Effect of Chemical Structure on the Adsorption of Amino Acids with Aliphatic and Aromatic Substitution Groups: In Situ STM Study

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The adsorption of three amino acids, L-valine (Val), L-leucine (Leu), and L-phenylalanine (Phe), bearing different substituted groups was investigated on a Cu(111) electrode by in situ electrochemical scanning tunneling microscopy (ECSTM). All three molecules are found to form well-ordered adlayers on the surface. Two stable adlayers of L-Val with the symmetry of (3×3) and $(2 \times \sqrt{3})$ were found at the investigated potential range, while L-Leu and L-Phe form similar (4×4) adlayer structures. With high-resolution STM images, the internal structural information of adlayers was obtained and the structural models for the three adlayers were proposed.

Introduction

The adsorption of amino acids represents the model system for the understanding of the interactions of biofunctional molecules, such as peptides and proteins, with surfaces.[1] The investigation of protein interactions is of special importance for the study of the biocompatibility of artificial biomaterials, biocatalysis processes, and the fabrication of biosensors. For example, the homocysteine-modified Au electrode has been found to have special electrochemical response toward Mb and may be applicable in chemisensors or biosensors.[2] To elucidate the interactions between amino acids and solid surface, it is necessary to investigate the microscopic structure of amino acids' adlayer on a surface. Recently, a number of works focusing on this topic were carried out. The bindings and electronic status of amino acids on Au, Cu, and other metal single-crystal surface have been obtained in UHV (ultrhigh vacuum) by LEED (low energy electron diffraction), RAIRS (reflection adsorption infrared spectra), XPD (X photoelectron diffraction), and STM (scanning tunneling microscopy).[3−10] For example, the adsorption geometry and domain structure of glycine on Cu(110) has been extensively investigated by several groups with different surface characterization methods.[3−7] Two coverage-dependent chemisorbed phases of L-alanine on Cu(110) were determined by RAIRS,[8] while the surface faceting of Cu(001) by L-alanine was observed by STM.[9] On the other hand, the adsorption of amino acids at the electrode/electrolyte interface is also emphasized, because of its close relationship with the application of the self-assembly of amino acids in catalysis, sensor, molecular devices, and so on. Several different adlayer structures of L-cysteine on Au(111) were reported by different groups.[11−13] The binding geometry of proline, phenylalanine, and tyrosine on Au and Pt electrode has been investigated by FTIR.[14,15] As a result, it is generally believed that the adsorption of amino acids depends strongly on the molecular structure. In the present study, amino acids with similar structure but different functional groups were chosen to investigate the effect of the different functional group on the adsorption geometry and configuration of the amino acids. As the first step in the study, the adsorption of L-Val, L-Leu, and L-Phe on Cu(111) electrodes was studied by in situ STM. The difference in chemical structure of the three molecules is that L-Val and L-Leu have aliphatic side chain and L-Phe has an aromatic substituted group. The effect of aliphatic and aromatic groups on the adsorption registry of amino acids was compared.

Experimental Section

A commercial Cu(111) single-crystal disk with a diameter of 10 mm (from MaTeck) was used as a working electrode for both electrochemical measurement and in situ STM observation. The well-defined single-crystal Cu(111) surfaces were prepared by electrochemical polishing. All solutions were prepared by diluting ultrapure $HClO_4$ and reagent grade L-Val, L-Leu, and L-Phe (Cica-Merck, Kanto Chemicals) with Millipore water. A homemade electrochemical cell with a reversible hydrogen electrode (RHE) in 0.1 M $HClO_4$ and a Pt counter electrode was employed for electrochemical measurement. The in situ STM apparatus used was a Nanoscope E (Digital Instrument Inc.). W tips were electrochemically etched in 0.6 M KOH. All electrode potentials are reported with respect to the RHE. The details of the experiment were the same as those described in our previous paper.[16]

Result and Discussion

Electrochemical Measurement. The Cu(111) electrode was initially examined by cyclic voltammetry (Figure 1a) in 0.1 M $HClO_4$ in the absence of organic molecules for comparison with the published result. After the examination, the electrode was transferred into a 0.1 M $HClO_4$ solution containing 1 mM L-Val, L-Leu, and L-Phe. Figure 1b−d shows the cyclic voltammograms (CVs) of Cu(111) in the solutions containing the three molecules, respectively. The overall shape of three CVs are almost the same as that of the bare Cu(111) obtained in 0.1 M $HClO_4$. Only the electric charge involved in the double-layer potential range becomes smaller due to the adsorption of molecules. The similar CV feature disclosed indicates the similar electrochemical behavior of these three molecules on Cu(111) electrode. In contrast, the orientation change of L-Phe on Au(111) with the

