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Direct observation of surface structure of D-alanine and D-/L-valine crystals by atomic force microscopy and comparison with X-ray diffraction analysis

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Abstract

A direct observation of surface morphology characterization of the crystals of D-alanine, D-valine and L-valine at molecular scale was presented by atomic force microscopy (AFM). The crystal structures of D-alanine, L-alanine and L-valine have been determined by X-ray diffraction methods. D- and L-alanine crystallize in the orthorhombic space group $P2_12_12_1$, $Z = 4$ with the unit-cell dimensions $a = 6.0073(5)$ Å/6.0095(5) Å, $b = 12.3030(7)$ Å/12.3388(7) Å, $c = 5.7732(4)$ Å/5.7904(3) Å, $\alpha = \beta = \gamma = 90°$ and L-valine crystallizes in the monoclinic space group $P2_1$, $Z = 4$ with lattice constants $a = 9.6737(5)$ Å, $b = 5.2664(3)$ Å, $c = 12.0196(6)$ Å, $\alpha = \gamma = 90°$, $\beta = 90.722(3)°$ at 270 K. The L-valine molecule was found to take two different conformations—*gauche* **I** and *trans* form. AFM images clearly show an ordered molecular morphology of orthorhombic alanine and monoclinic valine unit cells, which are in fair agreement with the X-ray diffraction data.

Keywords: Atomic force microscopy; Single crystal surfaces; Biological molecules – nucleic acids; X-ray scattering, diffraction, and reflection

Atomic force microscopy (AFM) is widely used to image biomolecules, from whole cells down to smaller structures, such as membranes, proteins and nucleic acids. High-resolution imaging of individual biomolecular surface is one of the most challenging task [1]. The resolution in scanning force microscopy is determined by the sharpness of the tip and is typically between 5 and 10 nm. The scanning probe microscope is the only microscope that can achieve nanometer-scale resolution on biological samples under native condition [2].

Alanine and valine are the essential biomolecules, which could evolve in the ice mantles of dust grains in interstellar space. The first unifying principle in biochemistry is that the key molecules

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have the same handedness or chirality [3]. Recent discoveries of excess of L-amino acids in the Murchison meteorite represent the first definite identification of exochirality and demonstrate a prebiotic chiral influence [4]. Up to now, almost all studies focus on the L-amino acids. Yang and coworkers [5,6] have studied the absorption of glycine, L-alanine and DL-alanine on Cu(0 0 1) by scanning tunneling microscopy. Simpson and March [7] studied the X-ray diffraction of L-alanine at 298 K. Destro et al. [8] and Gatti et al. [9] studied L-alanine at 23 K. Torii and Ittaka [10] studied the crystal structure of L-valine and Dalhus and Görbctz [11] studied L-valine at 120 K. The present work is emphasized to provide the AFM image of D-alanine, D-valine and L-valine and the data of X-ray diffraction of D-/L-alanine and L-valine for comparison.

AFM has proven an easy and powerful method for conducting data storage of nonconductive amino acid at nanometer scale. Nanoscope IIIa produced by Digital Instruments Company was used for direct observation of surface structure of D-alanine, D-valine and L-valine crystals. Such images are obtained by recording the Z coordinate of the tip as it scans the surface in contact mode with deflection set-point from -2 to -3 V, scan rate 30.52 Hz and scan size 10.12 nm. Images are obtained using a selected oxide-sharpened silicon nitride probe. AFM images show the surface morphology of the zwitterionic form of D-alanine (Fig. 1), D-valine (Fig. 2) and L-valine (Fig. 3).

D-alanine and L-alanine crystals were obtained by crystallization from slow evaporation of saturated aqueous solutions, producing well-formed crystals elongated along the c axis and with principal {1 1 0} faces. The X-ray crystallographic data for D-alanine and L-alanine single crystals were collected at 270 K on a Rigaku RAXIS-RAPID imaging plate diffractometer with graphite monochromated Mo Kα radiation ($\lambda = 0.71069$ Å). D-valine and L-valine crystals were grown from a warm saturated solution by slow cooling. They are colorless elongated along the b axis with well-developed {0 0 1} faces, and the X-ray crystallographic data of L-valine was collected at 270 K on a Nonius Kappa CCD diffractometer with graphite monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) in Table 1.

