

# Monolayer Film Behavior of Lipopolysaccharide from *Pseudomonas aeruginosa* at the Air–Water Interface

Thomas Abraham,<sup>\*,†,‡</sup> Sarah R. Schooling,<sup>§,⊥</sup> Terry J. Beveridge,<sup>§,⊥</sup> and John Katsaras<sup>†,⊥,#,○</sup>

Canadian Neutron Beam Center, Chalk River, Ontario, Canada, James Hogg iCAPTURE Centre, St. Paul's Hospital, 1081 Burrard Street, Vancouver, British Columbia, Canada, Department of Molecular and Cellular Biology, University of Guelph, Ontario, N1G 2W1, Canada, Advanced Food and Materials Network-Networks of Centres of Excellence (AFMnet-NCE), Guelph, Ontario, N1G 2W1, Canada, Biophysics Interdepartmental Group and Guelph-Waterloo Physics Institute, University of Guelph, Guelph, Ontario, N1G 2W1, Canada, Department of Physics, Brock University, St. Catharines, Ontario, L2S 3A1, Canada

Received May 22, 2008; Revised Manuscript Received July 25, 2008

Lipopolysaccharide (LPS) is an essential biomacromolecule making up approximately 50% of the outer membrane of Gram-negative bacteria. LPS chemistry facilitates cellular barrier and permeability functions and mediates interactions between the cell and its environment. To better understand the local interactions within LPS membranes, the monolayer film behavior of LPS extracted from *Pseudomonas aeruginosa*, an opportunistic pathogen of medical importance, was investigated by Langmuir film balance. LPS formed stable monolayers at the air–water interface and the measured lateral stresses and modulus (rigidity) of the LPS film in the compressed monolayer region were found to be appreciable. Scaling theories for two-dimensional (2D) polymer chain conformations were used to describe the  $\pi$ - $A$  profile, in particular, the high lateral stress region suggested that the polysaccharide segments reside at the 2D air–water interface. Although the addition of monovalent and divalent salts caused LPS molecules to adopt a compact conformation at the air–water interface, they did not appear to have any influence on the modulus (rigidity) of the LPS monolayer film under biologically relevant stressed conditions. With increasing divalent salt ( $\text{CaCl}_2$ ) content in the subphase, however, there is a progressive reduction of the LPS monolayer's collapse pressure, signifying that, at high concentrations, divalent salts weaken the ability of the membrane to withstand elevated stress. Finally, based on the measured viscoelastic response of the LPS films, we hypothesize that this property of LPS-rich outer membranes of bacteria permits the deformation of the membrane and may consequently protect bacteria from catastrophic structural failure when under mechanical-stress.

## Introduction

Lipopolysaccharide (LPS) is an essential macromolecular component of the outer membrane of Gram-negative bacteria, such as *Pseudomonas aeruginosa*.<sup>1,2</sup> As a component of the bacterium's outermost face, LPS contributes significantly to the bacterium's cell surface properties and influences interactions with extracellular components. Examples of this include the uptake or impermeability of certain antimicrobial agents, the formation of fine-grain precipitates and minerals on the cell surface,<sup>3</sup> as well as adhesion to surfaces during colonization of tissues and the initial steps of Biofilm formation.<sup>4,5</sup> The interfacial and monolayer properties of LPS are therefore of significant scientific and industrial interest, with practical applications.

Chemically, LPS is composed of three distinct domains:<sup>1,6</sup> (1) a lipid moiety with acyl chains and oligosaccharide headgroups, commonly referred to as lipid A, which firmly embeds the LPS molecule into the hydrophobic region of the

outer membrane; (2) a core oligosaccharide region immediately above the lipid A moiety, possessing constituents such as phosphate (often attached to heptose) and ketodeoxyoctonate; and (3) an O-side chain attached to the core oligosaccharide consisting of a chain of repeating sugar residues. The O-side chain can be neutral or possess a net charge, but generally, the core and O-side chain together form a hydrophilic moiety that extends into the aqueous medium surrounding the bacteria. Depending upon the presence or the absence of the O-antigen attachment in the core oligosaccharide region, there are two types of LPS commonly referred as the capped and uncapped LPS, respectively. Based on the presence or the absence of O-side chains, the LPS molecules are also classified into smooth or rough chemotypes, respectively. Several techniques have been used to study LPS isolated from various micro-organisms in order to better understand their chemistry and physical properties.<sup>7</sup> Despite these experimental developments, information pertaining to LPS's interfacial and monolayer properties refers predominantly to rough and deep-rough LPS forms; in comparison, studies of smooth LPS are noticeably absent in the literature, with an exception being the recent neutron diffraction studies.<sup>8,30</sup> Smooth LPS is difficult to manipulate, primarily due to its chemical heterogeneity; apart from the range of length within the side chain, more than one type of side chain chemistry may exist, as is the case with *P. aeruginosa*.<sup>6</sup> Moreover, while a cell may be referred to as possessing smooth LPS, it is most

\* To whom correspondence should be addressed. E-mail: tabraham@mrl.ubc.ca.

