the wild-type bacteria or rough mutants, the deep rough mutants (Rd) have lower resistance to certain hydrophobic antibiotics [10] and antimicrobial peptides [4].

It is well-known that the core of LPS is responsible for the *E. coli* negative electric charge in aqueous solutions [2]. This region is of great importance for the bacterial interactions. For example, the negative charges of LPS allow the binding to the cationic antimicrobial peptides (CAMPs) produced by the immune system to fight pathogens. Also, the removal of the core phosphate groups allows the bacteria to avoid the immune response and to resist toward antibiotics [11]. Therefore, the investigation of the electrostatic interactions between LPS molecules and model charged particles and interfaces in fluid media may mimic the in vivo-interactions of the bacteria inside living organisms. Such studies can shed light on finding new approaches for the efficient registration and possible inactivation of these pathogenic agents in aqueous media.

Some pathways for the detection and removal of LPS from aqueous media have been reported in Ref. [12]. Widely-used endotoxin detection systems are based on Limulus Amoebocyte Lysate (LAL) assay [13], which is derived from horseshoe crab blood, Limulus polyphemus, and it clots upon exposure to endotoxin. However, none of these procedures appears to be broadly applicable. In the recent years, two-phase aqueous micellar systems have also been applied for the purification and/or concentration of biological molecules [14], and of LPS in particular. The method is based on spontaneous separation of aqueous solutions into two immiscible aqueous phases with the one containing higher micelle concentration than the other. The endotoxin is then captured in the micelle-rich (bottom) phase. But despite these developments, innovative methods are needed, especially aimed at higher LPS-capture efficiency.

In the present study a novel approach to the detection and capture of LPS in aqueous media is put forward. A synthetic four-antennary oligoglycine $[Gly_7–NHCH_2–]_4C$ (T-4) is proposed as a captive agent. T-4 pertains to a special type of small molecules – $[Gly_n–NHCH_2–]_4C$ – and their structure is characterized by the presence of symmetric hydrophilic tails consisting of several oligoglycine units linked to a common center [15]. It has been established that the performance of these star-like molecules in aqueous solutions depends on the number of glycine units within the hydrophilic tails (n), the temperature, pH, etc. For example, the oligoglycine molecules with $n \geq 7$ can self-organize into various supramolecular assemblies in aqueous solutions: bulk hydrophilic nanoplatforms called tectomers or extra-regular 2D adsorption layers of monomolecular thickness at solid interfaces [15–17]. Evidences for the formation of tectomers in aqueous solutions may be found in Ref. [18]. The authors of this study have established that ~24 h are needed for the formation of equilibrium nanoplatforms in aqueous solutions of these oligoglycines. The innate reason for the onset of self-assembly is the formation of intra- and intermolecular hydrogen bonds [15–17]. It should be emphasized that T-4 molecules, as well as the tectomers, are not surface active. Due to the amine termini of the tails, the tectomers can acquire positive charge in aqueous solutions within a wide range of pH-values. The obtained nanostructures are biocompatible and nontoxic for living organisms and are therefore, suitable for medical applications, waste water purification, etc. For example, the tectomers have been verified to possess antiviral activity because they inhibit the adhesion of the influenza virus to host cells [19].

The main goal of the present study is to verify the onset of efficient capture of LPS traces by the T-4 nanoplatforms (tectomers) in aqueous solution. The evidences are extracted from measurements on the surface tension and surface dilational rheology at the solution/air interface of systems containing mixtures of Ra-LPS and $[Gly_7–NHCH_2–]_4C$. The interfacial measurements are combined with detailed characterization of microscopic foam films obtained from the same solutions. The experiments are focused on the outline of the proper conditions for possible application of the oligoglycine tectomers for the detection and capture of endotoxins in aqueous media.

2. Materials and instrumentation

The four-tailed antennary oligoglycine $[Gly_7–NHCH_2–]_4C$ (T-4) is purchased from PlasmaChem GmbH (Berlin, Germany). Lipopolysaccharides (LPS) from bacteria *Escherichia coli* EH100 with longer polysaccharide chains (Ra mutant, rough strain) are provided by Sigma Aldrich (purity ≤ 97%, purified by phenol extraction). Triply distilled water is used for the preparation of the aqueous solutions. For single tests measurements of T-4 solutions ultra pure water is also applied (CHROMASOLV® Plus, for HPLC, Sigma-Aldrich).

The investigated systems are aqueous solutions of T-4, LPS and LPS/T-4 mixtures. The T-4 solutions are prepared 24 h before the start of the measurements. The concentration range is C(T-4) = 5×10^{-5}–5×10^{-4} mol/l. LPS is added to the oligoglycine solution after the 24-hours incubation period of the T-4 solution, immediately before the start of the tensiometric measurements. Its concentration is 0.1 μg/l in all experiments. The temperature is kept strictly at 20.0 ± 0.1 °C during the experiments. For several test measurements in the case of C(T-4) = 1×10^{-4} mol/l the 24-hours incubation stage before adding LPS is skipped and LPS is added directly to the freshly prepared T-4 solution.

The interfacial properties are investigated by a Profile Analysis Tensiometer (PAT-1, Sinterface, Berlin, Germany). The option of an emergent bubble is applied (see e.g. [20,21]). The surface tension dynamics is studied in the course of 48 h. Aside from measuring the surface tension as a function of time, PAT-1 allows the possibility to invoke periodic changes of the bubble's volume. The interrelations of the dynamic surface tension and the changes in the bubble interfacial area are used for the extraction of surface rheological characteristics: dilatational elasticities and dilational viscosities. The measurements are performed within the low-frequency range of 0.005–0.2 Hz. Oscillation amplitudes are kept within the range of 5–10% of the bubble area during the experiments.

The foam films are obtained and investigated with the microinterferometric thin liquid film setup equipped with Scheludko–Exerowa cell. The details about this instrumentation might be found e.g. in [22,23]. The samples for the microinterferometric measurements are taken from the same initial solution of the LPS/T-4 mixture. The experiments are performed 24 h after the preparation of the LPS/T-4 solution (thus we have an overall 48-hours incubation period following the moment of T-4 solution preparation). For every composition of the system at least two sets