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The Influence of Molecular Dipole Moment on the Redox-Induced Reorganization of α -Helical Peptide Self-Assembled Monolayers: An Electrochemical SPR Investigation

Andrew J. Wain[†], Huy N. L. Do[†], Himadri S. Mandal^{‡,†}, Heinz-Bernhard Kraatz[‡], and Feimeng Zhou^{*,†}

[†]Department of Chemistry and Biochemistry, California State University Los Angeles, Los Angeles, California 90032

[‡]Department of Chemistry, University of Western Ontario, London, ON N6A 5B7, Canada

Abstract

Self-assembled monolayers (SAMs) of ferrocene-labeled α -helical peptides were prepared on gold surfaces and studied using electrochemical surface plasmon resonance (EC-SPR). The leucine-rich peptides were synthesized with a cysteine sulfhydryl group either at the C- or N-terminus, enabling their immobilization onto gold surfaces with control of the direction of the molecular dipole moment. Two electroactive SAMs were studied, one in which all of the peptide dipole moments are oriented in the same direction (SAM1), and the other in which the peptide dipole moment of one peptide is aligned in the opposite direction to that of its surrounding peptide molecules (SAM2). Cyclic voltammetry combined with SPR measurements revealed that SAM reorientations concomitant with the oxidation of the ferrocene label were more significant in SAM2 than in SAM1. The substantially greater change in the peptide film thickness in the case of SAM2 is attributed to the electrostatic repulsion between the electrogenerated ferrocenium moiety and the positively charged gold surface. The greater permeability of SAM1 to electrolyte anions, on the other hand, appears to effectively neutralize this electrostatic repulsion. The film thickness change in SAM2 was estimated to be 0.25 ± 0.05 nm using numerical simulation. The timescale of the redox-induced SPR changes was established by chronoamperometry and time-resolved SPR measurements, followed by fitting of the SPR response to a stretched exponential function. The time constants measured for the anodic process were 16 and 6 ms for SAM1 and SAM2 respectively, indicating that the SAM thickness changes are notably fast.

1. Introduction

The study of electron transfer (ET) across helical biomolecules, including DNA molecules and peptides, has been the focus of a multitude of investigations employing an ensemble of physical techniques.^{1–5} The motivation behind much of the work in this field has been to gain a deeper understanding of the mechanism of ET in biological processes such as photochemical reactions, oxygen transport and enzyme catalysis.^{3,6–9} In particular, the preparation of self-assembled monolayers (SAMs) of peptide molecules for investigations by electrochemical and surface methods has emerged as a unique approach to probe intramolecular ET.^{10–14} In addition to providing a platform for fundamental biological studies, such monolayers are also interesting from a technological perspective, potentially forming the basis of molecular electronics.^{15–}

*Corresponding author, E-mail address: fzhou@calstatela.edu; Tel: 323-343-2390; Fax: 323-343-6490.

[†]Present address: Department of Chemistry, University of Waterloo, Waterloo, ON N2L 3G1, Canada

¹⁷ In electrochemical studies, the immobilized peptide molecules are typically labeled with an electroactive moiety and interfacial ET is driven via the application of a potential to the substrate underlying the SAM. Whilst a great deal of information may be yielded from this methodology, an aspect that has not been thoroughly studied is the effect of the SAM composition on redox-induced film reorganization.