<sup>†</sup> Canadian Neutron Beam Center.

<sup>‡</sup> James Hogg iCAPTURE Centre.

<sup>§</sup> Department of Molecular and Cellular Biology, University of Guelph.

<sup>⊥</sup> Foods and Materials Network - Networks of Centers of Excellence.

<sup>#</sup> Biophysics Interdepartmental Group and Guelph-Waterloo Physics Institute, University of Guelph.

<sup>○</sup> Department of Physics, Brock University.

likely a mixture of both rough and smooth LPS, and an efficient LPS isolation must show good representation of all chemistries.<sup>9</sup> Collectively, purification, manipulation, experimentation, and the derivation of sensible conclusions from such a complex grouping of chemistries is not a facile process. Yet, given the prevalence and importance of such a system, there is all the more reason to pursue and understand “native” LPS behavior.

Here we report on Langmuir film balance studies of native LPS isolated from *P. aeruginosa*, an opportunistic pathogen associated with morbidity of cystic fibrosis patients,<sup>10</sup> infections of burn victims and immunosuppressed individuals,<sup>11,12</sup> and a major contributor of nosocomially acquired infections.<sup>13</sup> The pathogen’s importance to the medical field and the health care dollars associated in treating its medical consequences are, thus, beyond doubt. We found that smooth LPS reconstituted at the air–water interface of water and water with differing ionic conditions formed stable monolayers. Langmuir isotherm plots of surface pressure or lateral stress ( $\pi$ ) versus surface area per unit weight ( $A$ ) revealed  $\pi$ – $A$  profiles strikingly similar to those of polymers<sup>14</sup> but very different from those of phospholipids<sup>15</sup> and glycolipids,<sup>16</sup> including  $\pi$ – $A$  profiles from rough type LPS.<sup>17,18</sup> We also noted that the measured lateral stresses and the modulus (rigidity) of the LPS film in the compressed monolayer region were generally substantial. We analyzed the  $\pi$ – $A$  profiles using scaling theories describing polymer interactions in 2D space. From this analysis, we established that LPS polysaccharide chains are constrained at the air–water interface. Moreover, LPS molecules at the air–water interface exhibit typical polymeric behavior (excluded volume model), and the relaxation behavior of the LPS film, below its collapse region, was found to be viscoelastic.

## Materials and Methods

**Lipopolysaccharide Extraction and Purification.** LPS from *P. aeruginosa* PAO1 (serotype O5) was isolated using a hot phenol extraction based on the method described by Westphal and Jann.<sup>21</sup> In brief, cultures were grown to an early stationary phase in trypticase soy broth (BBL: 37 °C, 125 r.p.m.), harvested by centrifugation and washed twice in 0.9% NaCl (w/v). Cells were lyophilized and 20 g of dried cell mass was suspended into prewarmed water (70 °C). An equivalent volume of 90% phenol was gradually added to the cell suspension while being stirred, taking care that a good emulsion was formed. During the course of the extraction the temperature was maintained at 70 °C. After 3 h, the solution was allowed to cool down on ice, promoting phase separation, then centrifuged at 10000 *g* for 20 min. After centrifugation, the upper aqueous phase was retained and dialysed against water until phenol could no longer be detected. LPS was pelleted from solution by ultracentrifugation at 100000 *g* (18 h) in a Beckman Ti 45 rotor and washed a minimum of two times. The pellet was then taken up in a small quantity of water and freeze-dried. LPS was assessed for DNA, Kdo, and protein content by PAGE following the methods described by Darveau and Hancock.<sup>9</sup>

**Langmuir Film Balance Measurements.** The Langmuir film balance is an instrument that controls the molecular density or area of a floating monolayer at the air–water interface, while simultaneously measuring its surface pressure or lateral stress. This commonly available technique is used to study the interfacial properties of self-assembled monolayers of amphiphilic materials, such as lipids, polymers, and surfactants.<sup>22,23</sup> The amphiphilic molecules orient at the air–water interface in such a way that their polar head groups dissolve in the water subphase while their hydrophobic tails protrude into the air.

