NONCOVALENT BONDING IN MIXED MONOMOLECULAR FILMS OF ACRYLIC POLYMERS AND LYSOZYME AND FIBRINOGEN

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Goals

- Investigate the effects of polymer structure on surfactant properties
- Study binding between polymer films and proteins
- Study the effect of protein size and structure with polymers

Introduction

Surfactant compounds have molecular structures that feature hydrophilic and hydrophobic functional groups that allow them to spread on a liquid surface without dissolution. This characteristic allows these molecules to assemble on a liquid surface a form a film that is only one-molecule thick.

A Langmuir trough is used to compress and expand surfactant molecules on a liquid subphase and simultaneously measure the surface tension, γ, as a function of surface area. The difference between the surface tension of a clean surface, γ_0, and the surface with film, γ, is called the surface pressure, π.

$$\pi = \gamma_0 - \gamma$$

The shape of the isotherm provides clues about the interaction between the film components, and structure of molecules in the film. Extrapolation of the isotherm to the x-axis allows determination of the area occupied by molecules in the film called the limiting area.

Experimental

Apparatus: A Langmuir Trough shown in Figure 1 (Kibron MicroTrough S) made of a glass-coated metal container holds the liquid subphase. Two Teflon barriers are used to compress and expand the film between them. Surface tension is measured by a platinum rod in contact with the subphase as the barriers move.

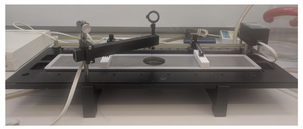
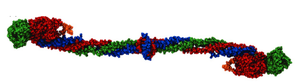
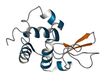


Figure 1. Kibron MicroTrough S with Teflon Barriers

Proteins: Both proteins were sourced from Sigma Aldrich.



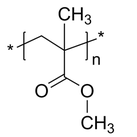
Fibrinogen
340 kDa



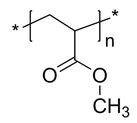
Lysozyme
14 kDa

Figure 2. Molecular structures of fibrinogen and lysozyme.

Polymers:



Poly (methyl methacrylate)
[PMMA]
M_W = 102,600, M_N = 48,300
(AIdrich Chemical Company)



Poly(methyl acrylate)
[PMA]
M_W = 30,700, M_N = 10,600
(Scientific Polymer Products)

Figure 3. Structure and source of PMMA and PMA.

Results

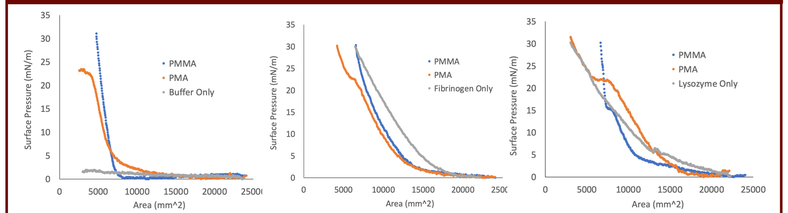


Figure 2. The compression isotherms of PMA and PMMA on buffer (left), fibrinogen (center), and lysozyme (right) are shown above.

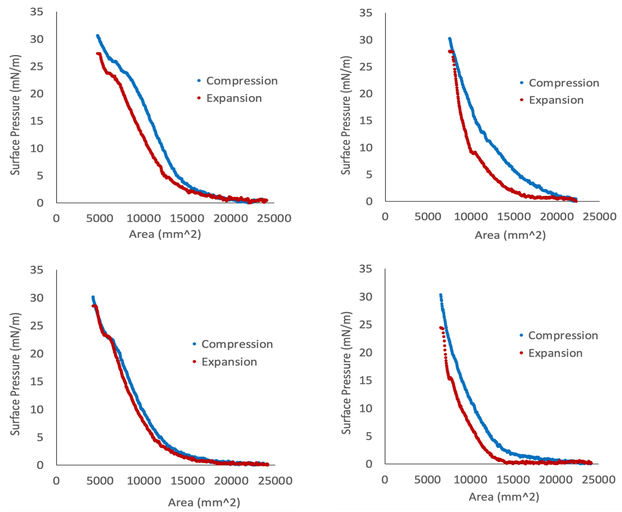


Figure 3. The compression and expansion isotherms for lysozyme (top) and fibrinogen (bottom) show the impact of protein size on the reversibility of binding. The isotherms for PMA (left) and PMMA (right) show the difference the flexibility or rigidity of polymer backbones has on the reversibility of binding.

Experimental cont.

Protocol:
Salinated potassium phosphate buffer (pH=7.4, 140 mM NaCl) was prepared and protein (0.7 mg/L) was dissolved as needed. Following thorough cleaning and rinsing, pure buffer or protein subphase was added to the trough. Approximately 2 µL of 1 mg/mL polymer solutions in chloroform (HPLC grade, Sigma-Aldrich) solvent were administered to the subphase between the Teflon barriers using a gas-tight 10 µL syringe (Trajan). Waited 5-minutes to allow solvent to evaporate. A compression isotherm was measured. Waited 5 minutes to allow film material to reorient. An expansion isotherm was measured. Each measurement was repeated in triplicate.

Results and Discussion

Compression isotherms indicate that PMA films are more flexible than PMMA due to effect of steric hindrance introduced by the methyl group. (Figure 2a)

The polymers form mixed monomolecular films with lysozyme and fibrinogen as shown in Figures 2b and 2c. Proteins enter the film from the subphase, whereas the polymers are introduced to the surface and do not dissolve in the subphase. Fibrinogen forms a miscible monolayer only with PMMA as shown by the smooth isotherm in Figure 2b. All other isotherms in Figures 2b and 2c have inflection points indicating that the mixed monolayers are immiscible.

The compression and expansion isotherms in Figure 3 are not perfectly superimposed. This feature is called hysteresis. Negative hysteresis occurs when molecules adhere due to intermolecular forces and surface pressures are lower for the expansion isotherm than for the corresponding compression isotherm.

Hysteresis is larger for mixed monolayer films composed of polymer and the smaller lysozyme protein than for the films containing polymer and the much larger fibrinogen protein. This suggests that binding interactions between the polymer and protein are effective at changing the film properties only when the protein is relatively small. The much larger protein is unaffected by the presence of polymer.

References

Ratner, B.; Hoffman, A.; Schoen, F.; Lemons, J. *Biomaterials Science*. Academic Press: San Diego, 1996.