Quasi-Anisotropic Magnetic Field Effect on Protoplasmic Streaming of Chara Braunii

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The velocity of protoplasmic stream in Chara braunii was measured in superconducting magnet. Under magnetic field parallel to the major axis of the cell, the velocity increased up to 15%. These enhancements were observed at the position of the maximum magnetic field and also at both of the inflection points. The perpendicular magnetic field did not affect the velocity. These findings demonstrate the chemo-mechanical conversion efficiency of the myosin motor depends on the magnetic field, which is caused by the magnetic orientation of the walking myosin molecules over the embedded actin filaments.

KEYWORDS: myosin motor, magnetic orientation, protoplasmic streaming, magnetic field, chara myosin

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Super conducting magnet is a challenging new tool for biophysics.[1,2] High quality protein single crystal is critically important for structural biosciences. The crystallization under microgravity induced by high gradient magnetic field has been successfully reported the increase in the quality.[3-6] In the field of single molecular biophysics, the magnetic beads technique is the most straightforward method to evaluate the mechanochemical performances of rotational molecular motor; F_1-ATPase.[7] Thus, the magnetic field has been recognized as the powerful tool to investigate the function and the structure of biological components. However, for living things, *in vivo* magnetic field effects are still under preliminary investigations.

In this article, the macroscopic fluid motion inside around an aquatic plant cell; Chara braunii, was investigated in superconducting magnet. The magnet (Japan Superconductor Technology, JMTD-10T150M) has the bore of 15 cm in the diameter. A single branch of Chara braunii was set between couple of glass plates filled with air-saturated water. The gap between the plates was about 0.5 mm. Since the sample cell was sealed from the ambient atmosphere, the concentration of oxygen was not artificially controlled. But, the sealed holder sufficiently prevents additional magnetic field effects neither on the magnetic induced oxygen dissolution nor on the water evaporation. The CCD camera was coupled with a relay lens and a specially designed objective lens (Mitutoyo M Plan Apo 10, The working distance=200 mm). The spatial positioning of the microscope was controlled by a x-y-z stage located outside of the magnet. All of the imaging data were stored in videotape and transferred to PC for detailed analysis. Optical irradiation to the sample was achieved by 250 W Xe lamp coupled with optical fiber (PNEUM Liquid light guide) through 10 mm water filter. The temperature of the sample was kept constant $23 \pm 0.5\ ^{o}C$.

The focal plane of the objective lens slices the real three-dimensional motion of the protoplasmic streaming as a two-dimensional fluid motion. By tracing the movement of small particles (organelles), the streaming velocity was measured. The spatial velocity distribution in the cell was inhomogeneous. The velocity distribution was shown in Fig. 1 as a function of the position along the minor axis. The inset in Fig. 1 is the schematic protoplasmic streaming image observed in the cell. An aspect ratio of the cell, which is the ratio between the cell length along the major axis and that along the minor axis, is about 100. The velocity was saturated at both sides near the cell walls. The direction of the stream was inverted at the center. The apparent velocity at the center was zero. Thus, as shown in Fig. 1, the spatial profile of the velocity was like a step function. In this article, the streaming velocity was defined as that observed in the saturated region.

The distribution of the magnetic field strength in the vertical direction is Gaussian like shape, whereas it is almost constant in the axial direction. Then, in the vertical direction, there are three specific positions; the upper and the lower inflection points, and the maximum point at the center. At both of the inflection points, the slopes of the field strength are the steepest. But, at the maximum point, the field gradient is zero. The magnet used has the characteristic values; $\boldsymbol{B}_{max}$=10 T and $\boldsymbol{B}(grad\boldsymbol{B})$=0 T^2/m at the center, and $\boldsymbol{B}$=6 T and $\boldsymbol{B}(grad\boldsymbol{B})_{max}$=290 T^2/m at the inflection points. The magnetic force, $\boldsymbol{F}_m$ is proportional to $\boldsymbol{B}$ ($grad\ \boldsymbol{B}$), then, $\boldsymbol{F}_m$ are the maximum at both of the inflection points but it is zero at the center. Comparison with the magnetic effects obtained at these three positions enables to distinguish whether $\boldsymbol{B}$ or $\boldsymbol{F}_m$ causes the effect.

An actual physical walk of a motor molecule induces the protoplasmic streaming; myosin is walking over the actin cables laid on the plasma membrane of the cell.[8-10)]

The motion is sustained by the repeated cycles of ATP hydrolysis occurring on the head part of the molecule. This head part (the central component of the chemo-mechanical converter) binds to a filament composed of polymerized amino acid forming the coiled-coil. The coiled-coil made of two α helices works as an extended moving rod (an oar) in cellular liquid, the protoplasm. This moving rod carries membrane-enclosed organelles and also induces the protoplasmic streaming. Thus, the velocity of the streaming might be depending on both the chemical kinetics of the ATP hydrolysis and/or the fluid dynamical friction i.e., the load for the motion.

