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Electrochemical titration of carboxylic acid terminated SAMs on evaporated gold: Understanding the ferricyanide electrochemistry at the electrode surface

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ABSTRACT

Ordered 11-mercaptoundecanoic acid (MUA) structures formed from self-assembly on evaporated gold electrodes were probed with potassium ferricyanide through electrochemical titration. The ferricyanide response was limited at these highly blocking films at low salt concentrations necessitating addition of 1 M KCl electrolyte. Voltammetry results strongly support the penetration of ferricyanide probe molecules into the SAM films for electron transfer. Recent models for electrochemical titration of SAMs, and interactions in weak polyelectrolytes are evoked for data analysis. The pK_a determined using the electrochemical titration model was 5.6 ± 0.2 for 50% MUA/50% 6-mercaptohexanol monolayers and 5.8 ± 0.2 for the 100% MUA films. Utilizing a statistical mechanical model for weak polyelectrolytes at the water–solid interface $(1 - pK)$ and a pK of 5.7, an intermolecular interaction component $(\beta\,\bar{E})$ of 2 was obtained for the 100% COOH films and $\beta\,\bar{E}$ of 1 for the 50% COOH films, which suggests a more deprotonated 50% MUA film with less intermolecular interactions compared to pure MUA films.

1. Introduction

Carboxylic acid self-assembled monolayers (SAMs) are routinely incorporated into devices to enhance protein/enzyme attachment to electrode surfaces due a favorable electrostatic nature of the monolayer at physiological pHs. Proteins are either electrostatically adsorbed or covalently attached to the SAM surfaces using amine/carboxylic acid chemistry [1–14]. The surface confined proteins adhered to carboxylic acid SAMs provide varied responses that are attributed to the many factors affecting the local microenvironment at the protein/SAM interaction zone including gold topography [7] as well as SAM and diluent thiol chain length [8,9]. To better understand the surface chemistry of the SAM, an acid dissociation constant (K_a) is measured and is often presented in the logarithmic form, pK_a. Understanding the surface pK_a for acid/base SAMs is essential due to the integral role of electrostatics in intermolecular interactions. Characterizing the pK_a of specific electrode materials has been of interest to surface scientists for decades [15–30]. Due to prior evidence of disparities between surface and solution pK_as, independent characterization of each modified surface is required. A plethora of techniques including contact angle titration [15], spectroscopy [16], FTIR-ATR [17], laser-induced temperature jump [18], atomic force microscopy [19], electrochemistry [20–29], and quartz crystal microbalance [30] have been employed to explore the surface chemistry of alkanethiols on gold. In spite of the myriad of procedures for characterization of the surface pK_a of SAMs, consistency is an issue. Surface pK_as of 11-mercaptoundecanoic acid have been reported ranging from 3.0 to 10.3 [19,21,24,26–30] illustrating the importance of measurement conditions in pK_a determination.

The goal of this work is to measure the carboxylic acid surface pK_a in mixed SAMs using a probe molecule $(Fe(CN)_6^{3-})$ in a simple, non-destructive, low cost electrochemical titration method. However, the well-ordered assembly of carboxylic acid SAMs on evaporated gold films exhibit a drastically different $Fe(CN)_6^{3-}$ response compared to SAMs formed on bulk gold. Experiments are conducted to better understand the principle mechanism of the ferricyanide electrochemical response at SAM modified gold electrodes.

2. Experimental

2.1. Reagents and materials

Water was purified on a Milli-Q/Organex-Q (Millipore) water purification system (resistivity of 18 MΩ cm at delivery). The alkanethiol derivatives 11-mercaptoundecanoic acid ($HS(CH_2)_{10}$-COOH, $C_{10}COOH$, 95%), 6-mercapto-1-hexanol ($HS(CH_2)_5COH$, C_6OH, 97%), and 1-nonanethiol ($HS(CH_2)_8CH_3$, C_8CH_3, 99%) were purchased from Aldrich and used without further purification. All other chemicals were reagent grade. $K_3Fe(CN)_6$ and $Ru(NH_3)_6Cl_3$ solutions (10 mM) were prepared in 200 mM potassium phosphate

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buffer (KPB) with 1 M KCl and pH'd individually with 6 M NaOH or 6 M HCl. Cytochrome *c* (Sigma Type VI, horse heart) was purified on a cation exchange column (Whatman, CM-52, carboxymethylcellulose) and stored in 4.4 mM phosphate buffer [31]. Purification and storage temperature was 4–5 °C.

2.2. Electrochemistry

Cyclic voltammetry (CV) was performed using a CH Instruments, Inc., 660C potentiostat (Austin, Tx). Formal potentials were calculated from CV peak positions, i.e., $E^{o\prime} = (E_{p,a} + E_{p,c})/2$. The three-electrode electrochemical cell [32] employed a platinum wire counter electrode, Ag/AgCl (1 M KCl) reference electrode, and SAM-modified gold working electrode. The Ag/AgCl reference electrode was calibrated daily against a saturated calomel electrode before experimentation. CVs collected for electrochemical titration plots were conducted at 100 mV/s.

2.3. Electrode preparation

Evaporated gold film electrodes (1000 Å Au/50 Å Ti on glass) were purchased from Evaporated Metal Films (Ithaca, NY). Electrodes were first heated in concentrated nitric acid until boiling, rinsed in room temperature Millipore water, followed by rinsing with absolute ethanol and then immersed immediately into an ethanolic alkanethiol solution. For carboxylic acid terminated SAMs, either pure 1 mM $C_{10}COOH$ or a 1:1 mixture of 2 mM C_{10}-COOH and 2 mM C_6OH was used. All SAMs were allowed to self-assemble for at least 24 h. Each electrode was rinsed with ethanol, mounted into the electrochemical cell, and rinsed with buffer. Background voltammograms, consisting of 200 mM KPB/1 M KCl as the membrane-bathing solution, were obtained prior to initial experiments and throughout the experimentation to track changes in the SAM.

