Electrodeposition of ferrocenoyl peptide disulfides†

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Using electrodeposition of cyclic and acyclic Fc–peptide disulfides tightly-packed Fc–peptide monolayers were conveniently formed, which exhibit significant differences in their electron transfer kinetics.

Self-assembled monolayers (SAMs) have been extensively studied over the last 20 years.[1–5] It was shown that the formation of alkylthiol SAMs can be aided by electrodeposition of the film on the gold surface,[6] cutting the time from days to minutes for the preparation of a monolayer. Additionally, the packing of a monolayer prepared in this fashion is denser and appears to lack some of the disorder associated with an incomplete monolayer formation. An electrodeposition step for the corresponding disulfides, which are often used to prepare SAMs has not been reported and usually takes several days.[7,8]

Our aim was twofold: (a) to develop an electrodeposition method for disulfides onto gold surfaces, (b) to investigate the electrochemical properties of these monolayers. We made use of two classes of Fc–peptides: acyclic ferrocenoyl (Fc)–peptide disulfides[9] and cyclic 1,1′-Fc–peptide disulfides,[10] which upon deposition onto a gold surface should give rise to two different structures on the surface, as indicated in Fig. 1. Acyclic systems will have the Fc group linked to the surface by a single amino acid linker, whereas the cyclic system can link the Fc group to the surface using both amino acid spacers. This would suggest differences in the rigidity of the attached molecule, which in turn may influence the electron transfer (ET) kinetics of the film.

We investigated the electrodeposition of the acyclic $[Fc–CSA]_2$ (**1-a**), $[Fc–Gly–CSA]_2$(**2-a**), $[Fc–Ala–CSA]_2$ (**3-a**), $[Fc–Val–CSA]_2$ (**4-a**) and $[Fc–Leu–CSA]_2$ (**5-a**)[9] and of the corresponding cyclic 1,1′-Fc$[CSA]_2$ (**1-c**), 1,1′-Fc$[Gly–CSA]_2$ (**2-c**), 1,1′-Fc$[Ala–CSA]_2$ (**3-c**). 1,1′-Fc$[Val–CSA]_2$ (**4-c**) and 1,1′-Fc$[Leu–CSA]_2$ (**5-c**)[10] and the properties of the corresponding films.

The electrodeposition was accomplished by placing a freshly oxidized (electrochemical cycling from 0.2 V to 1.6 V *vs.* Ag/AgCl in 0.5 M H_2SO_4) microelectrode (diameter: 25 μm) into a 10 mM ethanolic Fc–peptide disulfide solution and applying −1.3 V for 30 min. Note the absence of supporting electrolyte. Longer applied potential times were tested, but afforded no change in monolayer coverage and more anodic potentials did not result in monolayer formation. The large negative potential is known to reduce disulfides to thiolate anions,[11] which readies the system for monolayer formation. We compared these results with conventional incubation of the microelectrodes in a 1 mM ethanolic Fc–peptide solution for 5 days at room temperature. The resulting films were assessed electrochemically by cyclic voltammetry (CV), chronoamperometry (CA) and differential pulse voltammetry (DPV) (see ESI†). The film thickness was measured by ellipsometry and gave values of 9(3) Å for both electrodeposited and incubated monolayers (excluding **1-a** and **2-a**), which compares well with the calculated value for film thickness of 9(2) Å (n_S = 0.25 and K_S = 3.46 for the substrate, η = 1.40, see ESI†). The surfaces were also characterized by X-ray photoelectron spectroscopy (XPS) showing identical signals for both the electrodeposited and incubated film (see ESI†). CV was carried out on a custom-built potentiostat and CA was carried out using CHInstruments potentiostat model 660B. All electrochemical measurements were carried out in water using at least 5 different Fc–peptide modified gold microelectrodes to ensure reproducibility