Langmuir isotherm experiments were carried out on an automated, temperature controlled KSV LB5000 trough (KSV-Instruments Inc., Finland). The trough is made out of polytetrafluoroethylene (PTFE), a chemically inert and nontoxic material, while the two movable barriers

are of Delrin, a hydrophilic material preventing any leakage of the monolayer beneath the barrier. The barriers are used to compress the molecular monolayer and control its packing density. A platinum Wilhelmy plate monitored lateral stress by measuring the force due to surface tension on the partially submerged plate. Knowing the physical dimensions of the Wilhelmy, the force was converted into surface tension (mN/m).

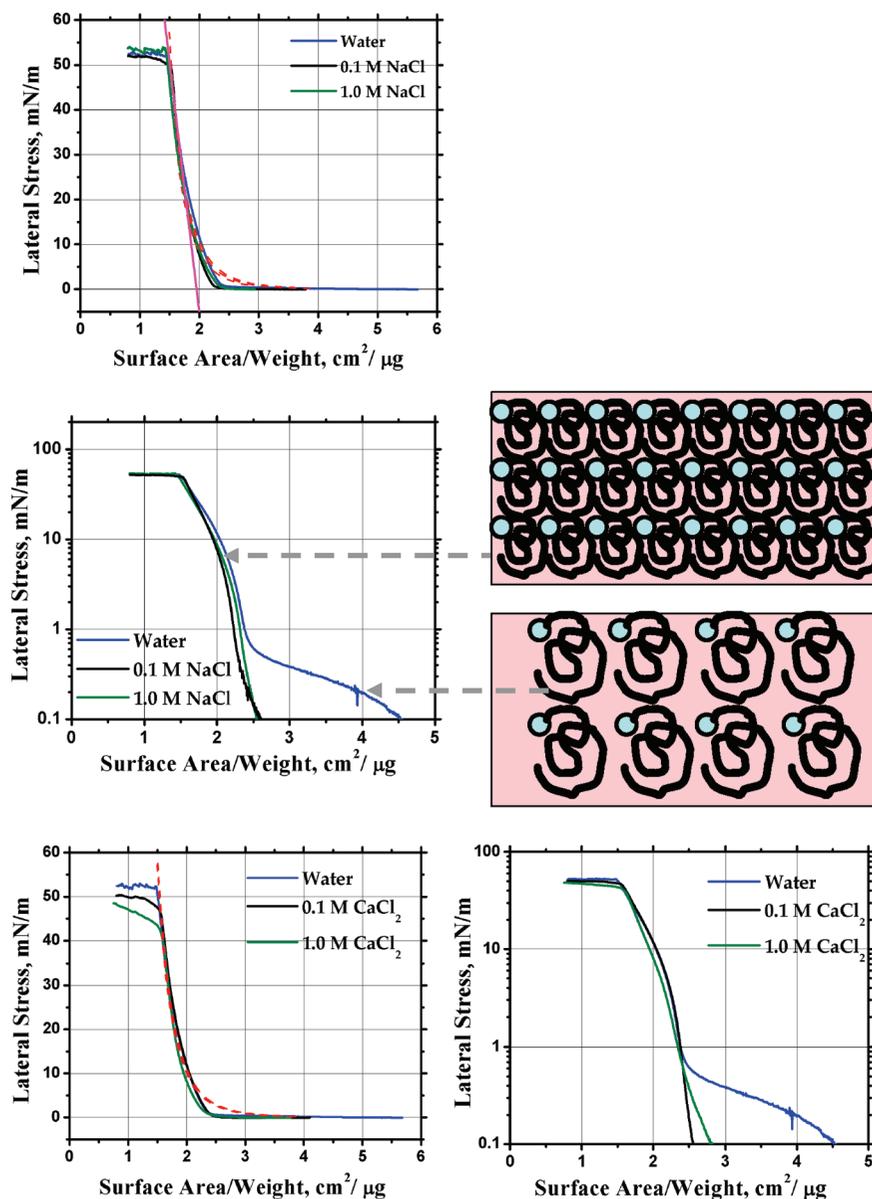
Molecules at a free surface (e.g., water–air interface) have fewer nearest neighbors than those same molecules in the bulk. The difference between their free energies is known as the excess free energy. The surface tension of a plane interface is given by the partial derivative  $\gamma = (\partial G/\partial S)_{T,P,n}$ , where  $G$  is the Gibbs free energy of the system,  $S$  is the surface area,  $T$  is the temperature,  $P$  is the pressure, and  $n$  is its composition. For a plane surface at equilibrium,  $\pi = \gamma_0 - \gamma$ , where  $\gamma_0$  is the surface tension of pure water and  $\gamma$  the value with the monolayer present. The characteristic description of an insoluble monolayer is usually expressed in terms of its lateral stress, area curve ( $\pi$ – $A$ ), or isotherm. Such isotherms contain information about the interfacial structure, phase transitions, and conformational transformations taking place effectively in two-dimensional space.