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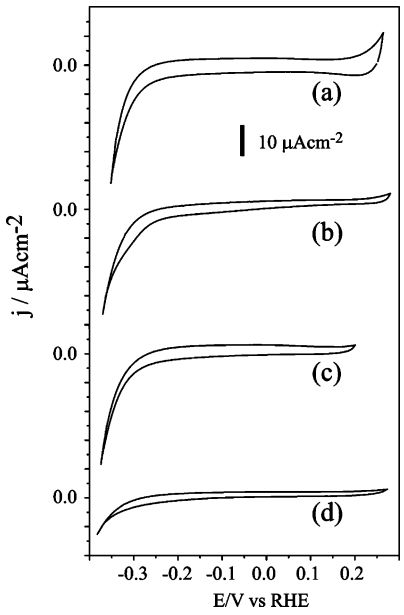

Figure 1. Cyclic voltammograms of Cu(111) electrode in 0.1 M $HClO_4$ in the absence (a) and presence of 1 mM L-Val (b), 1 mM L-Leu (c), and 1 mM L-Phe (d). The scan rate was 50 mV/s.

potential was confirmed by the electrochemical and FTIR results.[14] The details of the adsorption structures of these three molecules will be investigated by in situ STM.

STM Measurement. *L-Val Adlayer*. The atomic image of the Cu(111)−(1 × 1) structure was routinely discerned on an atomically flat Cu(111) surface in the absence of the molecules for the ease of determining the registry of the molecular adlayers to the underlying Cu(111) lattice. After resolving an atomically flat Cu(111) surface and Cu(111)−(1 × 1) structure in $HClO_4$, a droplet of L-Val molecule was directly injected into the STM electrochemical cell. The average concentration of L-Val in 0.1 M $HClO_4$ was ca. 1 mM. A few minutes later, the uniformed adlayer of L-Val was clearly resolved in the wide extended terrace. Figure 2a shows a typical STM image of an L-Val adlayer acquired at −0.2 V. A well-ordered defect-free molecular array is seen. The molecular rows cross each other at an angle of either 60° or 120° within an experiment error of ±2°. From comparison with the Cu(111)−(1 × 1) atomic image, it is found that all molecular rows are parallel to the ⟨110⟩ orientation of underlying Cu(111) lattice. The intermolecular distance along the ⟨110⟩ orientation is measured to be ca. 0.76 nm, about three times of the lattice distance of Cu(111). On the basis of the orientation of molecular rows and the intermolecular distance, we conclude that the observed structure of the L-Val adlayer is (3 × 3), as shown in Figure 2a by the superimposed drawn unit cell.

At this potential, other domains with smaller unit cells were also observed. Figure 2b shows the typical STM image of one more close-packed domain. It is found that the molecular rows intersect at the angle of 90° ± 2°. The molecular rows labeled ***a*** in Figure 2b are in the orientation of ⟨110⟩, while the rows ***b*** are in the ⟨121⟩ orientation of the Cu(111) lattice after comparing with the Cu(111) atomic image. The intermolecular distance along orientation ***a*** is measured to be 0.51 ± 0.02 nm, about twice the lattice distance along ⟨110⟩ directions, while the distance along orientation *b* is measured to be 0.44 ± 0.02 nm, close to the lattice distance in the $\sqrt{3}$ direction. On the basis of the above measurement, the adlayer structure is concluded to be (2 × $\sqrt{3}$).

