Significant Effects of Magnetic and Gravitational Fields on the Morphology of Protein Crystals (Orthorhombic Lysozyme Crystals Grown Using $NiCl_2$ as Crystallization Agent)

Da-Chuan Yin,[†,‡] Nobuko I. Wakayama,*[,†] Hitoshi Wada,[†] and Wei-Dong Huang[‡]

Tsukuba Magnet Laboratory, National Institute for Materials Science, Sakura 3-13, Tsukuba, Ibaraki 305-0003, Japan, and State Key Laboratory of Solidification Processing, Northwestern Polytechnical University, Xi'an 710072, Shaanxi, PR China

Received: June 5, 2003; In Final Form: August 29, 2003

Magnetic fields (either horizontal or vertical) were found to drastically change the morphology of protein (orthorhombic lysozyme) crystals. A pair of {011} faces evident in crystal grown in the absence of a magnetic field disappeared when crystals were grown in the presence of a 10 T magnetic field. This fact suggests that a magnetic field makes the growth rate of {011} faces faster than that of {101} faces and influences the kinetics of crystal growth. Horizontal and vertical magnetic fields tended to align the *c*-axis of the crystals along the direction of the field. The combination of magnetic orientation of crystals and buoyancy-driven convection due to gravitational field caused the difference in the growth environments for the {110} and $\{1\bar{1}0\}$ faces, resulting in differences in growth rates of these faces and thus differences in the crystal morphologies. The analysis of the crystal shapes showed that the ratio of the crystal growth rate of the top surface to that of the side surface was 0.2−1. Thus, our study shows quantitatively for the first time that the gravitational field influences the crystal morphology via buoyancy-driven convection.

1. Introduction

Obtaining high quality protein crystals to determine the protein structure by using X-ray diffraction analysis is critical. Magnetic fields are thought to affect the growth of protein crystals. These effects have therefore recently been studied extensively,[1] revealing phenomena such as improvement in crystal quality,[2,3] magnetic orientation of crystals,[4−7] decrease in both the crystal growth rate[8,9] and the dissolution rate,[8,10] and damping of natural convection.[11] However, there have been few studies about the influences of magnetic fields on crystal morphology. Recently, we reported[12] that orthorhombic hen egg-white lysozyme (HEWL) crystals ($P2_12_12_1$, with $a = 79.0$ Å, $b = 80.8$ Å, and $c = 37.5$ Å) were formed when the crystals were grown by using paramagnetic $NiCl_2$ as the crystallization agent, and the crystals looked rooflike when crystals grew in a vertical magnetic field of 10 T. In this paper, we investigated the significant effects of magnetic fields (both horizontal and vertical) on the morphology of this orthorhombic crystal. Then, we discussed the effects of magnetic fields on crystal morphology, considering the growth rates of different crystallographic faces, the magnetic orientation of the crystals, and buoyancy-driven convection caused by gravitational field.

2. Experimental Section

Hen egg-white lysozyme (Seikagaku Kogyo, recrystallized six times) was used without further purification. A supersaturated solution was prepared by mixing an equal volume of the lysozyme aqueous solution (80 mg/mL) and a $NiCl_2$ solution (160 mg/mL). The pH was controlled at 4.60 by adding 1 N HCl and measured by using a pH-meter (PH8111-J, Yokogawa Inc.). The solution was then filtered through a filter with a pore size of 0.22 μm. The crystallization was carried out batchwise in a glass vessel (18 mm i.d. × 50 mm), and the solution height in the vessel was kept at about 10 mm. The vessel was set inside a copper water jacket to control the temperature at 21 °C by temperature-controlled water flowing through the jacket. Orthorhombic type lysozyme crystals were grown for 2 days in the absence and presence of a 10 T superconducting magnet (JMTD-10T100M, Japan Magnet Technology, Inc.). The direction of the magnetic field, which was parallel to the central axis of the solenoid coil of the superconducting magnet, was kept horizontal or vertical. The morphology of the crystals was analyzed using a stereomicroscope (SZX-ILLK100, Olympus Inc.).

