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Surface structures of DL-valine and L-alanine crystals observed by atomic force microscopy at a molecular resolution

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Abstract

Molecular structures at the surfaces of DL-valine and L-alanine single crystals are observed by atomic force microscopy (AFM). The AFM image of the (0 0 1) surface of DL-valine crystal reveals highly ordered lattices with twofold symmetry. And for the L-alanine crystal, the parallel molecular chains and a periodic structure in the (1 2 0) plane are clearly resolved. The AFM images with molecular resolution are quite consistent with the bulk termination of our crystal models. No reconstruction is evident in the AFM images. The present results also demonstrate that AFM has the ability to probe the surface structures of biological crystals at a molecular resolution in both the lateral and longitudinal dimensions.

Keywords: Biological molecules – proteins; Single crystal surfaces; Atomic force microscopy; Surface structure, morphology, roughness, and topography

1. Introduction

Studies on the crystal structures of amino acids have been of increasing significance to understand both the structures of proteins and the origin of life on earth [1–3]. Techniques employed to determine amino acid structures have so far been limited mainly to X-ray diffraction methods and focused on the bulk representation [4–7], while investigation of the surfaces of amino acids is not only important for characterization of their crystal structures but also surface properties and crystallization processes [8,9]. Atomic force microscopy (AFM) is unique in their ability to image the surface morphologies of a variety of organic and biological specimens at the nanometer scale directly. Some surface lattice structures of amino acid crystals have been achieved successfully by AFM since its invention [10–12]. However, many difficulties and problems such as the artifact features are usually encountered due to the instability of tip–sample interactions during measurement, and which must be identified and eliminated carefully. It is still a great challenge to obtain high-resolution images at the molecular level on amino acids surfaces by AFM.

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Most recently, the surface lattice structures of L-valine, D-valine and D-alanine crystals have been obtained by AFM in our previous work [13]. In this paper, we use the AFM to image for the first time the surface ordered lattices of DL-valine and L-alanine single crystals. The molecular resolved images correspond compellingly to the simulated models. The results show the unique ability of AFM in probing the surface molecular structures of amino acids crystals in both the lateral and longitudinal dimensions. By comparison with the X-ray data of crystals, the surface structures of DL-valine and L-alanine crystals are studied.

2. Experiment

D-valine, L-valine and L-alanine of high purity were purchased from Sigma Chemical Company, and the single crystals were grown by crystallization from slow evaporation of saturated aqueous solutions. The well formed surfaces of single crystalline samples were used for AFM imaging.

The AFM experiments were performed on a Nanoscope IIIa system produced by Digital Instruments Company. The instrument operated in contact mode, using standard silicon nitride cantilevers with integrated pyramidal tips. All the AFM images in this paper were made from deflection signal, which is more sensitive to the fine surface details than height image and can reflect the surface molecular topographies as well. In order to verify the crystal plane indexes of the imaged surfaces, X-ray diffraction measurement was carried out on the same surfaces of DL-valine and L-alanine samples, which was recorded on a Scintag θ–2θ diffractometer (Cu Kα, $\lambda = 0.15405$ nm). The XRD patterns showed that the imaged surfaces were (0 0 1) face for DL-valine and (1 2 0) face for L-alanine crystals.

The crystal models of DL-valine and L-alanine were built in the Materials Studio program (Accelrys Inc.). The atomic coordinates of DL-valine crystal were obtained from the data of Dalhus and Görbitz [7] and that of L-alanine were from Simpson and Marsh [14]. The (0 0 1) face of DL-valine and (1 2 0) face of L-alanine crystals were then cleaved from these bulk structures.

