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Membrane protein resistance of oligo(ethylene oxide) self-assembled monolayers

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ABSTRACT

As part of an effort to develop biointerfaces for structure-function studies of integral membrane proteins (IMPs) a series of oligo(ethylene oxide) self-assembled monolayers (OEO-SAMs) were evaluated for their resistance to protein adsorption (RPA) of IMPs on Au and Pt. Spectroscopic ellipsometry (SE) was used to determine SAM thicknesses and compare the RPA of $HS(CH_2)_3O(CH_2CH_2O)_6CH_3$ (**1**), $HS(CH_2)_3O(CH_2CH_2O)_6H$ (**2**), $[HS(CH_2)_3]_2CHO(CH_2CH_2O)_6CH_3$ (**3**) and $[HS(CH_2)_3]_2CHO(CH_2CH_2O)_6H$ (**4**), assembled from water. For both substrates, SAM thicknesses for **1** to **4** were found to be comparable indicating SAMs with similar surface coverages and OEO chain order and packing densities. Fibrinogen (Fb), a soluble plasma protein, and rhodopsin (Rd), an integral membrane G-protein coupled receptor, adsorbed to the SAMs of **1**, as expected from previous reports, but not to the hydroxy-terminated SAMs of **2** and **4**. The methoxy-terminated SAMs of **3** were resistant to Fb but, surprisingly, not to Rd. The stark difference between the adsorption of Rd to the SAMs of **3** and **4** clearly indicate that a hydroxy-terminus of the OEO chain is essential for high RPA of IMPs. The similar thicknesses and high RPA of the SAMs of **2** and **4** show the conditions of protein resistance (screening the underlying substrate, packing densities, SAM order, and conformational mobility of the OEO chains) defined from previous studies on Au are applicable to Pt. In addition, the SAMs of **4**, exhibiting the highest resistance to Fb and Rd, were placed in contact with undiluted fetal bovine serum for 2 h. Low protein adsorption (≈12.4 ng/cm^2), obtained under these more challenging conditions, denote a high potential of the SAMs of **4** for various applications requiring the suppression of non-specific protein adsorption.

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1. Introduction

Surfaces resistant to nonspecific protein adsorption are an essential component of non-crystallographic structure-function studies of integral membrane proteins (IMPs). IMPs comprise a significant portion of the human genome (20–30%) and play vital roles in many functions of the cell [1]. Our objective is the creation of a biosurface containing oriented IMPs embedded in a membrane-like matrix on a high surface area substrate. Such membrane-functionalized constructs enable the application of neutron scattering and solid-state nuclear magnetic resonance (NMR) techniques to study proteins that are not amenable to crystallization as well as protein–antigen, protein–agonist, and protein–protein interactions in biologically-relevant milieus. Construction of the desired biosurface (Fig. 1) involves several steps. Briefly, an anodized aluminum oxide (AAO) surface is initially

Abbreviations: RPA, resistance to protein adsorption; OEO, oligo(ethylene oxide); TLC, thin layer chromatography; SAM, self-assembled monolayer; AAO, anodized aluminum oxide; IMP, integral membrane protein; FBS, fetal bovine serum; Fb, fibrinogen; Rd, rhodopsin; CA, contact angle.

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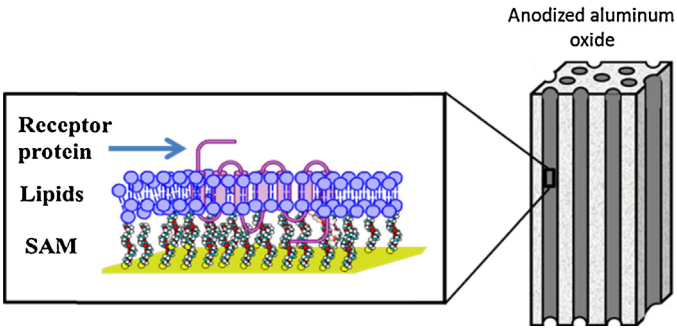

Fig. 1. Schematic of biosurface containing an oriented IMP such as a G-protein coupled receptor (GPCR) in a lipid-like bilayer on Pt-coated AAO membranes.

coated with a metal such as Pt, to which is chemisorbed a mixed self-assembled monolayer (SAM) containing a capture agent, in our case compounds with a nitrilotriacetic acid (NTA) terminus, laterally diluted with molecules that exhibit high resistance to protein adsorption (RPA) [2]. Exposure of the capturing SAM to polyhistidine-tagged (his-tag) IMPs, after activation by nickel to the NTA–Ni^+ complex, results in the well-established oriented protein capture *via* the strong NTA–Ni^+–his-tag interaction. Finally, a bilayer is constructed around the captured IMP to sustain it in its native (active) conformation. It is imperative that nonspecific protein adsorption be minimized in the protein capture step for meaningful interpretation of the scattering or NMR data.

Many surfaces modified by polymers or SAMs have been shown to be protein resistant [3–15]. Surfaces modified with chains of the ethylene oxide [EO] motif, *i.e.*, $(CH_2CH_2O)_n$, are the most well studied and understood and, generally, are the surfaces to which other surfaces are ultimately compared. However, the EO motif does not create a wholly sufficient condition for high RPA, *i.e.*, not all oligo(ethylene oxide) [OEO] or poly(ethylene oxide) [PEO]/poly(ethylene glycol [PEG] surfaces are protein resistant [16–19] and because polymer–protein interactions are inherently difficult to define [20], the mechanism of protein resistance has lacked clarity for the larger PEGs.

