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A Preliminary Study of Influence of the Chain Length of Alkanethiols on the Electrochemical Behavior of Redox Self-Assembled Multilayer Thin Films

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A Preliminary Study of Influence of the Chain Length of Alkanethiols on the Electrochemical Behavior of Redox Self-Assembled Multilayer Thin Films

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Abstract: Alkanethiols and ferrocene derivates were used to construct redox self-assembled double- or triplex-layer thin films, which acted as a model of redox self-assembled multilayer thin films (SAMTFs) to study the influence of alkanethiols chain length on the electrochemical behavior of the SAMTFs The rate of electron transfer reaction decreased exponentially with the distance between the Fc groups and the underlying electrodes The influence of chain length on the electrochemical behaviors of the SAMTFs was inconsistent under different situations On the experimental situation of this work, when electroactive groups were closer to the electrodes, SAMTFs by longer chains of alkanethiols displayed stronger electrochemical responses, while when distance between electroactive groups and electrodes was extended, SAMTFs by shorter chain would present more obvious electrochemical responses

Key words: Self-assembled multiplayer thin films, Cyclic voltammetry, Chain length, Alkanethiols, Electron transfer, Ferrocene

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1 Introduction

Alkanethiols can be self-assembled onto metal surfaces to form ordered and compact monolayers Self-assembled monolayers (SAMs) of substituted alkanethiols with terminal functional groups were often regarded as an attractive bionic membrane to study the interfacial processes[1,2] or as a functional interface to create self-assembled multiplayer thin films (SAMTFs), making use of cross-linking reaction or electrostatic attraction[3,4]. For the redox SAMs of alkanethiol, the influence of chain length on the electron transfer (ET) and the electrochemical behavior of the SAMs have already been extensively studied[5], while less work has been reported about those on the characters and electrochemical behavior of redox SAMTFs, which have great connection with a hot topic-constructing applied chemosensors and biosensors When the electroactive groups situated at the terminal of SAMTFs, the chain length would affect the electrochemical behavior of the SAMTFs on two aspects One hand, the stability and order of alkanethiol monolayer increased with the chain length and so did the attachment of electroactive groups[6]. On the other hand, alkanethiols acted as a potential energy barrier for the ET reaction between the electrodes and electroactive groups and the rate of ET decreased exponentially with the chain length[7]. Therefore, there was a compromise between the attachment increase of

electroactive groups and rate decrease of ET with chain length extending. In the present study, self-assembled bilayer and trilayer thin films with ferrocene groups situated at the termini of the multilayers were applied to investigate the integrated influence of alkyl chain length on the electrochemical behavior of redox SAM TFs.

2 Experimental

2.1 Reagents

1, 3-propanedithiol (HSC$_3$SH), 1, 6-hexanedithiol (HSC$_6$SH) and 1, 9-nonanedithiol (HSC$_9$SH) were purchased from Sigma-Aldrich. 3, 3'-dithiobis (succinimidylpropionate) (C$_3$-NHS, Sigma-Aldrich), reduced form of glutathione (GSH, Amresco) and aminoferrocene (FcNH$_2$, Tokyo Kasei Kogyo Co., LTD) were also acquired commercially. The hexapeptide, which sequence is Lys-Cys-Thr-Cys-Cys-Ala (FT), was synthesized by Sangon Co., (Shanghai, China). 11, 11'-dithio-bis(succinimidyl undecanoate) (C$_{11}$-NHS), -ferrocenylethylamine (FcC$_2$NH$_2$), and N-(2-ethyl-ferrocene) maleimide (Fc-Mi) were synthesized according to literature procedures[8, 9].

2.2 Constructing Self-Assembled Multilayers on Gold Eectrodes

The first monolayers (FMs) were fabricated by immersing the gold electrodes into respective solutions for various lengths of time. C$_3$-and C$_{11}$-NHS were used to form the NHS-terminated FMs and HSC$_3$SH, HSC$_6$SH, and HSC$_9$SH to form the sulphydryl-terminated FMs[10]. The modified electrodes were fully washed with respective solvent, and then were immersed in other solutions, as above description, to get further modification.