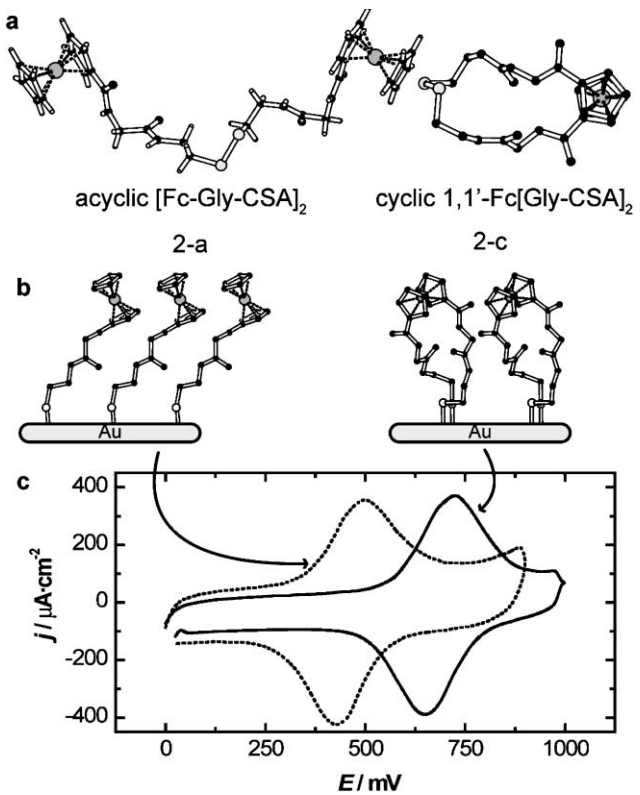

Fig. 1 (a) Crystal structure of acyclic $[Fc–Gly–CSA]_2$ (**2-a**) and cyclic 1,1′-Fc$[Gly–CSA]_2$ (**2-c**) (b) Schematic representation of the resulting Fc–peptide surfaces. (c) Cyclic voltammograms of **2-c** (solid line) and **2-a** (broken line) films on Au microelectrodes (d = 25 μm). 2.0 M $NaClO_4$ supporting electrolyte, scan rate 1000 V s^{-1}, Pt mesh auxiliary and Ag/AgCl (3.5 M KCl) reference electrode.

† Electronic supplementary information (ESI) available: Figs. S1–S6 and Tables S1–S4. See http://www.rsc.org/suppdata/cc/b4/b415278f/

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Table 1 Summary of electrochemical parameters analyzed by CV and CA. Values in parentheses are the standard deviations from 5 electrode measurements

	Electrodeposition from EtOH			
Compound	$E^{0\prime}$/mV	$k_{ET} \times 10^3$/s^{-1a}	Specific Area/Å^2 molecule^{-1}	Surf. Conc. $\times 10^{-10}$/mol cm^{-2}
1-c	670(7)	9.5	45(7)	3.7
1-a	465(9)	8.0	40(7)	4.2
2-c	688(6)	14.0	47(8)	3.5
2-a	464(6)	13.5	50(8)	3.3
3-c	635(6)	12.0	68(9)	2.4
3-a	490(7)	6.0	36(5)	4.6
4-c	670(7)	12.0	60(9)	2.8
4-a	488(7)	9.5	65(8)	2.6
5-c	686(8)	17.0	60(8)	2.8
5-a	484(7)	11.0	72(8)	2.3

[a] Error for k_{ET} calculations was 1.5×10^3 s^{-1}.

(2 M $NaClO_4$ supporting electrolyte, reference electrode: Ag/AgCl/3.5 M KCl, Pt mesh auxiliary electrode).

The electron withdrawing capability of the amides makes the disubstituted Fc more difficult to oxidize. All electrochemical parameters are included in Table 1. Integration of the Faradaic current provides the Fc surface concentration,[12] from which a specific area per molecule can be calculated. The theoretical area (calculated from crystal structure data)[9,10] of the acylic Fc–peptides and 1,1′-cyclo-Fc–peptides are ~30 and ~40 Å^2 molecule^{-1}, respectively.

Electrodeposited films of Fc–peptides, gave consistently higher surface concentration compared to films obtained by conventional incubation, suggesting that electrodeposition gives a tighter packed film. It is noteworthy that the difference in the molecular footprint obtained for 1,1′-Fc–peptide films prepared by electrodeposition and standard incubation are large. For acyclic Fc–peptides this difference is still significant. The electrodeposition of the 1,1′-cyclo-Fc–peptides results in a 2 to 3 times greater surface coverage than the incubation method. It appears that packing is significantly less tight if the films are prepared by incubation.