It is known that the binding of organic substances on a metal surface is determined by the interactions between the functional groups and the substrate. Obviously, the binding of amino acids involves the amino and carboxyl groups. In fact, the most common feature of the adsorption geometries proposed for the superstructures of the amino acids/Cu(110) and Cu(001) systems studied so far is that two the oxygen atoms of the carboxyl group bind on the atop sites of the substrate directly.[3−9,17] The special feature of L-Val is the isopropyl group. The interaction of this group with the substrate will be weaker than that of the amino group and carboxyl group with the Cu surface. On the basis of above consideration, two models for two adlayer structures observed are tentatively proposed in parts c and d of Figure 2, respectively. Figure 2c shows a model of the L-Val adlayer in the flat-on configuration. The two oxygen atoms of the carboxyl group binds directly on the top site of the Cu lattice, while the amino group locates on the hollow sites. The isopropyl group stretches freely on the surface. The intermolecular distance and the molecular row direction are in agreement with the unit cell parameters obtained from Figure 2a. Figure 2d shows the structural model for the L-Val adlayer in the vertical-on configuration. The only binding interaction of the adsorbates with substrate involves the carboxyl group on the top site. So the only STM feature of L-Val is a bright spot. In addition, the proposed different configurations of L-Val in models correlate with the larger apparent height of L-Val in Figure 2b than in Figure 2a. The different binding geometry of L-Val in the two models affects the adsorption energy of L-Val. For example, the vertical-on configuration has smaller adsorption energy than that in the flat-on configuration, because of the absence of the interaction between amino groups with the substrate. However, this energy can be compensated by the closer packing of the adsorbates in the vertical-on configuration, because the area per molecule occupied is smaller in the vertical-on configuration than that in the flat-on configuration. It is clear that other in situ experimental techniques and theoretical simulations could supply further structural information on the adlayer structure and surface coordination of L-Val on a Cu(111) surface.

The two ordered adlayer structures were consistently observed in the potential range from −0.35 to 0 V, indicating that the two structures have close stability on Cu(111) electrodes. This result is consistent with the CV measurement.

L-Leu Adlayer. With a similar procedure, the L-Leu adlayer was prepared on Cu(111). A well-defined adlayer is seen to extend on the wide terrace of Cu(111). From comparing the higher resolution STM image shown in Figure 3a with the Cu(111) atomic image, it is easy to conclude that the adlayer has (4 × 4) symmetry as the superimposed unit cell. Interestingly, the structural details of the molecules were also acquired in the high-resolution STM image. The linear STM feature is consistent with the structural feature of L-Leu. Zhao et al. studied the adsorption of L-lysine on the Cu(100).[17] It is proposed that the side chain ($(CH_2)_4NH_2$) parallels the substrate. The high-resolution STM images obtained in the present study also indicate that the side chain ($CH_2CH(CH_3)_2$) of L-Leu is parallel to the substrate. The tentatively proposed model is shown in Figure 3b. The carboxyl group also binds to the atop sites of the Cu(111). Large efforts have been made to obtain the close-packing structure of the L-Leu adlayer like that of L-Val. However, only the above-described structure was found. The only difference between L-Val and L-Leu is the length of the aliphatic side chain. Obviously, the adsorption energy of Leu will increase while more methene groups interact with the substrate. Thus, it is possible that the contribution of the