LPS monolayers were prepared as follows: A mixture containing liquid phenol (9 part phenol/1 part water) was mixed with chloroform and petroleum ether in a volume ratio of 2.5:8, respectively. At first, the mixture appeared cloudy, but could be made clear by the addition of solid phenol. A bulk solution of 1.0 mg LPS/mL was prepared and all subsequent experiments were performed from this stock solution. Purified water (Millipore, 18 M $\Omega$  cm resistivity) was used as the subphase and isotherm measurements were performed at  $21 \pm 0.5$  °C. All isotherms and hysteresis experiments were performed, unless otherwise noted, at a constant barrier speed of 0.5 cm<sup>2</sup>/sec. For measuring hysteresis, barrier movement was immediately reversed after the surface area/unit weight reached the desired value, while stress relaxation experiments were performed by compressing the film at a constant compression speed until the desired initial lateral stress had been reached. The film was then maintained at a constant area, while the stress was permitted to vary and recorded as a function of time.

## Results and Analysis

**Lateral Interactions of LPS Monolayers at the Air–Water Interface.** Figure 1 shows the formation of well-behaved LPS monolayers at the air–water interface. At a compression speed of 0.5 cm<sup>2</sup>/sec, the lateral stress onset at the beginning of this isotherm occurs at about 4.5 cm<sup>2</sup>/μg and the collapse at about 52 mN/m. Lateral stress increases monotonically with decreasing molecular area, and the slopes of the curves are indicative of either a highly compressible film or a film with high rigidity.

As described in the introduction, there is a polymeric chain in LPS that includes the core region and the O-side chain that is covalently attached to a lipid molecule. These covalently bound polysaccharide chains interact with the lipids and the air–water interface in various ways.<sup>24</sup> One possibility is that the polysaccharide chain adsorbs to the air–water interface and interacts (mixes) with the lipid. Another possibility is that the polysaccharide chain does not adsorb to the air–water interface, but dissolves in the water subphase. A third possibility is that the polysaccharide chain adsorbs at the air–water interface, but does not interact with the lipid at the air–water interface. From the pure water isotherm, it is clear that once the packing area per molecule has reached  $\sim 4.5$  cm<sup>2</sup>/μg ( $\sim 0.1$  mN/m), LPS molecules begin to interact laterally with each other. On further compression (Figure 1), the LPS chains increasingly pack together at the air–water interface. This increased packing is reflected as an inflection point, whereby lateral stress increases dramatically. Most likely, this inflection point indicates a critical lateral stress ( $\sim 1.0$  mN/m) at which the LPS polysaccharide



**Figure 1.** Linear and semilog plots of lateral stress and area isotherms of LPS in pure water, and for various monovalent and divalent salt concentrations. Broken red colored lines are a fit to the data using polymer scaling theory (eq 1). The solid pink line is a linear fit to the data in the high lateral stress regime (30–35 mN/m) and provides the interfacial elastic modulus of LPS monolayers at biologically relevant conditions. See text for details.

chain begins to experience steric interaction (excluded volume or segment–segment interaction) at the air–water interface.

Considering the polymeric behavior exhibited by LPS molecules, we can analyze the measured lateral stress (lateral repulsion) in terms of scaling theories of polymer segment interactions in 2D space.<sup>19,20</sup> For concentrations above the chain overlap concentration, the theory predicts that