The effect of molecular dipole moment on peptide SAM behavior has received a great deal of attention.^{1,17–20} It is well established that α -helical peptides exhibit a large macrodipole moment (approximately 3.5 D per residue)²¹ which is oriented parallel to the molecular axis, and the impact of the resulting electric field on protein structure and function has been highlighted.^{22–24} The direction of the molecular dipole moment has been demonstrated to be an important variable. For example Sek *et al.* illustrated that, for polyalanine SAMs on gold surfaces, ET occurs more rapidly in the C-terminus (δ^-) to N-terminus (δ^+) direction, than in the reverse direction.¹³ Kimura and co-workers have been especially active in this area.^{17, 25–27} Using scanning tunneling microscopy, they demonstrated that the length of immobilized peptides composed primarily of α -aminoisobutyric acid could be changed by the application of an external potential.²⁷ In this case the potential-induced molecular changes were attributed to switching between the α -helical and 3_{10} -helical forms of this peptide. Recently some of us studied the behavior of SAMs composed of leucine-rich α -helical peptides on gold surfaces, using a terminal ferrocene (Fc) label to investigate the ET properties of the peptides and relate them to the film composition and dipole orientation.¹⁹ Two different monolayers were prepared, one in which all of the molecular dipoles of the assembled peptides were aligned parallel and the other in which the dipole moment of one peptide was oriented in the opposite direction to that of its neighboring peptides that are modified differently, leading to an antiparallel dipole arrangement. Using cyclic voltammetry and electrochemical impedance spectroscopy (EIS), it was found that the ET kinetics were slower in the antiparallel than in the parallel dipole arrangement, which was ascribed to a more restricted motion due to stronger intermolecular interactions between opposing dipoles. Thus, a gated ET mechanism was proposed on the basis that the parallel dipole arrangement facilitates the ET between the gold surface to the Fc label. However, the effect of an applied potential on the organization of such mixed monolayers, and dynamics of the ET-induced SAM reorganization are features that have remained hitherto unaddressed.

Electrochemical surface plasmon resonance (EC-SPR) has been recognized as a highly valuable technique for the detection of redox-induced changes in molecules adsorbed at metal/solution interfaces.^{28–37} For example, Boussaad *et al.* studied ET-triggered conformational reorganization in immobilized cytochrome *c*, and measured sub-angstrom film thickness changes.³¹ Monolayer thickness changes have also been determined for the oxidation/reduction of SAMs composed of 11-ferrocenylundecanethiol using this technique in conjunction with electrochemical quartz crystal microbalance (EQCM) measurements.³⁴ In this case, the repulsion between the electrogenerated ferrocenium moiety and the positively charged gold electrode resulted in a more perpendicular molecular orientation relative to the surface, which could be readily measured by SPR. The notable sensitivity of SPR to structural changes at the metal-solution interface, combined with the versatility of electrochemical techniques for the study of interfacial ET reactions, renders SPR a powerful technique to probe redox-modulated ultrathin film reorganization.

In this work we employed EC-SPR to study redox-induced reorganization of Fc-modified leucine-rich α -helical peptide SAMs consisting of parallel and antiparallel dipole orientations, with the aim of gaining further insight into the processes triggered by ET. The SAMs studied are analogous to those used in previous work,¹⁹ consisting of one or more the following peptides: an Fc-labeled component with the sequence, Fc-KTAL₁₀NPC-NH₂ (Fc10L) and two unlabeled diluent peptides with the sequences of Ac-KTAL₁₀NPC-NH₂ (Ac10L) and Ac-

CTAL₁₀NPK-NH₂ (10LAc). The molecular structures are depicted in Figure 1. These peptides were synthesized with thiol-bearing Cys residues to allow their adsorption onto gold surfaces. Note that for both Fc10L and Ac10L, the Cys residue is positioned at the C-terminal, whereas for 10LAc, this residue is located at the N-terminal. Thus, when immobilized onto gold, the dipole moments of Fc10L and Ac10L run in the opposite direction to 10LAc. The Fc-labeled peptide is diluted in a matrix of unlabeled peptide molecules. SAM1 comprises only the Fc10L and Ac10L components (5:95), such that all of the peptide dipoles are aligned parallel, and SAM2 is composed of Fc10L, Ac10L and 10LAc (5:45:50), leading to an antiparallel dipole orientation (Figure 2). We show here that SAM thickness changes upon oxidation of the Fc moiety are likely the result of an electrostatic effect and are strongly dependent on the orientation of the peptide dipole moments. Using numerical simulation we quantify the film thickness change which occurs due to oxidation of the Fc label in SAM2. By measuring SPR angular changes concomitant with potential steps, we also determined a timescale for the rapid, redox-induced film reorganization events. Our findings are especially relevant from a methodological viewpoint, since a thorough understanding of the processes taking place during redox changes is crucial to the use of this method in the study of ET processes and the construction of biologically inspired molecular electronic devices.

