THERMOMECHANICAL PROPERTIES OF LANGMUIR-BLODGETT MONOLAYERS OF (*R*)-AND (*S*)-TUBERCULOSTEARIC ACID

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1. Introduction

Mycobacterium tuberculosis is a bacillus responsible for one of the most widespread diseases in the world – tuberculosis. Its uniqueness and high persistencelies partly in a relatively robust cell membrane consisting of glycerolipids composed of amixture of (*R*)-tuberculostearic acid [(R)-STA]and pure stearic acids in a mutual ratio 50/50. In this work we studied the mechanical and thermomechanical properties of tuberculostearic acid monolayers on a water surface utilizing the Langmuir-Blodgett (LB) technique. The known acids (stearic, palmitic and oleic) have been well examined [1–4], whereas the mechanical properties of tuberculostearic acids are not fully known. This is due to the fact that the synthesis of (R)-STA and (S)-STA is possible, but pure samples are only available in limited quantities [5].The present research is based on a comparison of properties of various fatty acids with similar hydrocarbon chains, such as oleic acid, palmitic acid, stearic acid, (R)-STA, (S)-STA and their mixtures in various molar ratios. Isotherms of the monolayers were measured and analyzed to find the conditions for the creation of separate material phases (gaseous, liquid, solid). The results are applicable to establish suitable models in numerical molecular dynamics of lipid bilayer membranes, or a possible future deposition on solid substrates. Moreover, the mechanical modulus of the materials was computed and finally, the Gibbs free energy of the mixtures was calculated to investigate the miscibility of the components. The mixture of (R)-STA acid and stearic acid with the molar fraction 0.5 (the natural ratio in the bacteria) shows a different elastic modulus and Gibbs free energy compared to the other ratios, which makes it ideal as a building element for the tuberculosis bacteria cell membrane.

2. Experimental notes

The experiments of layer compression and measurement of isotherms were carried out using the LB trough 611M from NIMA Technology. The trough is equipped with a motorized PC controlled mechanical barrier providing the compression speed of 10 cm^2/min for each experiment. Surface pressure was measured by aPS4 sensor – a microbalance with the Wilhelmy plate made of filtration paper with a porosity of 8–10 μm. Palmitic and stearic acids were purchased from Sigma-Aldrich, oleic acid from Fischer Scientific and (R)-STA, (S)-STA were synthetized [5]. Spectrophotometric grade chloroform from Sigma-Aldrich was used as a solvent.Solutions of fatty acids with a concentration of 1 mg/mL were prepared and applied on the subphase – deionized distilled water from a Millipore Simplicity device. A constant temperature of 20.5 °C was maintained by are circulating cooler Julabo FL300.

The substances were applied on the water surface by a micro syringe Hammilton with 50 μL volume.

3. Mechanical properties of straight-chain and bent-chain fatty acids monolayers

The comparison of two types of isotherms can be clearly seen in Fig. 2. The curve of stearic acid is in correspondence with the results in the literature[2, 3]. There are two groups of curves plotted in the graph corresponding to different types of molecules. The two left-shifted lines belong to stearic and palmitic acid. These molecules are simple straight-chain hydrocarbons with 18 carbons for stearic acid ($C_{17}H_{35}COOH$) and 16 carbons in the case of palmitic acid ($C_{15}H_{31}COOH$). The carboxylic group COOH is located at the end of the chains and forms a hydrophilic head of the molecule. It lies at the water surface in the LB experiment. On the other side, the hydrophobic carbon tails are stretched away from water. When the solid phase is reached all the chains are perpendicular to the water surface, mutually almost parallel and the molecules are in close vicinity to each other (the steepest parts of the left-shifted curves – Fig. 2). The second, right-shifted group of curves, describes the behavior of molecules with bent chains. Although the hydrocarbon chain of oleic acid ($CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$) has also 18 carbons like stearic acid, the presence of the double bound in the middle causes bending of the chain and determines its mechanical properties. The obtained isotherm is also consistent with the literature [4]. A similar effect is observed for (R)-TSA and (S)-TSA. Stearic acid is the chemical basis of the both molecules, to which a methyl group is attached in the middle of the hydrocarbon chain. This modification causes a very similar spatial arrangement of the chain compared to oleic acid. The chain is not straight and the physical properties of such a monolayer are completely different from the original stearic basis. Bent molecules are not able to stick in parallelout of the water surface. They are naturally placed and thermally moving in many different conformations depending on the surface pressure and temperature and thus cannot be compressed so as to occupy such a small area as stearic or palmitic acids.