3. Results and Discussion

3.1. Orthorhombic Lysozyme Crystals in the Presence and Absence of a Magnetic Field. Figure 1a−d shows microphotographs of orthorhombic lysozyme crystals crystallized by $NiCl_2$ in the absence and presence of the 10 T magnetic field. In the absence of the field (Figure 1a and b), crystals showed various morphologies, and the directions of their crystal axes were random. In the presence of the horizontal magnetic field (Figure 1c), however, the crystals showed more uniform morphology and all of the crystals were aligned with their *c*-axes along the horizontal magnetic field. The crystals grown in the vertical magnetic field showed much more uniform morphology (Figure 1d), namely, typical rooflike crystals reported previously,[12] and their *c*-axes were oriented along the direction of the vertical magnetic field. Thus, crystals grown in the absence of a magnetic field show diverse morphologies, whereas crystals grown in the presence of either a horizontal or vertical magnetic field of 10 T show more uniform morphology.

3.2. Comparison between Normal Tetragonal Morphology and Rooflike Morphology. According to X-ray diffraction

* To whom correspondence should be addressed. Phone: +81-29-851-3354, ext 5031. Fax: +81-29-863-5441. E-mail: wakayama.nobuko@nims.go.jp.

[†] Tsukuba Magnet Laboratory, National Institute for Materials Science.

[‡] State Key Laboratory of Solidification Processing, Northwestern Polytechnical University.

10.1021/jp035578k CCC: $25.00
Published on Web 11/19/2003

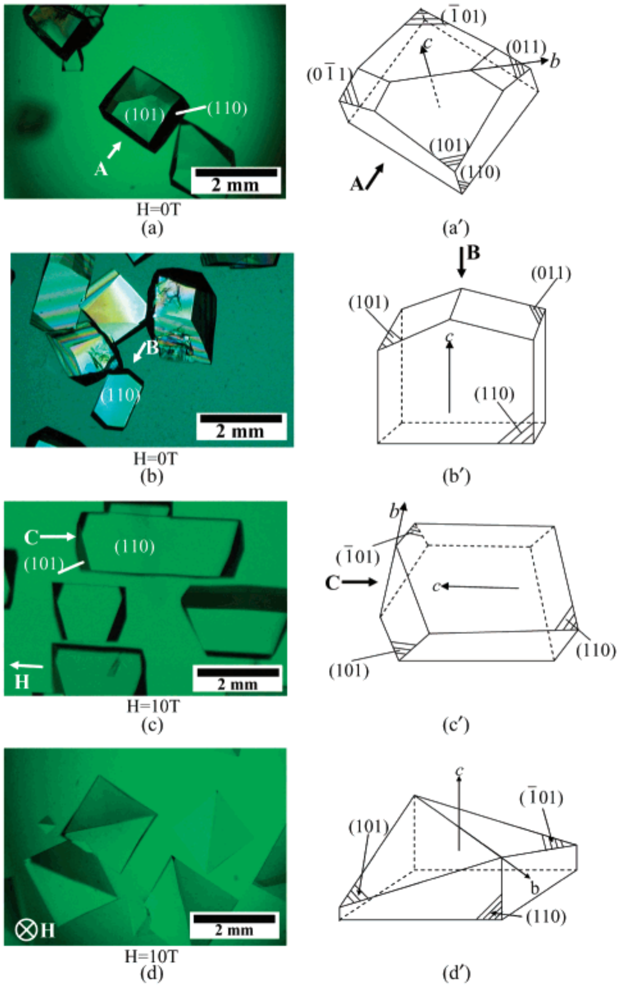

Figure 1. Habit of orthorhombic lysozyme crystals grown using $NiCl_2$ as crystallization agent: (a and b) in the absence of a magnetic field; (c) in the presence of a horizontal magnetic field (10 T); (d) in the presence of a vertical magnetic field (10 T). (a′−d′) Miller indices of the crystallographic faces. Vector **H** indicates the direction of the magnetic field.