Studies on SAMs of OEO-terminated compounds have led to a better definition of the structural and conformational requirements of the OEO segments such as chain length, packing density, conformational order, and, indirectly, the complex OEO–water interactions necessary for high RPA [17,19,21–24]. While full clarification is still lacking [25], it is now well-established that the condition of high RPA exists when the OEO segments uniformly cover the substrate and are (a) not tightly packed [3,16,17,23], (b) disordered, *i.e.*, composed of neither the 7/2 helical nor the all-*trans* extended conformation over any significant ensemble of molecules [17], (c) conformationally mobile [24], and (d) hydrated/interactive with water [16,26]. In addition, earlier reports established that RPA was largely independent of the OEO end group for $-(CH_2CH_2O)_nH$ (hydroxy-terminated) and $-(CH_2CH_2O)_nCH_3$ (methoxy-terminated) SAMs [27].

Maximum RPA was observed at approximately 55% surface coverage for SAMs of the general formula $HS(CH_2)_3O(CH_2CH_2O)_xCH_3$, {**1**, $x = 6$ (Fig. 2); $x = 5$ (Ref. [24])} on Au. However, these highly resistant SAMs of **1** were difficult to reproduce [short ($\leq$1 s), manually-performed immersion times in the SAM-forming solutions] and, more importantly, unstable. Protein adsorption was observed within hours of the initially-prepared SAMs as a result of surface reorganization forming ordered domains of **1** (OEO segment adopting the 7/2 helical conformation [24]) and poorly covered or bare Au patches. SAMs of the subsequent bis-sulfur OEO-compound, N,N-(bis 3′-thioacetylpropyl)-3,6,9,12,15,18-hexaoxanonadecanamide (BTHA) [28], in which the optimal OEO packing is encoded in the

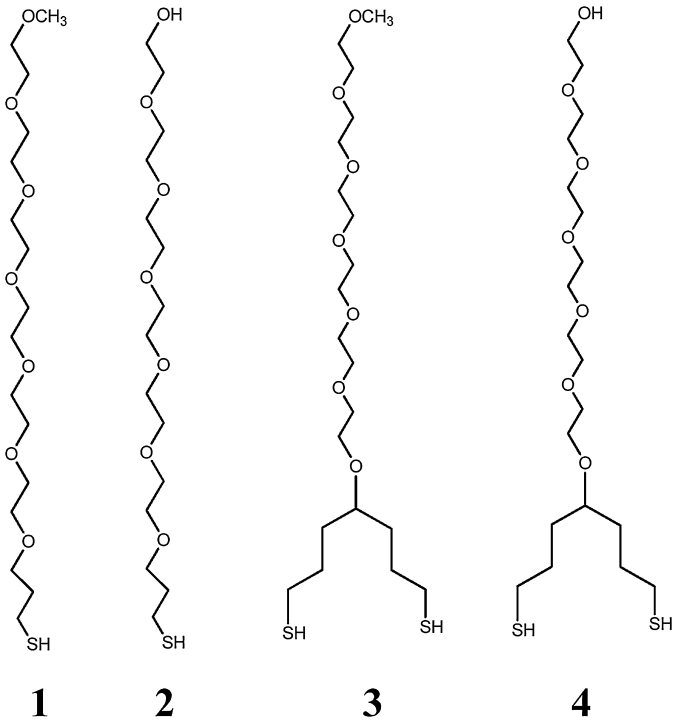

Fig. 2. Structures of the OEO compounds.

structure, required no exacting preparation conditions, sustained their physical properties when stored in preparatory solutions for weeks, and exhibited high RPA.

Essentially all of the previous protein adsorption studies of OEO SAMs utilized plasma soluble proteins, most frequently fibrinogen (Fb) and bovine serum albumin (BSA). Recognizing the need to establish SAMs that will minimize nonspecific protein adsorption for IMPs, this work reports the RPA properties of four OEO SAMs (Fig. 2): 1-mercaptopropyl-OEO **1** and **2** and 4-(1,7-dimercaptoheptan-4-oxy)-OEO **3** and **4** on Au and Pt substrates. For direct comparison to the plasma soluble protein literature we used Fb [(340 kD)] and for the IMP we used the readily available G-protein coupled receptor (GPCR) rhodopsin (Rd) [37 kD]. Compounds **1** and **2** provide a direct correlation to our previous work with monothiol OEO SAMs while **3** and **4** correlate to the BTHA SAMs, respectively [28]. We use *in situ* and *ex situ* spectroscopic ellipsometry (SE) to evaluate the RPA of the SAMs of **1**–**4**, on Au and Pt surfaces. The evaluation of Pt surfaces was necessitated by our ability to obtain more uniform Pt deposition in the AAO membrane pores using an organometallic vapor deposition procedure relative to Au deposited by plasma sputtering.

2. Experimental [29]

Materials and Reagents. Except for silica gel and FBS (fetal bovine serum), all chemicals, including fibrinogen and solvents, were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO) and were used as received. Silica gel (40 μm, 7024-02) was purchased from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ). FBS with Roswell Park Memorial Institute media and associated antibiotics were purchased from Life Technologies (Carlsbad, CA). Tetrahydrofuran (THF) was distilled under N_2 from CaH_2 immediately before use. Compounds **1** and **2** were prepared as outlined in Scheme 1 and compounds **3** and **4** as outlined in Scheme 2. The preparation of **1** [4,7,10,13,16,19,22-heptaoxatricosane-1-thiol (M=CH_3)] was described earlier [30]. Details of the preparation of **2** to **4** and spectral data for all compounds are given in the