3. Results and discussion

The crystal structure of DL-valine has been refined as triclinic space group $\boldsymbol{P}\bar{1}$ by Dalhus and Görbitz [7] in 1996, with lattice parameters $\boldsymbol{a} = 0.5222$ nm, $\boldsymbol{b} = 0.5406$ nm, $\boldsymbol{c} = 1.0838$ nm, $\boldsymbol{\alpha} = 90.89°$, $\boldsymbol{\beta} = 92.34°$, $\boldsymbol{\gamma} = 110.02°$, $\boldsymbol{Z} = 2$. The structural formula of valine molecules and the crystal structure of DL-valine in one unit cell are shown in Fig. 1a and b, respectively. The carboxylate and amino groups are ionized and the molecules exist in zwitterionic form in DL-valine crystal. Fig. 1c illustrates the crystal packing of triclinic forms of DL-valine viewed down the *a*-axis, and its unit cell is marked in the black box, containing one D-valine and one L-valine molecule. The structure is made up of double layers of molecules extending parallel to the (0 0 1) plane. Inside the double layers, each amino H atom of molecules is engaged in a single hydrogen bond [7], while outside the double layers, they are separated from each other by hydrocarbon side chains. So there form alternating hydrophobic and hydrophilic layers in the crystal.

The XRD pattern measured on the imaged surface of DL-valine crystal is shown in Fig. 2. The first peak at 8.14° is corresponding to (0 0 1) reflection and a series of peaks at higher 2θ angle are corresponding to the (0 0 l) reflections, which indicate that the surface for AFM imaging is a (0 0 1) face and the sample is a high-quality single crystal well grown along the *c*-axis.

Fig. 3a shows the AFM molecular image of DL-valine crystal, in which a dot of protrusion indicates a valine molecule and the ordered lattice at molecular resolution is observed. The measured lattices give *a*- and *b*-axes of 0.525 and 0.558 nm, and the angle between the two axes is 110.5°. The two-dimensional fast Fourier transform (FFT) pattern is given in the inset of the image, which shows twofold symmetric characterization in the plane. The surface molecular morphology of the (0 0 1) face of DL-valine crystal is simulated in Fig. 1d, in which only the isopropyl group close to the surface is visible for each molecule. It can be clearly seen that the topographic features are great agreement between the AFM image and the simulated image. We assume that the imaged (0 0 1)

