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SELF-ASSEMBLED MONOLAYERS AS MODELS FOR STUDYING PROTEIN ADSORPTION TO POLYMER SURFACES

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ABSTRACT

Self-assembled monolayers (SAMs) of functionalized alkanethiolates on gold are a well-characterized system for studying the interfacial properties of organic materials. We have used SAMs as models for the surfaces of organic polymers and used them to study the adsorption of proteins onto organic materials. We have formed SAMs from mixtures of alkanethiols in which one alkanethiol is hydrophobic and the other is terminated by a short ($2 \leq n \leq 17$) oligomer of poly(ethylene oxide). These "mixed" SAMs effectively resist the adsorption of fibrinogen from moderately concentrated (1 mg/mL) solutions. Protein adsorption begins when $\leq 5\%$ of the accessible area of the surface consists of hydrophobic groups. These findings suggest that real protein-resistant monolayers must present an almost defect-free surface of oligo(ethylene oxide) groups in order to eliminate adsorption.

INTRODUCTION

When long-chain alkanethiols [$HS(CH_2)_nR$, $n \geq 10$, R no larger than the cross-section of the polymethylene chain] adsorb from solution onto the surface of a metal (Au, Ag, Cu), they create an oriented, ordered monomolecular film [1, 2]. The properties of the interface between such a self-assembled monolayer (SAM) and the water or air in contact with it are dominated by the properties of the tail group, R [3]. The surface–air interface of the SAM can be varied widely by changing the tail groups, either by synthesis prior to assembly, or by the co-adsorption of two or more alkanethiols from solutions containing mixtures of alkanethiols [4–6]. The Au–S bond, in particular, is very specific: the Au–S bond is formed preferentially in the presence of a wide variety of tail groups, including many that are of biological importance (carboxylic acids [7], alcohols [3], amides [8], carbohydrates [9], etc.). SAMs are, therefore, potentially useful systems for studying the interactions of biological materials with the surfaces of organic solids.

We have reported [9] that SAMs can be used to study the non-specific adsorption of proteins to organic surfaces, a phenomenon of practical significance: non-specific adsorption has been implicated as one cause of failure in biomedical materials [10, 11] and in the fouling of membranes and other separation devices [12]. Furthermore, the design of sensors for use in biological milieux requires that these sensors not be fouled by the adsorption of undesired proteins.

In the last decade, several groups have shown that the amount of protein adsorbed to bulk hydrophobic polymers is dramatically reduced by grafting poly(ethylene oxide) (PEO) chains to the surface of the polymer [13–19]. The PEO is usually attached to the the surface at one end of the chain. We recently demonstrated that SAMs comprising mixtures of two alkanethiols ("mixed" SAMs) in which one component bore a hydrophobic tail group [$HS(CH_2)_{10}CH_3$] and the other a hexamer of

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ethylene oxide [$HS(CH_2)_{11}(OCH_2CH_2)_6OH$], resisted the adsorption of several proteins [9]. Figure 1 is a schematic representation of one of these mixed SAMs. We proposed to use mixed SAMs formed from $HS(CH_2)_{10}CH_3$ and $HS(CH_2)_{11}(OCH_2CH_2)_nOR$ to model the polymeric systems and to understand the influence of the length and number of oligo(ethylene oxide) chains in the interface upon the resistance of these materials to protein adsorption.

RESULTS

Figure 2 shows the synthetic scheme we used to prepare the oligo(ethylene oxide)-terminated alkanethiols, $HS(CH_2)_{11}(OCH_2CH_2)_nOR$. This scheme worked well for all the compounds we have prepared (R = H, n = 1–7; R = CH_3, n = 6, 17).

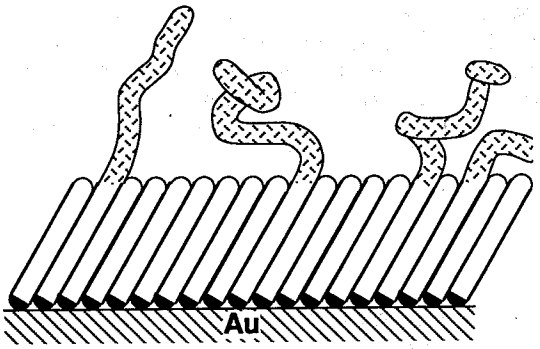
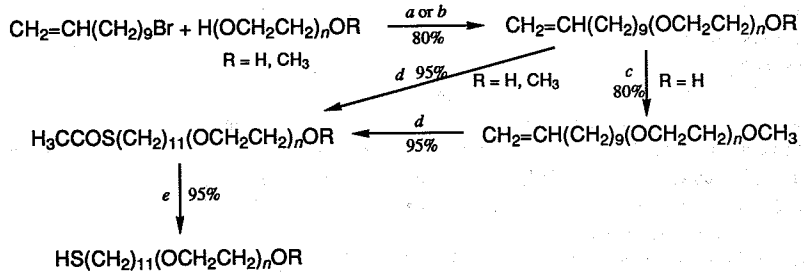


