Effects of Strong Magnetic Fields on Natural Convection in the Vicinity of a Growing Cubic Protein Crystal

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Recently, better quality protein crystals have been obtained by using a superconducting magnet. One of important factors that determine crystal quality is natural convection in the fluid where the crystals are grown. This paper presents a comprehensive, comparative numerical study of natural convection around a growing protein crystal when a vertical magnetization force caused by a gradient magnetic field acts on protein aqueous solution and the viscosity of the solution increases. The study shows that natural convection around a crystal can be damped most effectively and the crystal growth rate is reduced when an upward magnetization acts on a protein solution and the viscosity increases. The decrease of crystal growth is thought to contribute to the improvement of crystal quality. The technique of obtaining low gravity environments and damping natural convection in electric low- and non-conducting fluid will be useful for the preparation of many materials.

KEY WORDS: natural convection; magnetization force; Kelvin force; damping of convection; protein; numerical simulation; low gravity.

1. Introduction

Proteins are the elementary building units for living creatures, and the molecular structure of protein provides a basis for understanding the functions of biological macromolecules. Determining the structure of protein molecules is therefore crucial. The most powerful technique for determining protein structure is X-ray crystallography. Developments in beam technology, detectors, and computational crystallography have greatly accelerated the determination of structures. However, the production of crystals of adequate size and high quality is often the “bottleneck” for three-dimensional structure analysis of macromolecular crystals. Protein crystals are segregated from its aqueous solution. Since protein molecules are incorporated into the crystal during crystal growth, the solution density near the crystal surface becomes lower than that of the bulk solution and natural convection occurs. Recent crystallization experiments conducted on spaceshuttle have indicated that about 30% of protein crystals grown in the space yielded X-ray diffraction data of significantly higher resolution than the best crystals grown on the earth.1,2) An obvious difference between the space- and Earth-based experiments is the magnitude of gravity and buoyancy, and the natural convection is considered to be one of the important factors that determine crystal quality. If natural convection is damped during crystal growth, the quality of crystals will be improved.

Damping of natural convection in liquid metals and semiconductors by applying magnetic fields has been studied by many researchers since the 1960’s.3) Hartman number, Ha is generally used as a parameter to estimate the efficiency of magnetic damping: $Ha = \mu_0 H L \sqrt{\sigma/\rho \nu}$ where $\mu_0$ is vacuum permeability, $\sigma$ is electric conductivity, $L$ is the size of a container, $\rho$ is the density and $\nu$ is the kinematic viscosity. For most apparatus of molten silicon ($\sigma = 2 \times 10^6 \Omega^{-1} \text{m}^{-1}$), $Ha \approx 100–400$. On the other hand, for a typical protein crystal formation experiment, $\sigma = 5 \Omega^{-1} \text{m}^{-1}$, $\rho = 10^3 \text{kg} \cdot \text{m}^{-3}$, $\nu = 10^{-6} \text{m}^2 \cdot \text{s}$ and $L = 5 \times 10^{-3} \text{m}$. Even when $\mu_0 H = 10 \text{T}$, $Ha \approx 3.5$ and efficiency of magnetic damping is small. Qi et al.4) numerically simulated the natural convection in the vicinity of a growing cylindrical protein crystal (the diameter 1 mm, the height 1 mm) in a cylindrical cell (the diameter 10 mm, the height 10 mm) and concluded that the damping effects by Lorentz force is negligible even in 10 T. Thus, there have been few methods to damp natural convection efficiently in low- or non-conducting fluids.

Recently, a new method to control the magnitude of effective gravity (vertical acceleration) by applying a vertical magnetic field gradient and producing a vertical magnetization force has been proposed.5,6) Magnetization (Kelvin) force is a body force and acts on every material even if it is not ferromagnetic. Generally, a unit volume of a substance in a magnetic field gradient experiences a force:

$$F_m = \frac{1}{2} \mu_0 \chi \cdot \text{grad} H^2 = \frac{1}{2} \mu_0 \rho \chi_b \cdot \text{grad} H^2$$  \hspace{1cm} (1)

where $H$ is the magnetic field strength, $\chi$ is the volume magnetic susceptibility which is the product of density ($\rho$) and mass magnetic susceptibility ($\chi_b$). Under one-dimensional magnetic field gradient, Eq. (1) is rewritten as fol-
blood. Protein crystals were formed inside a commercially available vertical superconducting magnet, as shown in Fig. 1. At the center of the magnet (B), a magnetic field of 10 T is uniform, while \( \mu_0 H \) is 7 T and the absolute value of \( \mu_0^2 H(dH/dz) \) is 400 T²/m at the position A and C. At the position A, an upward \( F_M \) of 0.3 g acts and the effective gravity of 0.7 g is expected, while at the position C, 1.3 g is expected.

