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Self-Assembled Monolayers of an Oligo(ethylene oxide) Disulfide and Its Corresponding Thiol Assembled from Water: Characterization and Protein Resistance

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Self-assembled monolayers (SAMs) of the disulfide $[S(CH_2CH_2O)_6CH_3]_2$ ($[S(EO)_6]_2$) on Au from 95% ethanol and from 100% water are described. Spectroscopic ellipsometry and reflection-absorption infrared spectroscopy indicate that the $[S(EO)_6]_2$ films are similar to the disordered films of $HS(CH_2CH_2O)_6CH_3$ ($(EO)_6$) and $HS(CH_2)_3O(CH_2CH_2O)_5CH_3$ (C_3EO_5) at their protein adsorption minima. The $[S(EO)_6]_2$ SAMs exhibit constant film thickness (d) of 1.2 ± 0.2 nm over long immersion times (up to 20 days) and do not attain the highly ordered, 7/2 helical structure of the $(EO)_6$ and C_3EO_5 SAMs ($d = 2.0$ nm). Exposure of these self-limiting $[S(EO)_6]_2$ SAMs to bovine serum albumin show high resistance to protein adsorption.

Introduction

Surfaces that resist the adsorption of proteins are of significant value to the biotechnology, health care, energy, and maritime industries. The biofouling of surfaces initiated by the random, nonspecific adsorption of proteins results in (a) loss of useful lifespan or efficacy for prostheses,[1] implanted biosensors,[2] contact lenses,[3] and so forth, (b) decreased accuracy and detection level of protein and DNA arrays in drug discovery/clinical diagnoses,[4] (c) exogenous species invasion in isolated ecosystems,[5] and (d) increased costs for ship fuel consumption, ship hull cleaning, and power plant intake and outflow pipeline maintainance.[6] Surface modification with polymers or self-assembled monolayers (SAMs) is well-known to reduce such nonspecific adsorption; however, complete rejection of all proteins for extended periods has not been reported.[7,8] As argued earlier,[8] SAMs can provide insight into understanding, at the molecular level, the mechanism of protein adsorption because of the ability to control surface coverage and structural details and, hence, gain a more precise definition of the surface with which the protein interacts. From previous protein adsorption studies on SAMs, a number of key factors have emerged for resistance to protein adsorption to occur: an interior hydrophilic chemical structure in the chain is necessary,[9] the surface-bound molecules must be conformationally mobile,[10] and the free energies must be near zero or positive for the sum of the water, protein, and surface-bound molecules interacting with themselves and each other.[11] These characteristics are further supported by two additional observations. First, at high packing densities, with the surface-bound molecules confined to an ordered conformation, protein adsorption is always observed[10,12] regardless of the nature of the distal end-group.[13] Second, there is no correlation between protein adsorption and the surface energy as determined by macroscopic contact angle measurements, even though, in general, hydrophobic surfaces adsorb proteins.[14]

The majority of protein adsorption studies on Ag and Au have used oligo(ethylene oxide)-terminated (OEO) compounds, and the protein resistant properties of the resultant SAMs are typically those to which other structural motifs are compared.[15−18] Early studies used water insoluble OEO thiols that contain long polymethylene chains of the general formula $HS(CH_2)_wA(CH_2CH_2O)_xB$, with A = O or CONH, B = H or CH_3, $w \geq 9$, and $x = 1-6$, averages of 17, and averages of 45.[11] From the SAMs of some of these, it was hypothesized, with the aid of *ab initio* calculations, that surfaces with the OEO segment in a helical conformation would result in optimal resistance to protein adsorption.[20] This turned out not to be the case. Highly ordered

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SAMs with nearly all the OEO segments in a 7/2 helical conformation were first obtained using $HS(CH_2CH_2O)_6CH_3$ (abbreviated $(EO)_6$), which does not have a polymethylene chain (i.e., $w = 0$) and where the OEO segment is bonded directly to the thiol group. Protein adsorption experiments showed that these highly ordered SAMs were not protein resistant.[21,22] Subsequent studies of the related $HS(CH_2)_3O(CH_2CH_2O)_5CH_3$ SAMs (abbreviated C_3EO_5) showed that rejection of protein is correlated with the onset of self-rejection, that is, when the SAM packing density's increase begins to slow.[10] This suggested that the onset of self-limiting behavior indicates the existence of a layer of conformationally flexible, surface-bound molecules that adequately screens the underlying substrate. This condition not only provides a practical marker for the preparation protein rejecting surfaces but also may be the common state of protein resistant SAMs, which are *all* disordered.[8−10,12−18,21,22]