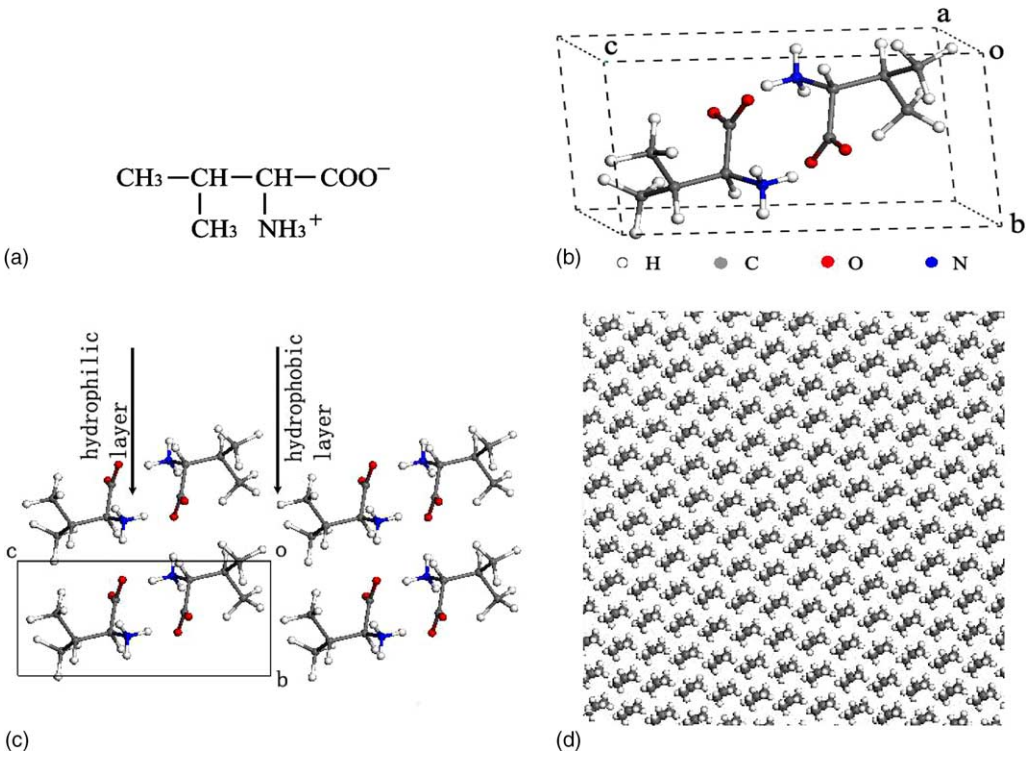

Fig. 1. (a) The chemical structure formula of valine molecules. The carboxylate and amino group are ionized and the molecule exists as a zwitterion in DL-valine crystal. (b) The crystal structure in the triclinic form of DL-valine. The unit cell contains one D-valine and one L-valine molecule. (c) The molecular packing of DL-valine polymorphs viewed down the *a*-axis. The unit cell is marked by the black box. Alternating hydrophobic and hydrophilic layers form parallel to the (0 0 1) face in the crystal. The hydrophobic layers contain the unpolar side chains, while the hydrophilic layers are composed of the charged carboxylate and amino groups. (d) The simulated surface molecular morphology of (0 0 1) face of DL-valine crystal. Only the isopropyl group is drawn (in CPK model) for each molecule because the group is close to and at the top site of the surface. The constants of the periodic cell are $\boldsymbol{a} = 0.5222$ nm, $\boldsymbol{b} = 0.5406$ nm, $\gamma = 110.02°$. (For color coding, see the online version.)

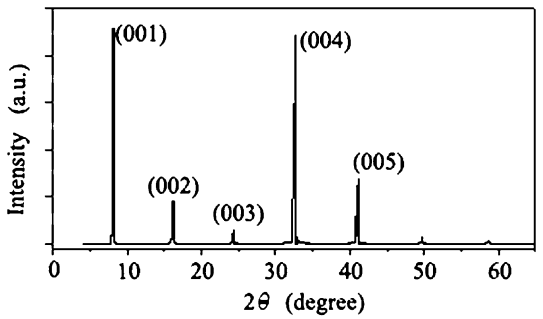

Fig. 2. The XRD pattern measured on the imaged surface of DL-valine crystal. Both the positions and intensities of the peaks are perfectly in accord with theoretical values of (00*l*) planes reflections, indicating that the surface for AFM imaging is (0 0 1) face in single crystal.

face should most likely be along the hydrophobic layer rather than the hydrophilic layer, because the van der Waals force between the layers containing non-polar groups is much weaker than the hydrogen bond that holds the double layer of carboxyl and amino groups together [12].

In the AFM image, the whole morphology of one valine molecule can be discriminated only as a single protrusion, and submolecular resolution cannot be achieved. We have tried to obtain more detailed and minute information from the AFM image with higher resolution (Fig. 3b), but it is difficult to give an accurate description of the interior structure of a valine molecule. Because all the isopropyl groups at the top of the surface have

