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# OPTICAL TWEEZERS STUDY OF LANGMUIR MONOLAYER LINE TENSION: EFFECTS OF PROTEIN AND SOLUBLE SURFACTANT

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## ABSTRACT

The line tension of a liquid expanded (LE)/gas (G)-phase boundary of methyl octadecanoate Langmuir monolayer (LM) at the air/water interface is investigated upon adsorption of protein or surfactant dissolved at different concentrations in the aqueous subphase. Optical tweezers experiments coupled with fluorescence microscopy were used to achieve this goal. We combined different theoretical models which view LMs as a 2d-fluid coupled with a 3d-subphase viscose barrier for better understanding of the LM properties such as viscosity and line tension. In particular, the line tension of the LE/G boundary has been investigated at nano-molar subphase concentrations and an increase in the line tension was observed. This result suggests that the presence of the aqueous subphase may affect the viscosity and the line tension of the monolayer and provides a direct test for the validity of existing theoretical models.

**Keywords**: Langmuir monolayers, line tension, optical tweezers.

## INTRODUCTION

Langmuir monolayer technique is a simple model used to study lipid bilayer-proteins or surfactant interactions at the air/water interface. Experiments with monolayers have the advantage of controlling the arrangement of the molecules by changing the molecular area and the pressure of the monolayer. In addition, the monolayer geometry makes it accessible to several optical techniques. In particular, fluorescence microscopy (Lösche and Möhwald, 1984; Peters and Beck, 1981; Tscharner and McConnell, 1981) and Brewster angle microscopy (Höning and Möbius, 1992; Hénon and Meunier, 1991) have been widely used to image LMs phase transitions. The study of this quasi-two dimensional interfacial fluid with the subphase fluid is essential for better understanding of the properties of biological membranes and membrane model systems (Stine and Knobler, 1991; Muller and Gallet, 1991; Benvengnu and McConnell, 1992). An important factor which affects the morphology of LMs is the line tension between the coexistence phases. The line tension is a measure of the excess free energy of molecules located in the transition region between the phases compared to the free energy of the interior of the phases. In fact, the appearance of the phases in LMs is mainly due to the competition between the short range line tension and the long range electrostatic interactions at the phase boundaries (Muller and Gallet, 1991). Over the last two decades, several experimental and theoretical techniques (Stine and Knobler, 1991; Benvengnu and McConnell, 1992) have been explored to measure the line tension between fluid Langmuir phases. Benvengnu and McConnell (1992) estimated the line tension in a mixed monolayer from the speed of approach of two bola ends. They adapted a simple hydrodynamic approximation assuming negligible surface viscosity and circular bola with no flow employing the results of Hughes *et al.* (1981) for a solid cylinder moving through a membrane. The line tension for various systems was also deduced from relaxation experiments (Stone and McConnell, 1995; Rivier *et al*., 1995; Mann *et al*., 1992; Mann *et al*., 1995; Läuger *et al*., 1995; Alexander *et al*., 2007) using the hydrodynamic approximation developed by Stone and McConnell (1995). Similarly, line tension estimations were obtained from the coalescence and the subsequent relaxation of two domains evolving in the monolayer (Steffen *et al*., 2001; Wintersmith *et al*., 2007; Zou *et al*., 2010). In other experiments, the domains were deformed directly using silica beads attached to domain edges by optical tweezers technique (Wurlitzer *et al*., 2000; Steffen *et al*., 2001).

Some of the aforementioned experimental investigations lack the coupling between the subphase viscosity and the monolayer viscosity which may affect the line tension values obtained experimentally. Few mathematical attempts which model the Langmuir film as a viscous 2d-fluid on a deeply viscous 3d-subphase were developed up to date (Hughes *et al.*, 1981; Stone and McConnell, 1995; Zou *et al*., 2010). Generally, these models assume that the interfacial film is of infinite extend with constant flow, where the domain deformations appear as a result of the competition between the line tension force and surface flow. In addition, these models neglected the dipole-

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dipole interactions between the different phases or within the phase. Recently, Zou *et al*. (2010) estimated the line tension from a gaseous hole closing in a polymer monolayer film in the LE/G coexistence region. These experiments were performed to test the hydrodynamic theories of a 2d-fluid coupled with the 3d-subphase developed by (Alexander *et al*., 2007).

