

# Behavior of Coexisting Growth and Dissolution of L-Alanine Crystals in the Presence of L-Methionine

Norihito Doki\*, Hiromi Nakamura, Sachi Honjo, Kenji Shimizu, Masaaki Yokota

Department of Chemistry and Bioengineering, Faculty of Science & Engineering, Iwate University, Morioka, Japan  
Email: \*doki@iwate-u.ac.jp

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## Abstract

Many research results have been reported on the effect of additives on crystal growth rate, but there are few examples comparing multiple crystal growth surfaces, and quantitative data is scarce. In this study, we investigated the effect of additives on crystal growth rate. Specifically, we investigated the effect of the additive L-methionine (L-Met) on the growth rate of L-alanine (L-Ala) crystals. We clarified that in the presence of an additive with a structure similar to that of the growing crystal, there are conditions under which crystal growth and dissolution coexist.

## Keywords

Crystal Growth, Amino Acid, Additive

## 1. Introduction

Most amino acids are produced by fermentation, and crude solutions contain diverse impurities, such as microbial enzymes, media components, and microbial metabolites, as well as the desired amino acid. The desired amino acid is commonly isolated by crystallization, and the impurities that have molecular structures similar to the desired amino acid affect crystallization behavior because of their strong interactions. Therefore, the effect of such impurities must be understood to control the crystallization process and crystal habit [1]-[3]. Addadi *et al.* [4] and Sano *et al.* [5] examined the effect of tailor-made additives on the crystal habit of amino acids. They demonstrated that other amino acids acted as tailor-made additives and changed the crystal habit of the crystallized amino acids. Thus, other amino acids are useful for designing the crystal habit of substrate amino acids. For this purpose, it is important to discuss the kinetic mechanism, which

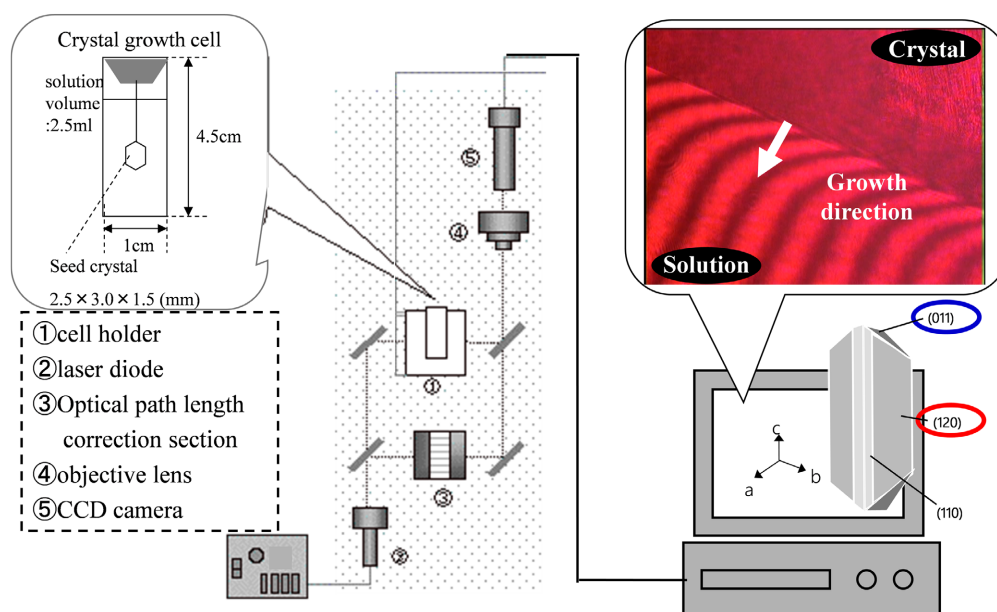
involves relationships among various factors, such as the crystal growth behavior of the specific crystal face, the additives, their concentration, and the degree of supersaturation [6]-[15]. However, although some studies of the kinetic mechanism of the effect of additives have been reported [6]-[8], no versatile model has been proposed to date. In this study, we investigated the effects of additives using an “*in-situ* observation device” that can observe the relationship between crystal growth rate and concentration distribution near the crystal surface in real time. Specifically, we investigated the effect of the additive L-methionine (L-Met) on the growth rate of L-alanine (L-Ala) crystals.