Here, we report on the SAMs of the OEO disulfide $[S(CH_2CH_2O)_6CH_3]_2$ (abbreviated $[S(EO)_6]_2$) with a direct comparison to its thiol counterpart $(EO)_6$. We show that the SAMs of $[S(EO)_6]_2$ assembled from water give markedly different monolayer properties from those of $(EO)_6$.[23] The $[S(EO)_6]_2$ SAMs do not attain high packing density and the concomitant order, but do have nearly identical thickness and spectral features of incomplete $(EO)_6$ SAMs that exhibit high resistance to protein adsorption.[10,21]

Materials and Methods[24]

Materials and Reagents. Except for silica gel, all chemicals used in reaction 1 and solvents for the purification of $[S(EO)_6]_2$ were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were used as received. Silica gel (40 μm, 10240-02) was purchased from J. T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ).

Synthesis. Pure $[S(EO)_6]_2$[25] was obtained by flash chromatography (Silica gel, 33 cm × 3 cm, 20% methanol/ethyl acetate) of materials obtained either from the oxidation of $(EO)_6$ either neat or in solution or via reaction 1, which favors disulfide formation at longer reaction times. The synthesis of $(EO)_6$ was described earlier.[21]

$$3Br(CH_2CH_2O)_6OCH_3 \xrightarrow[\text{MeOH, reflux}]{\text{xs } CH_3COS^-} \underset{(EO)_6}{HS(CH_2CH_2O)_6CH_3} + \underset{[S(EO)_6]_2}{[S(CH_2CH_2O)_6CH_3]_2} + 3Br^- \quad (1)$$

Sample Preparation. For spectroscopic ellipsometry on dried SAMs (*ex situ* SE) and reflection absorption infrared (RAIRS) measurements, silicon (100) wafers (Silicon, Inc, Boise, ID) were initially coated with chromium (∼2 nm) and then with gold (∼200 nm) by magnetron sputtering (Edwards Auto 306, U.K.) as described previously.[26] The Au-coated wafers were then immersed in 500, 50, or 5 μM solutions of $[S(EO)_6]_2$ in either 100% H_2O or 95% ethanol (hereafter simply referred to as water and ethanol).

Protein adsorption experiments (see below) were carried out on $[S(EO)_6]_2$ and $(EO)_6$ SE SAMs prepared on 100 nm Au substrates with a 5 nm Ti adhesion layer (Platypus Technologies, Madison, WI). The substrates were cleaned by a UV-ozone treatment for 15 min, soaked in 100% ethanol for 20 min,[27] rinsed with distilled water, and immersed in 0.5 mM aqueous solutions of either $(EO)_6$ or $[S(EO)_6]_2$ for > 18 h. Bare Au surfaces cleaned in the same manner were used as controls. The concentration of the protein solutions used in these experiments was 0.4 mg bovine serum albumin (BSA)/mL phosphate buffer (PBS; Sigma Chemical, St. Louis, MO).

***Ex Situ* SE.** Multiple wavelength *ex situ* ellipsometric measurements were performed on the SAMs using a J. A. Woollam Co., Inc. (Lincoln, NE) M-44 spectroscopic ellipsometer as described earlier.[26]

***In Situ* SE Protein Adsorption.** Multiple wavelength *in situ* ellipsometric measurements of protein adsorption on the SAMs were performed using a Woollam M2000D spectroscopic ellipsometer capable of simultaneously determining the ellipsometric angles Ψ and Δ for 508 wavelengths between 193 and 1000 nm. Measurements were made using the dynamic mode of the instrument, which allowed for real-time observation of developing phenomena. A three-phase model was used to analyze and interpret the data.[28] The model employs a substrate phase with dielectric function ϵ_s, a film with dielectric function ϵ_f and thickness t_m (modeled using the Cauchy function: $n = 1.45 + (0.01\ \text{nm}^2/\lambda^2)$), and the ambient PBS with a dielectric function ϵ_β (modeled with the Cauchy function: $n = 1.328 + (0.0021\ \text{nm}^2/\lambda^2)$). The optical constants for each substrate mounted in a custom-built *in situ* cell, subsequently filled with PBS, were derived by direct inversion of the ellipsometric data taken after conditioning[28] in PBS and prior to the introduction of BSA. These substrates were assumed to have an initial film thickness t_m of 0. Changes to the surface, such as roughening upon exposure to PBS, and the adsorption of various species were modeled by changes in t_m.[28] Model calculations were performed using vendor-supplied software.