In order to test the validity of the above theories experimentally, we choose to measure the effect of the Myelin basic protein (MBP) or sodium dodecyl sulfate (SDS) on the inter-phase line tension in LM at liquid/gas boundaries upon adsorption on the monolayer in the liquid/gas coexistence region. MBP is a highly basic protein with isoelectric point greater than 10.0 with a size of the order of 13 kDa (Campagoni, 1988). Depending on the surrounding conditions, the hydrophobic side of the MBP polypeptide main chain exhibits different structural conformations. MBP generates a random coil when dissolved in water and an α-helical or β-structure when electrostatically adsorbing to the lipid membrane (Smith, 1992; Krighaum and Hsu, 1975; Mendz *et al*., 1984, Keniry *et al*., 1981). This adsorbing results in an increase in the surface shear viscosity of the fluid membrane (Khattari *et al*., 2005). Furthermore, the SDS species are charged soluble organic surfactant penetrating the lipids membrane in specific sites in the mixed system. Such membrane-perturbing surfactants are commonly used to lyse cells to study their contents as well as to solubilize their membrane proteins (McConlogue *et al*., 1998). The selection of the lipid membrane, the protein, and the surfactant was based on their biological significance and their electrostatic properties.

In this study, the experiments were performed using fluorescence microscopy coupled with optical tweezers in order to compare the effect of MBP and SDS on the monolayer line tension (Wurlitzer *et al*., 2000). We assumed an infinitely deep subphase in which the protein or surfactant is dissolved homogeneously at nano-scale concentrations. Khattari *et al*. (2005) obtained experimentally the surface shear viscosity of LM upon adsorption of MBP or SDS. A linear increase of surface viscosity was measured using both solutes at nanomolar concentrations. The surface viscosity values of the above study were used in this work to investigate the line tension between the LE/G phases in a LM at various subphase concentrations.

## MATERIALS AND METHODS

Methyl octadecanoate, Lipid free MBP and SDS were purchased from Sigma and used as received. MBP is extracted from bovine central nervous system and is claimed to be 99% pure. The fluorescence dye hexadecylamino-7-nitrobenz-oxa-1,3-diazole was purchased from Molecular Probes. The methyl octadecanoate was dissolved in chloroform and mixed with 1% of the fluorescence dye. The monolayer was spread on an aqueous subphase, and left 30 min for equilibration. At temperature T=27.5±0.1°C, the monolayer was spread at an area per molecule of $A_{mol}$=38 $Å^2$/molecule. During the compression, the gaseous (G) phase was converted to liquid expanded phase (LE) and liquid condensed (LC) [between $A_{mol}$=(40-25) $Å^2$/molecule] such that all three or two phases coexist. The fraction of each phase depends on the average area per molecule. Without further purification, MBP (or SDS) has been dissolved in deionized water (Millipore Milli-Q, 18 MΩ, pH=5.5) at concentrations of 0.5 µM. In fluorescence microscopy experiments, it is added in the subphase very close to the objective. After adding the MBP or SDS, the monolayer was left for 30 min for homogeneous adsorption. Subphase concentrations were calculated under the assumption of homogeneous equilibration in the entire subphase.

