Creation and Electrochemical Desorption of Protein-Resistant SAMs

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Abstract

We studied self-assembled monolayers (SAMs) on gold of the alkanethiol $(EG)_7(CH_2)SH$, where EG represents a polyethylene glycol group, as well as mixed SAMs containing terminal groups ending in positive and negative charges (a "zwitterionic" SAM). We found that the polyethylene glycol-terminated thiol formed a protein-resistant surface, while a zwitterionic SAM composed of a 1:1 mixture of $N(CH_3)_3^+$-$C_{11}SH$ and SO_3^+-$C_{11}SH$ also formed an inert surface, as found previously[1]. Furthermore, these inert surfaces were found to lose their protein-resistance upon electrochemical reduction, corresponding to destruction of the SAM. However, they remained inert despite electrochemical oxidation.

Introduction

Self-assembled monolayers (SAMs) of alkanethiols on gold have long been used to study the interactions between proteins, cells, and surfaces. The alkanethiols form a densely packed, highly ordered monolayer on gold surfaces; their terminal ends may be functionalized with a wide variety of chemical groups, enabling the surface to exhibit a range of chemical properties. The creation of a variety of protein-resistant ("inert") SAMs has been a topic of recent interest, since it would allow further flexibility in studies of cell development on patterned surfaces.

We chose to study $(EG)_7(CH_2)SH$ (for brevity, referred to as "PP") and zwitterionic SAMs based on previous research indicating that inert SAMs tend to have overall electrically neutral surfaces. Inert SAMs also include terminal chemical groups that are hydrophilic, hydrogen bond acceptors, and not hydrogen bond donors.[2]

SAMs terminated in polyethylene glycol have been well-studied and found to be one of the most inert surfaces. PP presents a shorter alkane chain than previous EG-terminated alkanethiolates studied[3] (Figure 1).

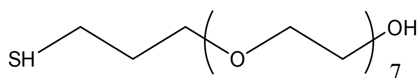

Figure 1: $(EG)_7(CH_2)SH$, known as "PP."

Holmlin et al. found that zwitterionic SAMs formed from a 1:1 ratio of $N(CH_3)_3^+$-$C_{11}SH$ (**A**) and SO_3^--$C_{11}SH$ (**B**) resisted the adsorption of the proteins fibrinogen and lysozyme.[1] In addition to studying the resistance of this surface to the protein bovine serum albumin (BSA), we also looked at mixtures of several other charged SAMs: NH_3^+-$C_{11}SH$ (**C**) and PO_3^{2-}-$C_{11}SH$ (**D**).

[1] Holmlin, R. E.; Chen, X.; Chapman, R. G.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 2841-2850.
[2] Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17,* 5605-5620
[3] Pale-Grosdemange, C.; Simon, E. S.; Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113,* 12-20

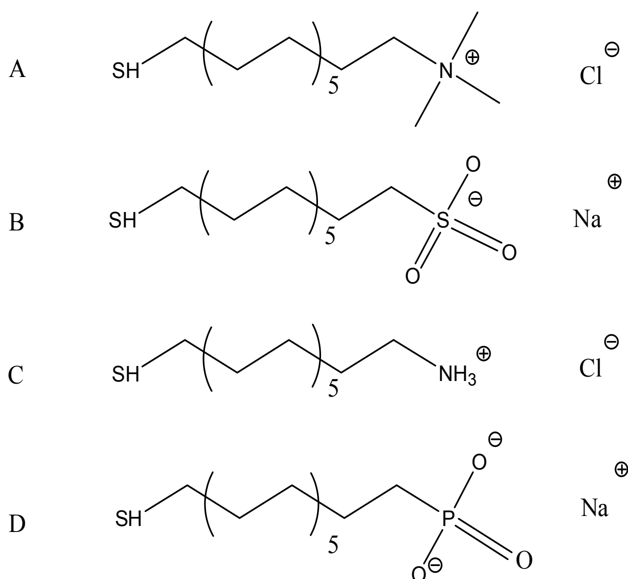

Figure 2: Charged alkanethiols used for zwitterionic SAMs.