2. Experimental

2.1 Reagents

Sodium perchlorate and 2,2,2-trifluoroethanol (TFE) were purchased from Fisher Scientific (Tustin, CA). All other reagents were acquired from commercial sources and used without further purification. Synthesis and characterization of the peptides Fc10L, Ac10L and 10LAc followed a published procedure.¹⁹ All aqueous solutions were prepared with deionized water purified with a Millipore Simplicity 185 system.

2.2 EC-SPR

EC-SPR experiments were conducted using a CHI 440 workstation (CH Instruments, Austin, TX) coupled with a BI SPR1000 spectrometer (Biosensing Instruments, Tempe, AZ). A polyetheretherketone (PEEK) electrochemical cell (Biosensing Instruments) was used, as reported previously,³⁴ employing a Ag/AgCl reference electrode (BAS, West Lafayette, IN) and a platinum wire counter electrode. Gold films, deposited onto BK7 glass slides (Fisher Scientific) following our published procedure,³⁴ were used as the SPR substrates. The active area of the gold film enclosed by the EC-SPR cell was determined via cyclic voltammetry, using a 1 mM solution of potassium ferricyanide in 0.1 M KNO₃. Peak current analysis over a range of scan rates, employing a ferricyanide diffusion coefficient of $7.1 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$,³⁸ revealed a surface area of 0.56 cm². This value is very close to the estimated geometric area ($\sim 0.5 \text{ cm}^2$), indicating that the gold surfaces employed were very smooth (consistent with our previous AFM and ellipsometric measurements).³⁹ Calibration of the SPR response was carried out frequently by adding aliquots of ethanol to the EC-SPR cell and comparing the angular changes to those calculated by Kolomenskii *et al.*⁴⁰ Experiments were conducted at $22 \pm 2^\circ\text{C}$.

2.3 Preparation of SAMs

SAMs were prepared by adsorption of the peptide at the cysteine thiol group onto the gold substrate. Briefly, each gold film was initially cleaned with a solution composed of 5 mL 30 % hydrogen peroxide, 5 mL ammonium hydroxide and 25 mL water, rinsed thoroughly with deionized water, and then dried under nitrogen. The substrate was then enclosed within a flow cell designed to allow a minimal volume of peptide solution dissolved in TFE (*ca.* 100 μL) to be pumped over the gold surface by syringe injection. Peptide solutions were prepared in TFE since this solvent is known to favor an α -helical conformation in peptides.⁴¹ The use of an

enclosed cell was required due to the high volatility of the TFE solvent needed for self assembly of α -helical peptide SAMs. After the surface was fully covered with solution, the injection was stopped and the substrate was immersed under the peptide solution for 24 h. Prior to EC-SPR, the SAM-modified substrate was rinsed with water and dried.

Two electroactive SAMs were prepared in this work, in addition to two electroinactive SAMs as controls. The peptide composition in each of the SAMs is given in Table 1. These SAMs were formed by exposing the gold surfaces to peptide solutions with the appropriate ratio of components, giving a total peptide concentration of 100 μ M. The surface characterization of analogous SAMs has been reported previously, in which the α -helical content was determined to be greater than 90 % in TFE.¹⁹ Although some structural variations may occur when immersed in aqueous solutions, it is reasonable to assume that the bulk of the SAMs remain homogeneous as a consequence of the peptide hydrophobicity. SAMs prepared with higher fractions (> 5 %) of the Fc-labeled peptide exhibited broadened voltammetry, suggesting a more complex behavior, possibly as a result of the Fc-Fc interaction. Such monolayers were thus not considered further.