2.3 Electrochemistry

Voltammetric analysis and Quartz Crystal Microbalance (QCM) frequency measurements were conducted at ambient temperature with a CHI 440 electrochemical workstation (CH Instruments, Austin, TX). A conventional three-electrode cell was used to carry out the cyclic voltammetry, with a platinum electrode and a Ag/AgCl electrode as the auxiliary and the reference electrodes, respectively. The working electrodes were gold disks with a diameter of 2-mm. Prior to each measurement, the Au electrodes were polished with diamond pastes and alumina slurry down to 0.05μm on a polishing cloth. The QCM frequency measurement was carried out in air. 9.995MHz AT-cut crystals coated with gold films were acquired from ICM Technologies (Oklahoma City, OK). After the pre-treated crystals were incubated in the solution of alkanethiols (or alkanedithiols) for enough time, crystals modified with the FMs were gotten and resonance frequencies were measured as the initial one. After the following attachment of the Fc derivatives or Fc labeled molecules, the final frequencies were recorded. Contrast between the frequencies was used to deduce the mass of attachment.

2.4 Mass Spectrometry

Matrix-assisted laser desorption-time mass spectrometer (MALDI-TOF, Biflex, Bruker) were used to analyze the composition of reactive products resulting from FT and Fc-Mi.

3 Results and Discussion

We used self-assembled bilayer or trilayer thin films to construct models with different average distance between the Fc groups and the Au electrodes, as shown in Fig 1. Alkanethiols with functional terminal groups were self-assembled to form the FMs. Fc derivatives anchored to the FMs indirectly (Fig 1a) or directly (Fig 1b).

Tunneling effect resulted in the ET reaction through the SAM TFs. The equation for the effect of tunneling on ET rate constant was $k_{(x)} = k_{(x=0)} \exp(-x)$[11], where x is the distance over which ET occurred and is a factor about nature of the medium. This equation revealed an exponential decrease in ET rate with x and . So the chain length influence on electrochemical responses of bilayer or trilayer system would be inconsistent.

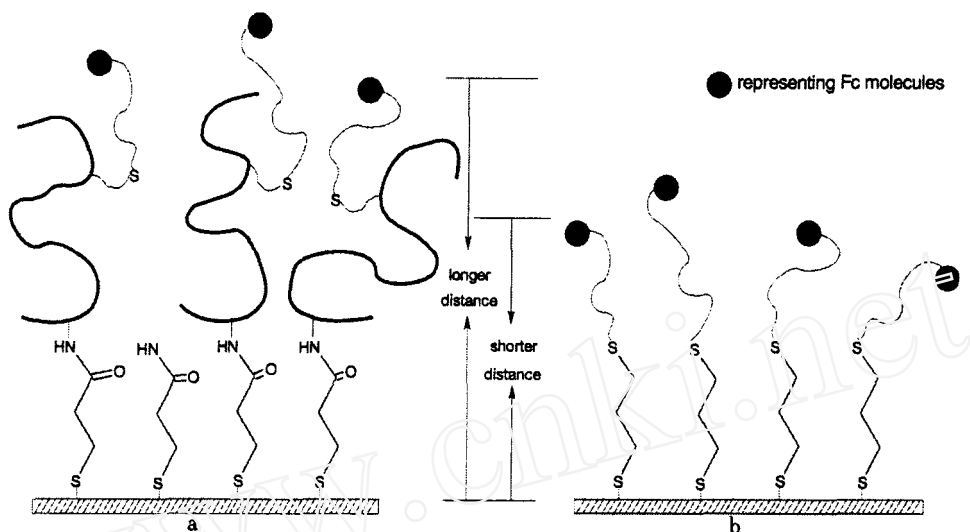Fig 2 was the cyclic voltammograms (CVs) of

