Dependence of the redox reaction of cytochrome \(c\) on the mixing state of binary self-assembled monolayers composed of 11-aminoundecanethiol and 10-mercaptoundecanoic acid on Au(1 1 1)

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Abstract

Electron transfer (ET) of horse heart cytochrome \(c\) (cyt \(c\)) immobilized on the artificially phase-separated binary self-assembled monolayers (SAMs) composed of 11-aminooctanethiolate (AUTe) and 10-carboxy-1-decanethiolate (MU Ae), (p-SAMs) has been investigated. It has been found that cyt \(c\) selectively adsorbs on domains of MU Ae of the p-SAMs by the electrostatic attraction and the ET of cyt \(c\) on p-SAMs is enhanced compared to that of cyt \(c\) adsorbed on the homogeneously mixed SAM of AUTe and MU Ae and also on the single-component MU SAM. This suggests a favorable orientation of cyt \(c\) on p-SAMs for ET with the electrode.

1. Introduction

Self-assembled monolayers of \(\omega\)-carboxyl-terminated thiolates that provide a negatively charged platform have been widely used for the study of the electron transfer (ET) reactions of horse heart cytochrome \(c\) (cyt \(c\)) because the protein immobilization can simply be achieved by electrostatic interaction through lysine-rich region of cyt \(c\) with the carboxyl-group exposed surface [1–21].

The SAMs of carboxyl-terminated thiolates have been frequently modified with a second thiolate species such as methyl- (CH\(_3\)) and hydroxyl (OH-) terminated thiolates for controlling the conformation [22–26] and orientation [25,27], for facilitating the ET of cyt \(c\) [28,29], and for selective immobilization into nanometer-scale regions [26]. On the other hand, carboxyl-terminated and the trimethylamino-terminated thiolates form binary SAMs that prevent the adsorption of cyt \(c\) [30]. This function has been ascribed to the formation of zwitterionic SAMs of amino- and carboxyl-functional groups [30]. These foregoing studies suggest that the presence of a second component in binary SAMs composed of carboxyl-terminated thiolates can greatly affect the way of the immobilization as well as ET of cyt \(c\) through the change in charged distribution and in the way the charged molecules are distributed on the surface.

In the present study, we report the effect of two-dimensional distribution of carboxyl-terminated SAMs on the electrochemical properties of cyt \(c\) adsorbed on the SAM. We employed binary SAMs composed of 11-aminoundecanethiol (AUTe) and 10-mercaptoundecanoic acid (MU Ae) having similar alkyl chain lengths. A simple coadsorption of the two thiols gives rise to a homogeneously mixed binary SAM at a molecular level [31]. To change the two-dimensional distribution of charged alkanethiolates, we used the electrochemical replacement method [32], which has been used for preparing artificially phase-separated SAMs having domains size in nanometer-scale [33–41]. We show that the two-dimensional distribution of the charged thiolates can be controlled by varying the structure of the binary SAMs from molecular- to nanometer-scale, and that the binary SAMs having two distinct types of nanometer-scale domains of AUTe and
MU Ae can be used for selective immobilization of cyt c for studying the ET between cyt c and the underlying electrode.

2. Experimental

11-Amino-1-undecanethiol hydrochloride (AUT) (Dongjinco), 10-carboxy-1-decanethiol (MUA) (Tokyo Kasei Kogyo), and 2-hydroxyethanethiol (MEL) (TIC) were used without further purification. Horse heart cyt c was purchased from Sigma and purified chromatographically [42]. Water was purified with a Milli-Q system (Millipore Co.). All other chemicals were of reagent grade and used without further purification. Gold substrates were prepared by vapor deposition of gold (99.99% purity) onto freshly cleaved mica sheets (Nilaco, Japan) which had been baked at 580°C prior to the desorption and maintained at 580°C during the deposition [43].