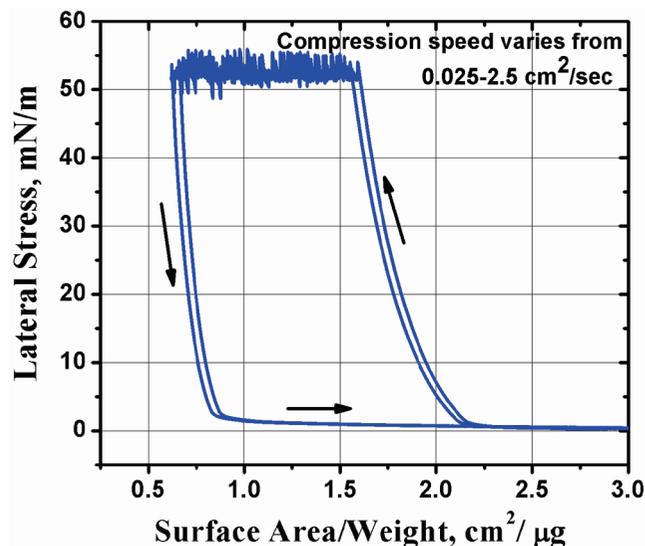
$$\pi = C^{2\nu/2\nu-1} \quad (1)$$

where  $C$  is the concentration or the number of chains per unit area, and  $\nu$  is the characteristic scaling exponent. For a given solvent, the scaling exponent  $\nu$  expresses the dependence of the polymer's radius of gyration (dimension) with respect to its MW, in this case, at the air/water interface. For a “good” solvent, the exponent  $\nu$  is calculated to be 0.77, and for a  $\theta$  solvent (“ideal” solvent)  $\nu$  is calculated to be 0.506.<sup>20</sup> As shown by the broken line in Figure 1, the isotherm is reasonably described by the proposed polymer scaling theory (eq 1). This suggests that LPS molecules at the air–water interface exhibit

typical polymeric behavior, which means that the polysaccharide segments reside at the 2D air–water interface. The general structural arrangements that we obtained from these monolayer studies is consistent with our recent neutron diffraction studies of LPS multilayers reported elsewhere.<sup>8</sup> The scattering length density profiles obtained from these neutron diffraction studies generally imply that the polysaccharide chains covalently linked to the LPS molecules are not fully extended, but rather crowded around the LPS core region.

De Gennes<sup>24</sup> hypothesized a similar situation where the polymer segments adsorb and mix with the lipid at the interface, resulting in a predicted enhancement of a lipid layer's rigidity at the air–water interface. This may explain the LPS film's high rigidity, as is clearly evident from the  $\pi$ – $A$  isotherms (Figure 1). For the present system, the experimental value of  $\nu$  is found to be 0.60, implying that for smooth LPS the air–water interface is in-between a good and an ideal ( $\theta$ ) solvent.

Lateral stress–area isotherms were also generated for sub-phases containing monovalent (NaCl) and divalent (CaCl<sub>2</sub>) salts



**Figure 2.** Hysteresis curves of LPS at compression speeds ranging from 0.025–2.5 cm<sup>2</sup>/sec.

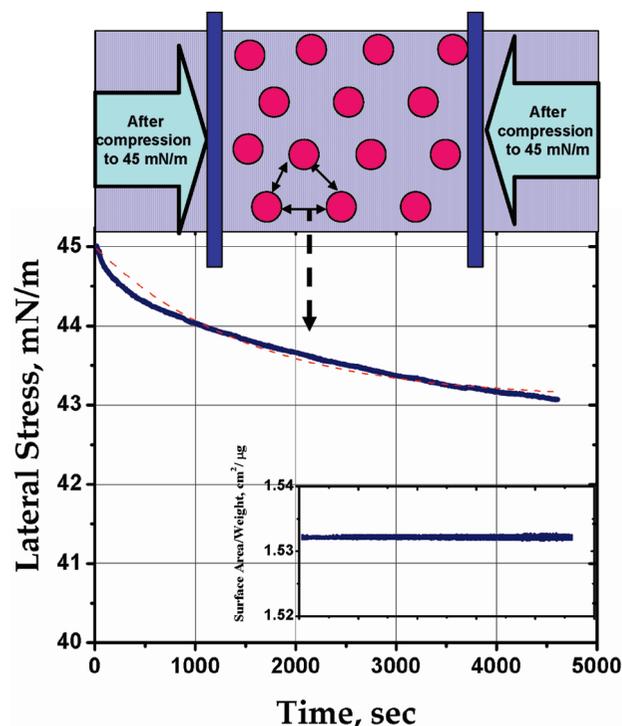
(Figure 1). In the high lateral stress region, the isotherms remain essentially the same, however, the low lateral stress region clearly shows differences. It seems that, in the presence of salts (both monovalent and divalent), the polysaccharide chain at the air–water interface adopts a compact conformation, most likely due to the electrostatic screening of anionic charges associated with the polysaccharide chain. Note that LPS is a polyanionic lipid, and its anionic groups reside mainly in the lipid A headgroup and core region. The  $\pi$ – $A$  profiles, after added salt, are again well described using the scaling description (dashed lines Figure 1) and results in a  $\nu$  of 0.60, implying that LPS at the air–water interface in the presence of NaCl or CaCl<sub>2</sub> is of intermediate solvent quality.