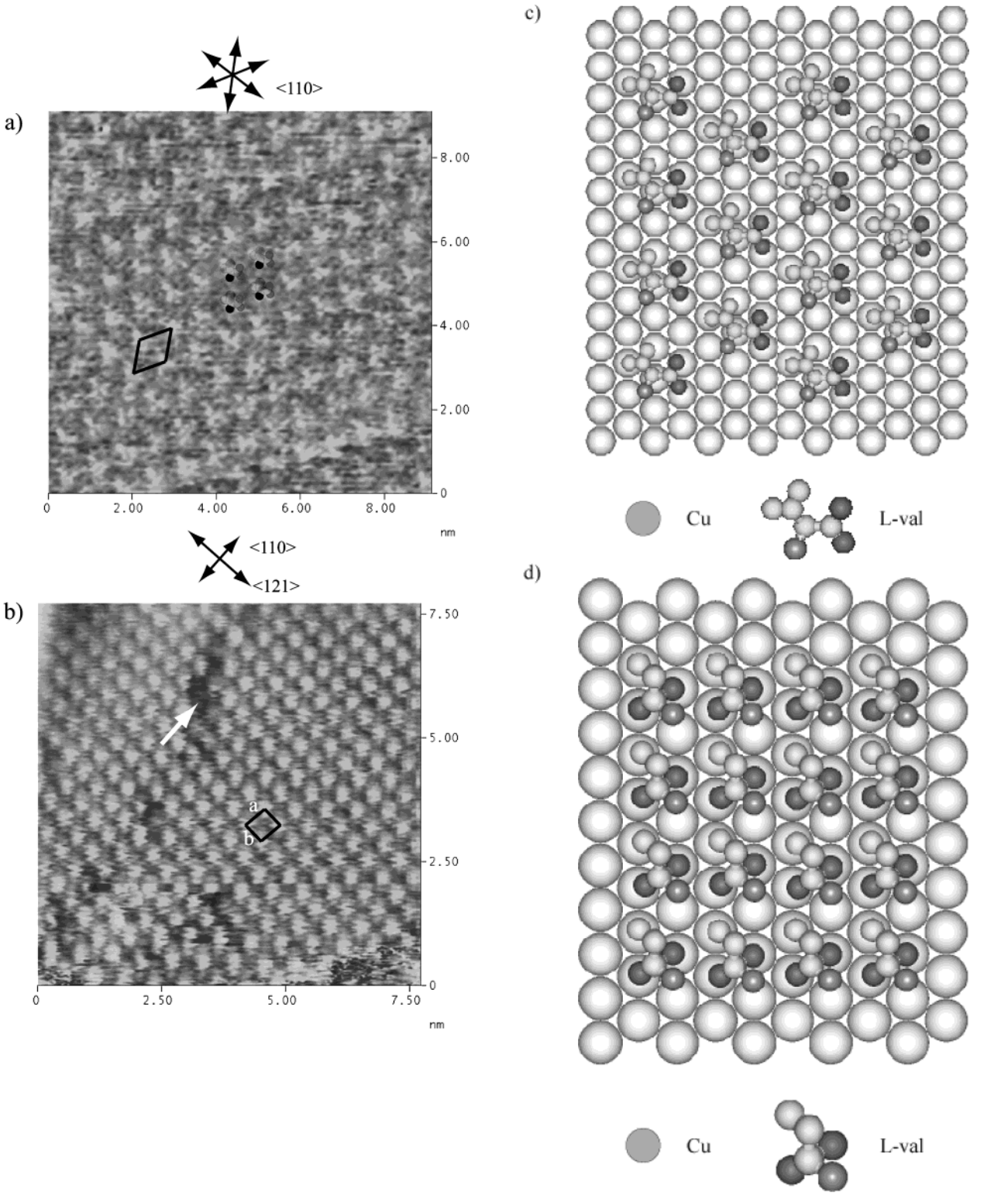

Figure 2. (a) STM top view of the L-Val adlayer with (3 × 3) symmetry on Cu(111) in 0.1 M $HClO_4$ + 1 mM L-Val obtained at −0.2 V. The tunneling current was 10 nA. The scanning rate was 13 Hz. (b) STM top view of L-Val adlayer with (2 × $\sqrt{3}$) symmetry on Cu(111) obtained at −0.21 V. The tunneling current was 10 nA. The scanning rate was 13 Hz. A defect is indicated by the arrow. (c) A schematic representation for the (3 × 3)-L-Val structure. (d) A schematic representation for the (2 × $\sqrt{3}$)-L-Val structure.

adsorption energy from the isobutyl moiety stabilizes the flat-on configuration of L-Leu.

The ordered (4 × 4) structure was consistently observed in the potential range from −0.35 to 0 V. At potentials more positive than 0 V, the well-ordered adlayer became disordered.

L-Phe Adlayer. Compared to the above two amino acids with an aliphatic side chain, the substituted group of L-Phe is benzyl. The difference between an aliphatic and aromatic group is hoped to affect the adsorption structure of amino acids on electrodes. Figure 4a shows a typical STM image of the L-Phe adlayer. The molecular rows are seen to extend at the terrace to form a well-defined adlayer. The symmetry of the adlayer is concluded to be (4 × 4) after comparing the adlayer unit cell with the Cu(111) atomic image. In the STM image shown in Figure 4a,