Electrochemical desorption of SAMs has been shown to be a means by which inert SAMs may be destroyed, allowing the gold surface to adsorb proteins. Specifically, Jiang et al. showed that reductive desorption of inert SAMs may be used to noninvasively release cells from the geometrical confinements imposed by surfaces patterned with regions of inert and protein-adsorbing SAMs[4]. We attempted both reductive and oxidative desorption of the PP and zwitterionic inert surfaces.

Methods

Formation of the SAM

PP was dissolved to a concentration of 2 mM in ethanol, while each charged alkanethiolate was dissolved to a 1 mM concentration in Millipore water. Gold-covered substrates were prepared by thermal evaporation of 1.5 nm of titanium, followed by 45 to 60 nm of gold, onto a glass slide. Once prepared, the slides were cut into squares of approximately 1 cm^2, then placed in a solution of the thiols. The slides were incubated in the thiol solution for various time intervals, and checked for inertness after incubation. A monolayer was shown to have formed completely when the surface was totally protein-resistant.

Surface Plasmon Resonance Spectroscopy

We determined the amount of protein binding to the SAMs using surface plasmon resonance spectroscopy (SPR). SPR is a real time, *in situ* optical technique that monitors the incident angle of light (the resonance angle) needed to excite surface plasmons at the interface of a thin film of gold and a dielectric layer, such as a SAM. As the thickness of the dielectric layer increases – for example, as proteins bind to a SAM – the resonance angle of the gold decreases[5].

[4] Jiang, X.; Ferrigno, R.; Mrksich, M.; Whitesides, G. M. *J. Am. Chem. Soc.* **2003**, *125,* 2366-2367

[5] Salamon, Z.; Macleod, H. A.; Tollin, G. *Biochimica et Biophysica Acta* **1997**, *1331,* 117-129

Thus the change in resonance angle enables easy quantification of the amount of protein adsorbing to a SAM.

In our experiments we first passed an 0.5% w/v solution of sodium dodecyl sulfate (SDS) in water over the SAM to clean debris from the chip surface. After this, we flowed phosphate-buffered saline (PBS, pH=7.4) over our surface, then replaced it with a solution of protein – either fibroblast growth media or 1 mg/mL BSA dissolved in PBS. After the protein injection, we again passed PBS over the surface to wash away proteins that were only weakly bound (Figure 3). The resonance angle was monitored throughout the experiment; by comparing the response units (RUs), 10 000 of which are equivalent to a shift of 1 degree in the resonance angle, before and after the protein injection, the amount of protein irreversibly bound to the surface could be quantified. A surface was considered inert if less than 50 RU of binding was observed, corresponding roughly to less than 1% of a monolayer.