Figure 1. A schematic representation of the structure of a mixed SAM containing $SC_{11}E_6OH$ and $SC_{10}CH_3$. The oligo(ethylene oxide) chains are flexible. In contact with water, a good solvent for OCH_2CH_2 groups, the chains are self-avoiding and tend to form *gauche* bonds (ref. 20). The areas of the hatched regions are approximately proportional to the cross-sectional area of the E_6OH chain.



[a]aq. NaOH, 100 °C [b]NaH, DMF [c]KOH, CH_3I, DMSO [d]CH_3COSH, AIBN, THF [e]HCl, MeOH.

Figure 2. Synthesis of oligo(ethylene oxide)-derived alkanethiols.

We prepared two-component mixed SAMs from $HS(CH_2)_{10}CH_3$ and an oligo(ethylene oxide)-terminated alkanethiol and determined their compositions using high-resolution X-ray photoelectron spectroscopy (XPS) of the O(1*s*) electrons. We then used ellipsometry to determine the amounts of several different proteins that adsorbed to each mixed SAM from aqueous solutions. The SAMs were immersed in 1-mg/mL solutions of protein in 0.01 M sodium phosphate buffer at pH 7.5 for 2 h at 20–25 °C, then removed from solution, rinsed with distilled water, and dried with a stream of nitrogen. The ellipsometric measurements were taken on the dry SAMs before and after immersion in the protein solution. Our measurements, therefore, represent the relative amounts of protein that remained adsorbed after the system had experienced a short-term (5 s) shear.

Figure 3 shows the amounts of fibrinogen adsorbed to four series of mixed SAMs derived respectively from $HS(CH_2)_{11}OH$ (E_0OH), $HS(CH_2)_{11}(OCH_2CH_2)_2OH$ (E_2OH), $HS(CH_2)_{11}(OCH_2CH_2)_6OH$ (E_6OH), and $HS(CH_2)_{11}(OCH_2CH_2)_{17}OCH_3$ ($E_{17}OCH_3$). Three significant observations arise from these data: First, SAMs that contained sufficiently high mole-fractions of oligo(ethylene oxide)-terminated alkanethiolates resisted the adsorption of proteins. The ellipsometric thicknesses, XPS spectra, and contact angles of these SAMs were unchanged by the immersion in protein solution. Second, at lower mole fractions, longer chains were more effective than shorter chains at reducting protein adsorption. Near $\chi = 0$, the slopes of the adsorption curves became progressively more negative as the length of the chain increased. Third, for $2 \leq n \leq 6$, the lowest mole fraction at which no protein adsorbed was nearly constant at approximately $\chi = 0.65$. This last observation suggests that some structural feature common to all these systems was responsible for their high resistance to protein adsorption.

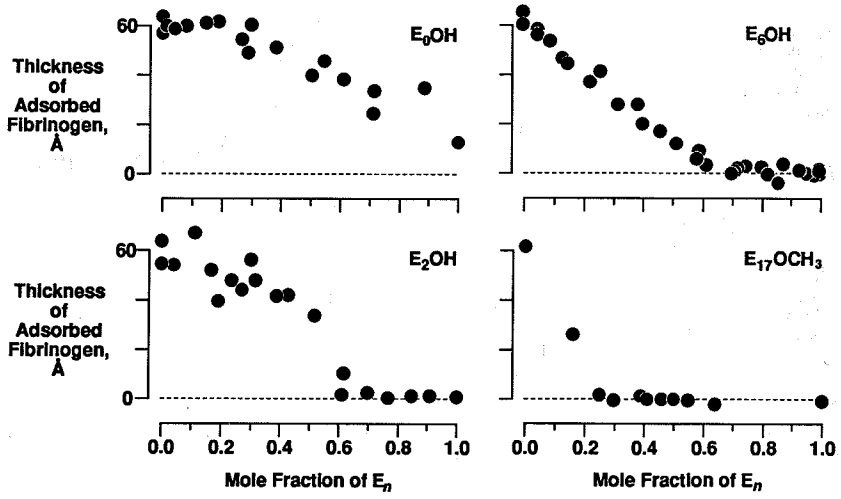


Figure 3. Adsorption of fibrinogen to mixed SAMs of $SC_{11}E_nOR$ decreases as the number or the length of the $SC_{11}E_nOR$ chains increases. The nominal thickness of the adsorbed films of fibrinogen on mixed SAMs containing $SC_{11}E_nOR$ are plotted as a function of the mole fraction of $SC_{11}E_nOR$ chains in the SAM. The values of thickness were determined by ellipsometry. The mole fractions were determined prior to adsorption of protein by X-ray photoelectron spectroscopy. The horizontal and vertical scales are uniform throughout the figure.