X-ray data show that D- and L-valine crystallize in monoclinic space groups with alternating hydrophobic and hydrophilic layers parallel to the xy plane. The hydrophobic layers contain the unpolar side chains, while the hydrophilic layers are composed of the charged carboxylate and amino groups. The layer consists of two identical

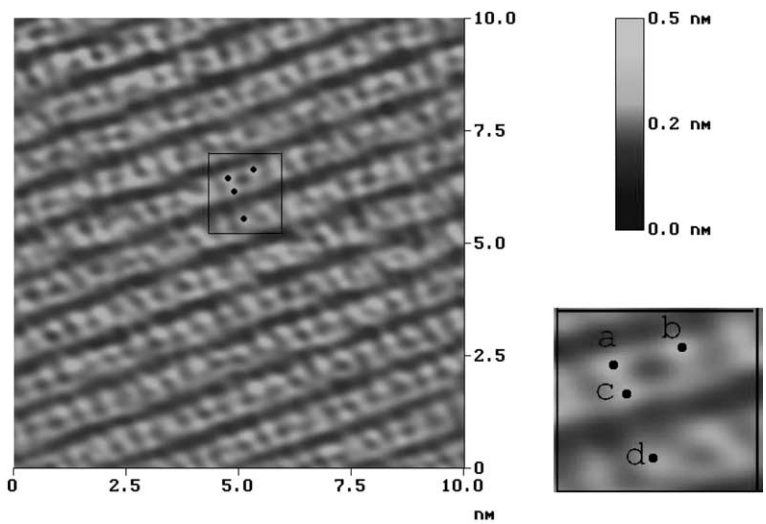

Fig. 1. AFM image of the D-alanine crystal. Scan rate: 30.52 Hz, scan size: 10.12 nm, number of samples: 512, data scale: 500.0 pm.

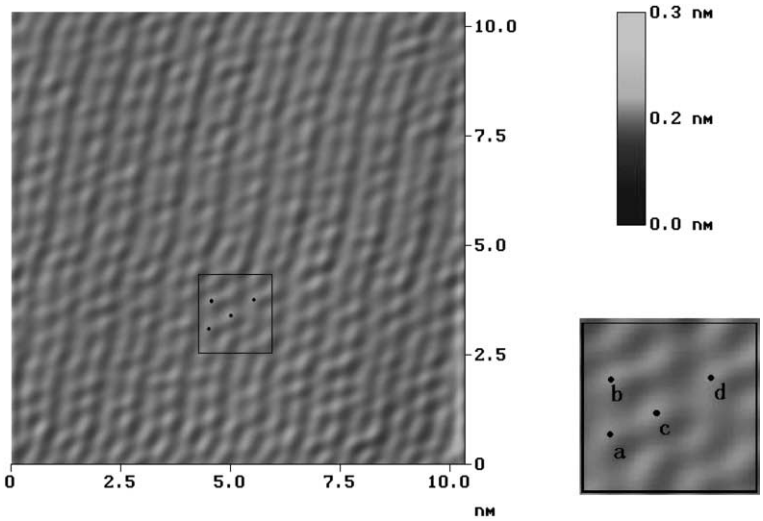

Fig. 2. AFM image of D-valine crystal. Scan rate: 20.35 Hz, scan size: 10.33 nm, number of samples: 512, data scale: 300.0 pm.

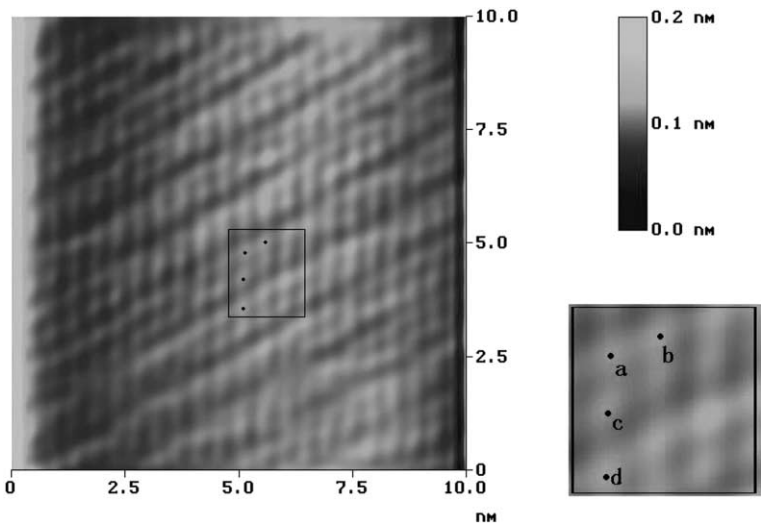

Fig. 3. AFM image of L-valine crystal. Scan rate: 20.35 Hz, scan size: 8.00 nm, number of samples: 512, data scale: 300.0 pm.