2.1. Preparation of the template phase-separated SAMs of AUTe–MELe

The template binary SAMs of AUTe–MELe were formed on Au(111) substrates by immersing gold substrates in the ethanol solution of AUT and MEL for more than 24 h, followed by rinsing with ethanol and drying in air [44]. In order to form sizeable domains, the total concentration of the thiols was set to be $1 \times 10^{-5}$ mol dm$^{-3}$ [45]. The composition of the template SAMs was controlled by varying the molar ratio of MEL, $\gamma_{\text{MEL}}^\text{soln}$, keeping $c_{\text{total}}$, the total constant, where $\gamma_{\text{MEL}}^\text{soln}$ is defined by $\gamma_{\text{MEL}}^\text{soln} = c_{\text{MEL}}^\text{soln}/c_{\text{total}}$, and $c_{\text{total}} = c_{\text{MEL}} + c_{\text{AUT}}$, $c_i$ being the molar concentration of $i$ ($i = \text{MEL}$ and AUT). The ratio of MEL adsorbed on the surface, $\gamma_{\text{MEL}}^\text{surf} = Q_{\text{MEL}}/(Q_{\text{MEL}} + Q_{\text{AUT}})$, was estimated from the charge under the voltammetric peaks of the reductive desorption [46,47] of the MEL domains ($Q_{\text{MEL}}$) and that of the AUT domains ($Q_{\text{AUT}}$).

2.2. Preparation of the final phase-separated SAMs of AUTe–MUAe

The selective replacement method [33,48] was used to form the phase-separated SAMs of AUTe–MUAe. MEL-rich domains were removed from the template binary SAMs by applying a potential of $-0.65$ V for 20 min in a 0.5 mol dm$^{-3}$ KOH solution. After the removal of MELe, the substrates were rinsed with ethanol and dried in air. The substrates were then immersed in $1 \times 10^{-3}$ mol dm$^{-3}$ MUA solution for 15–20 min to form the final phase-separated binary SAMs of AUTe–MUAe.

2.3. Cyt c immobilization on SAMs

Cyt c was immobilized on SAMs by immersing SAM-covered gold substrates into $5 \times 10^{-5}$ mol dm$^{-3}$ cyt c solution (10 mmol dm$^{-3}$ sodium phosphate buffer, pH 7.1) for 20 min. Excess cyt c was washed off by the buffer solution. Cyclic voltammograms (CVs) were recorded in the buffer solution without containing cyt c. The surface area of the electrode was estimated to be 0.126 cm$^2$. The potential was referred to an Ag/AgCl (saturated KCl) electrode. Scanning tunneling microscope (STM) images were taken under ambient conditions using a Nanoscope III (Digital Instruments). Mechanically cut Pt$_{80}$Ir$_{20}$ tips were used for STM imaging in air.

3. Results and discussion

Fig. 1A shows CVs for the reductive desorption of the template binary SAMs of AUTe–MELe in 0.5 mol dm$^{-3}$ aqueous KOH solutions. Curves (a)–(c) in Fig. 1A show the CVs for the binary SAMs formed in the thiols solution, $\gamma_{\text{MEL}}^\text{soln} = 0.5, 0.6$ and 0.8, respectively. The peak at $-0.65$ V grew with the increase of $\gamma_{\text{MEL}}^\text{soln}$, while the peak at $-0.95$ V was decreased. This suggests that the peak at the negative potential ($-0.95$ V) corresponds to the reductive desorption of AUTe-rich domains, and the peak at the positive potential ($-0.65$ V) corresponds to the reductive desorption of MELe-rich domains. The appearance of the two separated peaks indicates the phase separation in the template binary SAMs of AUTe–MELe [49].

Curves (a)–(c) in Fig. 1B are CVs for the reductive desorption of the SAMs of AUTe–MUAe prepared with the selective replacement method [33,48] from the corresponding template binary SAMs in Fig. 1A. The sharp single peaks were observed at the potential $-0.96$ V. This peak potential is close to the reductive desorption peak potential of MUAe of the phase-separated binary SAMs and to that of AUTe, and suggests that AUTe and MUAe molecules exist in the final SAMs.

Fig. 2 shows the STM images of the artificially phase-separated binary AUT–MUA SAMs prepared by the selective replacement method. These 100 × 100 nm images show the existence of bright and dark domains. Although AUT and MUA have similar alkyl chain lengths, the existence of bright and dark regions in the STM images shows the difference in the tunnelling characteristics of the terminal group AUT and MUA. The coverage of the bright regions was decreased with increasing $\gamma_{\text{MEL}}^\text{soln}$ as shown in Fig. 2(a)–(c), where the bright spots correspond to AUTe domains, and dark regions correspond to MUAe domains. These STM images ensure that the sizable phase separation of the final binary SAM takes place on Au(111).

Fig. 3 shows the dependence of the surface coverage of MELe on $\gamma_{\text{MEL}}^\text{soln}$ estimated from the area under the peak at $-0.65$ V in voltammograms of the redutive desorption [46,47]. Assuming that all adsorbed MELe molecules of the template binary SAMs are replaced with MUA from the solution, we can estimate the surface coverage of MUAe domains of the final binary SAMs of AUTe–MUAe from this figure.