Fig 1 Schematic layout of the SAM FMs with trilayers (a) and bilayers (b), representing the system having longer and shorter distance between the electrodes and Fc groups, respectively

Tab. 1 Voltammetric parameters and values of FcC_2NH_2 at NHS-terminated FMs

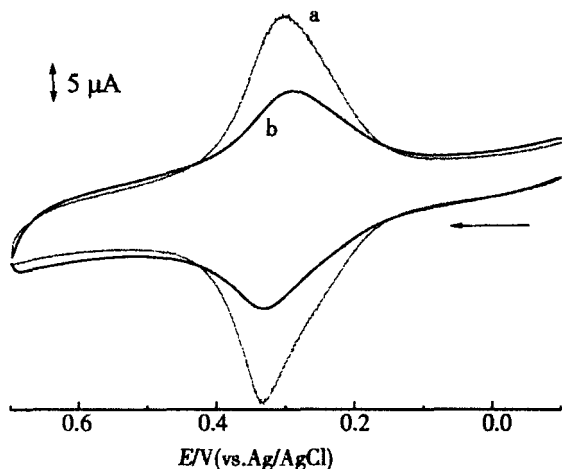| Reactions	E_{pa} /V	E_{pc} /V	E_p /V	i_{pa} /μA	Γ_{NHS} /nmol·cm^{-2}	Γ_{Fc} /nmol·cm^{-2}
C_{11}-NHS	0. 328	0. 301	0. 027	1. 10	0. 681 ± 0. 029	0. 429 ± 0. 014
C_3-NHS	0. 334	0. 299	0. 035	0. 95	0. 579 ± 0. 046	0. 326 ± 0. 020

Fig 2 Cyclic voltammograms of the FcC_2NH_2 attached onto a C_{11}-NHS FMs (a) and a C_3-NHS FMs (b) acquired in 0. 1mol/L HClO$_4$ solution

scan rate: 0. 1 V/s, the arrow indicated the scan direction

FcC_2NH_2 directly attached to the NHS-terminated FMs Peaks of FcC_2NH_2 immobilized on the C_{11}-NHS FMs (curve a) was more obvious than those on the C_3-NHS FMs (curve b) and the surface coverage of Fc (Γ_{Fc}) on C_{11}-NHS FMs was bigger than Γ_{Fc} on C_3-NHS FMs (Tab. 1). The values of Γ_{Fc} were calculated by the equation of $\Gamma = Q/nFA$, where Q is the area of the Fc oxidation peak at a definite sweep rate in CVs, n ($=1$) is the moles of electrons involved in the Fc oxidation, F is the Faraday's constant, and A is the area of the electrode. Because of the van der Waals force, C_{11}-NHS, with longer chain length, was prone to form the ordered and compact FMs[7], which proved by the value of Γ_{NHS} in Tab. 1, deduced from QCM measurements according to the formula of $\Gamma = -\Delta f/(C_f M_f)$, where M_f is the molecular weight of the alkanethiols, C_f is the sensitive factor of the crystal and Δf is the gap of the resonant frequencies before and after the alkanethiols modification. The denser NHS groups in C_{11}-NHS FMs naturally immobilized more FcC_2NH_2 than those in C_3-NHS FMs did[7]. The peak shape in Fig 2 revealed fairly facile electron transfer (ET), which meant dis-