analysis,[12] the lattice constants of rooflike crystals are similar to those of tetragonal crystals ($a = b = 79.20$ Å, $c = 37.94$ Å),[13] and the coordination of a Ni^{2+} ion to an Asp52Oδ2 atom in a lysozyme molecule distorts the normal tetragonal lattice into an orthorhombic lattice. We determined the evolution of the morphology from tetragonal to rooflike by considering the difference in growth rates between the {101} and {011} faces. Figure 2 compares the normal tetragonal morphology and the intermediate morphology between rooflike and tetragonal. As shown in Figure 2a and a′, the four {101} faces ((101), ($\bar{1}$01), (011), and (0$\bar{1}$1) faces) at the pyramid tip of the tetragonal lysozyme crystal were "equivalent" faces of relatively uniform size, indicating that the growth rates of these four faces were identical. However, this morphology differs when the growth rates of these four faces are dissimilar. Durbin et al.[14] found that tetragonal lysozyme crystals appeared less symmetric at low levels of supersaturation (supersaturation $c/s = 1.42$, where c was the concentration of the solution and s was the solubility). In our study, we also obtained such a less symmetric shape (Figure 2b) at much higher supersaturation ($c/s = 5.7$. The solubility was about 7 mg/mL when 80 mg/mL $NiCl_2$ was used[15]). We think this morphology is intermediate between the normal tetragonal crystals and rooflike crystals. Figure 2b′ clearly shows that when the growth rates of faces (011) and (0$\bar{1}$1) are larger than those of faces (101) and ($\bar{1}$01), such a less

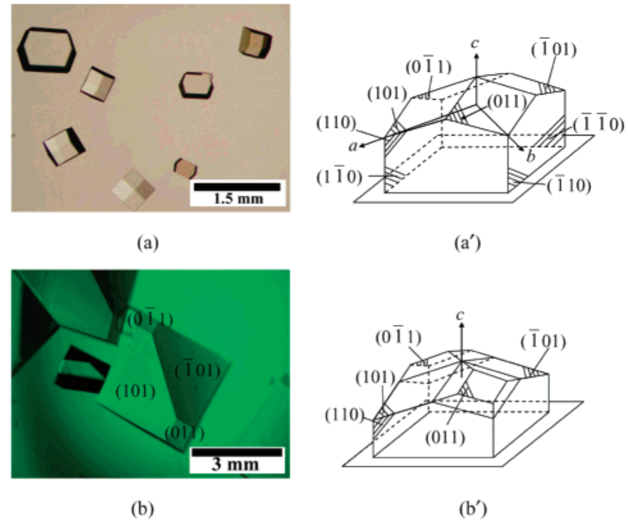

Figure 2. (a) Typical morphology of tetragonal lysozyme crystals grown from a solution (53 mg/mL HEWL, 35 mg/mL NaCl, pH = 4.60) at temperature 23 °C for 2 days in the absence of a magnetic field. (a′) Miller indices of tetragonal lysozyme crystals grown in the absence of a magnetic field. (b) Intermediate morphology between tetragonal and orthorhombic rooflike, grown using a solution (40 mg/mL HEWL, 80 mg/mL $NiCl_2$, pH = 4.60) at 19 °C for 2 days in the presence of a vertical magnetic field (10 T) and then in the absence of the magnetic filed and kept at room temperature (21 °C) for 2.5 months. (b′) Miller indices of the intermediate morphology.

symmetric morphology will appear. The difference in growth rates is thought to originate from the orthorhombic symmetry. When the difference in growth rate is large enough, faces (011) and (0$\bar{1}$1) will disappear, thus yielding a rooflike morphology as shown in Figure 1d. Figure 1d′ shows the Miller indices of such crystals. It is characteristic that only two faces ((101) and ($\bar{1}$01)) appear at the pyramid tip of the crystal.

3.3. Magnetic Field Effects on Crystal Morphology. The effects of a magnetic field on the crystal morphology were studied by using the Miller numbers shown in Figure 2b′. When crystals were grown in the absence of the magnetic field, various morphologies appeared (Figure 1a and b). In the crystal with a vertical c-axis (Figure 1a), two opposite {011} faces and two opposite {101} faces appeared at the pyramid tip, similar to the less symmetric intermediate crystal morphology (Figure 2b). In the crystal with a horizontal c-axis (Figure 1b), both the (101) and (011) faces appeared. Figure 1b′ shows the face indices of this crystal from the view direction of **B**. In the presence of a horizontal magnetic field (Figure 1c), however, the crystals had more uniform morphology compared with the case of those grown in the absence of the field. The major difference between these two types of crystals is that the crystal grown in the absence of a magnetic field had both the {011} and {101} faces at the pyramid tip. The number of faces at the pyramid tip ranged from 2 to 4 in the absence of a magnetic field, whereas, in the presence of a horizontal field, in general the number was 2, that is, two opposite {101} faces ((101) and ($\bar{1}$01) faces as shown in Figure 1c′). On the other hand, crystals grown in a vertical magnetic field showed the typical rooflike morphology (Figure 1d), and only two opposite {101} faces appeared at the pyramid tip, as shown in Figure 1d′. Thus, at the pyramid tip, the {011} faces disappeared in the presence of a magnetic field (either horizontal or vertical). This is the first report that a magnetic field itself significantly changes the morphology of a protein crystal.

