STEP VELOCITY ON THE {110} FACES OF TETRAGONAL LYSOZYME CRYSTALS UNDER HIGH PRESSURE

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Introduction

Three-dimensional structures of more than 30,000 proteins have been determined so far, mainly by X-ray crystallography. However, the protein crystallization process remains an obstacle to structural determination. In fact, only about 30% of purified proteins can be crystallized[1]; this rate has not increased significantly in the past 20 years.

High pressure is useful to control the protein crystallization. Visuri et al. [2] reported, for the first time, that the crystallization of glucose isomerase (GI) crystals was significantly enhanced with increasing pressure. Subsequently, Suzuki et al. studied effects of pressure on GI crystallization, and found that the solubility, C_e [3] decreased, and face growth rate, R [4], and step velocity, V_{step} [5, 6], with increasing pressure. These results indicate the kinetic acceleration of nucleation and growth rates of GI crystals with increasing pressure. However, we have not completely clarified the effect of high pressure in the elementary process of the crystallization.

To examine the elementary process of the crystallization, we need to observe the steps on protein crystals. Activation energy and activation volume $\Delta V^{\ddagger}$ are important factors to understand the crystallization process. Suzuki et al. calculated $\Delta V^{\ddagger}$ of GI crystal using V_{step} values under high pressure [7]. Although the molecular structure of GI in the solution is indispensable for further discussion, its molecular weight is much larger than the limit of NMR analysis. Therefore, we couldn't compare the change of molecular structure of GI with $\Delta V^{\ddagger}$. Thus, we focused hen egg-white lysozyme under high pressure, since its molecular structure in the solution under high pressure has been reported [8].

In this work, we measured V_{step} in the <110> direction of two dimensional islands on the {110} faces of tetragonal lysozyme crystals, and calculated step kinetic coefficients β_{step} under high pressure.

Experimental

Tetragonal crystals of hen egg-white lysozyme were grown from solutions containing six times recrystallized lysozyme (Seikagaku Kogyo Co. Ltd.). We used lysozyme without further purification. All other chemicals were of reagent grade. Lysozyme was dissolved in a 50mM sodium acetate buffer (pH 4.5). A sodium chloride solution of 50 mgmL^{-1} was prepared in the same 50mM acetate buffer. A supersaturated solution was prepared by mixing equal volumes of the lysozyme solution and the sodium chloride solution. The supersaturated solution (lysozyme: 100 mgmL^{-1}) was transferred into an *in situ* observation cell, and was incubated at 20.0˚C for a day to prepare suitable seed crystals on a sapphire window of 1 mm thickness. All the crystals were prepared under atmospheric pressure. Before each measurement of V_{step}, we replaced the solution in the cell with fresh one.

A laser confocal microscope combined with a differential interference contrast microscope (LCM-DIM) [9] (OLYMPUS, FV300, IX71) and an objective (OLYMPUS, SLCPlanFl 40x) were used for the step observation. *In situ* observation under high pressure was conducted by using a high-pressure vessel (Syn corporation Ltd., PC-100-MS). We observed steps on an upper surface of a lysozyme crystal through the sapphire window and the crystal itself. Solubility C_e of tetragonal lysozyme crystals were measured as previously described [10].

Results and Discussion

V_{step} in the <110> direction of two dimensional islands on the {110} faces at 0.1 and 50 MPa were measured in the range of protein concentrations C = 15.4 – 40.5 mgmL^{-1}. As shown in Figure 1 (a), V_{step} decreased with increasing pressure. The increase in V_{step} is attributed to both kinetic and thermodynamic contributions as [11],

$$V_{step} = \beta_{step}\Omega(C - C_e) \qquad (1)$$

where Ω is the volume of one lysozyme molecule inside a tetragonal crystals [12] and (C-C_e) is the number of molecules per unit volume.

To separate β_{step} from the thermodynamic one (C-C_e), we replotted V_{step} as a function of (C-C_e) (Figure 1(b)). β_{step} values thus obtained were (3.2 ± 0.1) × 10^{-7} and (2.1 ± 0.1) × 10^{-7} m s^{-1} at 0.1 and 50 MPa (here we assume that Ω at 50 MPa is same as that at 0.1 MPa [11]), respectively. β_{step} can be expressed as follows [13],

$$\beta_{step} = a\nu\left(\frac{\omega}{\lambda}\right)\exp\left(-\frac{\varepsilon_{kink}}{kT}\right) \qquad (2)$$

where a is the distance of advanced step per a molecule, ν is the vibrational frequency of adatoms, ω is the first neighbor distance between the molecule on a step, λ is the mean distance between kinks on the step, ε_{kink} is activation energy of the crystallization, k is Boltzmann constant. The decrease in β_{step} indicates the increase in ε_{kink} or λ. To distinguish the effects of these two

parameters, we have to measure the temperature dependence of β_{step} in future.

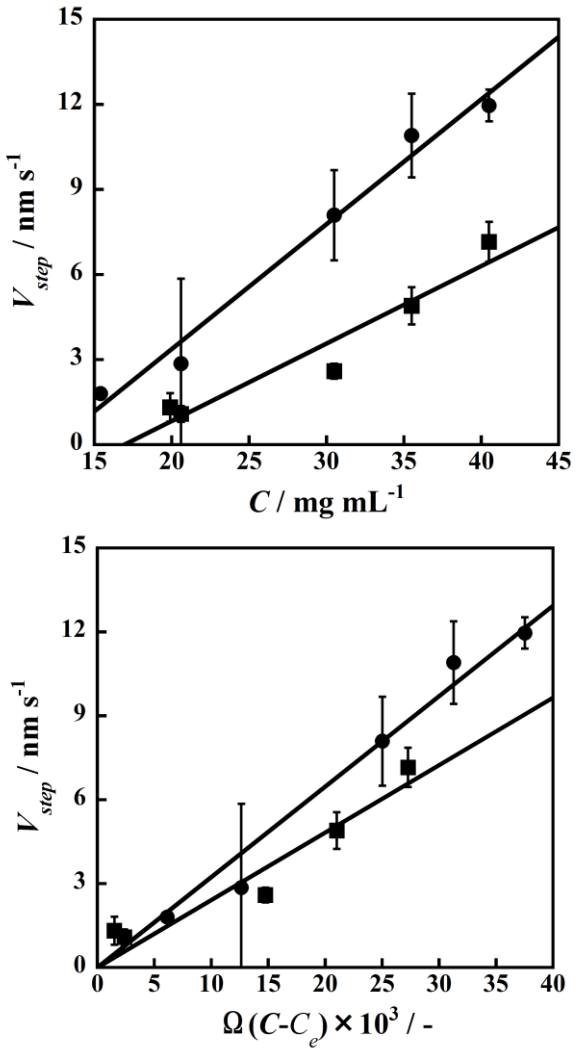

Fig. 1 V_{step} on the {110} faces of lysozyme crystals as a function of C (a) and C-C_e (b). V_{step} was measured at 0.1MPa (●) and 50 MPa (■). Temperature was 19.2 ˚C. The lines shown in (b) indicate the results of weighed linear fitting.

Conclusion

We measured V_{step} of tetragonal lysozyme crystals under high pressure, and calculated β_{step}. The key conclusions are as follows:

(1) V_{step} and β_{step} decreased with increasing pressure.

(2) The decrease in β_{step} indicates the increase in ε_{kink} or λ.

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