tance from Fc to electrode was suitable for effective ET. In this model, the more obvious electrochemical response of C_{11}-NHS FMs than C_3-NHS FMs should be mainly due to more Fc groups on C_{11}-NHS FMs
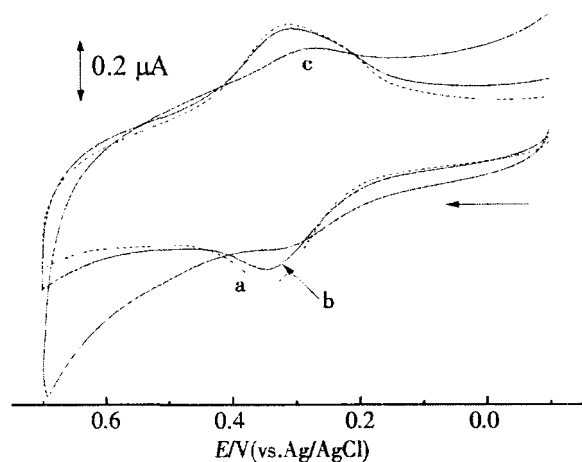

Fig 3   Cyclic voltammograms of the Fc-Mi anchored onto SAMs of HSC_9SH (a), HSC_6SH (b) and HSC_3SH (c) in 0. 1 mol/L HCO_4 solution

scan rate: 0. 1 V / s, the arrow indicated the scan direction

Figure 3 showed the CVs of Fc-Mi immobilized on the SH-terminated FMs, formed by alkanedithiols with chain length of 3, 6 and 9. The attachment of Fc-Mi came into being by the Michael addition of the Mi moieties to the sulphydryl groups[12]. Peaks on the CV of Fc-Mi stemming from HSC_3SH (curve c), were quite unobvious resulting from its lowest $_{Fc}$ (Tab. 2). With increase of the carbon chain, FMs of HSC_6SH and HSC_9SH became compact and immobilized more Fc (Tab 2). Relying on the facile ET reactions, just like the case of NHS-FMs, of Fc-Mi was still the main factor that influenced the electrochemical responses and longer chain was preferable for bigger response.

However, influence of the alkanethiol-FMs was thoroughly different when the distance between the underlying electrodes and the electroactive groups was enlarged a lot GSH, anchored on the NHS-FMs and bound Fc-Mi via sulphydryl groups, were introduced to construct a trilayer thin film (Fig 1a). Compared with the bilayer structure, the average distance was extended so much that it would affect the ET evidently, which was testified as below using the trilayer system. Carrying out QCM measurement to detect the total quantity of the immobilized Fc-Mi (72 ± 6 pmol/ cm^2) and integrating the anodic peak to deduce the number of Fc (62 ± 5 pmol/cm^2) bringing electrochemical response. The discrepancy was 10 pmol/ cm^2 and it would be even bigger using a larger oligopeptide-FT with three cysteine residues To avoid the influence from different labeling sites, we prepared fully labeled sample with the monitoring of MALDI-TOF.

Figure 4 was the data and the peaks of 1554. 7 and 1593. 8, showed the predominance of the fully labeled adduct and its complexing ion with kalium, respectively. Constructing SAMTFs with this adduct anchored onto C_3-NHS FMs, the quantity of immobilized Fc and those bringing electrochemical signals were calculated to be 52 ± 4 and 34 ± 2 pmol/cm^2, respectively.

The discrepancy was actually enlarged in comparison with that of GSH-SAMTFs, which could be attributed to the increase of the medium thickness[11]. Therefore, much different from the bilayer thin film, distance between Fc and electrodes in the constructed trilayer system were extended to the range that the cumbering of the SAMTFs to the tunneling effect became evident and the ET was sensitive to the distance changing The alkanethiols chain length would work on the of Fc, as well as on the ET in the model of

Tab. 2 Voltammetric parameters and   values of Fc-Mi attached onto HS-terminated FMs