Typical time courses of the protoplasmic streaming with and without magnetic field are shown in Fig. 2, where the sample was placed at the center; $\boldsymbol{B}$=10 T and *grad* $\boldsymbol{B}$=0. Since the intrinsic velocity was dependent on the sample; a branch of the Chara braunii, the velocity change by the magnetic field was measured by using the same branch. The branch investigated kept constant velocity more than 600 s. When the field direction was perpendicular to the major axis of the cell, Fig. 2(a) shows that the velocity was independent of the magnetic field, $\boldsymbol{v}_{\perp}$=76 μm/s. However, as shown in Fig. 2(b), the field parallel to the major axis affected the velocity; $\boldsymbol{v}_{/\!/}$=56 μm/s ($\boldsymbol{B}$=10 T), 42 μm/s ($\boldsymbol{B}$=0 T). The observations were accomplished including at the both of the inflection points as shown Figs. 3(a)-3(c). The spatial positions of the sample are at (a) the upper inflection point, (b) the center, and (c) the lower inflection point. The characteristic parameters of the magnetic field were (a) $\boldsymbol{B}$=6 T and $\boldsymbol{B}(grad\boldsymbol{B})$= -290 T^2/m, (b) $\boldsymbol{B}$=10 T and $\boldsymbol{B}(grad\boldsymbol{B})$=0 T^2/m, and (c) $\boldsymbol{B}$=6 T and $\boldsymbol{B}(grad\boldsymbol{B})$=290 T^2/m, where the spatial coordinate was positive to the direction upward . The magnetic field directions to the major axis of the cell are perpendicular and parallel in the left and the right columns, respectively. In all of these investigations, the field perpendicular to the

major axis did not affect the velocity, but that parallel to the axis increased the velocity. The magnetic field effect was obtained only in the parallel direction. Since the effect was observed at the center, the contribution of the magnetic force $\boldsymbol{F_m}$ can be ignored.

The anisotropic response also would expel the contribution of the magnetic field effect on the hydrolysis of ATP, if the reaction were the simple homogeneous chemical reaction. However, the ATP hydrolysis on myosin is different from common homogeneous chemical reaction. In the detailed study of myosin super family, their chemo-mechanical dynamics has been well investigated.[8-10] The velocity of the myosin walk is determined by the step size and the dwell time. The dwell time is the time interval between the walk motions of myosin molecule. The step size is about 20 nm. The dwell time is determined by two rate-limiting processes; the dissociation of ADP and the biding of ATP. The rate of ADP release from myosin after force-generation walk was dependent on the load.[8,9] As the load imposes on the walking myosin, the rate of the ADP release decreases, then the walking velocity also decreases. The dwell time was reported to increase 0.08 to 0.17 s, as the load increased 0 to 2 pN.[8]

The magnetic orientations of biological molecules have been reported so far.[11-13] The most of the orientation is attributed by the diamagnetic anisotropy of the molecule. In case of the rod shape molecule like fibrin polymers, flagellum, cilia and other microtubules, these molecules align parallel to the direction of the applied magnetic field.[11-13] The magnetic anisotropy energy for single molecule is given by[14]

$$U = -(H^2/2)(\chi_\perp + \Delta\chi\cos^2\theta) \qquad \text{----------(1)}$$

$$\Delta\chi = \chi_{II} - \chi_\perp$$

where diamagnetic susceptibilities, $\chi_{//}$ and $\chi_\perp$ are along the parallel and perpendicular to the major molecular axis, respectively. θ is the angle between the magnetic field $\boldsymbol{H}$ and

the major axis of the molecule. Most of the experimental works reported that the diamagnetic fibers are oriented along the magnetic field, i.e., $\Delta\chi>0$.[11-13] The major part of the myosin molecule is the 150 nm filament made by coiled-coil of two α helices, which is most probably oriented along the direction of the applied magnetic field.

The magnetic orientation of myosin can consistently explain the obtained results. As schematically shown in Fig. 4, the magnetic field decreases θ. As shown in eq. (1), the magnetic orientation is governed by $\boldsymbol{B}$ not by $\boldsymbol{F_m} \propto \boldsymbol{B}grad(\boldsymbol{B})$, which is consistent with the fact that the magnetic field effect was observed at the center. This magnetic orientation decreases the hydrodynamic cross section towards the protoplasmic streaming, which is equivalent to decrease the load of the myosin walk. Since the ADP release rate increase as the load decrease, the magnetic orientation shortens the dwell time and increase the walking velocity. Thus, the increased velocity of the moving wall in the cell causes the velocity enhancement in the protoplasmic streaming, which was due to the magnetic orientation of myosin molecules walking on the actin fibers. Probably, the orientation reduces the velocity saturation region as shown in Fig. 1 but the total spatial distribution change in the velocity field in the cell has not been evident yet.