Measurements were carried out using optical tweezers coupled with fluorescence microscopy [for details and schematic drawing see Khattari *et al*. (2005)]. Briefly, two simultaneous operations are performed by a 100x water film immersion objective, numerical aperture 1.0, built into the bottom of a temperature-controlled balance. First, it projects a fluorescence image of the monolayer onto a SIT camera (Hamamatsu C 3077-01). Fluorescence is excited in the monolayer by a linearly polarized argon ion laser (488 nm, 150 mW). The excitation light is blocked by a filter in front of the detector. The IR laser beam (1064 nm), coupled into the optical path using a dichroic mirror (transparent for fluorescence light) which directs the beam onto the interface. Finally, the beam is focused by the objective onto the monolayer. The IR laser power is adjustable between 2 mW-2 W depending on the experimental conditions. The diameter of the beam fits the back aperture of the microscope objective to get the smallest possible illumination spot in order to maximize the lateral optical force. The absorption of the laser in the water causes an increase in temperature of the order of 10 K/W (Wurlitzer *et al*., 2000). The fluorescence dye in the monolayer being illuminated by either the weakly focused $Ar^+$ laser or the strongly focused IR laser is not a significant heat source.

## RESULTS AND DISCUSSION

Figure 1 shows the relaxation of the LE froth in the gas phase, where the LE facet is cut with the tweezers. A local deformation of the facet (Fig. 1, t= -0.2 s and t= 0.08 s) is achieved by increasing the IR-laser power up to 250 mW, where local heating causes dilatational flow of the monolayer. The induced bending increases until the facet ruptures at t=0.2 s and shortening of the dangling end is observed for t >0.2 (see Fig. 1). Here, we assumed that LE facet is relaxing with a dangling end of a disc shape in

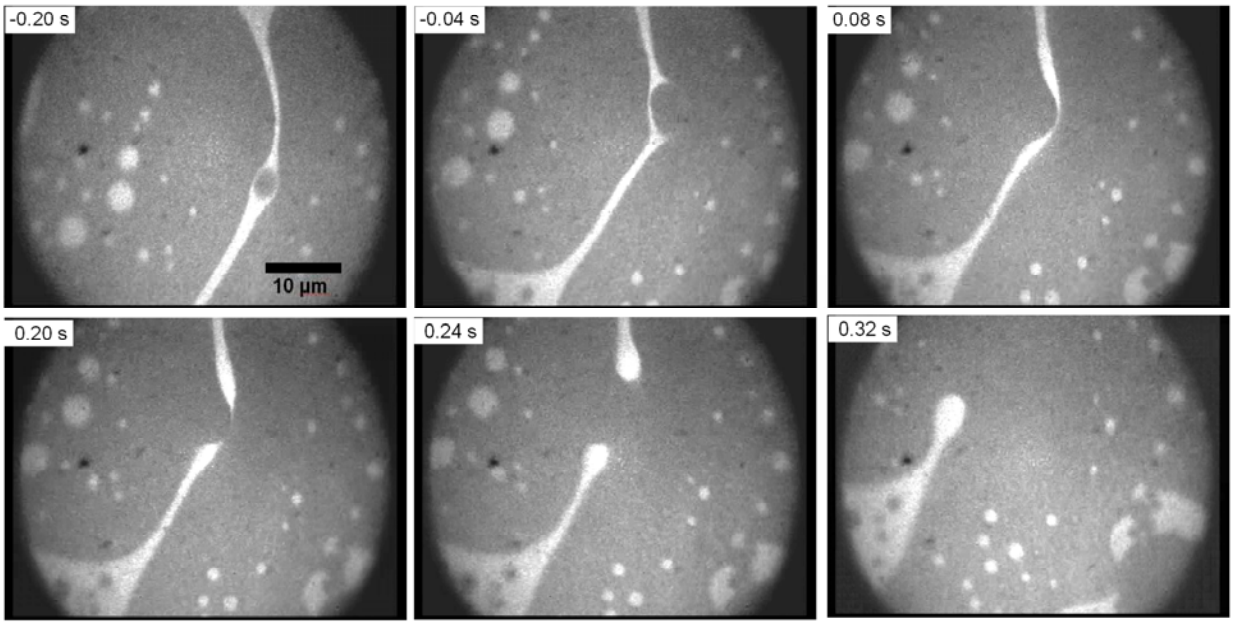


Fig. 1. Fluorescence microscope image of a methyl octadecanoate monolayer in the LE/G coexistence region at T=27.5°C and A=38 Å$^2$/molecule. A LE-facet separating two G areas (t=-0.2 s) is deformed by local heating at t=-0.08 s. The rupturing and shortening of the facet is observed between t=0.2-0.32 s.

the G-phase. Based on these images, we calculated the relaxation velocity of the disc by measuring the LE-facet length as a function of time. A linear relationship between the relaxed LE length and time was observed, which allows one to deduce a constant shortening velocity for each aqueous subphase concentration (to be commented on below).