2.4 Fitting Procedure

Time-resolved SPR angular changes acquired simultaneously with potential steps were fit to the stretched exponential function:

$$\Delta\theta \propto \exp \left[-(t/\tau)^\beta \right] \quad (\text{Equation 1})$$

where τ is the characteristic relaxation time, or time constant, of the exponential response and β is the stretching parameter (~ 0.5).³² A non-linear least squares regression was performed using Microcal Origin 6.0, with a fixed value of β (in the range 0.4 – 0.6) to determine the time constant.

2.5 Determination of SAM Thickness Changes

SAM thickness changes were calculated from SPR angular shifts using numerical simulation software (Winspall).⁴² A four phase model was employed, consisting of a BK7 prism (refractive index, $n = 1.515$), a gold film (real and imaginary refractive indices, $n = 0.154$ and $k = 3.55$ respectively, thickness 37 nm), the peptide SAM ($n = 1.5$) and the aqueous electrolyte solution ($n = 1.33$).⁴³ The peptide length was estimated by summing the helix length (0.15 nm per leucine residue) and the non-helical chain length (0.125 nm per bond along the chain axis), giving a total length of 4.0 nm.⁴⁴ The size of the Fc group was excluded from the calculation of peptide length, since only ~ 5 % of the SAM molecules were labeled with this moiety. The initial thickness of SAM2 was calculated as 3.6 nm, based on the peptide tilt angle of 26° determined previously for an analogous SAM.¹⁹ The addition of a fifth phase accounting for the accumulation of electrolyte ions at the top of the SAM surface was considered unnecessary given that resulting deviation of the refractive index compared to the bulk electrolyte should be minimal.³⁴

3. Results

Figure 3a depicts typical cyclic voltammograms (CVs) observed for gold surfaces modified with α -helical peptide SAMs in 0.2 M NaClO₄. The SAMs exhibited reversible redox behavior due to the oxidation and reduction of the Fc moiety, centered at a formal potential close to 0.35 V vs. Ag/AgCl. Analysis of the anodic and cathodic peak currents as a function of scan rate in the range 5 – 50 mV/s revealed a linear dependence, indicative of surface-confined electrochemistry. At a scan rate of 50 mV/s the peak-to-peak separation (ΔE_p) was measured to be 100 mV for SAM1 and 140 mV for SAM2. The electrochemical behavior described is in good agreement with that reported previously for analogous monolayers composed of α -

helical peptides consisting of 18-leucine residues, immobilized on gold microelectrodes.¹⁹ The difference in peak separation has been attributed to slower electron transfer kinetics in SAM2 than in SAM1 resulting from restricted motion in the more rigid anti-parallel dipole arrangement.

The Faradaic charge ($1.1 \pm 0.5 \mu\text{C}$), determined by integration of the voltammetric peaks, was used to estimate the coverage of the Fc-labeled peptide (Fc10L), based on the gold surface area (see Experimental section). An average coverage of $2 \times 10^{-11} \text{ mol cm}^{-2}$ ($1.2 \times 10^{13} \text{ molecules cm}^{-2}$) was calculated for the labeled SAM component. If we assume that this represents 5 % of the peptide molecules at the surface, a total peptide coverage of $2.4 \times 10^{14} \text{ molecules/cm}^2$ can be estimated. The coverage is comparable to that typically observed for ferrocenylalkanethiol SAMs ($2.7 \times 10^{14} \text{ molecules/cm}^2$),^{45,46} although this should be interpreted with caution, since the fraction of electroactive component within the monolayer does not necessarily reflect the composition of the self-assembly solution.^{47,48}

Figure 3b shows the simultaneously measured SPR angular changes. As the potential is swept in the anodic direction, an increase in the SPR angle ($\Delta\theta$) is observed which begins to plateau before the sweep direction is reversed. Although there is some hysteresis between the forward and reverse sweeps, in both cases the SPR angle returns approximately to its original value upon completion of the CV, suggesting that any redox-induced surface changes are reversible. The SPR angle-potential profiles for SAM1 and SAM2 are qualitatively similar, though it is clear that the magnitude of the angular changes is greater, by over a factor of two, for SAM2 than for SAM1. Although some film-to-film variation was evident, presumably due to slightly different surface coverage, comparable behavior was observed over a number of peptide-modified gold films.