3.4. Conditions for Rooflike Crystals To Appear. The conditions at which rooflike crystals appear depend on the

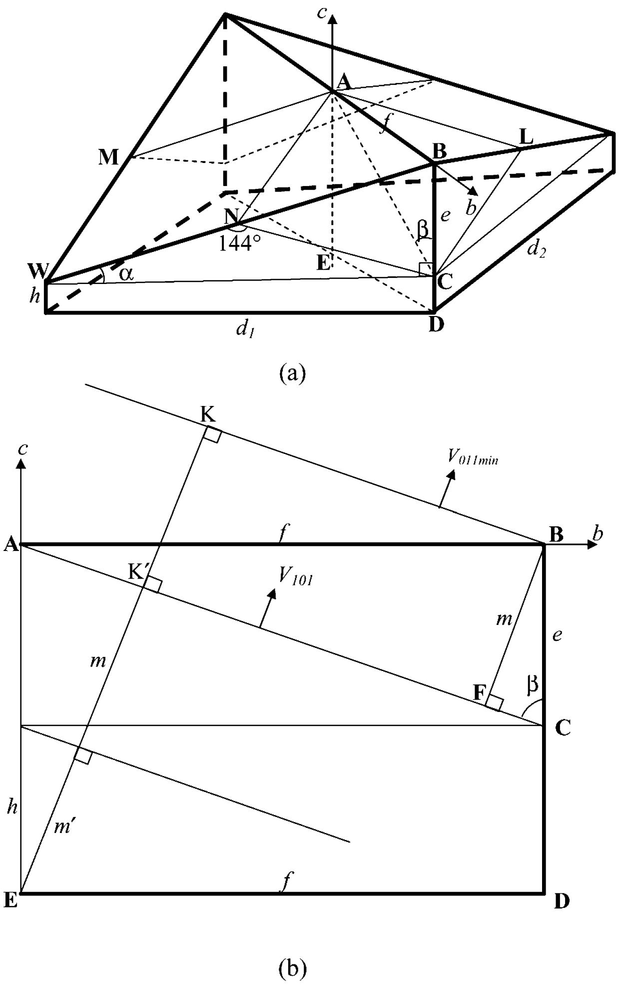

Figure 3. Schematics used to derive the growth rate conditions for attaining the rooflike morphology: (a) three-dimensional schematic of a rooflike crystal; (b) half of the vertical intersection of the crystal through the central roof-line (*b*-axis).

relationship among the growth rates of the {011}, {101}, {110}, and {1$\bar{1}$0} faces. Here, we estimate the growth rate conditions for attaining rooflike morphology. Figure 3a schematically illustrates the geometrical structure of a rooflike crystal. Judging from the square shape of the rooflike crystals shown in Figure 1d, we assume that the side lengths, d_1 and d_2, of the crystal are equal, and thus $d_1 = d_2 = d$, and that the growth rates of both the {1$\bar{1}$0} and {110} faces, V_{110} and $V_{1\bar{1}0}$, respectively, are equal, and thus $V_{110} = V_{1\bar{1}0}$. If the height difference between the highest and lowest points of the roof is BC and half of the length of the central roof-line is AB, then

$$\tan\beta = \frac{\mathrm{AB}}{\mathrm{BC}} = \frac{\frac{d}{2}\sqrt{2}}{d\tan\alpha} \qquad (1)$$

where α and β are the angles illustrated in Figure 3. Because α of the rooflike crystal structure is nearly the same as that for a tetragonal crystal structure, that is, $\alpha \approx 18°$,[16] eq 1 yields $\beta \approx 65°$.