**Surface Compressive Modulus (Rigidity) of LPS Monolayers.** Having described the general features of LPS monolayers at the air–water interface and commented on the rigidity of the LPS film, we quantified the surface compressive modulus of the LPS monolayer from the experimentally measured  $\pi$ – $A$  profile. The surface compressive modulus ( $E_s$ ), that is, the reciprocal of monolayer compressibility ( $C_s$ ) can be obtained from the  $\pi$ – $A$  isotherm from the following equation<sup>25</sup>

$$E_s = \frac{1}{C_s} = -A \left( \frac{d\pi}{dA} \right) \quad (2)$$

where  $A$  is the area per unit weight at a given lateral stress and  $\pi$  is the corresponding lateral stress. At high lateral stresses, that is,  $>30$  mN/m, mimicking biologically relevant membrane conditions,<sup>26</sup> the surface compressive modulus is found to be  $\sim 225$  mN/m, suggesting that this particular LPS monolayer is very rigid (stiff) due to the confinement of the polysaccharide segments at the air–water interface. As can be seen from the isotherms obtained in the presence of the monovalent and divalent salts (Figure 1), the added salts do not appear to have any influence on the modulus (rigidity) of the LPS monolayer film under the biologically relevant stressed conditions.

**Hysteresis of LPS Monolayers.** Hysteresis curves for three different compression speeds are shown in Figure 2. For these experiments, the monolayer is compressed far into the film’s collapse region and then expanded. The isotherms are indistinguishable and are independent of the compression speed (0.025–2.5 cm<sup>2</sup>/sec). From this we may infer that the relaxation processes, which occur while the molecules are compressed,



**Figure 3.** Lateral stress relaxation of an LPS monolayer after compression to a surface area of  $\sim 1.53$  cm<sup>2</sup>/μg. The dashed line is the fit to the data using the viscoelastic relationship (eq 2). The inserted figure shows that surface area per unit weight was kept constant during the measurements.

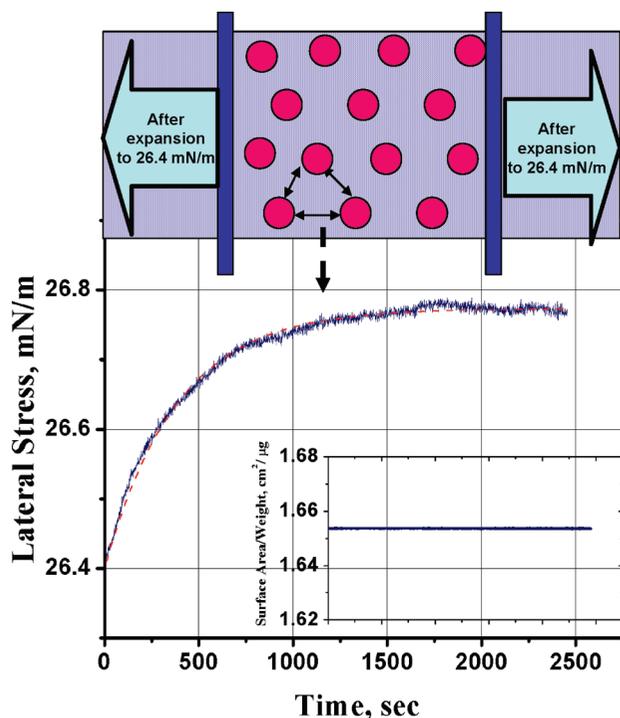
are taking place on a time scale smaller than the fastest compression speed. In the plateau region, that is, decreased molecular area, molecules from the monolayer most likely migrate into the aqueous subphase. Maintaining a constant lateral stress does not affect the structure of the monolayer. On expansion, all curves merge with the initial compression curves at high areas. Note, that the change in compression speed only affects how far the system is driven away from equilibrium.

**Stress Relaxation and Viscoelastic Behavior of the LPS Monolayer.** The stress–relaxation response following compression of the monolayer film was assessed (Figure 3). A lateral stress of 45 mN/m was applied (this value lies within the solid phase region of the isotherm) and the lateral stress decay upon relaxation was followed. The isotherm for this “stress-relaxed” LPS monolayer displayed identical collapse pressures as the nonstressed monolayer, implying that the observed continuous reduction in lateral stress was not the result of LPS monolayer film collapse.