The line tension between the LE/G phases is calculated by solving the hydrodynamics equations of the system as follows: the relaxation of the LE facet was described by the balance of static (line tension $F_\lambda$ and dipolar force $F_{dd}$) and viscous forces (drag force of the dangling LE end $F_\eta$). The force of the line tension of both sides $F_\lambda = 2\lambda$ ($\lambda$ denotes the line tension) on the strip is the driving force for the relaxation, while the dipole-dipole and drag forces oppose the shortening of the facet (Wurlitzer *et al*., 2000):

$$F_\eta + F_{dd} - F_\lambda = 0 \qquad (1)$$

The hydrodynamic drag force on the dangling end, moved at an air/water interface, is given by $F_\eta = fa\eta_{sub}(dL/dt)$, where $a$ denotes the radius of the dangling end, $\eta_{sub}$ the viscosity of the subphase, $v=dL/dt$ is the dangling end velocity, and $f$ a dimensionless friction coefficient. The friction coefficient depends on the detailed rheological properties of the monolayer and the subphase. The expression for the dimensionless friction coefficient $f$ which takes into account the coupling between the monolayer of viscosity $\eta_S$ and the subphase was calculated theoretically by De Koker (1996) as:

$$f = \frac{\pi}{\int_0^\infty \frac{J_1^2(x)}{x^2(1+Bx)}dx} \qquad (2)$$

where $B = \eta_s / \eta_{sub} a$ is the Boussinesq number. Assuming that the electrostatic contribution to the hydrodynamics is negligible (for detailed discussion see (Wurlitzer *et al*., 2000)) then Eq. (1) simplifies to

$$\lambda = \frac{f}{2}\eta_{sub} a v \qquad (3)$$

The results of the line tension of an octadecanoate monolayer on a pure subphase (*i.e.* with no MBP or SDS dissolved in the subphase) have been measured in references (Wurlitzer *et al*., 2000; Steffen *et al*., 2001). The estimated value of the line tension at LE/G boundaries is less than 10 pN. This result is in good agreement with the measured line tension data of other groups (McConlogue *et al*., 1998; De Koker, 1996).

Effects like shear flow of the LE facet during relaxation, shear viscosity of the LE and G phase, the presence of neighboring facets and the subphase coupling have been

neglected in these experiments. In this study, the effects of relaxation and shear viscosity of the LE phase at different subphase solute concentrations have been taken into account to determine the line tension values. The values of the Boussinesq number were derived from Figure 5 presented in (Khattari *et al.*, 2005). Then the values of the dimensionless friction coefficient *f* were calculated from Eq.(2). Finally, the obtained values of *f* were inserted in Eq.(3) to determine the values of the line tension.

The surface pressure-area isotherms (Fig. 2) at T=27.5°C of pure methyl octadecanoate and of methyl octadecanoate with MBP or SDS absorbed from 1.0 nM aqueous subphase give an idea about changes in the phase behavior due to the adsorption. We performed our experiments at an area per molecule of 38 $Å^2$ in the phase coexistence region of LE/G. The isotherms, presented in figure 2 indicate no significant effect of either solute on the phase behavior. In the phase coexistence region of the LE- and G-phase at an area per molecule of ≈ 38 Å per molecule, the surface pressure is below the resolution of the Wilhelmy system, hence no effect of MBP or SDS was observed on the phase diagram.

In this experiment, one LE facet is cut with optical tweezers and the subsequent relaxation is monitored as a function of time. The relaxation length of the LE facet seen in figure 1 was plotted as a function of time in figure 3.

