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Adlayer structures of DL-homocysteine and L-homocysteine thiolactone on Au(1 1 1) surface: an in situ STM study

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Abstract

The adsorption of DL-homocysteine (Hcy) and L-homocysteine thiolactone (HTL) on Au(1 1 1) electrode was investigated in 0.1 M $HClO_4$ by cyclic voltammetry and in situ scanning tunneling microscopy (STM). Hcy and HTL molecules formed highly ordered adlayers on Au(1 1 1) surface. High-resolution STM images revealed the orientation and packing arrangement in the ordered adlayers. Hcy molecules formed $(2\sqrt{3} \times 3\sqrt{3})R30^\circ$ adlayer structure and H-bonds between carboxyl groups were assumed to be responsible for the origin of tail-to-tail or head-to-head molecular arrangement, while HTL molecules formed (4×6) adlayer structure, and two different orientations and appearances in the ordered adlayer were found. Structural models were proposed for the two adlayers.

Keywords: Scanning tunneling microscopy; Cyclic voltammetry; Au(1 1 1) surface; DL-Homocysteine; L-Homocysteine thiolactone

1. Introduction

Understanding interactions between proteins and surfaces is important in many fields of biomedical sciences [1,2], such as biocatalysis processes and biosensors. The adsorption of amimo acids provides the model system for understanding the interactions of biofunctional molecules, such as peptides and proteins, with surfaces [3]. Cysteine is a small and zwitterionic amino acid. Additionally, as a thiol molecule, the self-assembled monolayers of cysteine molecules on gold substrates by sulfur–gold bonds were used to immobilize proteins [4–6], which has been employed to fabricate artificial protein arrays and biosensors. So the molecular feature and adlayer structures of cysteine on gold surfaces have been investigated by scanning tunneling microscopy (STM), spectroscopic techniques and secondary ion mass spectrometry [7–12]. Intramolecular and intermolecular hydrogen bonds play a role for the adlayer structures of cysteine molecules. Homocysteine (shown in Fig. 1a), which is the other amino acid that includes thiol group in its chemical structure, possesses similar chemical structure with a methylene difference to cysteine. Homocysteine can release homocysteine thiolactone (shown in Fig. 1b) by a metabolic error-editing process [13]. Homocysteine thiolactone is the product of the cyclization of carboxyl and thiol groups of homocysteine molecules. It has been shown that myoglobin strongly binds to homocysteine self-assembled monolayer electrode through electrostatic associate and some H-bond and the quasi-reversible electrochemical process of myoglobin has been obtained at homocysteine self-assembled monolayer Au electrode [14], though it is difficult at the bare electrode. But the structures of organized layers of homocysteine and homocysteine thiolactone on well-ordered gold surfaces have not been reported.

Scanning tunneling microscopy has been recognized as an important in situ technique for the structure study of adlayer on well-defined electrode surface in electrolyte solution. It has been used to observe the adlayer structures of some biomolecules including proteins [15,16] and amino acids [3,7–9].

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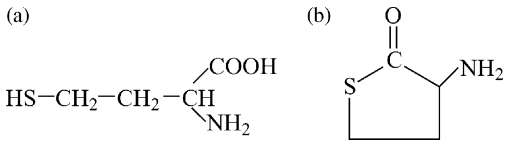

Fig. 1. Chemical structures of Hcy (a) and HTL (b).

In this paper, we investigated the electrochemical properties and adlayer structures of Hcy and HTL on Au(1 1 1) surface by in situ STM in 0.1 M $HClO_4$ solution.

2. Experimental section

In situ STM measurements were carried out with a Nanoscope E (Digital Instrument Inc., Santa Barbara, CA) in 0.1 mol/l $HClO_4$. Tunneling tips were prepared by electrochemical etching of a tungsten wire (0.25 mm in diameter) and sealed with transparent nail polish to minimize the Faradic currents. All the STM images were collected in the constant-current mode. An Au(1 1 1) facet of a single-crystal bead, prepared by melting an Au wire (99.999%), was used as the working electrode. Before each measurement, the Au(1 1 1) electrode was further annealed in a hydrogen–oxygen flame and quenched in ultrapure Milli-Q water saturated with hydrogen. Two platinum wires were used as reference and counter electrodes, respectively.