## 2. Experimental

The *in-situ* observation device shown in **Figure 1** was used to observe crystal growth behavior. First, an L-Ala saturated solution with a predetermined concentration was prepared, placed in a cell, and an L-Ala seed crystal ( $2.5 \times 3.0 \times 1.5$  mm) was fixed and immersed therein. Thereafter, the solution was cooled to a measurement temperature of  $35^\circ\text{C}$ , and the growth rate of each crystal plane and interference fringes near the surface of the growing crystal were measured. In addition, an L-Ala solution containing 0.1 mol% of L-Met was prepared and replaced with the solution in the cell, and a similar experiment was conducted. Atomic force micrographs were recorded on a Digital Instruments Dimension3000 controller. Deflection and height images of crystals in air were obtained using AFM contact mode with 0.12 N/m force constant silicon nitride tips.

### Seed crystal preparation method

A three-neck flask (PYREX 2000 ml) containing distilled water and excess L-Ala was placed in a thermostatic bath maintained at  $40^\circ\text{C}$  and stirred for 2 hours. After stopping the stirring and leaving it to stand for a few minutes, the supernatant



**Figure 1.** Experiment apparatus.

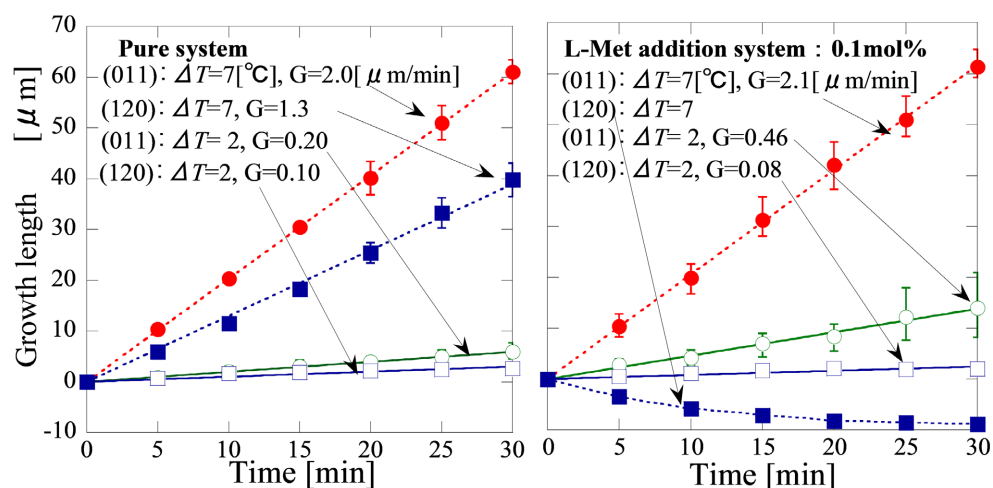
solution was filtered using a membrane filter, and the solution was transferred to a petri dish and left to stand while evaporating the solution at room temperature. From the precipitated crystals, crystals with relatively good shape and size of  $2.5 \times 3.0 \times 1.5$  mm were selected and used as seed crystals. After removing moisture from the crystal surface, a 0.4 mm stainless steel wire was attached so that the c-axis direction of the L-Ala seed crystal was the same as the direction of gravity.

### Crystal growth observation

2.5 ml of sample solution at a given temperature was injected into a  $1 \times 1 \times 4.5$  mm square quartz cell using a syringe, and the cell was placed in the cell holder of the device, which was set to a given temperature by a temperature control device. A seed crystal was then fixed in the cell. At this time, a thermocouple was placed near the seed crystal in the solution. The cell temperature was controlled to a measurement temperature of  $35^\circ\text{C}$ , and when the solution reached  $35^\circ\text{C}$ , it was left to stand for 5 minutes. Growth observation was then carried out for 30 minutes.