RAIRS. The RAIRS data were obtained using a Nicolet Magna-IR model 570 Series II spectrometer (Thermo Nicolet, Madison, WI) with a model FT-85 (85° grazing angle) Spectra-Tech external reflection accessory (Thermo Spectra-Tech, Shelton, CT) as described previously.[29]

Results and Discussion

Table 1 shows the thicknesses for the $[S(EO)_6]_2$ and $(EO)_6$ SAMs from both ethanol and water. Except for the assembly of $[S(EO)_6]_2$ and $(EO)_6$ from water at 5 μM and 500 μM, respectively, all SAMs reach their constant thickness (final state) after 1 day and do not change thereafter in either solvent. As can be seen from the data in Table 1, the final state of the $[S(EO)_6]_2$ films (SE = 1.2 ± 0.2 nm) remains constant over many days, indicating stable, self-limiting films.

RAIRS data of the $[S(EO)_6]_2$ and $(EO)_6$ SAMs from 1400 to 900 cm^{-1} and from 3100 to 2700 cm^{-1} are shown in Figure 1 A and B, respectively. The $[S(EO)_6]_2$ films from ethanol (black) and from water (red) exhibit the spectral features of the disordered $(EO)_6$ films obtained after 1 h from ethanol (blue; $c = 500$ μM; also see Table 2 for RAIRS data of ordered and disordered OEO SAMs).[30] The SE and RAIRS data indicate that the $[S(EO)_6]_2$

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(23) This is in contrast to SAMs of water insoluble disulfide/thiol pairs of the general formulas $[S(CH_2)_wM]_2$ and $HS(CH_2)_wM$, respectively, where $w \geq 9$ and M = end group, that give essentially the same packing densities and thicknesses, regardless of the nature of M. For example, for M = CH_3, see Jung, C.; Dannenberger, O.; Xu, Y.; Buck, M.; Grunze, M. *Langmuir* **1998**, *14*, 1103−1107 and, for M = OEO, see Houseman, B. T.; Gawalt, E. S.; Mrksich, M. *Langmuir* **2003**, *19*, 1522−1531.

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(25) Pure $[S(EO)_6]_2$: 270 MHz ^1H NMR ($CDCl_3$), δ (relative to tetramethylsilane) 3.78 (4H, t, $J = 7$ Hz, $-OCH_2CH_2SSCH_2CH_2O-$), 3.75−3.52 (20H, m, centered at 3.67, {$[SCH_2CH_2O-(CH_2CH_2O)_5-CH_3]_2$}), 3.38 (6H, $[S(CH_2CH_2O)_6CH_3]_2$, 2.88 (4H, t, $J = 7$ Hz, $-OCH_2CH_2SSCH_2CH_2O-$). Low resolution MS (fast atom bombardment): 622.35 (M^+) accompanied with 645.10 ($M + Na)^+$.

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(30) The spectral characteristics of OEO segments in both the ordered 7/2 helical and disordered conformations are discussed in detail in refs 21 and 22. In addition, the data in Figure 1A and B do not show the RAIRS characteristics of ordered SAMs with the OEO segments in all-*trans* conformations obtained for the A = O, B = CH_3, $w = 11$, and $x = 3$ compounds (see ref 12).

Table 1. *Ex Situ* Spectroscopic Ellipsometry Thickness (nm ± 0.1 nm)[a] of $[S(EO)_6]_2$ and $(EO)_6$ SAMs from Ethanol and Water as a Function of Immersion Time and at Various Concentrations

	$[S(EO)_6]_2$						$(EO)_6$			
	μM in H_2O			μM in EtOH			μM in H_2O		μM in EtOH	
immersion time (days)	500	50	5	500	50	5	500	50	500	50
1	1.3	1.3	1.0	1.0	1.2	1.3	1.4	1.7	2.0[c]	1.7
2	1.2	1.3	1.4					1.7	2.0	1.7
5	1.2	1.2	1.4	1.1	1.2	1.3	1.7	1.7		1.8
20	1.1	1.2	1.3		1.0[b]					

[a] Uncertainty = 1 standard deviation. [b] This thickness decrease is indicative of film degradation (RAIRS data not shown). Degradation was seen only from ethanol solutions and generally observed after 5−7 days for both the $(EO)_6$ and $[S(EO)_6]_2$ SAMs. Disulfide degradation does not occur in water. For example, a SAM thickness of 1.1 nm was obtained using a 2-year-old aqueous $[S(EO)_6]_2$ solution (500 μM). ^{1}H NMR data of the subsequently recovered $[S(EO)_6]_2$ (2.4 mg) was identical to that given in ref 25. [c] Thickness for highly ordered, densely packed $(EO)_6$ SAMs with the EO segments in a 7/2 helical conformation (ref 21).