The molecular models for Hcy and HTL were built by Hyperchem 6.0 software. The molecular conformation was optimized using AM1 method until root mean square is less than 0.01 kcal/Å. The values for molecules such as interatomic distance were estimated from the molecular models.

Cyclic voltammetric experiments were performed by the so-called hanging meniscus method using an EG&G PAR (Princeton Applied Research) Basic Electrochemical System controlled by a microcomputer with EG&G PARC M250 software. A reversible hydrogen electrode (RHE) and platinum wire were used as reference and counter electrodes, respectively. The working electrode was an Au(1 1 1) single-crystal. The solutions were deaerated with high purity nitrogen before each measurement.

All potentials were reported with respect to RHE. All solutions were prepared from ultrapure Milli-Q water. DL-Homocysteine (Hcy) and L-homocysteine thiolactone (HTL) were obtained from Sigma and used as received. The supporting electrolyte was 0.1 mol/l $HClO_4$ (Cica-Merck, Kanto Chemicals).

3. Results and discussion

3.1. Cyclic voltammetry of Hcy and HTL

Previous electrochemical investigation of Hcy was carried out using mercury electrode [16], which showed that the electrochemical behavior of Hcy is the same as that of cysteine. Here, the electrochemical response of Hcy on Au(1 1 1) was studied.

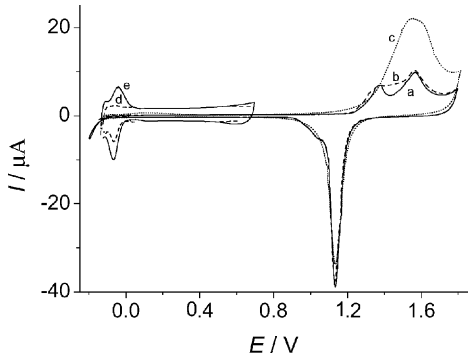

Fig. 2. Cyclic voltammograms of Au(1 1 1) in 0.1 mol/l $HClO_4$ containing homocysteine (a) 0, (b) 1.0×10^{-6}, (c, d) 1.0×10^{-4}, (e) 1.0×10^{-3} mol/l in (a–c) wide potential ranges and (e, d) narrow potential ranges (×10 enlarged charging lines). Scan rate: 100 mV/s.

Fig. 2 shows cyclic voltammograms (CVs) of Au(1 1 1) in the absence and presence of Hcy in 0.1 mol/l $HClO_4$. It can be seen that the double layer region of a bare Au(1 1 1) electrode (Fig. 2a) extends from 0 to 1.2 V. In subsequent anodic scan, two well-separated oxidation peaks were found at 1.3 and 1.5 V, indicating that the surface is a well-defined Au(1 1 1) free from contamination.

In the presence of Hcy with different concentrations, CVs of Au(1 1 1) electrode were significantly changed as shown in Fig. 2b and c. It can be seen that the two successive oxidation peaks at 1.3 and 1.5 V became one peak. The current involved in the reduction peak at 1.15 V decreased. Moreover, a pair of redox waves appeared at −0.050 V. To avoid damaging the well-defined Au(1 1 1) surface, the potential scan was limited to +0.70 V (Fig. 2d and e). The redox peak potential is associated with pH. This pair of redox peaks, which is ascribed to the one-electron transfer associated with thiol, is in agreement with that at mercury electrode [17]. The fact that the redox peak currents are almost not changed after the fifth cycle scanning and linearly dependent on scan rate suggests that both reactant and product are strongly adsorbed on Au(1 1 1) surface.

Fig. 3 shows cyclic voltammograms of Au(1 1 1) in the absence and presence of HTL. With the addition of HTL, no new peak was observed in the double layer region. The addition of HTL molecules only results in the two oxidation peaks of bare Au(1 1 1) electrode becoming one peak and the reduction peak at 1.15 V decreasing, which suggests that the Faradic process of the electrode surface is suppressed by the adsorbed HTL molecules.