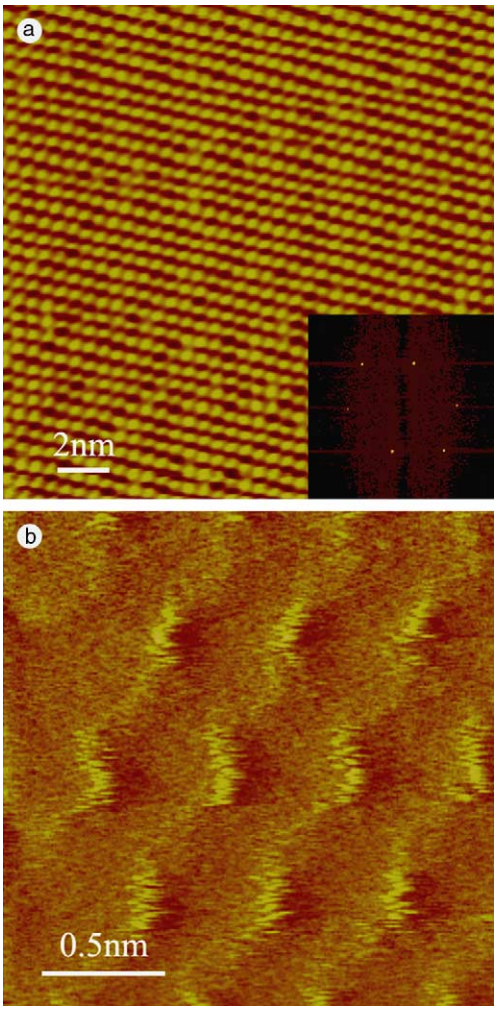

Fig. 3. (a) AFM molecular image of DL-valine crystal in (0 0 1) face (deflection mode). Inset of the image gives the fast Fourier transform (FFT) pattern of real space image, which shows twofold symmetric characterization in the plane. The measured lattices are 0.525 and 0.558 nm for *a*- and *b*-axes, respectively, and the angle between the two axes is 110.5°, which corresponds to the crystal constants in (0 0 1) plane of DL-valine single crystal. (b) AFM molecular image of (0 0 1) face in DL-valine crystal with higher resolution (deflection mode), scanning area: 2 nm × 2 nm.

the same height and orientation, the AFM image shows ordered protrusions with the same brightness and size. However, the following AFM images of L-alanine can give us more complex information, because there are various groups and atoms at the top site of the (1 2 0) face and they are situated at different heights.

The AFM image of L-alanine crystal is shown in Fig. 4a. The parallel molecular chains of different brightness can be easily resolved, and four molecular chains form a periodic structure. The white box outlines a unit cell in the plane, which contains four alanine molecules. The Fourier transform pattern of data is shown in the inset of the image, in which three pairs of clearly defined peaks in the straight line correspond to the three periodic structures perpendicular to the chain direction with periods of one, two and four times the molecular spacing, respectively. To clearly discern the periodic features, the original image is Fourier filtered and the processed image is shown in Fig. 4b, in which only the spectral peaks in the Fourier transform are preserved, and therefore high frequency noise is eliminated. Fig. 5 gives the XRD pattern from the same sample surface, and the unique peak at 20.62° corresponds exactly to the (1 2 0) reflection of the L-alanine crystal. It demonstrates that the imaged surface is a (1 2 0) face in the single crystal sample.

The crystal structure of L-alanine has been determined and refined as orthorhombic with space group $\boldsymbol{P}2_12_12_1$ by Simpson and Marsh [14], and the lattice parameters are $\boldsymbol{a} = 0.6032$ nm, $\boldsymbol{b} = 1.2343$ nm, $\boldsymbol{c} = 0.5784$ nm, $\mathbf{Z} = 4$. The chemical structure and the crystal structure in one unit cell of L-alanine are shown in Fig. 6a and b, respectively. All the molecules exist in zwitterions form in the L-alanine crystal as well, and all three protons in the amino group are used to form hydrogen bonds. One of the three hydrogen bonds links molecules together to form chains along the *c*-axis of the crystal, while the other two hydrogen bonds bind these chains together in a three-dimensional network [14].

Fig. 6c shows the top view of the molecular morphology pattern of the (1 2 0) face in the L-alanine crystal, in which the molecular chains along the *c* direction are clearly illustrated. The four molecules shown in the black box make up one unit cell of (1 2 0) plane with lattice constants 1.7250 and 0.5784 nm, compared to the measured sizes of 1.716 and 0.568 nm in the AFM image, respectively. The lateral view of the unit cell is