**Figure 2** shows the relationship between the effective gravity and the X-ray resolution of crystals. The crystals formed inside the magnet (△) showed a higher resolution width with decreasing effective gravity from 1.3 to 0.7 g. The resolution of the crystals formed in 0.7–0.8 g was 3.06±0.14 Å, while the crystals grown outside the magnet (○) showed 4.24±1.24 Å. The resolution of crystals formed in 0.7–0.8 g is improved by about 30% compared with the crystals formed outside the magnet. Thus, when protein crystals were formed under low gravity environment where natural convection was partially damped, the crystal quality was superior to the quality of the crystals formed in the absence of the field. Furthermore, in Fig. 2, the improvement was smaller than that at a reduced gravity, the crystal quality was also improved by applying a uniform field of 10 T.

On the other hand, Sato et al. reported that the application of a 10 T uniform magnetic field improved the quality of orthorhombic lysozyme crystals.\(^{3,10}\) The growth rate of protein crystals was observed to decrease under 4 T\(^{9}\) and 10 T\(^{10}\) of uniform magnetic fields, compared with the growth rate in the absence of a magnetic field. The dissolution rate of crystals were also observed to be reduced under 10 T.\(^{9,11}\) These facts suggest that the damping of natural convection occurs even in a uniform magnetic field. However, the damping efficiency of Lorentz force under 10 T is small in a low-conducting protein solution.\(^{4}\) Therefore, considering another possibility of damping natural convection by magnetic fields, we measured the viscosity of a protein solution inside a vertical superconducting magnet and found that the viscosity increased by 10–30% under 10 T.\(^{12}\) The increase of viscosity is also considered to contribute to the damping of natural convection during protein crystal growth.

In the present paper, in order to explain the observed phenomena,\(^{7,10}\) we numerically studied the damping of natural convection in the vicinity of a growing protein crystal, considering the effects of a vertical magnetization force, a magnetically induced increase of viscosity and Lorentz force.

### 2. Mathematical Model

#### 2.1. Theoretical Model

**Figure 3**(a) shows the top view of a mathematical model and the shaded area is the simulation domain. We assumed that many cubic crystals with edge length \( h \) are growing at the bottom of a vessel. The distance between the central points of two crystals is 1 mm in horizontal X- and Y-direction. The height of a protein solution was 2 mm. An external magnetic field along Z-direction is applied. The convective flow caused by the concentration gradient is three-di-
The nomenclature of symbols is: \( V \) is the velocity vector, \( H \) is the external magnetic field, \( \nabla \) is the kinematical viscosity of solution, \( p \) is the pressure, \( \rho \) is the density of solution, \( t \) is the time, \( \nabla \) is the operator of Laplacian, \( C \) is the concentration of protein, \( D \) is the diffusivity of protein, \( g \) is the gravitational acceleration, \( k \) is the unit vector of z-direction, \( \phi \) is the electric scalar potential, \( E \) is the electric field intensity, \( j \) is the induced electrical current, \( B \) is external magnetic flux density. Considering the direction of \( H \) and selecting proper scale values, the non-dimensional equations show as followings in tensor form.

\[
\frac{\partial U_i}{\partial x_i} = 0 \quad \text{(10)}
\]

The external force, \( F \) is:

\[
F = F_g + F_M + F_L \quad \text{(7)}
\]

\[
F_g = -\rho \frac{\partial \phi}{\partial t}, \quad F_M = \frac{1}{2} \mu_0 \mu_{s} V \nabla^2 H, \quad F_L = j \times B, \quad \text{(8)}
\]

\[
j = \sigma(E + V \times B), \quad E = \nabla \phi, \quad \nabla^2 \phi = -\nabla \cdot (E \times B) \quad \text{(9)}
\]

The mathematical model of this system consists of equations of mass balance, mass transfer, momentum balance, and using the Boussinesq approximation the following equation are used to describe physical problem:

\[
\nabla \cdot \rho V = 0 \quad \text{(4)}
\]

\[
\rho \frac{\partial V}{\partial t} + \rho (V \cdot \nabla)V = -\nabla p + \rho \nu \nabla^2 V + F \quad \text{(5)}
\]

\[
\nabla \cdot (V \cdot \nabla)C = D \nabla^2 C \quad \text{(6)}
\]