2.4. Electrostatically immobilized cyt c

Cytochrome *c* was initially adsorbed at room temperature to carboxylic acid electrodes surface by 0.5 h immersion in low ionic strength phosphate buffer (4.4 mM, pH 7.0, I = 10 mM, 30 μM cyt *c*) and yielded approximate monolayer coverage (~15 pmol/cm^2). A partial desorption was then performed by rinsing with pH 7.0, 40 mM phosphate buffer for 5–10 s, leaving behind a cyt *c* submonolayer of ca. 3–5 pmol/cm^2. A narrower $E^{o\prime}$ distribution was obtained, in agreement with prior findings [31].

3. Results and discussion

3.1. Determining the pK_a of the carboxylic acid in $C_{10}COOH$ pure and mixed SAMs

Measuring and understanding the pK_a of carboxylic acid terminated SAMs has been a goal of many research groups over the past two decades [15–30]. Determining the pK_a has been nontrivial because of the observed sensitivity of the carboxylic acid terminal group to variant individual solution conditions and electrode preparation. Therefore, using published –COOH SAM pK_a values would only provide general trends due to the inherent variables in monolayer preparation and solution conditions, which result in changes in microenvironments at the SAM interaction surface. To accurately characterize the surface pK_a for an individual SAM composite electrode, the value must be measured.

The protonation state of a self-assembled monolayer is readily obtained through electrochemical titration. Electrochemical titration on bulk gold has been reported for pure carboxylic acid terminated SAM surfaces [24,26]. Both negatively and positively charged probe molecules are effective for probing the acidic SAMs due to the influence of electrostatics on the process. Using ferricyanide, $Fe(CN_6)^{3-}$, there is a gradual decrease in charge transfer relative to increasing pH for carboxylic acid SAMs due to a gradual increase in the surface concentration of carboxylate ion [24,27]. The reciprocal effect is observed when a positively charged probe molecule, ruthenium hexamine $Ru(NH_3)_6^{3+}$, is employed with a gradual increase in charge transfer relative to increasing pH [26]. Therefore, it is reasonable to assume that the observed change in ferricyanide peak current reflects the change in the degree of the dissociation of the carboxylic acid group in the SAM, $HS(CH_2)_xCOOH$.

Theory developed from previous work explains the current response at –COOH SAM composite electrodes [24,26,27].

$$\text{Acid dissociation HA} \leftrightarrow \text{H}^+ + \text{A}^- \tag{1}$$

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} \tag{2}$$

In the monolayer system, the current measured is from the probe transferring electrons, which is effected by the surface concentration of the acidic and basic SAM components. If total current measured is assumed to be composed of two parts, a current from the probe molecule interacting with the dissociated SAM (–COO$^-$, A$^-$) and non-dissociated SAM (–COOH, HA) components, then the total current would be $i_{total} = i_A–[A^-]+i_{HA}$ [HA], where [A$^-$] + [HA] = 1 ([A$^-$] and [HA] are surface concentrations with the total monolayer coverage being the unit concentration). The pK_a is derived from Eq. (2).

$$\text{p}K_a = \text{pH} - \log\frac{[\text{A}^-]}{[\text{HA}]} \tag{3}$$

The current response, assuming [A$^-$] + [HA] = 1 and $i = i_{A^-}[A^-] + i_{HA}[HA]$, is defined by the expression

$$\text{p}K_a = \text{pH} - \log\left[\frac{i_{HA} - i_{A^-}}{i - i_{A^-}} - 1\right] \tag{4}$$

Solving Eq. (4) for i and choosing a pK_a allows the experimental data to be fitted. The currents for the protonated (i_{HA}) and deprotonated (i_{A^-}) states are taken from the plateau region of the titration plot at acidic pH and basic pH, respectively.

Tominaga et al. [26] used a similar model to characterize nanoparticle modified gold/SAM surfaces. Again assuming i_{total} = i_A–[A$^-$] + i_{HA} [HA], where [A$^-$] + [HA] = 1 and incorporating the 1 – pK model used for characterizing weak polyelectrolytes [21,26,33].

$$\theta = \frac{[\text{A}^-]}{[\text{A}^-] + [\text{AH}]} \tag{5}$$

$$i_p = i_A - [\theta] + i_{HA}[1 - \theta] \tag{6}$$

$$\text{pH} = \log\frac{\theta}{1-\theta} + \frac{\beta\,\bar{\text{E}}\theta}{2.303} + \text{p}K \tag{7}$$

The titration curves are fitted using Eqs. (5)–(7) where pK is the intrinsic pK in the absence of intermolecular interactions between thiols, θ is the degree of acid dissociation, β is 1/RT, and $\bar{\text{E}}$ is the potential of mean force due to electrostatic interactions.

In this work, the –COOH SAM pK_a on evaporated Au is characterized by the two models. Ferricyanide electrochemical responses at SAMs on evaporated Au are found to be dramatically different from the published $Fe(CN)_6^{3-}$. Zhao et al. [24], and Dai and Ju [27] present quasireversible voltammetry at SAM modified bulk gold electrodes while our ferricyanide responses at SAM modified