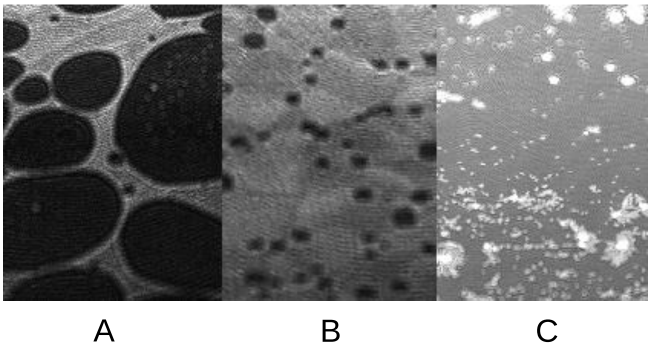

Fig. 1: Pictures from a Brewster angle microscope (BAM) of different phases during the compression of stearic acid monolayer on water surface: A) condensed liquid; B) solid phase; C) situation just after the monolayer collapse.

Three clearly distinguishable regions can be identified in the stearic or palmitic acids isotherms in Fig. 2. The first one runs to a molecular surface area of25 $Å^2$ and represents the gaseous phase where the molecules do not interact. Second, from 25 to 20 $Å^2$ is the condensed liquid phase –weak intermolecular interactions start and the forming layer is observed by BAM (Fig. 1A). The steepest part of the isotherm corresponding to the solid state (BAM photo Fig. 1B). Subsequently, the monolayer collapses at the pressure of ~60 mN/m in the case of stearic acid. Individual floes of the cracked monolayer overlap and separated islands of coagulated substance appear on the surface (Fig. 1C, white grains).

Further, the isotherm rapidly falls down as a consequence of sudden releasing of the surface pressure.

The oleic acid, (R)-TSA as well as (S)-TSA start with their liquid phase much earlier, at ~50 Å^2. A bent-chain molecule occupies larger surface area and is able to interact with its neighbors at a greater distance. However, this interaction is much weaker than that for the straight-chain molecule. The three right-shifted isotherms arise slowly and the phase transitions are smooth. The monolayer never reaches solid phase, it transfers from so-called condensed liquid to expanded liquid phase and finally, at a pressure ~30 mN/m, the substance smoothly changes into a limp multilayer structure. Further, the pressure remains practically constant against the area, not mention that the term "area per molecule" is meaningless when we are not speaking about a monolayer.

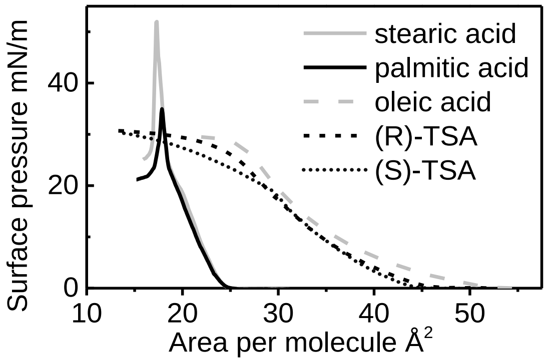

Fig. 2: Experimentally obtained isotherms of monolayers of the fatty acids.