The time, velocity, pressure, \( B \) and \( \phi \) are scaled using as reference quantities \( U, V, \rho \), \( L, \rho \), \( \nu L, \rho \), \( D \), \( \Phi \), respectively. \( U_i \) and \( x_i \) represent each of the three dimensionless velocity components \( U, V, \) and \( W \) and coordinates of \( X, Y, Z \). \( B_0 \) is the magnetic flux density of the point where \( \nabla \Phi^2 \) takes a maximum value. \( Sc = \frac{\nu L}{D} \) is the solutal Schmidt number defined as \( Sc = \frac{\nu L}{D} \). \( Ra \) is solutal Rayleigh number of concentration defined as \( Ra = \rho \Delta \rho \frac{U L}{\mu D} \), and \( \Delta \rho \) is the density difference, \( \rho_w \) is the bulk solution density and \( D \) is the diffusion coefficient of protein molecules. The non-dimnensional concentration is defined as \( \Psi = \frac{C - C_m}{C_i - C_m} \) where \( C_i \) is the protein concentration at crystal interface and \( C_m \) is the protein concentration far away from the crystal surface (bulk concentration of the solution).

The velocities normal to the surfaces of four vertical boundaries were assumed to be zero. The boundary conditions on these four vertical surfaces were treated as periodical conditions. The crystal growth at the crystal surface was assumed to be constant, \( C_s \) far away from the crystal. The size of a growing crystal was assumed to be constant. The top surface of the solution was treated as no slip surface and the protein concentration therein was assumed to be \( C_m \). The size of the growing crystal was assumed to be constant, \( C_s \). No-slip condition for velocities and adiabatic condition for concentration were assumed at the bottom of a vessel.

The physical properties of fluid used in simulation are: \( X_s = -9 \times 10^{-4} \text{m}^3/\text{kg} \) and \( \beta = 2.5 \times 10^{-5} \text{K}^{-1} \). In the simulation, \( 0.5 \mu \text{m} \) diameter particles are assumed to be \( \frac{1}{2}\mu \text{m} H \text{dH/dx} \approx \pm 400 \cdot B_0^2 \nabla T \). Using \( B = \mu_0 \text{H} \), the magnetic field is

\[
B = B_0 \left( 1 - \frac{1}{2} \frac{\mu_0 H}{B_0} \frac{dH}{dx} \right)^{0.5} \quad \text{(18)}
\]
In this simulation, $B_0 = 7 \text{T}$. Natural convection occurs because the solution density in the vicinity of a crystal, $\rho_s$ is less than the bulk density $\rho$: $\Delta \rho / \rho = (\rho_s - \rho) / \rho = 0.00795$.\(^{13}\) The diffusion coefficient and kinematic viscosity of solution without a magnet field were assumed to be $D_0 = 7 \times 10^{-11} \text{m}^2/\text{s}$ and $v_0 = 10^{-4} \text{m}^2/\text{s}$, respectively. $Sc = 14286$. In the simulation process, we assumed that the viscosity of protein solution increases by 10 percent by applying the magnetic field\(^{22}\) and the relationship, $Dv = \text{Const}.$ exists.

According to the mass balance and Fick’s first law, the local crystal growth rate, $G_{\text{local}}$, at crystal surface is defined as:

$$G_{\text{local}} = \frac{D}{C_s} \cdot \frac{\partial C}{\partial \bar{r}} = \frac{D(C_s - C)}{C_s L_x} \left( -\frac{\partial \Psi}{\partial n} \right) \quad \text{......(19)}$$

where $C_s$ is the mass concentration of solute in the crystal, $n$ and $\bar{n}$ are non-dimensional and dimensional vector normal to the crystal surface.

2.2. Numerical Method

The governing equations were solved in primitive variable in a uniform, three-dimensional staggered grid based on the finite difference method.\(^{10}\) The QUICK scheme\(^{15}\) was used in the finite difference formulation of the convective terms to minimize numerical diffusion effects. The SIMPLE algorithm was used to solve the coupled heat transfer and fluid flow problem. The discretization of time is performed by explicit scheme. To ensure convergence of the numerical algorithm in the steady state, the maximum residual of mass flux in one control volume will be less than $10^{-10}$. To achieve high resolution of the velocity and concentration fields near the crystal interface, where the gradients are steep, a finer grid near the crystal interface as shown in Fig. 3(c) was used in the simulation.

