Observation of Crystal Growth of Lysozyme Protein Crystals by Atomic Force Microscopy

N. Gvozdev *, L. Rashkovich, I. Yaminsky

Department of Physics, Moscow State University, Vorob'evy Gory,
Moscow 119899, Russia
E-mail: gvozdev@polly.phys.msu.su

SUMMARY: Surface structure and the propagation of elementary growth layers over the (010) face of orthorhombic lysozyme crystal is examined at a molecular-scale resolution by the method of atomic force microscopy (AFM). The steps have a small number of kinks spaced by about 150 growth units. The step motion occurs via successive deposition of rows of growth units. The data obtained are discussed in terms of the model of one-dimensional nucleation.

Introduction

Recently, much progress has been made in the understanding of the protein crystal growth mechanisms [1, 2, 3]. Despite the structural complexity of the protein molecules, as well as the compositional complexity of protein solutions, there are pronounced similarities between the some mechanisms and kinetics underlying the crystallization of proteins and inorganic materials. The purpose of the given work was the study of lysozyme crystallization mechanism and comparison of the received data with the already available data on inorganic crystals.

Experimental and results

Lysozyme crystals were grown by the method of spontaneous crystallization by cooling the solution of 5% w/v hen-egg-white lysozyme (available from Seikagaku, Japan) and 5% w/v NaCl acidified with hydrochloric acid to pH 4.6. We studied the most development (010) faces of the crystal habit. Growth surfaces were examined on a Nanoscope-3 atomic force microscope in the height and deflection modes. A sample on a glass or mica substrate was immersed into a solution in a standard cell. The crystals were grown at ~25°C. Supersaturation was estimated from the solubility data [4] as $s = C/C_0-1 \approx 1$, where C and C_0

 CCC 1022-1360/00/$ 17.50+.50/0

are the actual and equilibrium concentrations of lysozyme. For a number of reasons, no more accurate estimation of s was possible.

It was found that the face growth on the layer-spiral mechanism by tangential moving of elementary growth layers. Screw dislocations on a crystal surface are the sources of elementary growth layers, which formed dislocation hillock. Fig. 1 presents three dislocation hillocks. The dislocation hillock form is defined by strong anisotropy of step velocity along crystallographic axes. In our case, step velocity along *C*-axis is ten times greater than perpendicular direction velocity.

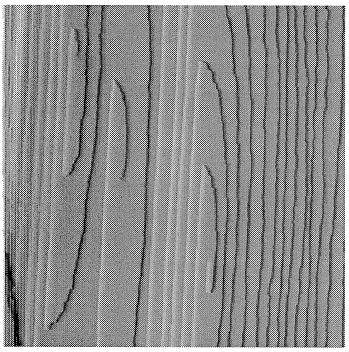

Fig. 1. The screw dislocations on the (010) face. The frame size is $11\times11\mu m^2$.

The (010) face with the molecular resolution is presented in Fig. 2. The steps moving to the left (see Fig. 1) contain straight regions oriented along the *C*-axis. These regions are separated by the kinks. The kink density is low. The distance between the kinks is equal to 150 lattice spacing along the *C*-axis.

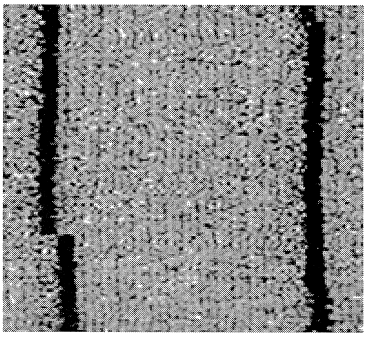

Fig. 2. Growth steps and kinks on the (010) face. The steps move to the left along the *a*-axis. The frame size is 220×380 nm^2.

The growth rate is a function of kink density. The kinks arise both as a result of one-dimensional nucleus formation and as a result of molecular diffusion. Usually, kink height and elementary step height are equal to one cell parameter along *A*-axis and *B*-axis respectively.

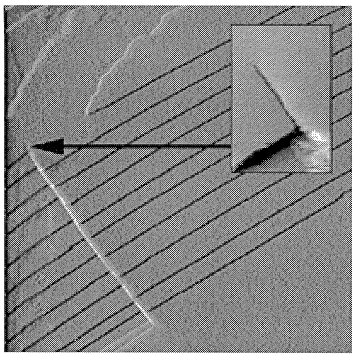

Fig. 3. Structure defect on the face. The frame size is 13×13 μm^2. The fragment size is 320×480 nm^2.

Besides the dislocations, we observed the molecule packing defects (see Fig. 3). These defects can be treated as an additional layer of the molecules appearing in a volume of a crystal. The height of this step is 1.5 times greater then the height of elementary steps. The elementary steps are moving around of this defect. During long time of observation, the form and size of defect didn't vary.

The basic quantitative parameters of a crystal growth such as: steps (V) and kinks (v) velocities, attachment (ω^+) and detachment (ω^-) frequencies and kink density (ρ), 1D nucleation rate (J) are determined [5] (V=0.19 nm/s, v=19.3 nm/s, ω^+=12.6 s^{-1}, ω^-=6.3 s^{-1}, $\rho=1.74\times10^{-3}$ nm^{-1}, $J=2.9\times10^{-5}$ $nm^{-1}s^{-1}$). Attachment and detachment frequencies and 1D nucleation rate were calculated using one-dimensional nucleation theory.