Implicit in the SPR angle-potential profiles in Figure 3b is information relating to the kinetics of the redox-induced SAM reorganization. However, it is difficult to deconvolute the potential- and time-dependent changes from such a potential sweep experiment due to the various processes that contribute to the overall SPR angular change (*vide infra*). Therefore, chronoamperometry and time-resolved SPR measurements were conducted in order to allow a more accurate measurement of the redox-induced SAM reorganization processes. The electrode potential was pulsed for 20 s between 0 and 0.5 V, switching the Fc-labeled peptides between oxidized and reduced states, and the time-resolved SPR angular changes were monitored simultaneously (Figure 4). As with the CV experiments, the SPR angular changes are clearly greater for SAM2 than SAM1, but interestingly the rate of change during the early stage of each pulse also appears to differ between the two SAMs. For example, in the anodic potential steps, the SPR angle increases more sharply for SAM2 than for SAM1. To investigate this further, we analyzed the initial pulse data (0 – 200 ms) by fitting to a stretched exponential function (Equation 1). Boussaad and Tao have demonstrated that by using a stretching parameter, β of close to 0.5, such a function can allow determination of the time constant, τ , from EC-SPR data, which gives an indication of the timescale of redox-induced changes.³² Such stretched exponential behavior is thought to originate from a broad distribution of activation energies, which in this case may result from a range of conformational states in the peptide molecules.³² Using this equation, excellent fits were obtained (see Figure 4b) using the parameters reported in Table 2. The table includes the angular shift, $\Delta\theta_{\text{max}}$, observed upon stepping from 0 to 0.5 V vs. Ag/AgCl. Control experiments were also undertaken using SAM1b and SAM2b, both of which do not contain the Fc label.

4. Discussion

In order to rationalize the above observations it is necessary to consider what factors contribute to the changing SPR angle. In the case of EC-SPR at SAM-modified surfaces, three major

processes are considered to affect the SPR angle, namely electron density changes at the metal surface ($\Delta\sigma$), monolayer thickness changes (Δd) and refractive index changes (Δn) within and adjacent to the SAM due to the ingress/egress of electrolyte ions and solvent molecules.

Algebraically, the SPR angular shift resulting from a potential perturbation, ΔV , may be written as:^{49,50}

$$\frac{\Delta\theta(\lambda)}{\Delta V} = c_1 \frac{\Delta n(\lambda)}{\Delta V} + c_2 \frac{\Delta d}{\Delta V} + c_3 \frac{\Delta\sigma}{\Delta V} \quad (\text{Equation 2})$$

where c_1 , c_2 , and c_3 are system-dependent constants and λ is the wavelength of the incident light (constant in this work). We will now consider each of the three terms in the context of the α -helical peptide SAM compositions and structures.

The SPR phenomenon naturally depends on the density of electrons at the metal surface, and thus any surface charging effect ($\Delta\sigma$) resulting from a varying potential will induce an angular change. The influence of potential on the surface polarity depends largely on the potential of zero charge (pzc) of the metal/electrolyte interface. Previous studies have indicated that polycrystalline gold immersed in perchlorate solution has a pzc close to 0 V vs. Ag/AgCl.⁵¹ It is thus reasonable to conclude that upon sweeping or stepping the gold electrode potential from 0 to 0.5 V vs. Ag/AgCl, the surface is being changed from approximately neutral to a positively charged or electron deficient state, resulting in a positive SPR angle shift. This process is considered to be the prevailing contribution to the SPR angle changes in SAM1b and SAM2b, where redox processes are absent. However, since the same potential changes were applied to SAM1 and SAM2 in the experiments above, the third term in Equation 2 is expected to be constant and so this charging factor is unlikely to contribute to their differing behaviors.