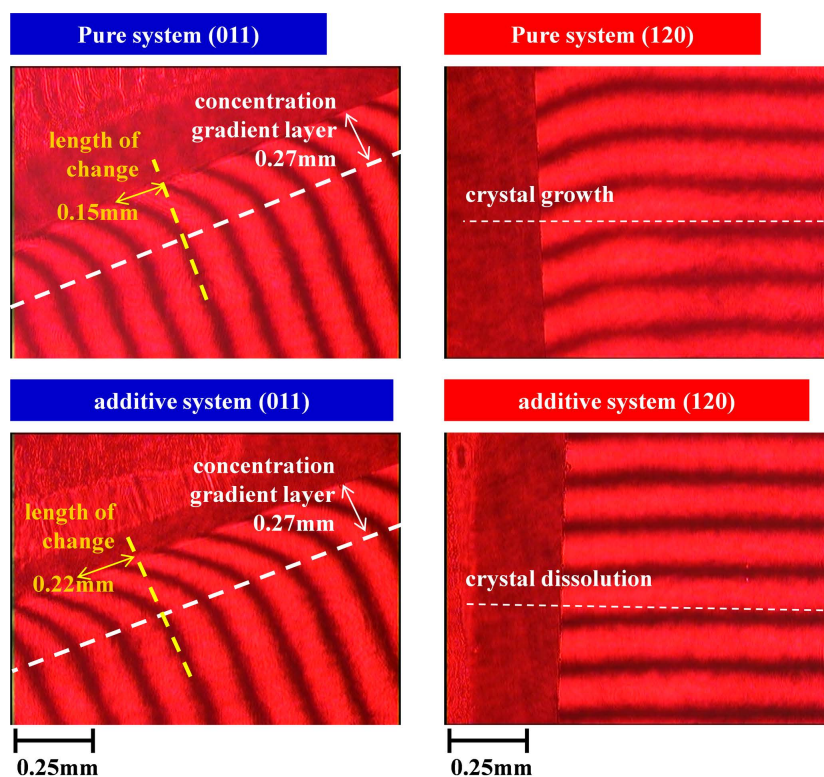
## 3. Results and Discussion

**Figure 2** shows the changes over time in the amount of crystal growth on each crystal plane at supercooling degrees  $\Delta T = 2$  and  $7$  in the pure system and the L-Met-added system (0.1 mol%). The amount of growth under the growth conditions increased linearly over time. In the pure system, the growth rate  $G$  is higher as  $\Delta T$  is larger, and the growth rate of the (011) plane is higher than that of the (120) plane. In the L-Met-added system, when  $\Delta T = 7$ , the growth rate of the (011) plane was no different from that of the pure system, but crystal dissolution occurred on the (120) plane. At  $\Delta T = 2$ , the growth rate of the (011) plane was higher than that of the pure system, but there was no difference between the growth rate of the (120) plane and the pure system. In this way, it was found that the effect of additives on the crystal growth rate was reversed depending on  $\Delta T$ , and furthermore, it was found that there is a condition ( $\Delta T = 7$ ) in which crystal growth and dissolution coexist.