sheets, related by a twofold screw axis. The valine crystal contains two crystallographically independent molecules A and B with different conformations in the asymmetric unit and take the *gauche* **I** and *trans* form respectively (Fig. 4). The carboxyl groups of the A and B molecules are coplanar with their respective C_α atoms. The four amino hydrogen atoms (two each from molecules A and B) in *gauche* **I** and *trans* positions relative to the carboxylate group form five hydrogen bonds in each sheet. The nitrogen atom is surrounded by four oxygen atoms at short distances. In molecule A the two oxygen atoms O_B (11)[0 1 0] and O_A (2)[0 1 0] are situated in approximate tetrahedral

Table 1
Crystallographic data of D-/L-alanine and L-valine crystals at 270 K

Sample	D-alanine		L-alanine	L-valine
Empirical formula		$C_3H_7NO_2$		$C_5H_{11}NO_2$
Formula weight		89.10		117.15
Radiation		Mo Kα		Mo Kα
Wavelength (Å)		0.71069		0.71073
Crystal system		Orthorhombic		Monoclinic
Space group		$P2_12_12_1$		$P2_1$
Unit cell dimensions				
a (Å)	6.0073(5)		6.0095(5)	9.6737(5)
b (Å)	12.3030(7)		12.3388(7)	5.2664(3)
c (Å)	5.7732(4)		5.7904(3)	12.0196(6)
α		90°		90°
β		90°		90.722(3)°
γ		90°		90°
Crystal dimensions (mm)	$1.00 \times 1.00 \times 0.70$		$0.30 \times 0.40 \times 0.50$	$0.5 \times 0.5 \times 0.13$
Z		4		4
D_{calc} (mg m^{-3})	1.387		1.378	1.271
Volume (Å^3)	426.69(5)		429.36(5)	612.30(6)
$2\theta_{max}$	54.9°		54.9°	60.04°
No. of reflections measured	3867		4092	12, 905
Structure solution		Direct Methods (MITHRIL 84)		Direct Methods
Refinement		Full-matrix least-squares		Full-matrix least-squares
Function minimized		$\sum w(F_o^2 - F_c^2)^2$		$\sum w(F_o^2 - F_c^2)^2$
Least squares weights		$W = 1/\sigma^2(F_o^2)$		$W = 1/\sigma^2(F_o^2)$
No. observations($I > -10.00\sigma(I)$)	598		604	2854
Residuals: R; Rw	0.063; 0.078		0.060; 0.077	0.0542; 0.0831
Residuals: $R1$	0.029		0.028	0.0372
Goodness of fit indicator	1.33		1.33	1.043
Max shift/error in final cycle		0.000		0.000
Maximum peak in final diff. map	0.25 e$^-$/Å^3		0.22 e$^-$/Å^3	0.191 e$^-$/Å^3
Minimum peak in final diff. map	−0.15 e$^-$/Å^3		−0.14 e$^-$/Å^3	−0.139 e$^-$/Å^3

directions and the two amino hydrogen atoms will lie respectively nearly on the lines joining the amino nitrogen atom and two oxygen atoms [10].

AFM images of D-valine and L-valine crystal surfaces are shown in Figs. 2 and 3. A scattering of protrusions, about 0.2 nm in diameter, indicates a morphology characterization of molecules. The feature of Fig. 1 is the presence of several little pits. These pits have the characteristic rectangular shape of D-alanine with several atoms deep and a few angstroms wide. Note that both images of D-valine and L-valine seem like a pair of antipode forming the asymmetric units. In both images, 2D undulations of asymmetric units are observed.

The structure of chiral lattices in two and three dimensions may provide insights into chiral discrimination, and chiral phases play an important part in the physics and applications of liquid–crystal and amphiphilic films [12]. AFM shows that surface molecules of enantiomer crystals have long-range order, but molecular resolution is not achieved. According to Torii data, the packing distance is 4.1 Å between side-chain carbon atoms. By comparison with the AFM image and the X-ray figure of D-alanine and L-valine, it is clearly showed that the alanine and valine molecules all are in zwitterionic form with the carboxylate groups close to the surface and with oxygen atoms at the top site (Fig. 5, Table 2).