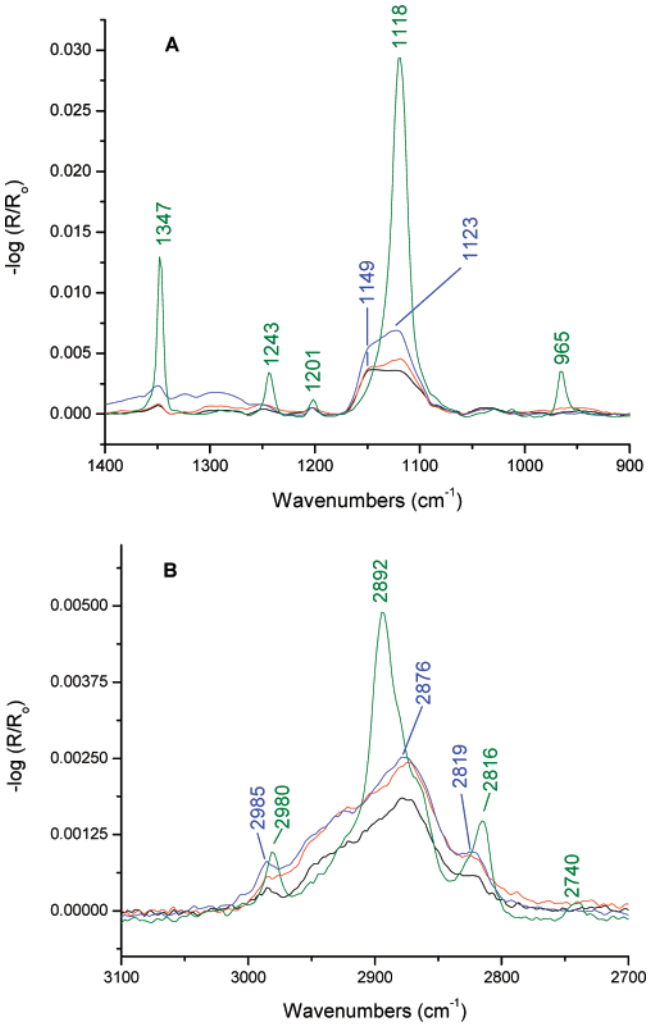

Figure 1. RAIRS spectra of $[S(EO)_6]_2$ films from ethanol and from water (black and red, respectively) after 5 days and of $(EO)_6$ from ethanol after 1 h and 1 day (blue and green, respectively) from (A) 1400−900 cm^{-1} and (B) 3100−2700 cm^{-1}.

SAMs are disordered with reduced packing densities relative to that observed earlier for the fully formed SAMs of $(EO)_6$ and $C_3(EO)_5$.[10,21] Because half of the disulfide is the same length as the thiol, and approximately the same length as C_3EO_5, the $[S(EO)_6]_2$ SAM thickness of 1.2 ± 0.1 nm corresponds to surface coverages of 50−70%,[31] where resistance to protein adsorption was found to be maximized.[10]

Table 2. Spectral Mode Assignments of Ordered (Helical) and Disordered OEO SAMs on Au

		band position, wavenumbers (cm^{-1})	
spectral mode assignment[32]		OEO segment ordered, 7/2 helix	OEO segment disordered
CH_3, stretch, asym		2980	2983−2985
CH_3, stretch, sym		2819	2814−2816
CH_3, rocking		1201	1203
CH_2, stretching (sym)		2892	2876[b]
OEO combination[33]		2741	—[c]
CH_2, wag	$A_2(4)$[34]	1347	—[c]
CH_2, twist	$A_2(5)$[34]	1243	—[c]
C−C & C−O stretch	$A_2(6)$[34]	1118[a]	>1120[d]
CH_2, rocking	$A_2(7)$[34]	965	—[c]

[a] Prominent band with no discernible higher wavenumber shoulder. [b] Broad band from 2950−2800 cm^{-1}. [c] Band in previous column absent or attenuated. [d] Variable, broad band from 1135 to 1120 cm^{-1} usually accompanied by a higher wavenumber shoulder at ∼1149 cm^{-1}.