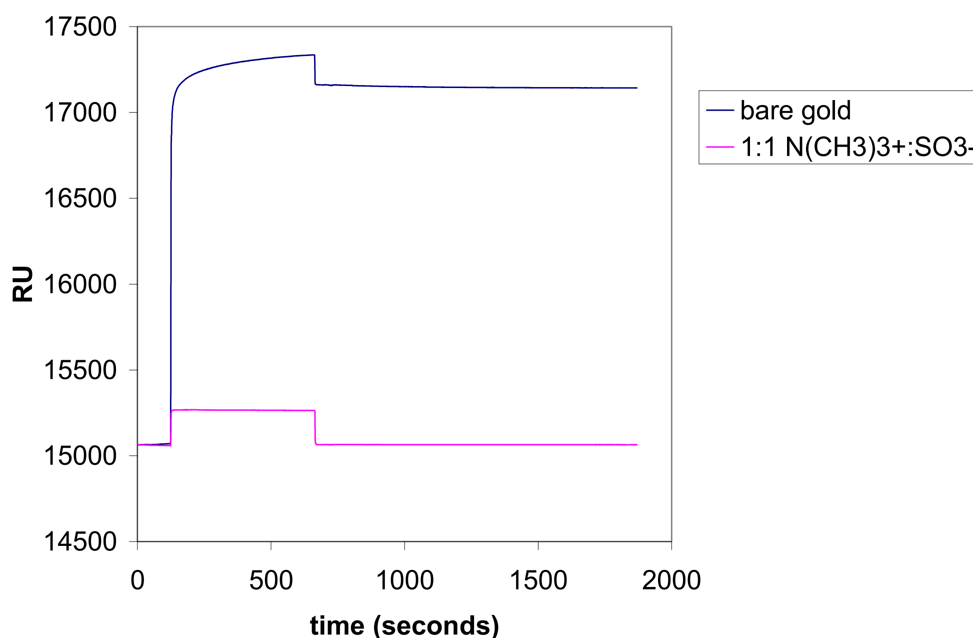

Figure 3: SPR sensorgram of protein injection, comparing a bare gold surface to an inert, zwitterionic surface of **A** and **B.** The flow rate was 5 uL/sec. PBS buffer was first passed across the surface for 2 minutes, followed by an injection of protein (in this case, 60 uL of protein, lasting 12 minutes). Subsequently, the surface was washed with PBS buffer to remove loosely bound proteins. A 1 mg/mL BSA solution was injected on the inert SAM, whereas a 10 mg/mL injection was used on the bare gold; hence the difference in the RU values during the protein injection.

Electrochemical Desorption

We immersed a SAM-covered gold surface in 2 mL of PBS in a cell culture plate. Two stainless steel electrodes were attached to a voltage source. For desorption, the voltage was set to a value between 1.00 V and 2.50 V; one electrode was held in contact with the chip surface, the other in solution, for time periods ranging from 1 minute to 10 minutes. After electrochemistry, the inertness of the SAM was again monitored by SPR.

Cyclic Voltammetry

Yang et al. had shown that oxidative desorption of a nonanethiol SAM occurs slowly at 0.5 V in a solution of 0.1 M KOH.[6] We performed a similar experiment on SAMs of PP, and, for comparative purposes, SAMs of C_{18} alkanethiol. Cyclic voltammetry was done at speeds of 5 mV/sec, 10 mV/sec, and 100 mV/sec, in solutions of PBS, 0.1 M KOH in Millipore water, and 0.1 M KCl in Millipore water, cycling from a voltage of -0.20 V to 1.20 V or 1.10 V, and then returning to -0.20 V. PBS was chosen as an electrolyte solution because all desorption experiments were done in this physiologically relevant medium; 0.1 M KOH was used as a comparison to Yang et al.'s experiment; and 0.1 M KCl was used because it has a similar ionic strength to the 0.1 M KOH, yet is at neutral pH, similar to the PBS. A silver/silver chloride electrode was used as a reference, a platinum gauze as the counter electrode, and the SAM-covered gold attached to a stainless steel strip as the working electrode.

Results

The SAMs of PP were found inert to the fibroblast growth media after incubation in the thiol solution for 1 hour. In contrast, the 1:1 combination of charged SAMs **A** and **B** took at least 6 hours to form a complete monolayer, and were incubated for 24 hours before further studies were done on these surfaces. The other zwitterionic SAMs - 1:1 **B** and **C**, 1.5:1 **B** and **C**, 2:1 **B** and **C**, 1:1 **A** and **D**, 1.38:1 **A** and **D**, 2:1 **A** and **D**, 3:1 **A** and **D** – still showed a change in the response units of between 300 and 800 RU, indicating that they were not yet completely inert, for incubation times up to 125 hours. Furthermore, the negatively charged SDS molecules also appeared to bind to the surface of these SAMs during the cleaning wash, probably indicating that the surface was not overall electrically neutral. In addition, there was a significant amount of crystal formation on the gold surfaces for the non-inert SAMs.

