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CHEMICAL INTERACTIONS BETWEEN PROTEIN MOLECULES
AND POLYMER MEMBRANE MATERIALS

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INTRODUCTION

During the past year, we have used the Surface Forces Apparatus (SFA) to measure the intermolecular forces between a model protein (hen egg-white lysozyme) and a model hydrophilic surface (mica), between lysozyme and itself and between lysozyme and a model hydrophobic surface composed of a crosslinked alkoxysilane surfactant (hexadecyltriethoxysilane, HTE). As expected, repulsive forces are dominant between the hydrophilic surfaces with the same charge (lysozyme-lysozyme) while attractive forces are dominant between oppositely charged surfaces (lysozyme-mica) and between the lysozyme and the hydrophobic surface. The DLVO theory for charged surfaces was found to agree with the results of the lysozyme-lysozyme interaction.

Efforts also have been focused on trying to create a well-formed, defect-free monolayer of the HTE on the surface of the mica using a Langmuir-Blodgett (LB) apparatus. A smooth, defect-free surface is desired for the intermolecular force studies. Atomic force microscopy has been used to determine the topography of the HTE films.

EXPERIMENTAL RESULTS

Langmuir-Blodgett monolayer formation

The Langmuir-Blodgett apparatus was used to place a monolayer of the HTE surfactant onto mica for use in the SFA. The LB apparatus consists of a trough with a dipping well, double barriers, a surface pressure measuring device and a dipping mechanism. For the experiment, the trough is filled with 10^{-2} M nitric acid. The mica substrate is then submerged below the air/water interface so that a single layer will be deposited. The HTE, dissolved in 95 % chloroform/5 % ethanol (v/v), is introduced to the interface. The acid is allowed to hydrolyze the HTE for a preset amount of time and then the double barriers are closed to obtain a preset surface pressure. This surface pressure is maintained for a certain amount of time to allow for the condensation reaction between the HTE monomers to create a cross-linked, two-dimensional film on the acid surface. The mica is then brought through the interface so that the film is deposited on the mica surface. The deposited film reacts with the

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mica surface which allows a chemical bond between the film and the mica to form. The reaction is completed by annealing the film under vacuum. It is important that the film is cross-linked and reacted to the mica so that when a SFA experiment is performed, the film will not delaminate from the mica and float away from the surface of interest.

- Originally, a different surfactant (octadecyltrimethoxysilane, OTM) was used in the study. Upon study of this surface in the SFA it was seen that purely electrostatic interactions were occurring between the mica and the OTM layer. Since the OTM reacts with the mica so that a fatty chain is extending from the surface, this was a bit puzzling. Atomic force microscopy was used to look at the surface and it was seen that the layer was very porous. The surface porosity of the film was estimated to be 16.5% with no large regions of continuous film. Based upon changes in the film upon changes in the temperature that they were formed led us to determine that the reaction was occurring much faster than desired. We then used the HTE monomer because the leaving group is a little bulkier. Upon optimization of the reaction times in the LB deposition step, we were able to achieve a film with a surface porosity of 1.7%. Defects were still evident on the surface, but they were not as numerous and there were much larger regions of the well-formed film. See Fig. 1. The contact area for force measurements in the SFA is $1 \mu\text{m}^2$ whereas the film had continuous regions 1 to 2 orders of magnitude larger, thus the film may be safely used for measurements in the SFA.

Surface Forces Apparatus measurements

The SFA was used to look at the interaction between the lysozyme and itself and between lysozyme and both a hydrophobic and hydrophilic surface. In the lysozyme-lysozyme experiment, the SFA was injected with a lysozyme solution so that both surfaces were covered. After 4 hours of adsorption, this solution was drained out of the SFA and was replaced by pure water. The interaction followed DLVO theory for electrostatic repulsion of two like charged surfaces. This is expected since the lysozyme ($pI=11$) has a large positive charge at the pH of the experiment ($pH=4$) which was chosen since lysozyme is known to have very little self-aggregation at low pH values. The adhesion between the two lysozyme layers was measured to be on the order of 3-4 mN/m which correlates with published data.

The lysozyme-mica interaction also followed an expected trend. This experiment was performed similar to the lysozyme-lysozyme interaction experiment, except that only one surface was introduced to the lysozyme solution. In water, mica is negatively charged so an attractive force should be seen at $pH=5$ when the lysozyme covered surface is brought into proximity of the mica surface. This attractive force was seen in the experiment along with an adhesion relating to the lysozyme on the mica of 40 mN/m.

The lysozyme-HTE interaction once again followed the expected trend. It is known that proteins adsorb to hydrophobic surfaces rather rapidly and extensively, thus we would expect an attractive force. For this experiment, the lysozyme was allowed to adsorb onto one surface as in the case for the lysozyme-mica interaction, while the HTE was placed on the other surface. The expected attractive force was seen and it was rather long range with the attractive force being evident as far away as $3000\text{\AA}$. The adhesion seen between the HTE and the lysozyme was 44 mN/m. See Fig. 2.

Low temperature plasma surface modifications

Water plasma treatment and plasma induced graft-polymerization were both used to modify the surfaces of two commercially available membranes. These same techniques have and will be used to prepare different surfaces for the SFA and the AFM studies.

For water plasma treatment, the membrane films (polyether sulfone and polyacrylonitrile) were exposed to H₂O/He plasma (0.2 Torr, 25 W, 30 s) and then exposed to air or water for different periods. The films or membranes were then not treated, acid treated (10 mM HNO₃), or covered with OTM and annealed (2 h, 100 °C).

For plasma induced graft-polymerization, the membrane films were exposed to He plasma (0.2 Torr, 25 W, 30 s) and exposed to air and then immersed in nitrogen degassed monomer solution (1 or 5 % HEMA in H₂O, 50 °C) with Soxhlet extraction in H₂O/EtOH 9/1, for 24 h.

All the membranes were characterised with ESCA, ATR/IR and contact angle and then tested for improved ultrafiltration performance with 1 g/l lysozyme solution at pH 4.5. Improved retention and lower fouling was observed for several treatment methods.

Publications during this period

The following papers relevant to this research were published and submitted:

1. Pincet, F., Perez, E. and Belfort, G. (1994), Do denatured proteins behave like polymers?, *Macromolecules*, 27 (12), 3424-3425.
2. Pincet, F., Perez, E. and Belfort, G. (1994), Molecular interactions between proteins and synthetic membrane polymer films, *Langmuir*, resubmitted after reviews.
3. Ulbricht, M and Belfort, G. (1994), Low Temperature Surface Modifications of Polyacrylonitrile Ultrafiltration Membranes - 1. Plasma Treatment Effects, *J. Applied Polym. Sci.*, submitted.

Figure 1. Atomic force micrograph of a model hydrophobic surface composed of a crosslinked alkoxy silane surfactant (hexadecyltriethoxysilane, HTE).

Figure 2. Normalized force versus distance of separation for mica surfaces covered with hexadecyltriethoxysilane, HTE and adsorbed lysozyme and immersed in a 50 mg/l lysozyme solution at 20 °C and pH 4.6 for 5.5 h.