Figure 3b shows half of the vertical intersection through the central roof-line (*b*-axis) of the crystal (rectangle ABDE in Figure 3a). If the "disappeared" faces possess a minimum growth rate $V_{011\mathrm{min}}$ to achieve the rooflike morphology, and the growth rate of the {101} faces is assumed to be V_{101}, then the length *l* to the faces grown at time *t* can be obtained from Figure 3b as follows:

Length *l* to the disappeared {011} faces at time *t* (EK):

$$l_{011} = m + m + m' = 2m + h\cos(90° - 65°) = 2m + 0.909h = V_{011\mathrm{min}}t \qquad (2\text{-}1)$$

Length *l* to the grown roof {101} faces at time *t* (in Figure 3, EK′ is the length to the imagined mirror face ANCL of the (101) face, that is, ANWM, and is equal to the length *l* to the grown rooflike face (101)):

$$l_{101} = m + m' = m + 0.909h = V_{101}t \qquad (2\text{-}2)$$

Length *l* to the grown {110} and {1$\bar{1}$0} faces at time *t*:

$$l_{110} = l_{1\bar{1}0} = 0.5d = V_{110}t \qquad (2\text{-}3)$$

where *m* is the length of BF in Figure 3b and *h* is the height of the lower point of the roof to the "ground", that is, the bottom of a container.

From the relationship between *m* and *d*, that is, $m = \mathrm{BC}\sin\beta = d\tan\alpha\sin\beta = 0.294d$, the growth rates of the faces as a function of the known size *d* and *h* can be expressed as

$$V_{011\mathrm{min}}:V_{101}:V_{110} = (0.589d + 0.909h):(0.294d + 0.909h):(0.500d) \qquad (3)$$

In a vertical magnetic field, if the growth rate of {110} and {1$\bar{1}$0}, V_{110}, is large enough so that the lowest point of the "roof" touches the bottom of a container, that is, $h = 0$, then the growth rate of {011}, $V_{011\mathrm{min}}$, should be at least twice V_{101} and about 18% faster than V_{110}. If V_{110} is comparable to the growth rate of {101} faces V_{101}, that is, $h/d \approx 0.226$, then to maintain the roof morphology, $V_{011\mathrm{min}}$ should be at least 59% faster than V_{101} and V_{110}.

On the other hand, for crystals grown in the presence of a horizontal magnetic field, the side lengths of the crystal d_1 and d_2 are different from each other. However, we can still use eq 3 to roughly estimate the ratio between the crystal growth rates. Judging from the crystals in Figure 1c, the lengths along and perpendicular to the *c*-axis are 2*h* and d_1, respectively. If we assume $d_1 \approx h$, then $V_{011\mathrm{min}}$ will be at least 24% faster than V_{101} and two times faster than V_{110} (the growth rate of the face facing upward).

Thus, the disappearance of the {011} faces in the presence of either a horizontal or vertical magnetic field suggests that the magnetic field makes the growth rate of the {011} faces larger than that of the {101} faces. Previous studies report that the growth rates of protein crystals decreased when grown in a magnetic field,[8,9] but only the effect of the magnetic field on the mass transport was proposed as the mechanism. Because the mass transport environments of the {101} and {011} faces are similar to each other in either a vertical or horizontal magnetic field, the difference in growth rate between these two faces indicates an unexpected magnetic field effect on crystal growth, that is, magnetic field effects on growth kinetics.

At present, we observed the rooflike crystals, that is, the disappearance of some crystal faces by the application of a magnetic field, only in the system using $NiCl_2$ as crystallization agent. When paramagnetic salts such as $CoCl_2$ or $MnCl_2$ were used as crystallization agents and the magnetic field was applied, the disappearance of any crystal faces was not observed.[12] When the nonparamagnetic salt NaCl was used as crystallization agent, such a phenomenon was also not observed. Further research is needed to clarify the mechanism of this effect.