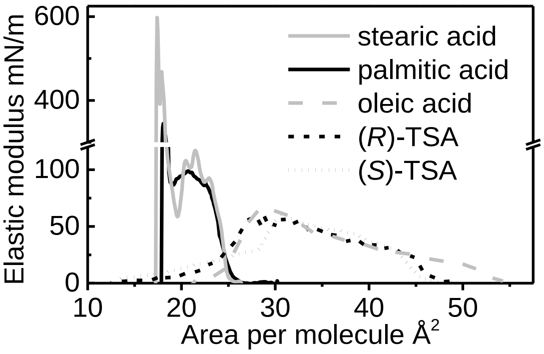

Fig. 3: Elastic moduli of the examined substances.

The mechanical similarities are also apparent from Fig. 3, where the elastic modulus of the monolayers in each phase are plotted. These moduli were calculated from the experimentally obtained isotherms using following formula [6]:

$$E = -A\left(\frac{\partial \pi}{\partial A}\right)_T \tag{1}$$

where E is the elastic modulus, A is the area per molecule, π is the surface pressure and T is temperature. While the straight-chain molecules havea high modulus (from 100 mN/m to 400 mN/m in the solid phase), the maximum modulus of the bent-chain molecules is ~60 mN/m at an area ~30 Å^2/molecule. This suggests, that the solid state is not reached and the monolayer remains in a so-called expanded liquid state, as it was observed e.g. by Mao [4]. Further compression does not increase the modulus, because the monolayer does not exist anymore. Remarkable is the fact, that the elastic modulus of the three molecules (oleic acid, (R)-STA and (S)-STA) has almost the same value.

4. Mixtures of (*R*)-tuberculostearic and stearic acid

To examine the properties of the mixture of (R)-TSA and stearic acid, which represents the fatty acid composition of the cellular membrane of *Mycobacterium tuberculosis*, mixtures with an (R)-TSA molar fraction 0, 1/4, 1/2, 3/4 and 1 were prepared and measured. The concentration of the solutions in chloroform were always 1 mg/mL. The obtained isotherms are plotted in Fig. 4. The extreme mixtures (pure stearic acid – molar fraction 0 and pure (R)-TSA – molar fraction 1) have known isotherms with recognizable phases and monolayer collapse in the stearic acid isotherm and slow smooth raising with a terminate constant plateau in the (R)-STA curve. However, the presence of stearic acid is obvious in the mixtures with molar fractions of (R)-STA 0.5 and 0.75 as a later monolayer collapse.

The physical interaction of substituents in mixtures can be estimated by analysis of surface excess and Gibbs free energy.The Gibbs free energy ΔG of the mixture is given by [6]

$$\Delta G = \int_{0}^{\pi} \left(A_{12} - x_1 A_1 - x_2 A_2 \right) \mathrm{d}\pi \qquad (2)$$

where A_{12} is molecular area of the mixture at a given pressure, A_1 and A_2 are occupied molecular areas of single monolayers, x_1 and x_2 are molar fraction of the mixture substituents.

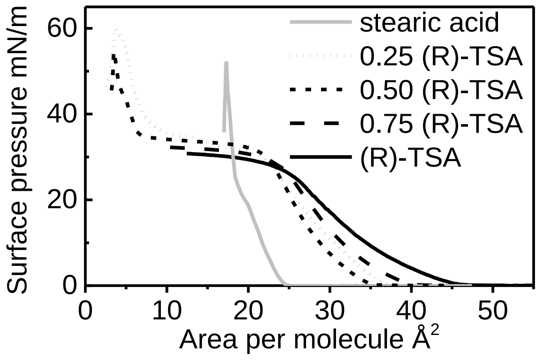

Fig. 4.: Isotherms of the mixtures and the pure substances (R)-TSA and stearic acid.

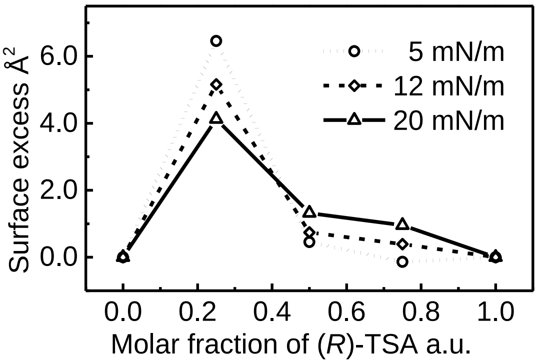

Fig. 5: Area excess of the mixtures of (R)-TSA and stearic acid.