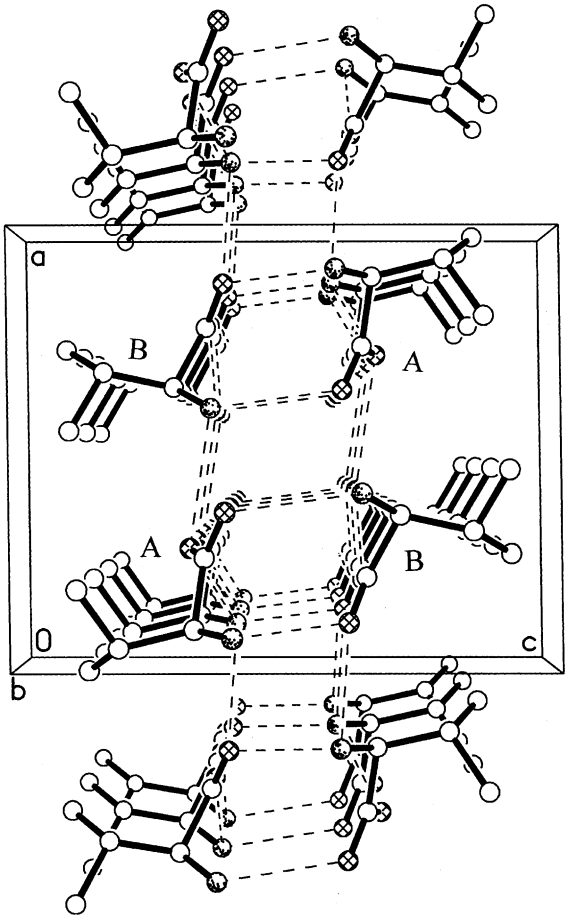

Fig. 4. The molecular packing of L-valine in unit cell view down the *b* axis.

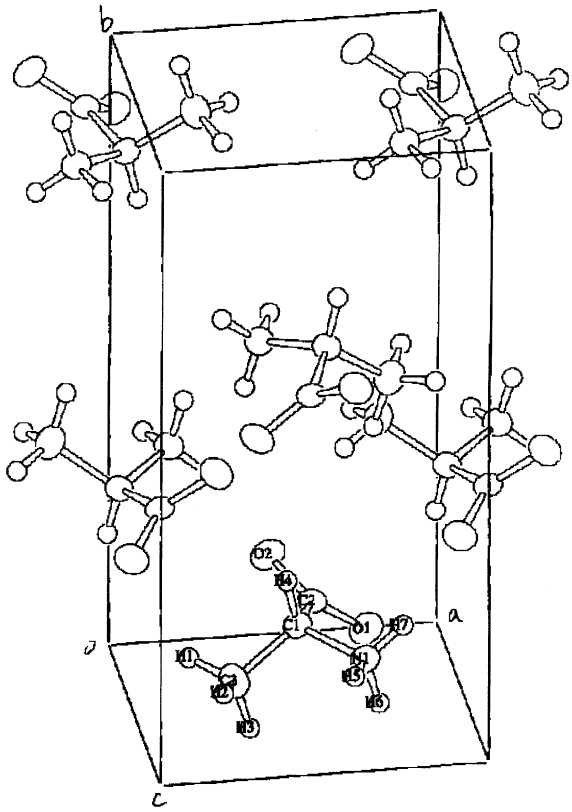

Fig. 5. Projection of the crystal structure of D-alanine along the *b* axis.

Table 2
AFM data of D-alanine, D-valine and L-valine

Crystal	Distance of O atoms (molecule A to B, *ab*, Å)	Distance of two unit cells (*ac*, Å)	Distance of O atoms (molecule A to A, *cd*, Å)
D-alanine	5.3	3.4	5.8
D-valine	6.0	4.2	5.1
L-valine	5.4	4.1	6.4

In summary, we have directly observed the surface structure of D-alanine, L-valine and D-valine crystals at molecular scale. The unrecorded region shows the periodical arrangement. We can easily discriminate the crystals of alanine and valine from the AFM data about the distance of two unit cells. The present study is the innovative work to provide the figure of AFM real image by direct observation and compare with the X-ray diffraction image of D-alanine and L-valine obtained by the global refinement of all collected reflections. It seems that AFM can discriminate the surface molecules of enantiomer crystals based on long-range order.

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