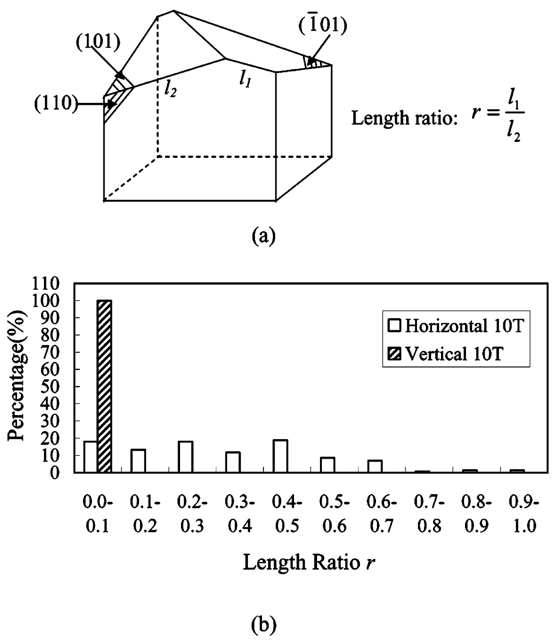

Figure 4. Distribution of length ratio r of the crystals grown in the presence of a vertical ($n = 33$; n is the total number of crystals for data collection) and horizontal magnetic field ($n = 127$). Length ratio r is defined as the ratio of the shorter length l_1 to the longer length l_2 of the edge lines between the {110} faces and the {101} faces.

3.5. Effects of Magnetic Orientation on Crystal Morphology. Although rooflike crystals, that is, crystals with only two opposite {101} faces, appeared when grown in the presence of either a horizontal or vertical magnetic field, the crystals grown in a vertical magnetic field (Figure 1d) show a much more uniform habit than those grown in a horizontal field (Figure 1c). To quantify the diversity in habit, we chose the length ratio of the edge lines between the {101} and {110} faces as a parameter for comparison. As shown in Figure 4a, we defined this length ratio r as the ratio of the smaller length l_1 to the larger length l_2, so that r was always less than 1. In Figure 4b, the distribution of r for our experimental results is given. The habit of the crystals grown in the vertical magnetic field was uniform, and their r concentrated at around ∼0, whereas, in the presence of the horizontal field, r clustered in the range 0−0.7. Next, we show that this difference in the r distribution is correlated with the growth rates of {110} and $\{1\bar{1}0\}$ faces when the crystals are the rooflike type.

Generally, the process of protein crystal growth is affected by buoyancy-driven convection caused by a gravitational field.[17] According to the numerical simulation by Qi et al.,[18] the contribution of natural convection to crystal growth rate increases with the crystal size, and the ratio of the side-surface growth rate to the top-surface growth rate increases with crystal size. For example, when the crystal size is 100 μm and the solution height is 5 mm, the calculated side-surface growth rate is about 4 times as fast as that of the top-surface (Figure 14 in ref 18) because of buoyancy-driven convection. Such a difference in growth rate will also affect the crystal habit.

As shown in Figure 5, we consider crystal growth in vertical (a) and horizontal magnetic fields (b). When crystals are grown in a vertical magnetic field (Figure 5a), all of the four {110} and $\{1\bar{1}0\}$ faces are vertical. When the growth rate of the $(1\bar{1}0)$ and $(\bar{1}10)$ faces, V_1, is faster than that of the $(\bar{1}10)$ and $(1\bar{1}0)$ faces, V_2, a rooflike morphology will not appear (Figure 5a-i). Only when $V_1 = V_2$ will a rooflike morphology appear (Figure 5a-ii). Therefore, our experimental result that all of the crystals