Attempts were made to fit the experimental data using the viscoelastic relationship, previously used to describe similar monolayer behavior.<sup>27</sup> According to this equation, the relaxation could be described by

$$\pi_t = \pi_\infty + (\pi_0 - \pi_\infty) e^{-t/\tau} \quad (3)$$

where  $\pi_t$  is the lateral stress at time  $t$ , and  $\pi_0$  and  $\pi_\infty$  are the lateral stresses at  $t = 0$  and after a time where the system is no longer undergoing change. The characteristic time of the relaxation is denoted by  $\tau$ , which was found to be  $\sim 1659$  s. This slow relaxation process can be associated with the rearrangement of LPS molecules, including their polysaccharide chains, at the air–water interface. Similar to the hysteresis behavior, we found that compression speed had almost no effect on the relaxation time. However, after expanding the LPS



**Figure 4.** Relaxation of lateral stress after expanding the LPS monolayer film to a surface area of  $\sim 1.65 \text{ cm}^2/\mu\text{g}$ . The dashed line is the viscoelastic fit (eq 3) to the data. The inserted figure shows that surface area per unit weight was kept constant during the measurements.

monolayer, the lateral stress was found to increase as soon as the barriers stopped moving (Figure 4). Here again, the viscoelastic relaxation process can be well described using eq 3; in this case,  $\tau$  was determined to be  $\sim 380 \text{ s}$ . It is interesting to note the difference between stressed (1659 s) and stress-relieved (380 s) LPS monolayers. Compared to the stressed LPS film, the one that was stress-relieved took a relatively shorter time to attain its equilibrium conformation at the air–water interface.

### Discussion and Conclusions

Langmuir film balance studies demonstrated that smooth type LPS forms stable monolayers at the air–water interface. The strong lateral interactions exhibited by LPS were clearly manifested in the high lateral stress region and this may be characteristic of the lateral stresses present in the membranes of *P. aeruginosa*. In the low lateral stress region, the addition of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  to the subphase considerably reduced the area/molecule, denoting a change in the molecule's arrangement and conformation, as a result of changes in lateral interactions and salt bridging. For instance, at  $0.1 \text{ mN/m}$  the addition of  $0.1 \text{ M}$   $\text{NaCl}$  to the subphase reduced the area per unit weight to  $\sim 2.5 \text{ cm}^2/\mu\text{g}$ , a value nearly half of that obtained for LPS monolayers residing in pure water. In agreement with current knowledge, the ionic environment clearly influenced the interfacial structure of LPS molecules, specifically by creating a more compact monolayer. Intriguingly, irrespective of the aqueous environment and ions present, the ability of this relevant membrane component to withstand highly stressed environments was clearly evident from the  $\pi$ – $A$  isotherms, as the rupture/collapse of LPS monolayers occurred only at relatively higher lateral pressures ( $42$ – $52 \text{ mN/m}$ ). However, the influence of the added salts, in particular, the divalent salt in reducing the rupture pressure of

LPS monolayers is clearly evident in the  $\pi$ – $A$  profiles. For instance, compared to pure water, the addition of  $1.0 \text{ M}$   $\text{CaCl}_2$  to the subphase reduced the collapse pressure of the LPS monolayers by 20%, suggesting that at high concentrations the divalent salt seemingly weakens the ability of the membrane to withstand elevated stress conditions. This may at first seem contradictory, yet it is well-known that high concentrations of divalent cations can increase the migration of DNA and proteins across the outer membrane, presumably by inducing “cracks” in the membrane's structure.<sup>28,29,31</sup>

What then would be the practical significance of the observed relaxation processes and viscoelastic behavior of LPS films at interfaces? The relaxation time is associated with the viscoelastic behavior of the LPS film and this material property distinguishes the solid behavior (elastic) from the time dependent fluid (viscous) response to the applied lateral stress (surface pressure). In the case of Gram-negative bacteria, LPS is involved in adhesive interactions with substrate during colonization and subsequent Biofilm formation. These bacterial surfaces are found in widely differing environments and also exhibit a variety of physical properties. For successful and persistent colonization of bacterial surfaces in mechanically stressed environments, the bacterial surfaces, of which the LPS molecules are the major component, must be able to adapt to fluctuations in mechanical stresses. In particular, in order to avoid prolonged exposure to mechanical stress, bacterial surfaces must be able to “deform” or risk disastrous structural failure. Since LPS resides on the outer face of the *P. aeruginosa*'s outer membrane, it would be highly interactive with an adherent surface and our monolayer studies, being analogous to this face, are highly relevant. The viscoelastic response of LPS monolayers could protect the bacteria from catastrophic structural failure, possibly buying time during which the bacteria can generate an adaptive phenotypic response to prevent detachment from the substrate.

**Acknowledgment.** The authors thank A. Saxena (University of Guelph) for helping characterize the LPS used in this study. The work by S.R.S. and T.J.B. was supported by funds through AFMnet-NCE. T.A. was supported through an NSERC Visiting Fellowship Award.