Changes in the thickness of the SAM (Δd) are expected to shift the SPR angle, and this is likely the dominant factor. Oxidation of the Fc moiety yields cationic ferrocenium (Fc^+), which is electrostatically repelled from the positively charged gold surface, resulting in a smaller tilt angle ψ (i.e. the peptide molecules become more perpendicular to the surface; cf. Figure 2), and thus increasing the apparent monolayer thickness. It has also been shown that the application of a potential can result in a rotation of the ferrocene moiety,⁵² although EC-SPR measurements at ferrocenylalkanethiol monolayers would suggest that tilt angle variations have a greater influence on the observed SAM thickness change.³⁴ The tilt angles in an open-circuit have been determined previously for analogous SAMs, giving values of 22° and 26° for SAM1 and SAM2 respectively.¹⁹ The thickness increase during electrolysis should be translated to a positive shift in the SPR angle, suggesting that the SAM reorganization is significantly greater for SAM2 than for SAM1. This is conceivable if one considers the permeability of the SAMs to electrolyte ions (which, in itself, may also contribute to the refractive index of the SAM).³⁴ The intermolecular attraction between the opposing macro-dipoles in SAM2 makes it a more rigid and more tightly packed monolayer than SAM1, resulting in a lower permeability to electrolyte ions in the former. This is supported by EIS carried out on analogous SAMs, which indicated that the capacitance of SAM1 is greater than in SAM2.¹⁹ This fact is also evident from the charging currents in their CVs (Figure 3a). We propose that the greater ionic permeability of SAM1 causes a more effective shielding of the positive charges accumulated at the gold surface during the anodic potential sweep, by perchlorate anions that could be initially present in the film or penetrated into the film during the SAM reorganization. This would lead to a less pronounced electrostatic repulsion between the gold surface and the electrogenerated Fc^+ moiety in SAM1 compared to SAM2, and thus a smaller SAM thickness change. The greater thickness change in SAM2 may contribute to the greater peak separation (ΔE_p) in the CV, as the longer distance between the Fc^+ and the electrode surface could retard the ET process. An additional feature of the reorganization process that may contribute to the film thickness increase is the possibility that the Fc group penetrates into the SAM due to

enhanced solubility in this phase, whereas the Fc^+ moiety should be better solvated in the aqueous phase. Such behavior is evident from the strong diffusive component indicated by EIS carried out on similar SAMs.^{19,53} This effect is likely more prominent for SAM2 than SAM1 as a result of the more hydrophobic film interior, which further supports our interpretation.

The control experiments carried out in the absence of the Fc label are also consistent with the above proposal (see $\Delta\theta_{\text{max}}$ values in Table 2). Without the generation of a positively charged Fc^+ moiety at the peptide terminus, the electrostatic repulsion that results in the tilt angle changes is no longer present. Thus, SAM1b and SAM2b give comparable SPR angle changes, which are less substantial than that for SAM2, where the repulsive effect dominates. The fact that the maximum SPR angle shift for SAM1 is marginally smaller than those for SAM1b and SAM2b could suggest the operation of an additional effect, resulting from the presence of the Fc label and the parallel dipole alignment, which detracts from the SPR angular shift.

The motion of ions into and out of the SAMs intrinsically affects the refractive index (Δn) at the interface. The migration of solvated electrolyte ions into the SAMs would increase the refractive index and thus result in a positive SPR angle shift. Based on the above argument that permeation of ions through SAM2 is expected to be less than SAM1, this would lead us to predict a *higher* SPR angle change in SAM1. Since this is not the case, we conclude that, during oxidation, the contribution of ion motion *into* the film to the SPR angular changes is much less significant than the SAM thickness changes described above. As for a potential explanation for the smaller $\Delta\theta$ value for SAM1 than for SAM1b, it is possible that, in the case of SAM1, cation expulsion from the monolayer and/or anion incorporation from the solution may occur due to the generation of Fc^+ . If the cation egress is more prominent than anion ingress, this process would result in a net decrease in the monolayer refractive index during the anodic process, which in turn would decrease the SPR angle. Such competitive anion ingress and cation egress processes for charge compensation are well known for both conductive polymer and redox polymer thin films.^{54–57}