Comparison of the data in Figure 3 with the wettability of these SAMs provides insight into the structural origin of the protein resistance of oligo(ethylene oxide) films. Figure 4 shows the maximum advancing and minimum receding contact angles of water on the mixed SAMs from Figure 3. The most important observation derived from Figure 4 is that the concentrations at which the contact angle began to rise and that at which protein began to adsorb were closely related. Indeed, on the SAMs we studied, the observation of advancing contact angles of water greater than that of a SAM formed only from the oligo(ethylene oxide) component were always accompanied by the observation of adsorbed protein. No universal, quantitative correlation between the wettability and protein-resistance of SAMs can be derived from these data, however: SAMs that contained mostly E_0OH were both more hydrophilic and more adsorbent than those that contained mostly E_nOR. Furthermore, oligo(ethylene oxide) chains terminated by methyl ethers had different contact angles but indistinguishable adsorption properties from their OH-terminated analogs. What the data in Figures 3 and 4 *do* show is that the accessibility of hydrophobic regions to the solution phase is perhaps the most important single factor in determining the protein resistance of an oligo(ethylene oxide)-coated surface.

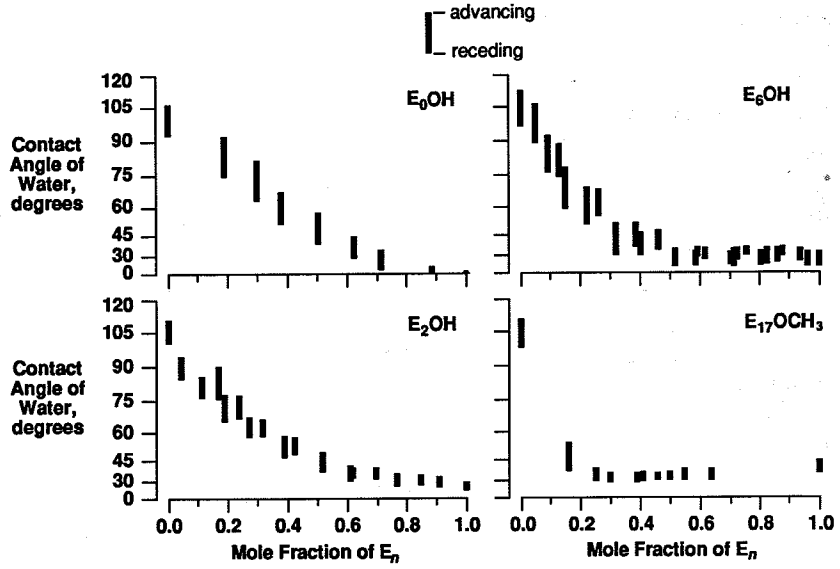


Figure 4. The wettability of mixed SAMs of $SC_{11}E_nOR$—as measured by $\cos \theta_a(H_2O)$ and $\cos \theta_r(H_2O)$—the maximum advancing and minimum receding contact angles (ref. 21) of water on the SAMs—as a function of the mole fraction of $SC_{11}E_nOR$ chains in the SAM. Each symbol in the plots is bounded above by the value of $\cos \theta_a(H_2O)$ and below by the value of $\cos \theta_r(H_2O)$. The length of the symbol represesents the hysteresis in the contact angle of water, $|\cos \theta_a(H_2O) - \cos \theta_r(H_2O)|$. The horizontal and vertical scales of the plots are uniform throughout the figure.

CONCLUSIONS

We observe similar results for each of the five proteins we have examined—fibrinogen, pyruvate kinase, lysozyme, ribonuclease, and chymotrypsinogen. We therefore draw two conclusions from this data: First, the interfacial properties of the surface predominate over those of the protein in determining whether a protein will adsorb to a particular surface. Second, protein resistance in these oligo(ethylene oxide)-derived systems is achieved by a continuous, water-swollen coating of oligo(ethylene oxide), regardless of the length of the oligomer. Whenever hydrophobic "defects" are present, protein adsorption occurs.

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