SAMs of PP were reduced at 1.50 V for 1 minute and at 2.00V for 5 minutes. The 1.50 V, 1 minute samples showed from 192.4929 RU to 396.1305 RU of binding to the fibroblast growth media after desorption, while the 2.00V, 5 minutes samples showed between 533 to 2353 RU of binding. However, oxidation of the PP surfaces had little effect - most surfaces showed binding under 50 RU, comparable to an unoxidized PP surface. Bare gold showed adsorption of the fibroblast growth media between about 2400 and 3000 RU.

Zwitterionic SAMs of 1:1 **A** and **B** were also electrochemically reduced, at 1.00 V for 2 minutes, 1.00 V for 5 minutes, 1.50 V for 2 minutes, and 1.50 V for 5 minutes. All samples showed adsorption of 1 mg/mL BSA of between 500 RU and 2000 RU. The spread of adsorption values within a group was large, revealed by the representative histograms of the reductive desorption results for 1.00V, 2 minutes and 1.50 V, 5 minutes (Figure 4). Bare gold around 2000 to 2500 RU of 1 mg/mL BSA.

[6] Yang, D. F.; Al-Maznai, H.; Morin, M. *J. Phys. Chem. B* **1997**, *101,* 1158-1166

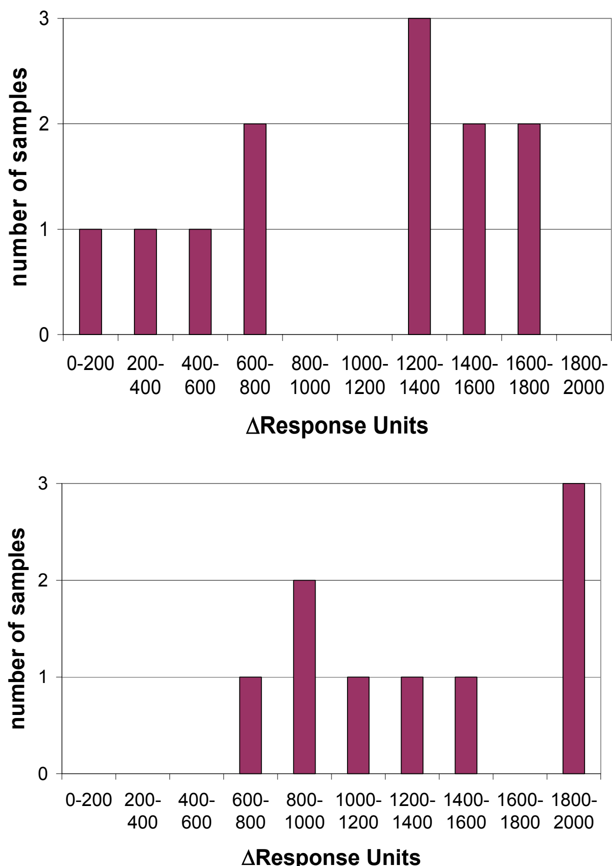

Figure 4: Results of reductive desorption of 1:1 **A** and **B** SAMs, at a) 1.00 V for 2 minutes and b) 1.50 V for 5 minutes.

As with the PP surfaces, oxidation of the 1:1 **A** and **B** surfaces at 1.00V for 2 minutes, 1.50 V for 2 minutes, 1.50 V for 5 minutes, and 2.00 V for 5 minutes showed binding under 20 RU, indicating that the SAM remained inert.

Cyclic voltammetry studies on PP surfaces in 0.1 M KOH, at scan speeds of 5 mV/sec and 10 mV/sec, revealed a small positive peak on the first scan at 0.5 V, followed by a positive rise in current at 0.7 V. Subsequent scans on the same surface showed a peak with a maximum at 0.9 V, which increased in height with each scan (Figure 5a). These subsequent scans no longer revealed the peak at 0.5 V, although all scans showed a negative dip at about -0.14 V. CV scans on PP at 100 mV/sec appeared similar, except that the 0.5 mV/sec peak was very low. Similar results were obtained with C_{18} at the same scan speeds. Cyclic voltammetry on both PP (Figure 5b) and C_{18} surfaces in PBS and in 0.1 M KCl revealed no positive peak in the region of 0.5 V and no negative dip around -0.14 V. Only the rise in current remained, beginning around 1.00 V. We observed that, after the first scan in 0.1 M KCl, the gold on the working electrode literally peeled off the glass slide. No such dramatic transformation was observed in either PBS or 0.1 M KOH, although occasionally the gold appeared slightly blistered after several scans.