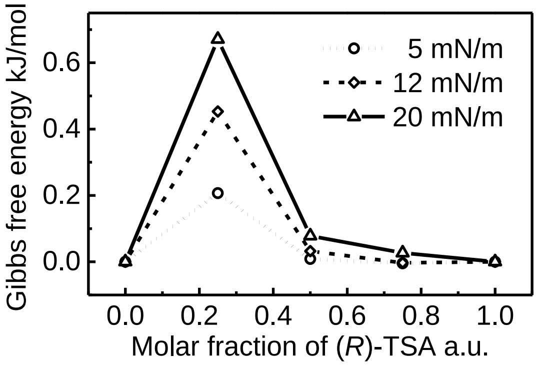

Fig. 6.: The Gibbs free energy of the (R)-TSA and stearic acid mixtures with different molar fractions at different surface pressures.

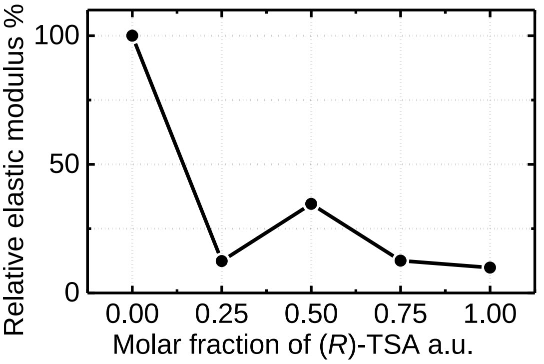

Fig. 7: Comparison of the maximum elastic moduli of the (R)-TSA and stearic acid mixtures. The values for the pure substances can be also found seen in Fig. 3.

High surface excess and high positive Gibbs free energy indicates a weak miscibility, repulsion forces prevail in the monolayer interactions and the substituents are held largely in separate domains of the surface. As can be seen from the graphs in Fig. 5 and Fig. 6, such a situation is significant at the (R)-STA molar fraction 0.25. The surface excess decreases with increasing pressure, the Gibbs energy rises markedly. A different situation occurs for the higher molar fractions: from 0.5 the Gibbs free energy is close to 0 and neither repulsion nor attraction forces dominate, resulting in near ideal mixture. The Gibbs free energy as well as the surface excess increase only slightly with the rising pressure. The noteworthy outcome follows from Fig. 7 where the maximum elastic moduli of the individual mixtures relative to the modulus of pure stearic acid are plotted. Except for that, the molar fraction 0.5 exhibits evidently greater elastic modulus than the other mixtures. Meaning that, a membrane of such mixture could be stiff enough even though the phase state is not purely solid.

5. Conclusion

Comparing the similarities in the isotherms of fatty acids leads to the deduction that the formation of a monolayer is affected mainly by the spatial shape of a hydrocarbon chain. Whilst the linear straight chains comprise condensed liquid and solid phase, bent-chain acids can achieve only expanded liquid states. From there, the unknown substances in molecular modeling of mechanical properties of lipid bilayer membranes can be probably represented by the models with the similarly shaped molecular chain. By examining the mixtures of *(R)*-tuberculostearic acid and stearic acid we found that the maximum elastic modulus is reached for the molar fraction equal to 0.5 compared to the other mixtures. The Gibbs free energy was near to zero for the mixtures with the value of molar fraction 0.5 and higher indicating a thermodynamically balanced composition. Taking these findings into account, such molar fraction seems to be ideal for the construction of the cell membrane of the extremely persistent organism *Mycobacterium tuberculosis*. However, this hypothesis needs to be verified in further experiments with mixtures at various temperatures including the physiological temperature of the human body.

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