Taken together, it is reasonable to assume that the first and third terms of Equation 2 are comparable for SAM2 and SAM2b. Therefore, by comparing the SPR angle changes for each, we have a means to quantify the contribution film reorganization alone, and to estimate the SAM thickness change (Δd) upon oxidation of the Fc label. After the potential sweep/step from 0 to 0.5 V, the difference in $\Delta\theta$ between SAM2 and SAM2b is approximately 45 mdeg. The conversion of this measured SPR angular change to an absolute SAM thickness change may be achieved by solving the Fresnel equations using a numerical simulation.^{34,43} Based on a four layer model (see Experimental section), Δd was estimated at 0.25 ± 0.05 nm, suggesting a decrease in tilt angle from 26° to approximately 16° during this potential perturbation. This value is larger than the monolayer thickness changes observed for ferrocenylundecanethiol SAMs (~ 0.1 nm), determined using EC-SPR and EQCM measurements.³⁴ This suggests a greater repulsive effect in the case of the peptide SAM, which may result from either a more tightly packed peptide SAM and/or a greater ionic permeability in the alkanethiol SAM. Both factors are to be expected since (1) the ferrocenylundecanethiol SAMs were prepared without a diluent (i.e. they consisted of 100 % Fc -labeled alkanethiol), and so the repulsion between the bulky head-groups is likely to have resulted in a slightly less tightly packed film and (2) the stronger intermolecular forces in the peptide SAM allow fewer solvated ions to penetrate than the ferrocenylundecanethiol SAMs. The latter argument can be understood in light of the intermolecular interactions playing a role in SAM structure and rigidity.⁵⁸ In the case of alkanethiol SAMs, only weak Van der Waals forces hold adjacent molecules together, whereas the additional dipole-dipole interactions in SAM2 are expected to be significantly stronger. Combined with the greater hydrophobicity generated by the leucine residues, and the presence of intramolecular hydrogen bonding in the peptide molecules, this results in a much more rigid SAM that is relatively impenetrable to electrolyte ions and their hydration spheres. Although

only a fraction of the electroactive peptide SAMs were Fc-labeled, the significant SPR angle changes observed are a good indication that redox-induced SAM reorganization involves movement of the unlabeled peptides also, which is a further manifestation of the SAM rigidity. It has been demonstrated previously that an applied electric field may influence the length of immobilized peptide molecules as a consequence of their dipole moment, which may further contribute to the observed SAM thickness changes.²⁷ Although such effects cannot be excluded, the influence on the measured SPR angle change must be small, since the maximum angle changes observed for SAM1b and SAM2b, which have very different dipole compositions, are comparable.

Finally, our EC-SPR approach can conveniently deduce the dynamics of the peptide SAM reorganization processes (see data in Table 2). The time constants determined are very fast compared to those reported by Boussaad and Tao for electrochemically induced conformational changes in cytochrome *c* immobilized on mercaptopropionic acid SAMs, which were in the hundreds of milliseconds.³² This is understandable, since conformational re-structuring of the heme center in cytochrome *c* adsorbed through electrostatic interactions is expected to be significantly slower than redox-induced changes in a relatively compact, covalently bound, peptide monolayer. Comparison of the time constants for the oxidative pulses for SAM1 and SAM2 indicates that the redox-induced surface changes in SAM2 occur more quickly than in SAM1. The shorter time constant measured for SAM2 may result from the dominating influence of the tilt angle change, suggesting that this is a very fast process compared to the other contributions. Similarly, the longer time constant of SAM1 could reflect the relatively slow affect of ion motion into/out of the monolayer during the anodic pulse.