3. Results and Discussion

3.1. Velocity and Concentration Field around a Growing Crystal in the Absence of a Magnetic Field

First, we numerically simulated the natural convection in the absence of the magnetic field (the external force $F = F_0$ in Eq. (7)). The three dimensional presentations of velocity contour surface and non-dimensional concentration contour surface are depicted in Fig. 4. The shape of non-dimensional concentration contour surface ($\Psi = 0.1$) looks like a mushroom (Fig. 4(a)). The shape of vertical velocity contour surface of $w = 15 \mu \text{m/s}$ looks like a heat balloon (see Fig. 4(b)). These are the main characteristics of concentration and velocity distributions when a protein crystal is growing in normal gravity. It is characteristic that natural convection caused by protein crystal growth is localized. Under micro-gravity such natural convection does not exist.

The velocity vector and non-dimensional concentration fields are presented in Fig. 5(a) for $Y = 0.5 \text{mm}$ plate and Fig. 5(b) for enlarged map. Figure shows that the strong natural convection occurs and the steep concentration gradient exists around the crystal. The upward velocity takes a maximum at around $z = 0.4 \text{mm}$, i.e., about three times of crystal size above the crystal top surface (see also $\bigcirc$ in Fig. 8(a)). The enlarged map shows that the concentration gradient near the vertical crystal surface tends to decrease near the bottom of a container.

3.2. Effects of Lorentz Force on Velocity and Concentration Field around a Growing Crystal

We numerically estimated the damping of natural convection by Lorentz force (the external force $F = F_0 + F_L$ in Eq. (7)) in uniform magnetic fields of 10 and 20 T. The crystal size was 100 $\mu \text{m}$, $\sigma = 15 \Omega^{-1} \text{m}^{-1}$, and the viscosity was not affected by the magnetic field. Figure 6 shows the effects of magnetic fields on the distribution of vertical velocity along the intersection line of plates $X = 0.5 \text{mm}$ and $Y = 0.5 \text{mm}$. The maximum velocity was nearly independent of $H$, and the damping efficiency of natural convection is negligible. Hereafter, we neglected the term of Lorentz force.

3.3. Effects of Magnetic Fields on Velocity and Concentration Field in the Vicinity of a Growing Crystal

We considered the effects of vertical magnetization force and the magnetic increase of viscosity (the external force
F = F_L + F_M in Eq. (7)). Figure 7(a) presents the effects of magnetic field gradient on vertical velocity around the crystal along the intersection line of plate Y = 0.5 mm and plate Z = 50 μm. According to the viscosity measurement, the viscosity was assumed to increase 10% by applying magnetic fields (ν = 1.1 ν₀). When the protein solution was set in a uniform magnetic field (the position B inside the magnet in Fig. 1) and the viscosity increases 10%, the maximum velocity decreases about 11% (⊙) compared with that in the absence of the field (○). When an upward magnetization force of 0.3ρg acts on the solution (the position A) and ν = 1.1 ν₀ (□), the maximum velocity decreases by about 30% compared with that in the absence of the field (○). On the other hand, when a downward magnetization force acts (the position C) and ν = 1.1 ν₀ (△), the maximum velocity increases about 11%. Thus, most efficient damping of natural convection occurs when the protein solution was set at the position A where an upward F_M acts on the solution. Figure 7(b) shows the distribution of non-dimensional concentration along the intersection line of plate Y = 0.5 mm and plate Z = 50 μm. No significant difference was observed in the presence and absence of the field.

Figure 8(a) shows the effects of magnetic fields on the distribution of vertical velocity along the intersection line of plates X = 0.5 mm and Y = 0.5 mm. When an upward magnetization force is added (the position A) and the viscosity increases by 10% (□), the maximum velocity is reduced about 28% compared with that in the absence of the magnetic field (○). When a downward magnetization force is applied (the position C) and ν = 1.1 ν₀ (△), the maximum velocity increases about 15%. At the center of the magnet where ν = 1.1 ν₀ (⊗), the maximum velocity decreases about 10%. Furthermore, the patterns of the vertical velocity distribution are different from each other. Fig. 8(b) presents the non-dimensional concentration distributions in the vicinity of the crystal. Even when an upward magnetization force acts and ν = 1.1 ν₀, any significant change does not appear in the distribution of non-dimensional concentration.