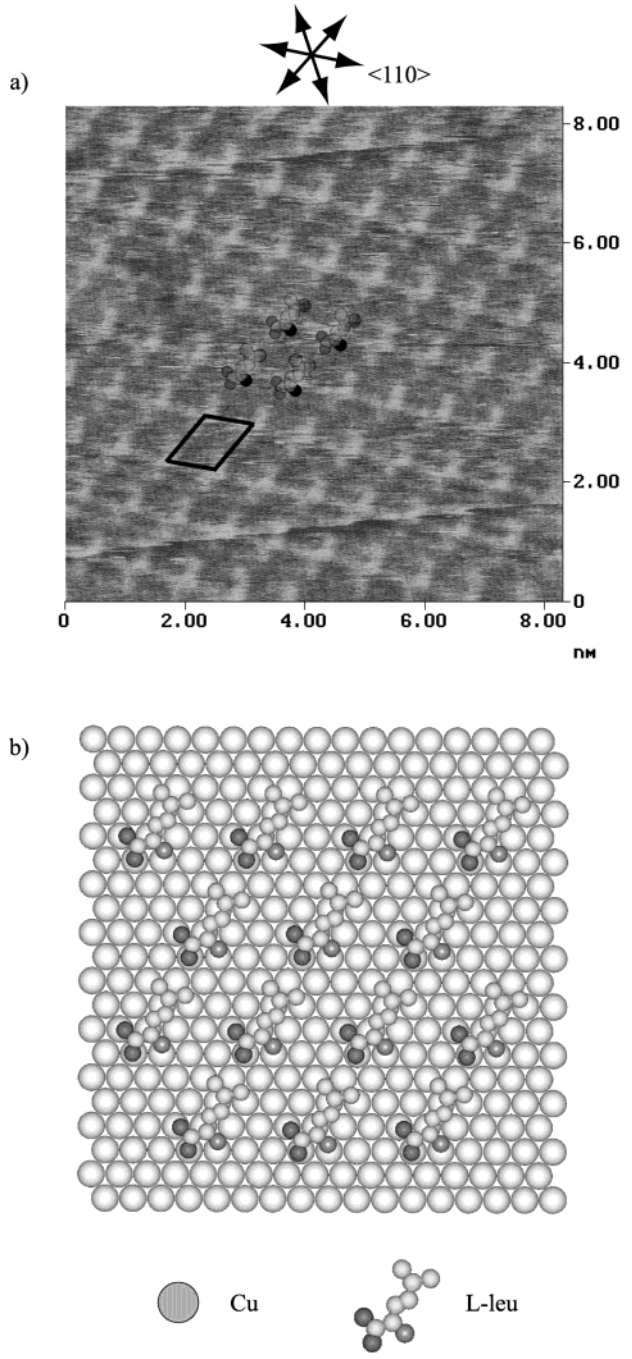

Figure 3. (a) STM top view of the L-Leu adlayer on Cu(111) in 0.1 M $HClO_4$ + 1 mM L-Leu at −0.2 V. The tunneling current was 10 nA. The scanning rate was 15 Hz. (b) A schematic representation for the (4 × 4)-L-Leu structure.