### References and Notes

- (1) Wilkinson, S. G. *Prog. Lipid Res.* **1996**, *35*, 283–343.
- (2) Nikaido, H. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 593–656.
- (3) Langley, S.; Beveridge, T. J. *Appl. Environ. Microbiol.* **1999**, *65*, 489–498.
- (4) Flemming, C. A.; Palmer, R. J.; Arrage, A. A.; van der Mei, H. C.; White, D. C. *Biofouling* **1998**, *13*, 213–222.
- (5) Makin, S. A.; Beveridge, T. J. *Microbiology* **1996**, *142*, 299–307.
- (6) Lam, J. S.; Matewish, M.; Poon, K. K. H. In *Pseudomonas*, Ramos, J. L. Ed.; Kluwer Press: Norwell, MA, 2004; Vol. 3, Chapter 1, pp 3–51.
- (7) Caroff, M.; Karibian, D. *Carbohydr. Res.* **2003**, *338*, 2431–2447.
- (8) Abraham, T.; Schooling, S. R.; Nieh, M.-P.; Kucerka, N.; Beveridge, T. J.; Katsaras, J. *J. Phys. Chem. B* **2007**, *111*, 2477–2483.
- (9) Darveau, R. P.; Hancock, R. E. W. *J. Bacteriol.* **1983**, *155*, 831–838.
- (10) Høiby, N.; Johansen, H. K.; Moser, C.; Song, Z.; Ciofu, O.; Kharazmi, A. *Microbes Infect.* **2001**, *3*, 23–35.
- (11) De Macedo, J. L.; Santos, J. B. *Mem. Inst. Oswaldo Cruz.* **2005**, *100*, 535–539.
- (12) Aloush, V.; Navon-Venezia, S.; Seigman-Igra, Y.; Cabili, S.; Carmeli, Y. *Antimicrob. Agents Chemother.* **2006**, *50*, 43–8.
- (13) Sligl, W.; Taylor, G.; Brindley, P. G. *Int. J. Infect. Dis.* **2006**, *10*, 320–325.
- (14) O'Brien, K. C.; Lando, J. B. *Langmuir* **1985**, *1*, 453–455.
- (15) Helm, C.; Laxhuber, L.; Losche, M.; Mohwald, H. *Colloid Polym. Sci.* **1988**, *264*, 46–55.
- (16) Masakatsu, H.; Minamikawa, H.; Tamada, K.; Baba, T.; Tanabe, Y. *Adv. Colloid Interface Sci.* **1999**, *80*, 233–270.

- (17) Brandenburg, K.; Seydel, U. *Biochem. Biophys. Acta* **1984**, 775, 225–238.
- (18) Roes, S.; Seydel, U.; Gutschmann, T. *Langmuir* **2005**, 21, 6970–6978.
- (19) De Gennes, P. G. *Scaling Concepts in Polymer Physics*; Cornell University Press: New York, 1979.
- (20) Vilanove, R.; Rondelez, F. *Phys. Rev. Lett.* **1980**, 45, 1502–1506.
- (21) Westphal, O.; Jann, K. *Methods Carbohydr. Chem.* **1965**, 5, 83–91.
- (22) Gaines, G. L., Jr. *Insoluble monolayers at gas liquid interfaces*; Interscience: New York, 1966.
- (23) Roberts G. G. *Langmuir-Blodgett films*; Plenum Press: New York, 1990.
- (24) De Gennes, P. G. *J. Phys. Chem.* **1990**, 94, 8407–8413.
- (25) Behroozi, F. *Langmuir* **1996**, 12, 2289–2291.
- (26) Marsh, D. *Biochim. Biophys. Acta* **1996**, 1286, 183–223.
- (27) Bois, A. G.; Baret, J. F.; Panaiotov, I. I. *Chem. Phys. Lipids* **1984**, 34, 265–277.
- (28) Cohen, S. N.; Chang, A. C.; Hsu, L. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, 69, 2110–2114.
- (29) Papahadjopoulos, D.; Potris, A.; Pangborn, W. *Ann. N.Y. Acad. Sci.* **1978**, 308, 50–66.
- (30) Kučerka, N.; Papp-Szabo, E.; Nieh, M.-P.; Harroun, T. A.; Schooling, S. R.; Pencer, J.; Nicholson, E. A.; Beveridge, T. J.; Katsaras, J. *J. Phys. Chem. B* **2008**, 112, 8057–8062.
- (31) Ferris, F. G.; Beveridge, T. J. *Can. J. Microbiol.* **1986**, 32, 52–55.

BM800562R