Fig. 4 illustrates AFM tip influence on the elementary growth steps. Image "a" is obtained while the tip moves toward the steps. The tip is scratching a part of growth layer 3.5 nm in thickness (these regions noted by the arrows).

It is half of parameter along *A*-axis. The tip doesn't destroy layers at the reverse movement (tip moving from up to down). It is not known why the tip scratches half of unit cell.

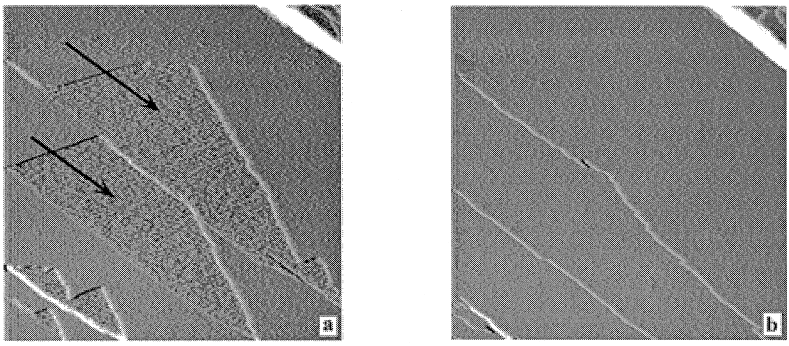

Fig. 4. AFM Tip Influence on the elementary growth steps. "a"-tip up, "b"-tip down. The frame area is 3.2×3.2 μm^2.

The region of a surface with doubling of the period of a lattice along A-axis is presented in Fig. 5. It means that, there is a difference between properties of neighbour cells along A-axis.

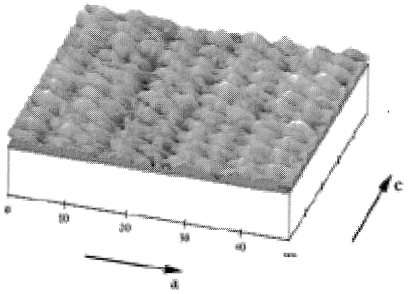

Fig. 5. Period doubling in the *a*-direction on the (010) face. The frame size is 50×50 nm^2.

This phenomenon can be treated as a surface reconstruction, which was found at the protein crystals for the first time. However, the period doubling effect disappears at decrease of "tip-

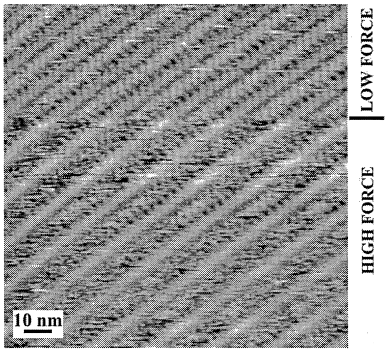

Fig. 6. AFM tip influence on the crystal surface. The frame size is 130×130 nm^2.

sample" force. In Fig. 6 we changed "tip-sample" force during scanning. The doubling has disappeared at decrease of force.

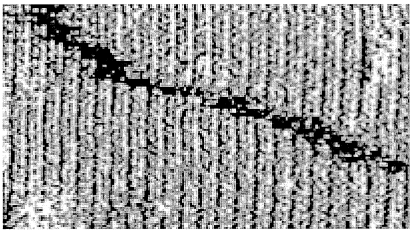

Fig. 7. Period doubling in the *b*-direction on the (010) face. The frame area is 230×120 nm^2

The coupled rows on the neighbour layers are shifted by half period along *A*-axis (see Fig. 7). This fact can be treated as a doubling of parameter along *B*-axis at least in two surface layers.

Conclusion

The surface morphology and growth kinetics of the (010) face of orthorhombic lysozyme crystal was investigated *in situ*. It was found that the face grows according to dislocation mechanism, similarly to inorganic crystals. 2D nuclei are unimportant for the growth. However, lysozyme elementary steps moves much more slowly in comparison with inorganic crystals. It was found that the steps contained a few of kinks and steps move by means of one-dimensional (1D) nuclei formation. 1D-nucleation mechanism was not found on inorganic crystals. Measurements of intermolecular distances show the evidence for surface reconstruction, which was found at the protein crystals for the first time. AFM tip influence on the crystal surface was discussed.

References

1. S. D. Durbin,W. E. Carlson, *J. Crystal Growth.* **122**, 71 (1992)
2. A. I. Malkin, Yu. G. Kuznetsov, W. Glantz and A. J. McPherson, *J. Phys.Chem.,* **100**, 11736 (1996)
3. J. D. Ng, Yu. G. Kuznetsov, A. I. Malkin, *et al., Nucleic Acids Res.,* **25**, 2582 (1997)
4. M. Ataka and M. Asai, *J. Crystal Growth,* **90**, 86 (1988)
5. L. N. Rashkovich, N. V. Gvozdev and I.V. Yaminsky, *Crystallogr. Rep.* **43**, 698 (1998)