3.4. Dependence of Crystal Size on the Averaged Velocity of Natural Convection
In order to evaluate the efficiency of magnetic damping...
of natural convection, the following average velocity in the solution is defined:

$$|V|_{\text{ave}} = \sqrt{\frac{1}{n^3} \sum_{i=1}^{n} \sum_{j=1}^{n} \sum_{k=1}^{n} |V|_{i,j,k}^2} \quad \text{(20)}$$

As the crystal grows and the crystal size, $h_c$, increases, the average velocity increases as shown in Fig. 9. When an upward magnetization force is added and the viscosity increases by 10% ($\triangleright$), the averaged velocity decreases even at small crystal size. When $h_c = 5 \mu m$, the average velocity decreases about 20%, while it decreases 27% at $h_c = 100 \mu m$, compared with velocity in the absence of a magnetic field ($\bigcirc$). Thus, the damping efficiency tends to increase with crystal size. When a downward magnetization force is added and the viscosity increases by 10% ($\triangle$), the averaged velocity is nearly as same as that in the absence of the field when the crystal size is below 20 $\mu m$. When the crystal size is above 20 $\mu m$, the average velocity becomes larger than that in the absence of a magnetic field.

3.5. Dependence of Crystal Size on Averaged Non-dimensional Concentration Gradient Normal to the Crystal Surface

The averaged crystal growth rate is proportional to the averaged normal non-dimensional concentration gradient. The definition of averaged normal non-dimensional concentration gradient is: $(-\partial \Psi/\partial n)_{n,\text{ave}} = \int (-\partial \Psi/\partial n)_{n} ds/s$ where $s$ is the area of a top surface or one of the side surfaces of the crystal. As shown in Fig. 10, the value of $(-\partial \Psi/\partial n)_{n,\text{ave}}$ shows the tendency to decrease with increasing the crystal size. When only the viscosity increases by 10% ($\triangledown$), the value of $(-\partial \Psi/\partial n)_{n,\text{ave}}$ is equal to the value in the absence of the field ($\bigcirc$). When a downward magnetization force is added and $v = 1.1 v_{0}$ ($\triangle$), the value of $(-\partial \Psi/\partial n)_{n,\text{ave}}$ for the crystal size of 100 $\mu m$ is higher about 7% than that in the absence of a magnetic field. On the other hand, when an upward magnetization force is added ($\bigodot$) and the viscosity increases by 10%, the value of $(-\partial \Psi/\partial n)_{n,\text{ave}}$ for the crystal size of 100 $\mu m$ is about 8% lower than that in the absence of the field ($\bigcirc$). As indicted by Eq. (19), the crystal growth rate is proportional to the product of $D$ and $(-\partial \Psi/\partial n)_{n,\text{ave}}$. Under a magnetic field, $D$ decreases about 9% because $\sqrt{D} = \text{Const}$. The values of crystal growth rate at the positions A, B and C decrease by 17, 9 and 3%, respectively compared with in the absence of the field. The decrease of crystal growth rate will contribute to the improvement of crystal quality. The simulation results explain the experimental results: when crystals were formed under various effective viscosity, the higher quality crystals were obtained with decreasing effective gravity as shown in Fig. 2.7) The simulation also agrees with the improvements of crystal quality5,6) and the decrease of crystal growth rate6,10) when crystals were formed in a uniform field.

In the present study, we used the same magnetic field strength and its gradient when the crystal formation experiments were conducted5) and 0.7 g was supplied. Recently developed magnetic force booster16) can supply 1335 $T^2/m$ (0.03 g) when it is attached to a commercially available 10 T magnet. A new type of superconducting magnet to supply 1350 $T^2/m$ (0 g) is also fabricated.17) If we can use low gravity obtained by these magnets, the efficiency of damping natural convection will increase significantly and better crystals will be obtained. This new technique of damping natural convection in low- and non-conducting fluids can be applied to not only protein crystal growth but also the preparation of many materials.

4. Conclusions

This paper presents a numerical study of natural convection around a growing protein crystal when a vertical magnetization force is added and the viscosity of the solution increases by 10%. The key points of the present study are:

1) The combination of an upward magnetization force and a magnetic increase of viscosity can damp natural convection in the vicinity of a growing protein crystal most effectively. The magnetic damping of natural convection occurs even for a small crystal such as $h_c = 5 \mu m$.

2) Both of the upward magnetization force and the magnetically induced increase of viscosity contribute to the decrease of the averaged normal non-dimensional concentration gradient near the crystal surface and hence the crystal growth rate.
The simulation results explain reasonably the magnetic improvement of crystal quality.\(^7,8\)

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REFERENCE