Fig. 4 shows (CVs) recorded for cyt c-immobilized SAMs of different surface compositions. A couple of redox peaks appeared in the forward and reverse scans (thick...
line) corresponding to the redox reactions of cyt c adsorbed on the phase-separated binary SAMs of AUT–MUA (p-SAMs), while no peak was observed for cyt c adsorbed on the single-component SAM of AUTe (long dashed line). This difference in voltammetric behavior suggests that cyt c selectively adsorbs on MUA domains of the p-SAMs through electrostatic attraction between cationic lysine residues with carboxylate terminals on the p-SAMs surface [9,12,14,15,19,26,50]. The surface concentration, \( G \), of electroactive cyt c adsorbed on p-SAMs estimated from the CV varied from 4.2 to 5 \( \mu \)mol cm\(^{-2} \): \( E \) changed from 0.2 to 0.8 (Fig. 5). In contrast, the electroactive cyt c adsorbed on a single-component MUAe SAM (dotted line) was 9 \( \pm 0.5 \) pmol cm\(^{-2} \), which is similar to the values reported for cyt c adsorbed on carboxyl-terminated SAMs [10,29].

A small couple of peaks (thin line) can be observed for cyt c adsorbed on homogeneously mixed binary SAMs of AUT–MUA (h-SAMs). Similarly, Whitesides et al. reported that binary SAMs composed of amino- and carboxyl-/sulfo- terminated thiols can form zwitterionic SAMs that can resist the adsorption of proteins [30]. The significant decrease in the amount of electroactive cyt c adsorbed on h-SAMs suggests that the adsorption of cyt c to the electrically neutral SAMs is much weaker in comparison with that to the phase-separated state of p-SAMs.

Fig. 6 shows the scan rate dependence of the peak separation, \( \Delta E_p \), of cyt c immobilized on different SAMs. The increase in \( \Delta E_p \) for the cyt c adsorbed on the p-SAMs with the scan rate was slightly greater than that for the single-component MUAe SAM. On the other hand, the increase for the h-SAMs was significantly greater than that for the p-SAMs. The variation in \( \Delta E_p \) with the scan rate reflects that the ET rate for cyt c immobilized on the p-SAMs is faster than that for cyt c immobilized on the single-component MUAe SAM and significantly faster than that on h-SAMs.

The ET rate constant of a protein adsorbed on a SAM is probably proportional to the probability of the electronic coupling across the SAM/protein interface [3]. Thus, one might expect that the increase in \( \Gamma \) of cyt c adsorbed on the single-component MUAe SAM compared with the amount adsorbed on h-SAMs leads to the increase in the ET rate. However, the increase in ET rate for cyt c adsorbed on the p-SAMs compared with those on the single-component
SAM of MUAe suggests that the orientation of cyt c also depends on the two-dimensional distribution of MUAe molecules. For the case of p-SAMs, cyt c molecules preferentially adsorb on MUAe domains, while AUTe domains block the adsorption of the protein. The appearance of AUTe domains alters the mixing state of MUAe and AUTe from the molecular level mixing in h-SAMs to the nanometer-scale phase separation in p-SAMs. This may be the reason for the change in the orientation of cyt c favorable for ET. Fig. 6 shows that for p-SAM the ET rate of adsorbed cyt c does not strongly depends on the surface composition of thiolates. This suggests that two-dimensional structure of MUAe SAM in nanometer-scale plays an important role in determining the orientation of cyt c.

The increase in the ET rate of proteins adsorbed on mixed SAMs compared with single-component SAMs of carboxy-thiolates was also reported [28]. For example, ET rate of yeast cyt c adsorbed on binary SAMs composed of 7-carboxy-1-heptanethiolate and 7-hydroxyl-1-heptanethiolate [29], or cyt c adsorbed on binary SAMs composed of
of cyt separated binary SAMs of AUTe–MUAe prompts the ET of cyt on the rate of the electron transfer.

4. Conclusion

The effect of the mixing state of binary SAMs composed of AUTe and MUAe on the rate of the electron transfer (ET) of cyt has been investigated. Artificially phase-separated binary SAMs of AUTe–MUAe prompts the ET of cyt in comparison with the homogeneously mixed binary SAMs and the single-component SAM of MUAe.

This suggests that the two-dimensional distribution of adsorbed MUAe SAM in nanometer-level is a crucial factor for efficient ET of adsorbed cyt.

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