5. Conclusions

EC-SPR has been employed for the first time in the investigation of redox-induced reorganization in α -helical peptide SAMs, and it has been demonstrated that this process is highly dependent on the molecular dipole orientation. The dipole-dipole interactions present in SAM2 create a more hydrophobic, relatively ion-free environment such that repulsive forces between electrogenerated Fc^+ and the positively charged gold surface are maximized, yielding a substantial increase in SAM thickness upon oxidation. In contrast, the anions within the more permeable SAM1 effectively neutralize the electrostatic repulsion, leading to a less significant thickness change. These differences are also reflected in the measured time constants. It is evident from our observations that the secondary structure of peptide molecules confers an additional level of control in terms of SAM properties, as compared to the more commonly studied alkanethiols. Our work suggests that intermolecular forces between peptide molecules produce a more rigid structure, which has a significant influence on its reorganization behavior upon redox modulation.

An understanding of the physical changes in SAMs brought about by the applied potential is fundamental to the use of electrochemistry in the study of ET through such monolayers. Thus, factors such as molecular packing and intermolecular forces that may influence the permeability of SAMs to electrolyte ions are inherently important. For example, film thickness changes should be accounted for in the case where scanning probe microscopy techniques are employed to study ET through SAMs, since such methods are highly distance dependent. Also, if ET through SAMs depends on the molecular orientation, changes in the tilt angle triggered by redox reactions and electrostatic repulsions may need to be considered if experimental observations are to be interpreted accurately. In addition to the impact on ET studies, the results presented here are especially interesting from the perspective of molecular electronics. The ability to control redox-induced monolayer thickness changes by adjusting the SAM composition is of no doubt a useful commodity in the fabrication of molecular switching devices. Similarly, the knowledge that such changes are fast (on the order of milliseconds) is

also of significance to this field. Finally, this work is a further demonstration of the power of EC-SPR in the study of notably fast redox-induced surface events, particularly those which cannot be solely addressed by electrochemical methods.

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Figure 1.
Molecular structures of the peptides Fc10L, Ac10L and 10LAc.

Figure 2.

Schematic depiction of peptide monolayers on gold surfaces, indicating the direction of the peptide dipole moments. (a) SAM1 and (b) SAM2. To highlight the difference in the two SAMs, 10Lac molecules are shown in red. The tilt angle of the peptide chains is denoted by ψ .

Figure 3.

Cyclic voltammetry (a) and simultaneous SPR (b) of gold surfaces modified with SAM1 (solid line) and SAM2 (dashed line). CVs were recorded in 0.2 M NaClO_4 at a scan rate of 50 mV/s.

Figure 4.

Time-resolved SPR response of SAM1 (solid line) and SAM2 (dashed line) during potential steps between 0 and 0.5 V vs. Ag/AgCl. (a) 20 s anodic and cathodic pulses. (b) Initial (0 – 200 ms) anodic response with data simulated using Equation 1 and the parameters listed in Table 2 (circles).

Table 1

SAM peptide compositions.

	Fc10L	Ac10L	10LAc
SAM1	5 %	95 %	-
SAM1b	-	100 %	-
SAM2	5 %	45 %	50 %
SAM2b	-	50 %	50 %

Table 2
Summary of EC-SPR results of different SAMs studied in this work

	Anodic Step		Cathodic Step		$\Delta\theta_{\text{max}}/\text{mdeg}$
	τ/ms	β	τ/ms	β	
SAM1	16 ± 2	0.58 ± 0.09	10 ± 3	0.48 ± 0.03	39 ± 10
SAM2	7 ± 2	0.61 ± 0.09	5 ± 1	0.46 ± 0.10	96 ± 20
SAM1b	10 ± 4	0.63 ± 0.07	6 ± 1	0.46 ± 0.04	55 ± 10
SAM2b	10 ± 3	0.62 ± 0.05	6 ± 2	0.49 ± 0.08	52 ± 10