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Magnetic Orientation of *Lysozyme* Crystals

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Magnetic orientation of *Lysozyme* crystals was studied in *horizontal* magnetic fields (< 1 T). The critical field and critical volume are estimated to be 0.5–0.8 T and ca. 33.6 μm^3, respectively. The experimental factors affecting magnetic orientation are discussed.

A magnetic field is a useful tool to orient crystals.[1] As for materials of biological importance, magnetic orientation of *Lysozyme* crystals has been reported by two groups.[2,3] The critical fields (H_c's) for two groups are about one order of magnitude different. Here H_c is tentatively defined as the field above which 80% of the crystals are oriented. In this paper, orientation of *Lysozyme* crystals is studied in *horizontal* magnetic fields to clarify experimental factors affecting magnetic orientation.

Experimental

The crystal preparation method was analogous to those reported.[2,3] Hen egg-white *Lysozyme* from Seikagaku Kogyo and other chemicals of the purest grade were used as supplied. Tetragonal *Lysozyme* crystals were prepared by mixing equal volumes of *Lysozyme* solution (100 mg/mL in 100 mM sodium acetate buffer, pH 4.5) and precipitant solution (65 mg/mL NaCl in the same buffer) and poured into glass Petri dishes (Sibata, ϕ19 mm×12 mm) and wells of plastic tissue culture plates (Iwaki 3860-096, ϕ7 mm×10 mm). The volumes of the solution were 1.4 mL in the former vessel and 100 µL in the latter. In the latter, wells were sealed with a covering of liquid paraffin (50 µL). Magnetic fields were applied by using a conventional electromagnet (Tokin, SEE-10) and a superconducting magnet (Oxford, SM-1000). The vessels were placed in horizontal magnetic fields at 20 °C for 10 h. The directions of crystals were observed by a microscope and recorded by a camera, after taking them out of the magnet.

Results and Discussion

Figure 1 shows tetragonal *Lysozyme* crystals grown in Petri dishes. At zero field, crystals grow with different shapes. They are classified roughly into two groups by their apparent crystal shapes as viewed from above: i.e., "hexagonal" and "square" crystals. In a "hexagonal" crystal, the *c*-axis, which is parallel to the long diagonal axis of the crystal, is in the horizontal plane, whereas it is perpendicular to this plane in a "square" crystal. At zero field, 60–70% of the crystals are "hexagonal" and 40–30% are "square" in shape. The fraction of "square" crystals decreases with increasing magnetic field. At 0.64 T, about 90% of crystals are "hexagonal" in shape and are oriented in such a way that their *c*-axes are parallel to the field. The "hexagonal" crystals are geometrically stable, compared with "square" ones, in magnetic fields as well as at zero field. Analogous magnetic field effects are observed in the crystals grown in wells of plastic plates.

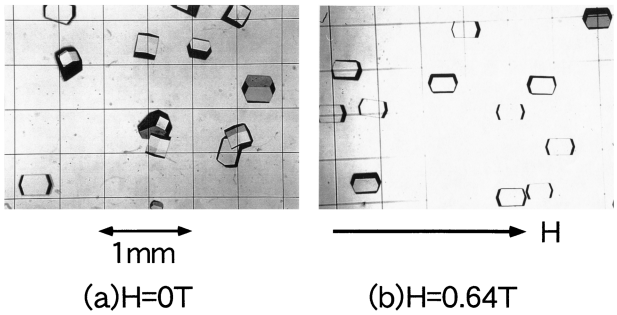

Fig. 1. Photographs of *Lysozyme* crystals grown in glass Petri dishes. (a) 0 T. (b) 0.64 T.

Magnetic orientation of *Lysozyme* crystals is explained in terms of magnetic anisotropy of crystals. The magnetic anisotropy energy $E(\theta, H)$ of a crystal is given by Eq. 1, when it is composed of a mole number n of molecules and has an magnetic symmetry axis z:[1]

$$E(\theta, H) = -(n/2)[\chi_\perp + (\chi_\parallel - \chi_\perp)\cos^2\theta]H^2 \quad (1)$$

where θ is the angle between the axis z and the field H, and $\chi_\parallel$ and $\chi_\perp$ are molar magnetic susceptibilities parallel and perpendicular to z. In the case of tetragonal *Lysozyme* crystals, the *c*-axis is the magnetic symmetry axis z (i.e., $\chi_\parallel = \chi_c$, $\chi_\perp = \chi_a = \chi_b$) and $\chi_\parallel - \chi_\perp = 76\times10^{-6}$ emu mol^{-1}.[3] Therefore, *Lysozyme* crystals are oriented in order to minimize their magnetic energies.

The "hexagonal" crystals whose *c*-axes are within ±7.5° with respect to the field are defined as "oriented" crystals and the fraction of "oriented" crystals is calculated as a function of magnetic field (Fig. 2a). The H_c's are about 0.5 T (glass dishes) and 0.8 T (wells of plastic plates). H_c is slightly larger for the crystals in wells of plastic plates than for those in glass dishes. The bottoms of each well are specially treated so that culture tissues can adhere there easily. This suggests that crystals on the bottom of glass dishes can rotate easily, compared to those on the bottom of wells.

Next, the critical volume, i.e., the volume of a crystal needed for magnetic orientation, is estimated from the angular distribution of crystal axes in magnetic fields. The probability $P(\theta, H)$ that the *c*-axis of a crystal is at angle of θ with respect to H is calculated by dividing the number of crystals whose *c*-axes are between θ −7.5° and θ +7.5° with respect to H by the total number of crystals. The angles θ are calculated at 15° intervals from +90° to −90° for 600–1200 crystals at each magnetic field. The results for crystals in glass dishes are given in Fig. 2b. It is obvious that magnetic orientation starts at the very low fields.