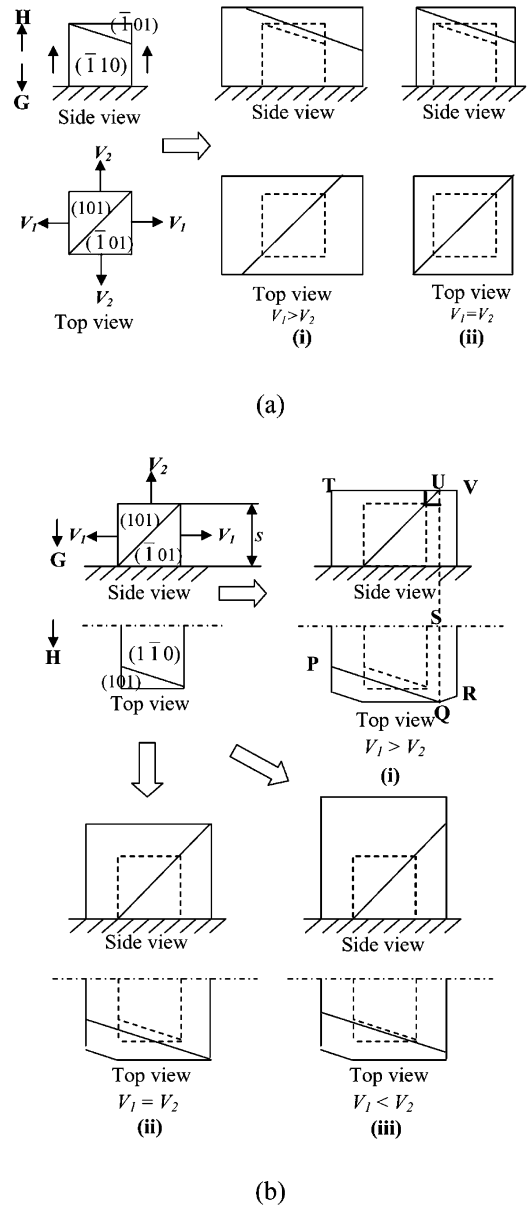

Figure 5. Schematic of the finally grown crystal habit as a function of growth rates of {110} and $\{1\bar{1}0\}$ faces, V_1 and V_2, respectively, in the presence of a (a) vertical and (b) horizontal magnetic field.

appear "rooflike" indicates that the vertical four faces had the same growth rate.

On the other hand, in a horizontal magnetic field (Figure 5b), the initial size and shape of the rooflike crystals (or nuclei) grown are assumed to be the same as those grown in a vertical magnetic field (Figure 5a). However, because the orientation of the crystals is affected by the magnetic field, the face attached to the bottom of the vessel will not grow. Because the mass transport conditions near the top and two side surfaces differ from each other, their growth rates might be affected by buoyancy-driven convection. We assume that the (110) and $(\bar{1}\bar{1}0)$ faces perpendicular to the bottom of the vessel have the same growth rate, V_1, while the growth rate of the $(1\bar{1}0)$ face facing upward is assumed to be V_2 (Figure 5b).

The habit of the crystals depends on the difference in growth rates V_1 and V_2. When $V_1 > V_2$, the habit shown in Figure 5b-i appears. In our experiments, when the field was applied horizontally, such a habit was frequently observed (Figure 1c), indicating that the faces perpendicular to the vessel bottom had larger growth rates than those of the faces facing upward. This observation agrees well with the numerical simulation by Qi et al.[18] that the growth rate of a side surface is larger than that of the top surface. When $V_1 = V_2$, the habit in Figure 5b-ii appears. We also observed such a habit experimentally, as in Figure 1c (r = 0−0.1; about 18% of the total number of the crystals appeared like this in Figure 4). When $V_1 < V_2$, the habit in Figure 5b-iii will appear. However, we did not observe such a habit experimentally. These results indicate that the orientation of the crystals causes differences in the growth environments of {110} and {1$\bar{1}$0} faces by buoyancy-driven convection, resulting in differences in the growth rates of {110} faces and thus yielding various habits.

3.6. Relationship between the Length Ratio (*r*) and the Growth Rate Ratio (V_2/V_1) of the {110} and {1$\bar{1}$0} Faces. Here, we discuss the relationship between r and V_2/V_1 in order to explain the microscopic appearance of crystals grown under a vertical or horizontal magnetic field. If we assume that the starting crystal is a nucleus, that is, $s \approx 0$ (where s is the side length of the seed crystal; Figure 5b), and that the two faces of "roofs" grow at the same rate, then the relationship between r and V_2/V_1 can be estimated.

In Figure 5b, the growth rates of the top face and the two side vertical faces are V_2 and V_1, respectively, and r is defined as QR/PQ. Because ∠SQR = ∠SQP, UV/QR = TU/PQ, we have

$$r = \mathrm{QR/PQ} = \mathrm{UV/TU} = \frac{V_1 t - V_2 t}{s + V_1 t + V_2 t} \qquad (4)$$

where t is the growth time.

If $s = 0$, then eq 4 becomes

$$\frac{V_2}{V_1} = \frac{1 - r}{1 + r} \qquad (5)$$

Judging from the distribution of r in Figure 4 together with eq 5, in a vertical magnetic field, $r = 0$, and thus, $V_2 = V_1$. In a horizontal magnetic field, $\{V_2\}/\{V_1\}$ ranged between 1 and 0.2 (r ranged between 0 and 0.7) and the averaged $\{V_2\}/\{V_1\}$ was 0.54. Thus, $\{V_2\}/\{V_1\}$can be determined experimentally. This result shows that buoyancy-driven convection due to a gravitational field also significantly affects the process of protein crystal growth.