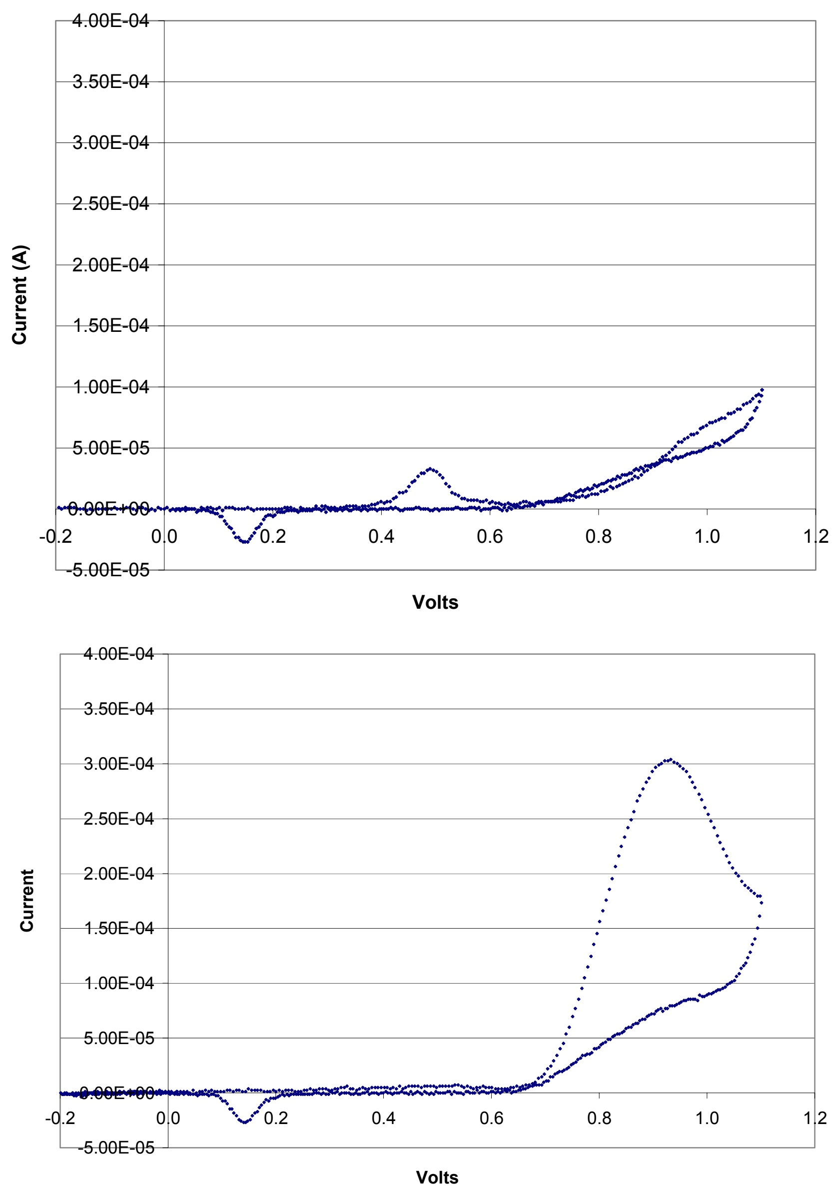

Figure 5a. First (top) and second (bottom) cyclic voltammetry scans at 5 mV/sec on inert PP surfaces in 0.1 M KOH. These scans cycled up from -0.20 V to 1.10 V and then returned to -0.20 V.