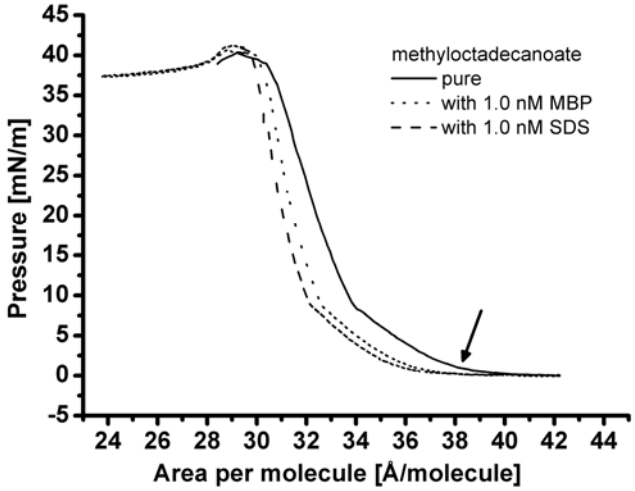


Fig. 2. Surface pressure-area isotherms of a methyl octadecanoate monolayer at T= 27.5°C without and with 1.0 nM of MBP or SDS dissolved in the subphase. All line tension measurements were performed when the area per molecule corresponds to the arrow indicated in the graph, well within the LE/G coexistence region.

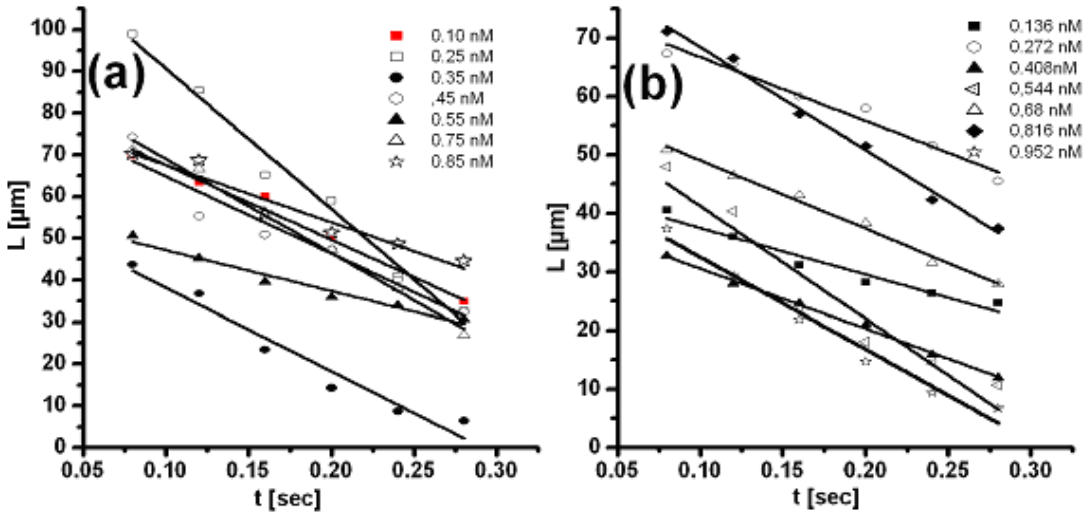


Fig. 3. Plot of the relaxation length of the LE facet as a function of time after, switching off the tweezers, for (a) MBP and (b) SDS at different concentrations. The shortening velocity is of the order of 200±20 µm/s.

Figure 3 shows the relaxation behavior of the LE facet of an octadecanoate monolayer with different concentrations of MBP or SDS dissolved in the subphase. The relaxation length depends on the solutes concentration indicating that both of them affect the rheological properties of the monolayer as they incorporated into it. A linear dependence of the relaxation length of the LE facet on time was observed. The shortening velocity $v=dL/dt$ was derived from the slopes of these lines lies in the range 180-220 μm/s depending on the subphase concentration.