3.2. STM observation

3.2.1. Adlayer structure of Hcy

An Au(1 1 1) facet formed on the single-crystal bead was investigated by in situ STM in 0.1 mol/l $HClO_4$ solution,

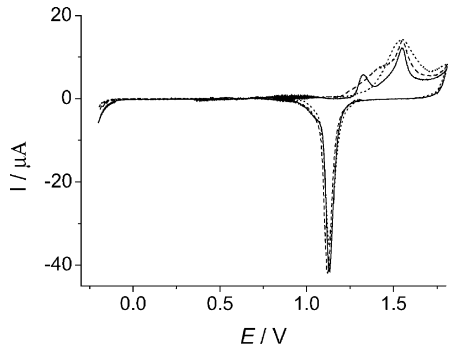

Fig. 3. Cyclic voltammograms of Au(1 1 1) in 0.1 mol/l $HClO_4$ containing homocysteine thiolactone (solid) 0, (dashed) 1.0×10^{-4}, (dotted) 1.0×10^{-3} mol/l. Scan rate: 100 mV/s.

revealing a (1 × 1) structure. After the examination of bare Au(1 1 1) surface, a drop of Hcy solution was directly added into the STM cell under the potential control of 0.5 V. The final concentration of Hcy molecules is about 1.0×10^{-6} mol/l. A few minutes later, highly ordered arrays of Hcy molecules were observed. Fig. 4a shows a typical large scale STM image of highly ordered Hcy adlayer. The molecular rows consist of bright spots.

More details of the orientation and packing arrangement of Hcy molecules in the ordered adlayer were revealed by higher resolution STM image as shown in Fig. 4b. It is clear that the adlayer is composed of molecular clusters. One cluster consists of four spots, as indicated by S_1, S_2, F_1, and F_2. There is brightness difference in the four spots. S_1 and S_2 appear bright, and F_1 and F_2 dark. The distance between S_1 and S_2 is measured to be ca. 0.53 ± 0.03 nm. The distance between S_1 and F_1 as well as S_2 and F_2 is 0.58 ± 0.03 nm, which is close to the distance between sulfur and oxygen (referred to the oxygen of –OH group) in an Hcy molecule. Now, it is clear that Hcy molecules appear as pairs as outlined in Fig. 4b. Each Hcy molecule appears as one bright and one dark spots. According to the literature [9,17], sulfur exhibits bright contrast. Therefore, the bright spot is assigned to sulfur and the dark one corresponds to amino and carboxyl groups. The molecular arrangement exhibits tail-to-tail or head-to-head organization. One direction A along ⟨1 2 1⟩ direction, gives a period of 1.5 ± 0.02 nm. The distance between the neighboring molecules along the molecular row B is measured to be 1.0 ± 0.02 nm. Moreover, direction B exhibits ca. $120 \pm 2^\circ$ rotation with respect to direction A. Thus, direction B also parallels to ⟨1 2 1⟩ direction of Au(1 1 1) substrate. The unit cell, is therefore, described as $(2\sqrt{3} \times 3\sqrt{3})R30^\circ$ as outlined in Fig. 4b. Each unit cell includes two Hcy molecules.

It has been reported that intermolecular and intramolecular hydrogen bonds among adsorbed cysteine or cystine molecules are responsible for the origin of the cluster–network structures for the adlayers [14]. Under the present experimental condition, the carboxyl terminal of Hcy molecule is non-charged (–COOH) and the amino group is protonated ($–NH_3^+$). It is difficult to form electrostatic interaction between molecules. However, H-bond may exist in the ordered adlayer. H-bonds between carboxyl groups should play a crucial role in the formation