The full-width-at-half-maximum, E_{fwhm} (see ESI†), is a useful parameter that assesses the homogeneity of the Fc environment. The redox signal for all Fc–peptide films prepared in this study exhibit widths that exceeds the ideal, E_{fwhm} of 90 mV,[13] indicating the presence of some lateral interactions between the molecules in these films. H-bonding presumably plays an important role as was shown before in films of the acyclic Fc–peptides.[13–18] Interestingly, there is little difference between the films formed from cyclic and acylic Fc–peptides. However, Fc–peptide films formed by electrodeposition have lower E_{fwhm} values (160(10) mV *versus* 210(20) mV). This difference points to a more uniform film if electrodeposition is used.

The ET kinetics of all films were assessed by CV and CA and are summarized in Table 1 and S3 (see ESI†). The methods were described before.[18] There are two key results of our kinetic study: (a) the k_{ET} for films prepared by electrodeposition or by incubation are the same; (b) the k_{ET}s for Fc–peptide films of cyclic Fc–peptides are higher compared to the corresponding acyclic systems. A probable explanation for the faster ET kinetics for the cyclic systems is their inherent ability to establish two Au–S linkages, allowing ET to proceed along both peptide spacers. It is also interesting to note that most amino acid systems exhibit faster k_{ET} compared to compounds **1-a** and **1-c** having only a cysteamine spacer. The amino acid chain may allow for better packing on the surface due to intermolecular H-bonding interactions thereby increasing the rigidity of the linker.

Confirmation that both sulfur atoms of the cyclo systems are bound to the gold comes form reductive desorption experiments. As stated above Fc is a one-electron redox probe and sulfur is known to undergo a one electron reductive desorption at sufficiently negative potentials. Thus, DPV experiments were carried out in H_2SO_4 for the Fc and KOH for the Au–S reduction due to the instability of Fc at high pH values. The integration of the Fc and sulfur reduction for both the acyclic and cyclo FcGly derivatives **2-a** and **2-c** are shown in Fig. 2. The ratio of the integrated area between the Fc and sulfur reduction peaks shows that the cyclo system has a 1 : 2 (Fc : S) ratio and the acyclic derivative has a 1 : 1 (Fc : S) ratio which, is evidence that both sulfur atoms of the cyclo derivatives were bound to the Au surface. The shoulder in Fig. 2b, at *ca.* −0.45 V, is attributed to the decomposition of Fc at high pH. Additionally, crystal structure data supports this claim because the cyclo derivatives participate in intramolecular H-bonding and the acyclic derivatives exhibit intermolecular H-bonding.

In summary, we have presented an electrochemical method to form Fc–peptide monolayers from Fc–peptide disulfides, giving rise to well-packed monolayers on gold. This method should find widespread applications for the formation of monolayers from disulfides. Our studies allowed a direct comparison of the ET

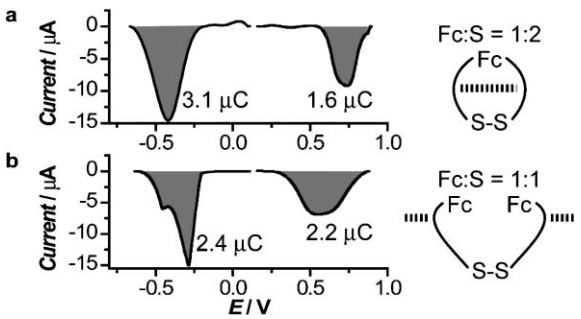

Fig. 2 DPVs of (a) cyclo and (b) acyclic, FcGly derivatives. Integrated peak currents for cyclo- and acyclic-derivatives are in a 1 : 2 and 1 : 1 ratio, respectively, indicating both sulfur atoms of the cyclo derivatives are bound to the Au surface. The hatched lines in the models represent H-bonding patterns found in the crystal structure.

kinetics of cyclic and acyclic Fc–peptide disulfide systems. Our results show faster ET kinetics for the cyclic systems compared to the acyclic systems, which may be the result of the enhanced rigidity of the molecules on the surface. We are now investigating this phenomenon in more detail and hope to compare our results to the growing number of ET studies on other Fc–peptide systems[18–21] in order to get additional mechanistic insight.

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