4. Conclusions

In this study, we determined the effect of magnetic field on the morphology of orthorhombic lysozyme crystals grown using $NiCl_2$ as a crystallization agent. The key conclusions are as follows.

(1) A pair of {011} faces disappear in the presence of a magnetic field (horizontal or vertical). This disappearance indicates that the growth rate of the "disappeared" faces becomes faster than that of the {101} faces due to the applied magnetic field. This indicates that a magnetic field can affect the growth kinetics of protein crystals.

(2) Magnetic orientation of crystals causes differences in the growth environments of {110} and {1$\bar{1}$0} faces due to buoyancy-driven convection, resulting in differences in growth rates and thus different crystal morphologies. The ratio of the crystal growth rate of the top surface to that of the side surface estimated on the basis of our experiments was 0.2−1. Thus, the gravitational field also influences the crystal morphology via buoyancy-driven convection.

Acknowledgment. This work was supported by the JSPS (Japan Society for the Promotion of Science) Postdoctoral Fellowship Foundation and by the EYTP (Excellent Young Teachers Program of the Ministry of Education, People's Republic of China). The authors also thank Dr. M. Goto at the National Institute of Advanced Industrial Science and Technology for useful discussions.

References and Notes

(1) Wakayama, N. I. *Cryst. Growth Des.* **2003**, *3*, 17−24 and references therein.
(2) Lin, S. X.; Zhou, M.; Azzi, A.; Xu, G. J.; Wakayama, N. I.; Ataka, M. *Biochem. Biophys. Res. Commun.* **2000**, *275*, 274−278.
(3) Sato, T.; Yamada, Y.; Saijo, S.; Hori, T.; Hirose, R.; Tanaka, N.; Sazaki, G.; Nakajima, K.; Igarashi, N.; Tanaka, M.; Matsuura, Y. *Acta Crystallogr.* **2000**, *D56*, 1079−1083.
(4) Sazaki, G.; Yoshida, E.; Komatsu, H.; Nakada, T.; Miyashita, S.; Watanabe, K. *J. Cryst. Growth* **1997**, *173*, 231−234.
(5) Ataka, M.; Katoh, E.; Wakayama, N. I. *J. Cryst. Growth* **1997**, *173*, 592−596.
(6) Astier, J. P.; Veesler, S.; Boistelle, R. *Acta Crystallogr.* **1998**, *D54*, 703−706.
(7) Tanimoto, Y.; Yamaguchi, R.; Kanazawa, Y.; Fujiwara, M. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 1133−1134.
(8) Yanagiya, S.; Sazaki, G.; Durbin, S. D.; Miyashita, S.; Nakajima, K.; Komatsu, H.; Watanabe, K.; Motokawa, M. *J. Cryst. Growth* **2000**, *208*, 645−650.
(9) Yin, D. C.; Inatomi, Y.; Kuribayashi, K. *J. Cryst. Growth* **2001**, *226*, 534−542.
(10) Yin, D. C.; Inatomi, Y.; Wakayama, N. I.; Huang, W. D.; Kuribayashi, K. *Acta Crystallogr.* **2002**, *D58*, 2024−2030.
(11) Qi, J. W.; Wakayama, N. I.; Ataka, M. *J. Cryst. Growth* **2001**, *232*, 132−137.
(12) Yin, D. C.; Oda, Y.; Wakayama, N. I.; Ataka, M. *J. Cryst. Growth* **2003**, *252*, 618−625.
(13) Datta, S.; Biswal, B. K.; Vijayan, M. *Acta Crystallogr.* **2001**, *D 57*, 1614−1620.
(14) Durbin, S. D.; Feher, G. *J. Cryst. Growth* **1986**, *76*, 583−592.
(15) Ataka, M.; Katsura, T. *Abstracts of the Fourth International Conference on Biophysics and Synchrotron Radiation, Tsukuba, Japan*, 1992; p 354.
(16) Jones, F. T. J. *Am. Chem. Soc.* **1946**, *68*, 854−857.
(17) Kundrot, C. D.; Judge, R. A.; Pusey, M. L.; Snell, E. H. *Cryst. Growth Des.* **2001**, *1*, 87−99.
(18) Qi, J. W.; Wakayama, N. I. *J. Cryst. Growth* **2000**, *219*, 465−476.