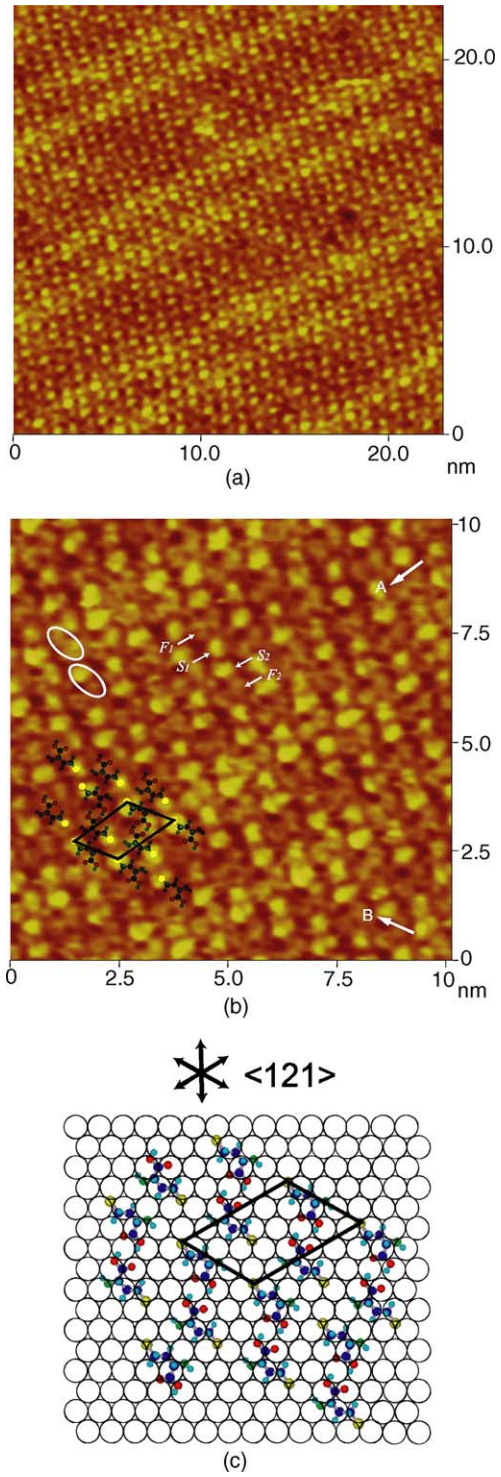

Fig. 4. In situ STM images of homocysteine on Au(1 1 1) in 0.1 mol/l $HClO_4$ + 1.0×10^{-6} mol/l homocysteine at 0.60 V vs. RHE. (a) $I_t = 0.8$ nA, $E_{bias} = 0.134$ V. (b) $I_t = 0.7$ nA, $E_{bias} = 0.134$ V. A corresponding structural model is presented in (c).

of the molecular adlayer on the substrate and are likely to be the direct origin of tail-to-tail or head-to-head molecular arrangement. Based on the above analysis, a structural model was proposed in Fig. 4c. The thiol group is located on a three-fold hollow site of the Au(1 1 1) lattice, while the other groups are subsequently arranged on the other positions such as bridge sites in accordance with their chemical structures. The distance between the nearest neighboring carboxyl groups matches the length of hydrogen bond.

3.2.2. Adlayer structure of HTL

Similar experiments were also carried out to investigate the adsorption of HTL molecules on Au(1 1 1) surface. It was found that HTL molecules form highly ordered adlayers as shown in Fig. 5a. The two bright molecular rows, denoted by arrows A and B, are rotated 120° with respect to each other. In the comparison with the underlying Au(1 1 1) lattice, both the molecular rows A and B are parallel to ⟨1 1 0⟩ direction.

Fig. 5b shows a higher resolution STM image. It can be seen that the adlayer consists of molecular clusters. Each cluster outlined by an ellipse can be recognized to contain two triangles composed of several spots in the STM image (shown in Fig. 5b). In the comparison with the chemical structure of HTL molecule, each triangular shape is assumed to be a molecule. Moreover, the distance between spots a and b (shown in Fig. 5b) is ca. 0.56 ± 0.02 nm, which is close to the distance between sulfur and nitrogen in an HTL molecule. The effect of elements and functional groups on STM image contrasts was investigated and the results showed the brightness sequence as sulfur > amino [9,17]. Hence, the brighter spot a should be assigned to sulfur atom and spot b amino group of HTL molecule. On the basis of the above analysis and the chemical structure of HTL molecule, these two HTL molecules are modeled as in Fig. 5b (indicated by an ellipse), which take two different orientations and appearances. The distance between S atoms in these two molecules is measured to be 0.7 ± 0.02 nm. The periodic distance along the A direction is ca. 1.15 ± 0.02 nm and B ca. 1.65 ± 0.02 nm. The unit cell, is therefore, described as (4×6) as shown in Fig. 5b. Each unit cell includes two HTL molecules. Based on the above analysis, a structural model was outlined in Fig. 5c. S atom is located on a three-fold hollow site of the Au(1 1 1) lattice, while the other groups are subsequently arranged on other positions such as two-fold bridge sites in accordance with their chemical structures. The angle α shown in Fig. 5c is 47°. The brightness difference between the two S atoms can be due to the different adsorption sites, which has been reported previously [18–21]. The bright spot in STM image corresponds to the S atom in fcc position and the dark one in hcp position.