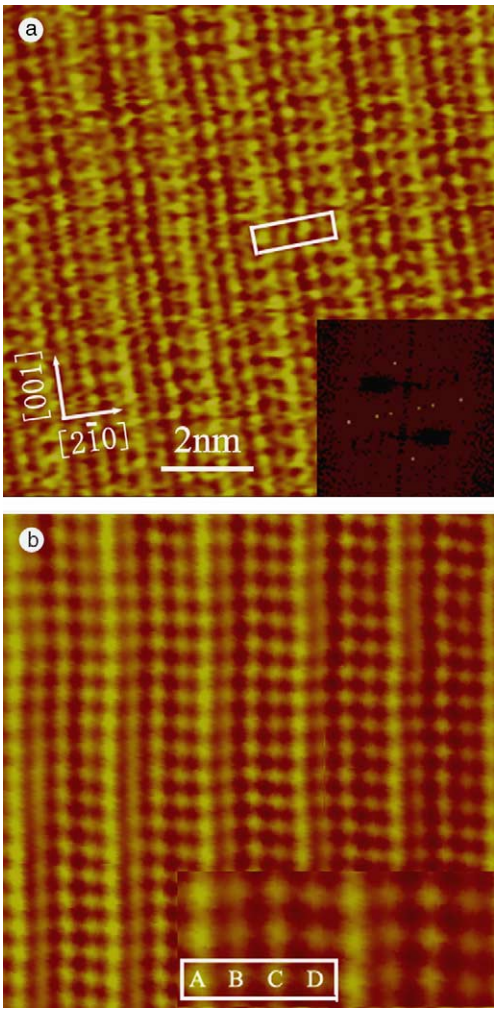

Fig. 4. (a) AFM molecular image of L-alanine crystal in (1 2 0) face (deflection mode). The parallel molecular chains with different brightness along [0 0 1] direction and a periodic structure in [2 $\bar{1}$ 0]. direction can be easily resolved. The white box containing four molecules indicates one unit cell in the plane. The dark inset gives the FFT pattern of this crystal face, in which three pairs of peaks in the straight line is corresponding to the periodic structures with one, two and four times of molecular spacing along [2 $\bar{1}$ 0] direction, respectively. (b) The Fourier filtered image after rotation and cropping from the original image (a). The white box in the enlarged area marks four molecules in the unit cell of this face.

given in Fig. 6d. It is clearly shown that the four alanine molecules in the unit cell are located at different heights and with varying orientation, and each pair of molecules form a symmetric unit. The

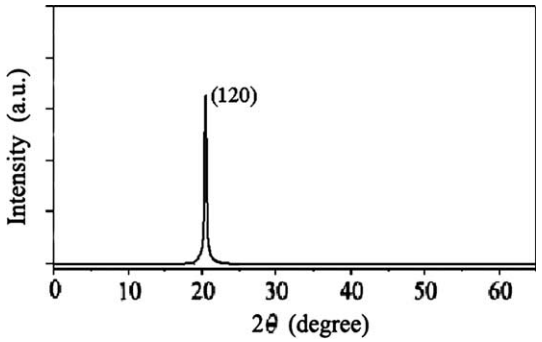

Fig. 5. The XRD pattern from the imaged surface of L-alanine crystal, in which the unique peak at 20.62° corresponds exactly to (1 2 0) reflection.

regular fluctuation topographies are also remarkably in agreement with the AFM image. The molecule A is the highest one in the unit cell, with a methyl group at the top site (Fig. 6d) corresponding to the brightest dot (A) in the AFM image (Fig. 4b); the third molecule C corresponds the brighter dot (C), with the carbonyl group and an oxygen atom close to the surface; the molecules B and D are located in the lowest position, compared to the darker dots (B) and (D), respectively. It is important to note that only heavy atoms are considered during comparison of the group heights in molecules, due to the fact that hydrogen atoms can make only a very limited contribution to the interaction between the molecules and AFM tips. The impressive agreement of topographic modulation both along and perpendicular to the chain direction between the real and model images is exciting. It indicates that AFM is a powerful method to study surface morphologies at molecular resolution in both the lateral and longitudinal dimensions.

We have known that different crystal faces have various growth rates during crystallization of amino acids, and the morphology of a crystal is usually determined by the slowest growing faces [8]. In our experiment, the well developed surfaces for imaging are the (0 0 1) face in DL-valine crystal and the (1 2 0) face for L-alanine. This can be explained by the "periodic bond chain" (PBC) theory, whose basic assumption is that crystal growth can be considered as the formation of bonds between the crystallizing molecules, and different