Interestingly, when final state disulfide $[S(EO)_6]_2$ SAMs were placed in 50 μM ethanol or water solutions of $(EO)_6$ for ∼1 day, the film thickness increased by 0.4 ± 0.1 nm and was comparable to that obtained for $(EO)_6$ SAMs on bare Au substrates (1 day, Table 1). These results might be obtainable either with desorption/substitution of the disulfide by the thiol or with the addition of the thiol (as thiolates on the Au) to the disulfide already present. In the first case, we must argue that the disulfide remains intact but that it binds with less free energy than the thiolates that replace it. In the second case, either the disulfides have reductively dissociated into thiolates or the added thiols (that become thiolates on the surface) can compress the disulfides, reaching a level of coverage above the disulfide self-limit. We believe the first case is the preferred explanation by comparison of our results with the SAMs of another thiol/disulfide pair, cysteine/cystine, assembled from water.[35] Two results from that work stand out. First, the disulfide linkage in cystine remains intact and cysteine does not form disulfides when bound to a Au (111) surface as seen from the electrochemical adsorption peak difference of 0.14 V (more positive for the disulfide). This difference reflects both the molar free energy of the reaction and the surface coverage, which brings up the second result: the final coverages also differ. At neutral pH in 0.1 M $KClO_4$, coverages for the L-cysteine, D,L-cysteine, and D,L-cystine were, respectively, 3.0×10^{-10}, 2.5×10^{-10}, and 1.2×10^{-10} mol/cm^2 (2.4×10^{-10} cysteine thiolate equivalents). In other words, the disulfide SAM has a lower packing density.

The larger Flory radius of the OEO segments in this thiol/disulfide pair amplifies the differences in coverage (∼20% for cysteine/cystine vs ∼40% for $(EO)_6/[S(EO)_6]_2$) and the self-limiting characteristic of this water soluble OEO disulfide. The parallels in the coverages between the cysteine/cystine and the $(EO)_6/[S(EO)_6]_2$ thiol/disulfide pairs suggest that the $[S(EO)_6]_2$ molecules are replaced by $(EO)_6$ molecules and form the same surfaces that $(EO)_6$ does when it alone is available. More direct confirmation of this conclusion requires modified compounds and will be the subject of a subsequent report.

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Table 3. *In Situ* SE Thickness of Adsorbed Bovine Serum Albumin (BSA) on Bare Au and on the $(EO)_6$ and $[S(EO)_6]_2$ SAMs

	surface		
	Au[a]	$(EO)_6$[b]	$[S(EO)_6]_2$
adsorbed BSA (nm)	2.1 ± 0.10	1.13 ± 0.10	0.05 ± 0.04

[a] Platypus Au. [b] Thickness ~ 1.7 nm (Table 1).

The 1.7 nm $(EO)_6$ (~85% coverage[31]) and 1.2 nm $[S(EO)_6]_2$ (~60% coverage[31]) SAMs were exposed to bovine serum albumin (BSA) with the results shown in Table 3. Relative to bare Au, BSA adsorption to the $[S(EO)_6]_2$ and $(EO)_6$ SAMs is ~2% and ~53%, respectively, in agreement with earlier results for the analogous C_3EO_5 SAMs (Figure 1, ref 10). Taken together, the SE, RAIRS, and protein adsorption data suggest that the final form $[S(EO)_6]_2$ SAMs are similar to the ~60% coverage C_3EO_5 SAMs. The near complete inhibition of adsorption of large molecules (proteins) and small molecules (self-rejection) by the lower packing density $[S(EO)_6]_2$ SAMs indicates that the underlying substrate is fully screened by conformationally mobile, surface-bound molecules.

Conclusions

The OEO disulfide $[S(EO)_6]_2$ forms SAMs different from its thiol counterpart $(EO)_6$. SE and RAIRS data indicate that the $[S(EO)_6]_2$ layer is self-limiting with disordered OEO chains and its thicknesses correspond to a surface coverage of 50−70%. These film characteristics are established with immersion times of approximately 1 day at concentrations as low as 5 μM from ethanol and from water and remain constant thereafter (up to 20 days in water). The final coverage for the $[S(EO)_6]_2$ films corresponds to that of previous films of conformationally mobile, surface-bound OEO segments that were found to screen the underlying substrate and minimize the adsorption of proteins. We show the OEO disulfide SAMs to be highly protein rejecting and present a paradigm for further development of ultrathin protein-resistant surfaces and matrixes.

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