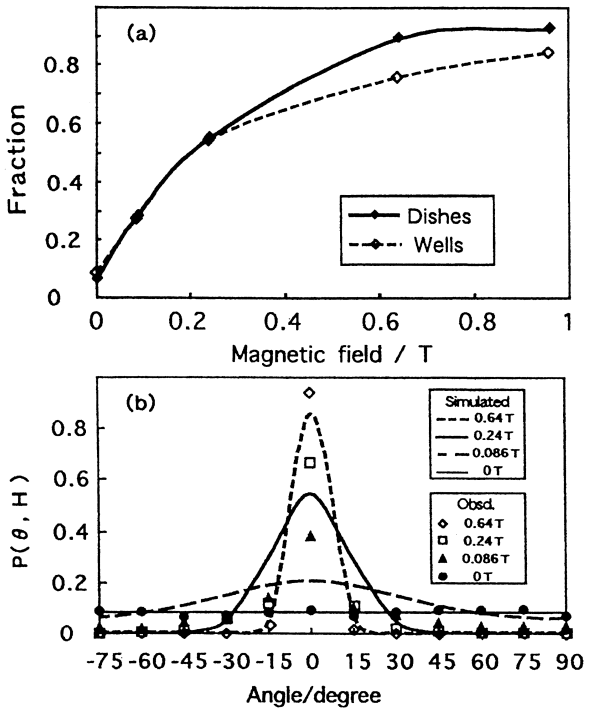

Fig. 2. (a) Magnetic field dependence of the fractions of "oriented" crystals for the crystals grown in Petri dishes (◆) and wells of plastic plates (◇). (b) Angular distribution of $P(\theta, H)$ for the crystals grown in Petri dishes. Lines are simulated ones. See text.

The probability $P(\theta, H)$ is given by the following equation, when magnetic orientation takes place within a horizontal plane:[1]

$$P(\theta, H)\, d\theta = \exp[-E(\theta, H)/kT]\, d\theta / \int_{-90^\circ}^{90^\circ} \exp[-E(\theta, H)/kT]\, d\theta \qquad (2)$$

where k is the Boltzmann constant, and T is the absolute temperature.

Simulated lines of $P(\theta, H)$ for *Lysozyme* crystals, grown in glass dishes, are shown in Fig. 2b. At 0.64 T the simulated line fits reasonably with the observed data, though at lower fields ($\leqq$ 0.24 T) simulated lines do not reproduce the observed ones. This is because orientation of crystals is three-dimensional at zero field, though it is two-dimensional in higher field, as is clear from Fig. 1. From the simulated lines shown in Fig. 2b, the mole number of molecules, n, is obtained to be 1.9×10^{-15} mol, leading the number of unit cells of 1.4×10^{8} and the critical volume of ca. 33.6 μm^3. Very similar values are obtained from the data using wells of plastic plates. The volume obtained here is about one order of magnitude larger than the reported ones.[2,3]

Finally, let us compare the present results with reported ones. Ataka et al.[2] studied orientation of *Lysozyme* crystals grown in a cylindrical glass vessel (ID = 15.6 mm) at 18 °C in *horizontal* magnetic fields and showed that H_c was ca. 0.5 T, though the definition of "oriented" crystals was not given. Yanagiya et al.[3] grew *Lysozyme* crystals in wells of the same plates as we used here under *vertical* fields. They defined the "oriented" crystals as the ones whose c-axes are within ±3° with respect to H and concluded that H_c was about 8–10 T. The H_c value obtained in this work is in good agreement with the field reported in Ref. 2 but is in disagreement with the one reported in Ref. 3. The definition of "oriented" crystals used in Ref. 2 would be similar to what we used here. Although there is a large difference between our value and that reported in Ref. 3, we re-calculate the fraction of "oriented" crystals under the same definition used in Ref. 3. Then it is obtained that the fractions are about 0.49 (0.64 T) and 0.76 (0.95 T) in Petri dishes and about 0.54 (0.64 T) and 0.57 (0.96 T) in wells of plastic plates. From these values H_c's are roughly estimated to be 1 T (dishes) and 2–3 T (wells). These values are about twice of the values obtained under the previous definition. Thus the definition of "oriented" crystals affects significantly the strength of H_c. Furthermore, it is estimated from the data in Ref. 3 that the fractions of "oriented" crystals are 0.15–0.20 (0.6 T) and 0.20–0.30 (1 T). These values are less than one half of the values obtained here under the same definition. Since, except for the directions of magnetic fields, our experimental conditions are analogous to those used in Ref. 3, the directions of magnetic fields would be responsible for the discrepancy. In vertical magnetic field, "square" crystals are the energetically stable ones, and therefore, oriented crystals are "square" in shape. However "square" crystals are geometrically less stable in shape than "hexagonal" ones. Thus "square" crystals must stand on the bottom of vessels in a geometrically unstable manner in *vertical* magnetic fields, in good contrast with the fact that "hexagonal" crystals with wide flat surfaces can lie stably on the bottom in *horizontal* magnetic fields. Therefore, an excess energy would be required for the orientation of "square" crystals in the *vertical* magnetic field, compared with that of "hexagonal" crystals in the *horizontal* field.

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