The calculated values of the line tension make use of the surface shear viscosity of the monolayer with different MBP and SDS concentrations from the data obtained in (Khattari *et al*., 2005) along with viscosity of water $\eta_w$ (T=27.5°C)= 0.7 mPa.s. We used this information about the monolayer reheological properties together with Eq.(3) to infer the line tension values at different concentrations. It should be noted here that the temperature difference between the experiments performed in this work and these presented in the above reference may cause an experimental error in the line tension values presented in figure 4.

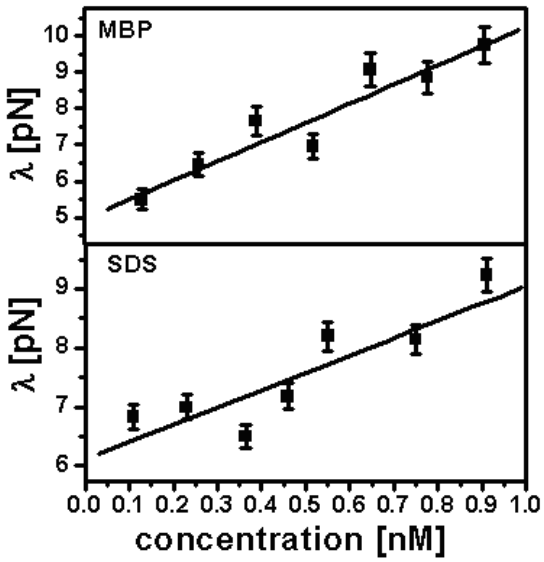


Fig. 4. Line tension of the LE/G phase boundaries of methyl octadecanoate upon adsorption of SDS and MBP. The data are extracted from the relaxation experiments presented in figure 3. An increase of the line tension as a function of MBP or SDS subphase concentration is observed.

Figure 4 shows the line tension values of the LE/G boundary as a function of the concentration, c, of MBP or SDS dissolved in the aqueous subphase of depth ≈ 3 mm. The figure reveals a linear increase in the line tension of LE/G boundary $\lambda=\gamma c$ ($\gamma$=0.8) with the subphase concentration. Assuming that all the MBP adsorbs to the monolayer when interacts electrostatically with the monolayer, one expects that MBP undergoes conformational changes from random coil in the aqueous solution to α-helix or β-sheets. Without such conformational changes upon adsorption, there is no way to explain how such a tiny amount of the protein could possibly have any effect on the inter-phase line tension. Also, the arrangement of MBP at the monolayer surface might not be entirely statistical. An arrangement of MBP in the form of 2d network could explain part of the line tension increase.

Unfolding of the protein at the bilayer membrane has been reported previously using different techniques (Keniry *et al*., 1981). This work shows that there is an intimate relation between structural properties of the protein (e.g. secondary and tertiary) and folding. It can be unraveled when working at concentrations below the overlap concentration of MBP, where entanglements between the proteins at the surface are negligible and do not yet affect the dynamic properties such as the surface rheological properties of the membranous lipid environment. It is well known that entanglements are impossible for a simple anionic highly viscous soluble surfactant like SDS (Khattari *et al*., 2005). However, the same linear increase in the line tension with the SDS subphase concentrations is observed similar to that of MBP. For solid particles or highly viscous 2d droplets immersed into a monolayer, the effective line tension of the suspension will depend on both the area fraction of suspended droplets and the effective area per molecule of the monolayer. At the same area fraction, smaller particles lead to higher effective line tensions than larger particles because a larger fraction of hydrodynamic interaction is mediated via the 2d monolayer, while larger particles mediate the hydrodynamic interaction more via the subphase (Keniry *et al*., 1981; Mendz *et al*., 1981).

## CONCLUSIONS

The presence of protein or surfactant at different concentrations in the subphase underneath the monolayer results in an increase in the line tension. Up to our knowledge, this is the first experimental work which takes into account the coupling between the aqueous subphase with the two-dimensional monolayer system at air/water interface. This work has merged the theoretical models developed by different groups to calculate the line tension in the presence of MBP or SDS in the aqueous subphase.

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