**Figure 2.** Changes in crystal growth over time in pure system and L-Met added system (0.1 mol%).

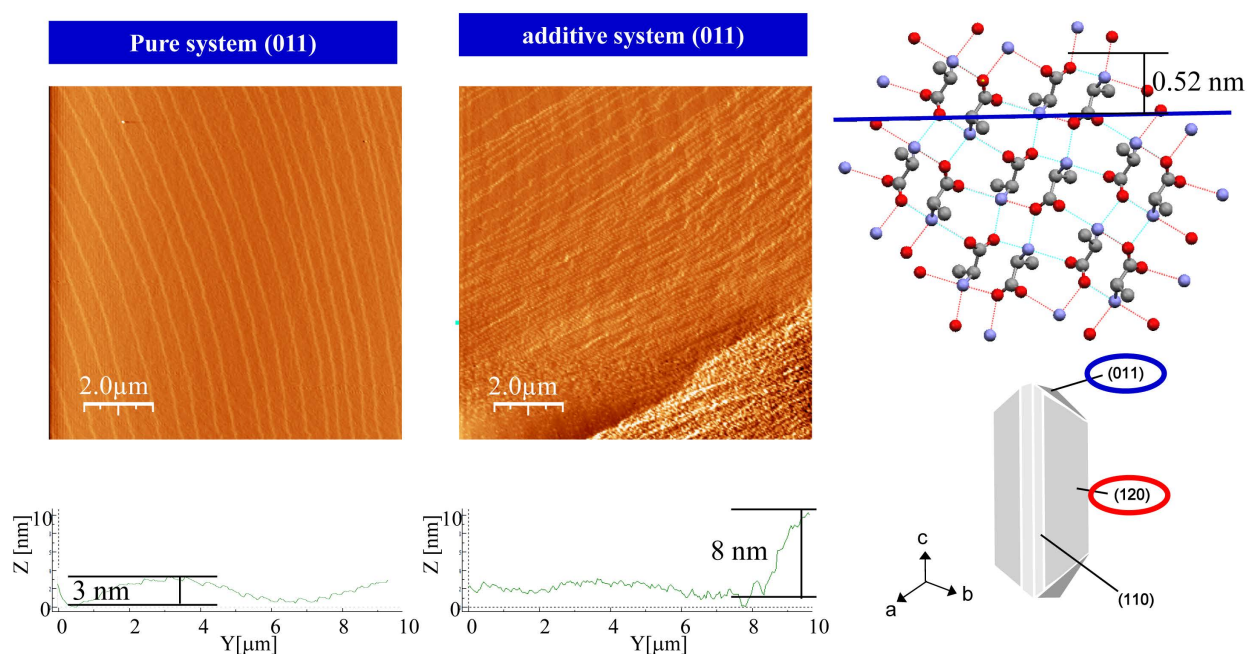
**Figure 3** shows the results of comparing interference fringes observed under additive-free and additive-added conditions. In the case of a pure system, it can be seen that the interference fringe changes on the (011) plane, which has a high growth rate, are larger than on the (120) plane. In the case of the L-Met additive-added system, the change in interference fringes on the (011) plane differs in the dotted line region compared to the pure system, although the growth rate is almost the same. The interference fringes near the crystal surface under conditions where the growth of the (011) plane and the dissolution of the (120) plane coexist are shown in comparison with the pure system. When the crystal surface grows or dissolves, the concentration near the crystal surface changes, and the refractive index changes accordingly, so this change in concentration appears as a shift in the interference fringes. Even though the additive system was dissolved at the (120) plane, it was slightly bent in the same direction as when the pure system was grown. In the case of an additive system, it is believed that the concentration of L-alanine on the crystal surface decreases due to the surface adsorption of L-methionine, which results in the region near the crystal surface becoming undersaturated, leading to the dissolution of the crystal surface. On the (011) face, the growth rates for the pure system and the additive-added system are almost the same, and the range of concentration changes is identical. However, the change in the interference fringes was slightly larger for the additive-added system compared to the pure system. The growth rate is likely due to the surface structure,



**Figure 3.** Interference fringes near the crystal surface (5 minutes after starting measurement,  $\Delta T = 7$ ).

given the possible differences in the solute uptake mechanisms.

**Figure 4** shows AFM images of the (011) surface after 20 min of growth under each condition, as well as the cross-sections of the steps and the step advancement rates. When AFM measurements were performed in liquid, straight steps were observed in the pure system, whereas in the additive system the steps were distorted and the surface was rough. Moreover, the height of the macro step was 3 nm for the pure system and 8 nm for the additive-added system. The fine irregularities in the cross-sectional view of the steps correspond to one molecule, and are considered to be steps of a monolayer. It can be seen that the macro steps of the pure system are equivalent to 6 monolayer steps, while the macro steps of the additive-added system are equivalent to 15 monolayer steps. The step forward speed was significantly slower in the additive-added system than in the pure system. On the (011) surface, the adsorption of additives on the surface stops the steps and slows down the rate of step advancement. Then step bunching occurs and the steps become higher. Once a certain height is exceeded, the additive is absorbed and growth continues, so it is thought that the additive has little effect on the growth rate of the (011) face.



**Figure 4.** Observation of the (120) surface by AFM.

#### 4. Conclusion

The mechanism of action of L-Met on L-Ala crystal growth, *i.e.*, the face-selective effect of L-Met on the L-Ala crystal growth rate in each direction, was investigated. It was found that the additive had no effect on the crystal growth of the (011) face under conditions of  $\Delta T = 2$  and 7. It was found that the (120) face dissolved in the additive-added system under conditions of  $\Delta T = 7$ . At that time, the (011) face was growing as a crystal. In this way, it was shown that there are conditions in

which crystal growth and dissolution coexist in the presence of an additive with a structure similar to that of the growing crystal.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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