It is well-known that the adlayer structure and ordering degree of molecule arrangement are dependent on the substrate material, solution pH, immersion time for forming the molecular adlayer and potentials applied. Different experimental conditions result in cysteine forming three different adlayer structures, $(\sqrt{3} \times \sqrt{3})$R30°, $(3\sqrt{3} \times 6)$R30° and

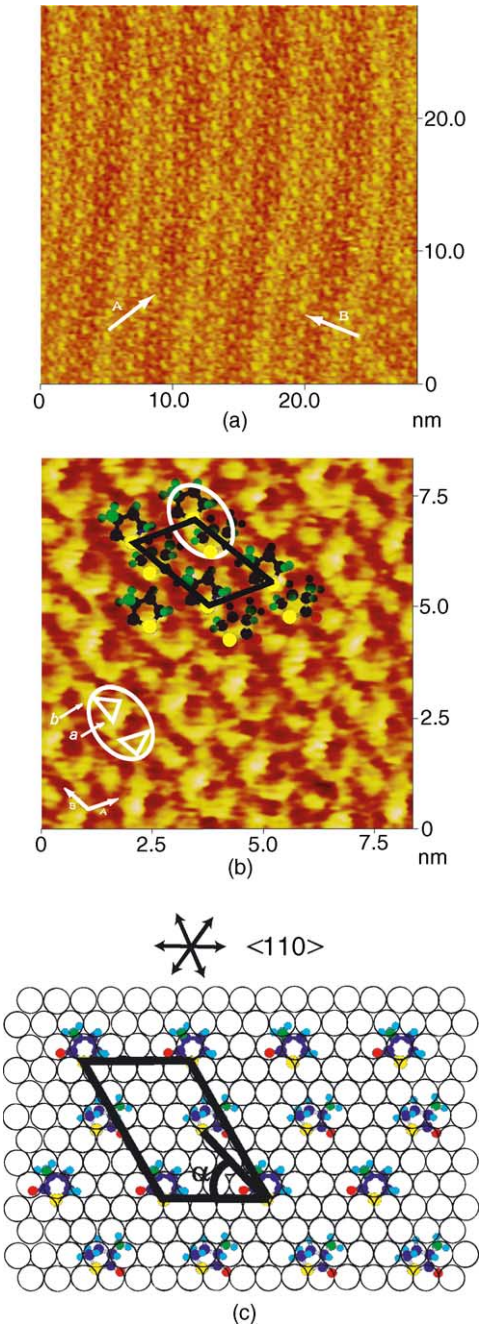

Fig. 5. In situ STM images of homocysteine thiolactone on Au(1 1 1) in 0.1 mol/l $HClO_4 + 1.0 \times 10^{-6}$ mol/l homocysteine thiolactone at 0.55 V vs. RHE. (a) $I_t = 0.8$ nA, $E_{bias} = 0.0950$ V. (b) $I_t = 0.5$ nA, $E_{bias} = 0.112$ V. The structural model is outlined in (c).

$(4 \times \sqrt{7})$R19° [13–15]. In addition, a low-density surface structure of thiophene having an intermolecular distance of 5 Å in a row and an interrow distance of 12 Å was obtained after a relatively shorter immersion time of 2.5 h and a longer immersion time of 24 h results in a densely packed thiophene monolayer at saturation coverage [22,23]. In the present research, under the potential control, the adsorptions of Hcy and HTL on Au(1 1 1) form $(2\sqrt{3} \times 3\sqrt{3})$R30° and (4×6) structures, respectively, and these adlayer structures

are stable. The models in Fig. 4c and Fig. 5c give surface coverages of 0.11 and 0.09, respectively.

In summary, in situ STM has been employed to investigate the adsorption of Hcy and HTL molecules on Au(1 1 1) electrode in 0.1 mol/l $HClO_4$ solution. These two molecules were found to form highly ordered adlayers on Au(1 1 1) surface. For Hcy molecule, the $(2\sqrt{3} \times 3\sqrt{3})R30^\circ$ structure is observed in the ordered adlayers. H-bonds between carboxyl groups are assumed to be responsible for the origin of tail-to-tail or head-to-head molecular arrangement. HTL molecules take two different orientations and appearances in the ordered adlayers and form (4×6) structure.

Acknowledgements

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