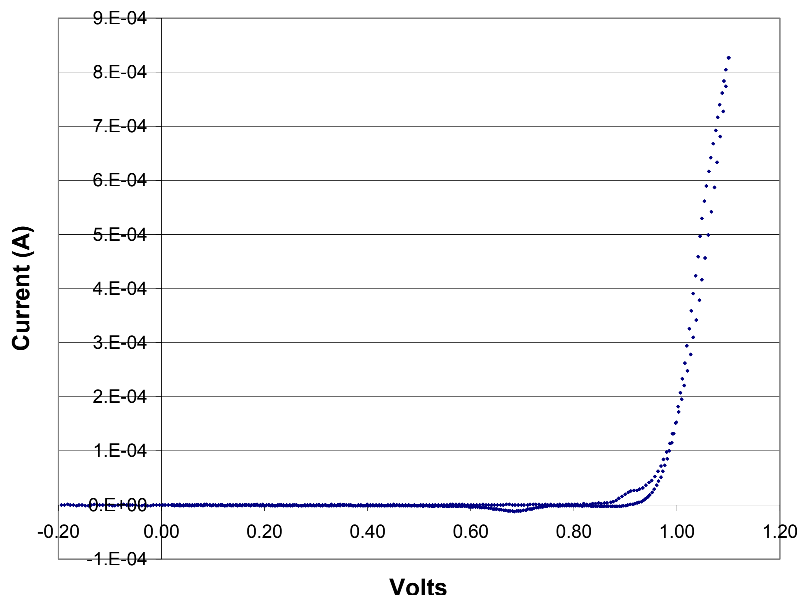

Figure 5b: First cyclic voltammetry scan on an inert PP surface in 0.1 M KCl at 5 mV/sec. After the first scan, the gold peeled off the surface of the working electrode, leaving only the titanium adhesion layer on glass.

Discussion

Inert surfaces were achieved using PP SAMs and zwitterionic SAMs made from 1:1 A and B. This reveals that shorter-chain alkanethiols terminated in several ethylene glycol units may form inert surfaces. Why the 1:1 **A**:**B** surface is protein-resistant remains unsolved: the fact that inert surfaces could not be formed from other combinations of charged alkanethiols hints that perhaps the inertness is due to other properties of **A** and **B** besides their charge and the overall electrical neutrality of the surface. However, the SDS binding points to a charge imbalance on these other zwitterionic surfaces, indicating that other ratios of thiols may yield electrically neutral and thus possibly inert surfaces.

SPR of reduced PP and 1:1 **A**:**B** SAMs showed protein binding, indicating that these surfaces were no longer inert and that the SAMs had been partially destroyed. Indeed, some of the binding values in RU for PP at 2.00 V for 5 minutes are comparable to those of bare gold (around 2000 RU), indicating that the SAM may be completely desorbed. The large spread in protein binding values for both surfaces indicates that inconsistencies still remain, however. Further refinement of experimental procedures – for example, making sure that the surface of the chip fully saturates with protein during the SPR – will reveal whether the inconsistencies are due to experimental conditions or whether the reductive desorption is inhomogenous.

Oxidative desorption appears to have no effect on inert SAMs at physiological pH. This was corroborated by cyclic voltammetry experiments in 0.1 M KCl and PBS. For these electrolyte solutions, the only rise in current was seen after 1.00 V, and seems to be associated with the oxidation of the gold surface which, in the case of KCl, may lead to complete peeling away of the gold from the glass slide. In basic solution, the peak at 0.5 V was attributed to the desorption of the SAM, followed by oxidation at 0.7 V of the now-unprotected gold surface. The fact that the peak at 0.5 V does not appear on subsequent scans indicates that the SAM is

now completely desorbed. The appearance of the peak in 0.1 M KOH corroborates with the results of Yang et al., who postulated that the oxidative desorption of SAMs is contingent upon hydroxide ions. The near-disappearance of the 0.5 V peak at scan speeds of 100 mV/sec further corroborates with Yang et al.'s observation that this oxidative desorption is slow. Thus we have further confirmed that the oxidative desorption of SAMs is pH-dependent, and cannot be differentiated from the oxidation of gold at neutral pH.