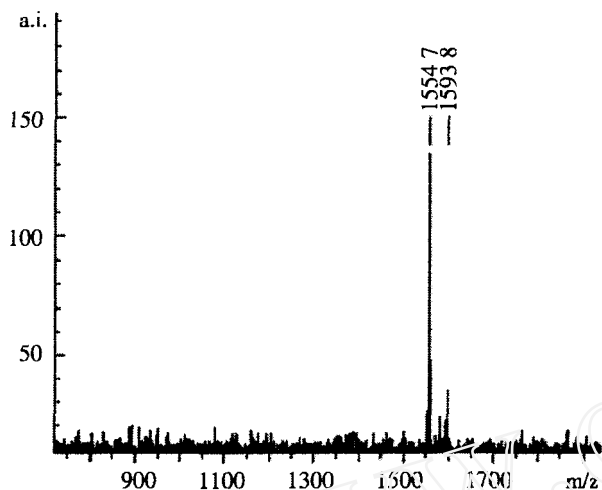| Reactions	E_{pa} /V	E_{pc} /V	E_p /V	i_{pa} /μA	$_{Fc}$ /nmol· cm^{-2}
HSC_3SH	0. 309	0. 280	0. 029	0. 046	0. 018 ± 0. 002
HSC_6SH	0. 327	0. 305	0. 022	0. 129	0. 051 ± 0. 005
HSC_9SH	0. 335	0. 317	0. 018	0. 170	0. 072 ± 0. 010

Fig 4　MALDI-TOF-MS of the products of Fc-Mi and FT in PBS solution, the measurement was carried out 20 h after the mix of reactants with the mol ratio of 25 ∶ 1 (Fc-Mi ∶ FT). The molecular weight of FT, FT + (Fc-Mi), FT + 2 (Fc-Mi) and FT + 3 (Fc-Mi) are 627. 8, 936. 4, 1245. 5 and 1554. 6, respectively

Fig 5　Cyclic voltammograms of the Fc-Mi-labeled GSH attached onto a C_3-NHS (a) and a C_{11}-NHS (b) SAM acquired in 0. 1 mol/L $KClO_4$ solution scan rate: 0. 1 V / s, the arrow indicated the scan direction

trilayer, thus the influence on the electrochemical behaviors of trilayer system was distinct

Figure 5 was the *CV* s of Fc-Mi immobilized onto the NHS-terminated FMs via GSH. Visibly, the peak current of the *CV* of Fc-Mi anchored to C_3-NHS FMs was higher than that of Fc-Mi stemming from C_{11}-NHS FMs, though C_{11}-NHS FMs still had the potential to immobilize more Fc-Mi. This phenomenon was just opposite to that of the bilayer system. Hence, the of Fc was not the main factor, but only one of the factors that could influence the electrochemical behaviors in these trilayer systems

Apparently, with respect to electrochemical investigation of the redox SAMTFs, which possibly to be thick from electrodes to electroactive groups, alkanethiols with longer carbon chain were not favorable for constructing the SAMTFs showing sensitive electrochemical responses

4　Conclusions

It was discovered that there was a distance range, within which longer chain alkanethiols behaved well at electrochemical responses such as in the case of SAMTFs wit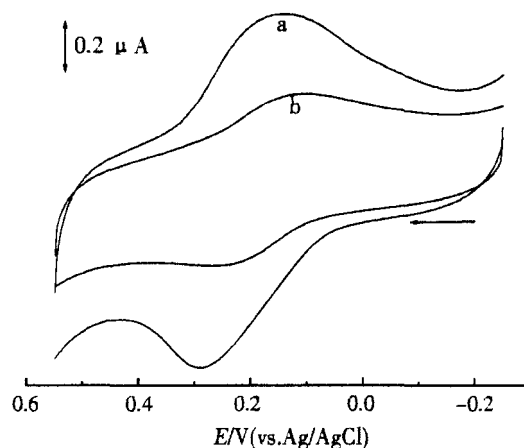h a bilayer structure or comprised of small molecules, and shorter chain alkanethiols behaved better electrochemically, beyond which the ET was too be cumbered to be perceptible. This study about the model of SAMTFs on electrodes would provide practicable groundwork for the construction of well-defined and effective chemosensors and biosensors. The further studies are in progress

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