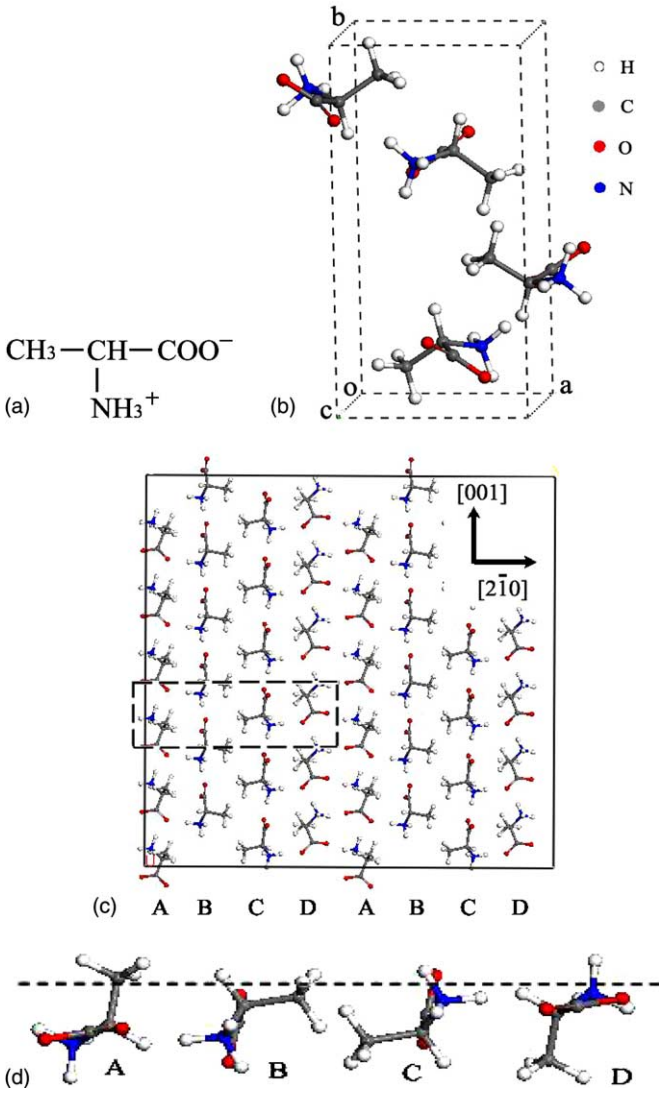

Fig. 6. (a) The structural formula of alanine molecules. All the molecules exist as zwitterionic form in L-alanine crystal. (b) The crystal structure in orthorhombic form of L-alanine. The unit cell contains four alanine molecules. (c) The top view of simulated molecular pattern of the (1 2 0) face in L-alanine crystal. The unit cell in this plane is composed of four molecules as well (marked in the black box). (d) The lateral view diagram of the four alanine molecules in one unit cell in (1 2 0) face. The four molecules are located at different heights and orientations, and each pair of molecules (A and D, B and C) forms a symmetrical cell. The highest molecule A in the unit cell with a methyl group at the top site corresponds to the brightest dot (A) in the AFM image (Fig. 4b); the third molecule C corresponds the brighter dot (C), with a carbonyl group and oxygen atom at the top site; the molecule B and D in the lowest position are compared to the darker dot (B) and (D), respectively. All hydrogen atoms in molecules are out of consideration during calculation of the group heights. The result shows remarkable agreement between the real and model images. (For color coding, see the online version.)

crystal faces can be classified according to the number of periodic bond chains (PBCs). The faces with two or more PBCs must be stepped faces complying with a layer mechanism, so that their growth rates are rather slow [15,16]. In the case of amino acid crystals, the bonds between the

molecules are mainly hydrogen bonds and van der Waals forces. According to the PBC theory, the (0 0 1) face of DL-valine and (1 2 0) face of L-alanine both have two PBCs, therefore, they are well formed as the morphologically important faces and can be observed on the crystal. Moreover, the measured surface molecular lattices in the AFM images are both in agreement with those of the given faces in the bulk crystals, which implies that no reconstruction occurs in these surfaces and their structures are simple terminations of the bulk.

4. Conclusions

We report on the direct observation of the surface lattice structures of DL-valine and L-alanine crystals by atomic force microscopy. The XRD analyses on the samples show that the imaged surfaces were (0 0 1) face for DL-valine and (1 2 0) face for L-alanine crystals. The AFM image of (0 0 1) face of DL-valine crystal reveals highly ordered lattices with twofold symmetry, while for L-alanine crystal, the parallel molecular chains and a periodical structure are clearly resolved in (1 2 0) face. The measured lattices agree well with the molecular structures in the bulk crystals, which implies that the lattice structures of the imaged surfaces correspond quite well to the bulk termination, and no reconstruction occurs. The present work demonstrates that AFM provides an effective way to measure the surface lattices of single crystals directly, and has the startling ability to probe the surface molecular structures in the three dimensions.

Acknowledgements

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