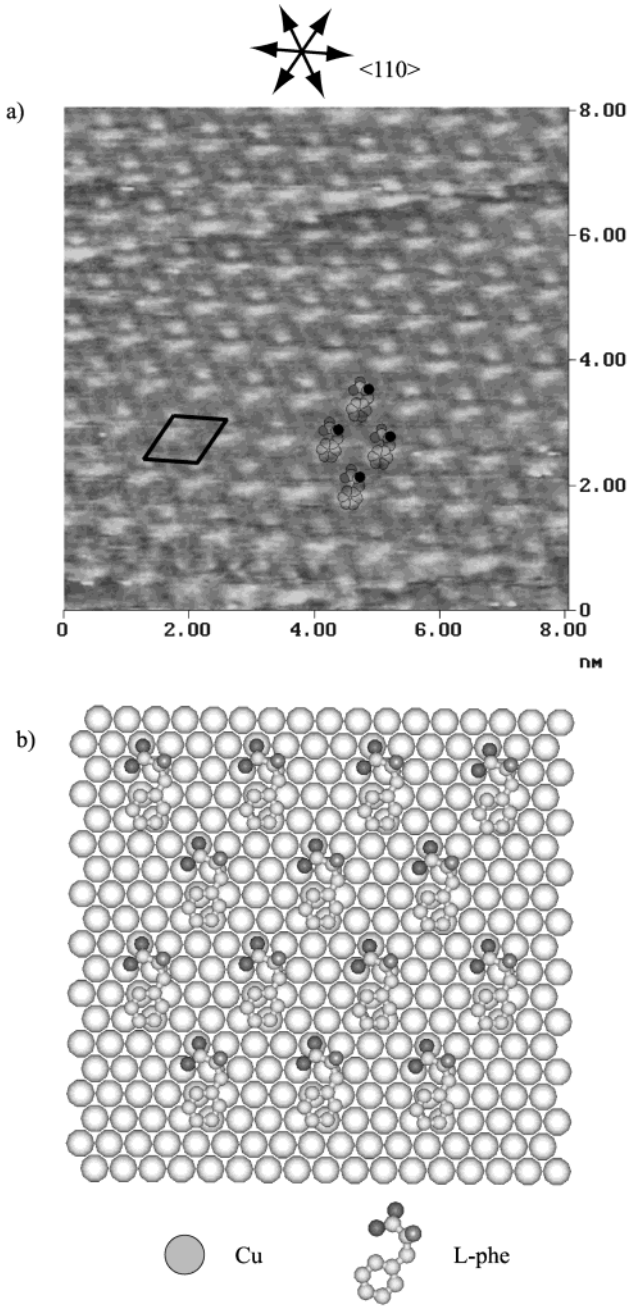

Figure 4. (a) STM top view of the L-Phe adlayer on Cu(111) in 0.1 M $HClO_4$ + 1 mM L-Phe at −0.2 V. The tunneling current was 10 nA. The scanning rate was 15 Hz. (b) A schematic representation for the (4 × 4)-L-Phe structure.

each L-Phe molecule appears as two spots with different contrast. These two spots are tentatively ascribed to the benzyl group and amino acid group by considering the chemical structure and electronic structure of L-Phe. Similar to the adsorption registry of the two amino acids discussed above, the carboxyl group of L-Phe also binds on the atop sites of Cu(111). On the other hand, the benzyl groups is hoped to adsorb on the Cu-(111) with flat-lying conformation to facilitate the interaction between π electrons and substrate. The recent in situ FTIR studies indicate that the adsorption of Tyr and Phe on Au and Pt electrodes involves the interaction of the carboxyl and π aromatic electrons of these two molecules with substrate.[14,15] In addition, π electrons bearing aromatic molecules always take a flat-on configuration on Cu(111) on the basis of our previous results.[16,18] On the basis of the above consideration and the obtained STM images, a tentatively proposed model for the adlayer of L-Phe on Cu(111) is depicted in Figure 4b. The bright spot is thought to correspond to the benzyl, while the weak spot is ascribed to the carboxyl groups binding on atop sites of Cu-(111) substrate.

The ordered adlayer of L-Phe was consistently observed at the potential range from −0.35 to 0 V. At potentials more positive than 0 V, the well-ordered adlayer became disordered, indicating the occurrence of desorption or other electrochemical process.

As disclosed by the in situ STM images, three amino acid molecules form well-ordered adlayers on the Cu(111) electrode surface. However, the detailed structural information obtained from the high-resolution STM images indicates that the amino

acids take different configurations upon adsorption. The adsorption configuration of molecules on a metal electrode surface depends on the interaction of different functional groups with the substrate. From the previous investigation on the adsorption of amino acids on Cu single-crystal both in UHV and solution environments, it is known that there is a strong interaction between the carboxyl of amino acids and the Cu substrate.[6,9] However, the other functional groups also affect the adsorption configurations of amino acids on substrate, considering the flexibility of amino acids. For L-Val, two adlayers were found at the potential range investigated and proposed to be formed by the molecules in flat-on and vertical-on configurations. Upon increasing of the length of the side chain of amino acids, only the flat-on configuration of L-Leu was found. The L-Phe also takes a flat-on configuration on Cu(111), because the interaction between the π electrons of the benzyl moiety and substrate can help the stability of the adsorption geometry. However, it is anticipated that other in situ experimental techniques and theoretical simulations could supply further structural information on the adlayer structure of amino acids on the metal surface to elucidate the interaction of different functional groups with the substrate.

In summary, the adsorption of L-Val, L-Leu, and L-Phe on Cu(111) electrode was studied by cyclic voltammetry and ECSTM. The similar CV features of Cu(111) in 0.1 M $HClO_4$ in the presence of amino acids indicate that the amino acids adsorb on the Cu(111) electrode. In situ STM investigation disclosed that all three molecules form an ordered adlayer on Cu(111) electrode surface. Two stable adlayers of L-Val with the symmetry of (3×3) and $(2 \times \sqrt{3})$ were found. While L-Leu and L-Phe form similar ordered (4×4) adlayers. With high-resolution STM images, the internal structural information on adlayers was obtained, and the structural models for the three adlayers were proposed. The effect of substituted groups on the adsorption structure of amino acids on the surface was discussed. Hopefully, the present study is useful in the further study of the interactions of a protein with metal surfaces and in the discrimination of individual amino acids on a surface by STM.

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