The observed anisotropy between the magnetic field directions to the cell is considered as the secondary affect, which is caused by the large aspect ratio of the Chara braunii cell. The cell shape is roughly rectangle, and the aspect ratio is about 100. Intrinsically, the molecular axis of myosin is perpendicular to the plasma membrane of the cell. When the direction of the magnetic field is parallel to the major axis of the cell, the total amount of the magnetically oriented myosin is 100 times larger than those oriented under the perpendicular magnetic field. As shown in Fig. 5, this large

difference in the number of the magnetically oriented myosin molecules is the origin of the observed anisotropy. The number of the oriented myosin under the perpendicular magnetic field is not enough to induce the velocity change in the total streaming.

In this article, the quasi-anisotropic magnetic field effect on protoplasmic streaming of Chara braunii was investigated. The essential contribution for these phenomena is the magnetic orientation of walking myosin molecule, which decreases the hydrodynamic friction and reduces the dwell time. The observed anisotropy in the magnetic field directions is caused by the difference in the number of the oriented myosin molecules. The proposed mechanism of the magnetic field effect is consisted with foregoing results obtained by the single myosin molecule experiments.[8)] The *in-vivo* magnetic field effects reported in this article are well explained by the constitutive experimental works *in-vitro*.

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Figure Captions

Fig. 1 Spatially resolved streaming velocity along the minor axis of the cell. Inset is the observed protoplasmic streaming image in the cell.

Fig. 2 Typical time course of the streaming velocity. Open and closed circles are the velocity with and without the magnetic field, respectively. The directions of the magnetic field are (a) perpendicular and (b) parallel to the major axis of the cell. The characteristic parameters of the applied magnetic field were $\boldsymbol{B}$=10 T and $\boldsymbol{B}(grad\boldsymbol{B})$=0.

Fig. 3 Magnetic field effects on the velocity of the protoplasmic streaming. The spatial positions of the sample are at (a) the upper inflection point, (b) the center, and (c) the lower inflection point in the bore of the superconducting magnet. The directions of the magnetic field to the major axis of the cell are perpendicular and parallel in the left and the right columns, respectively. The characteristic parameters of the magnetic field were (a) $\boldsymbol{B}$=6 T and $\boldsymbol{B}(grad\boldsymbol{B})$= -290 T^2/m, (b) $\boldsymbol{B}$=10 T and $\boldsymbol{B}(grad\boldsymbol{B})$=0 T^2/m, and (c) $\boldsymbol{B}$=6 T and $\boldsymbol{B}(grad\boldsymbol{B})$= 290 T^2/m.

Fig. 4 Schematic representations of the mechanism for the velocity enhancement induced by the magnetic field. The magnetic orientation of walking myosin molecule ($\theta_0 > \theta_H$) decreases the hydrodynamic friction, reduces the dwell time and increases the velocity. The dwell time ($\boldsymbol{d}_w$) is the time interval between the walk motions of the myosin molecule. Each walk motion is represented as a single step in the displacement ($\boldsymbol{D}$).

Fig.5 Schematic representation of the mechanism for the apparent anisotropy to the cell geometry in the magnetic field. Since the aspect ratio of the cell is larger than 100, the amount of the oriented myosin is much larger under the field parallel to the major axis of the cell. The direction of the magnetic field was vertical in both cases.

Fig.1

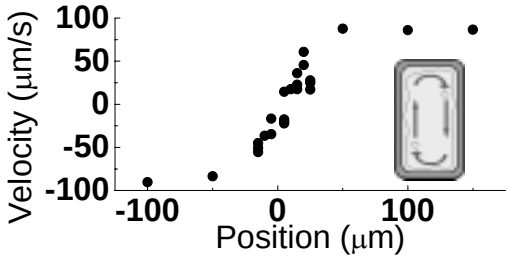


Fig.2

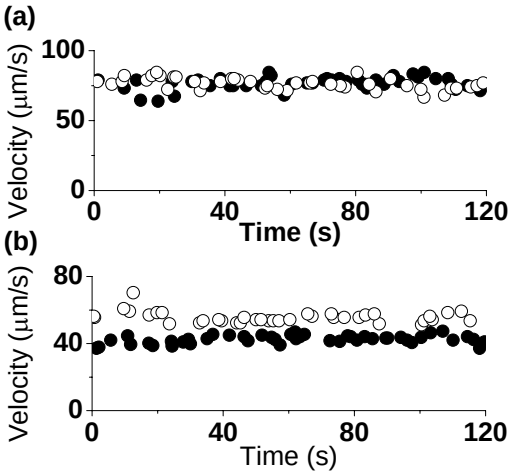
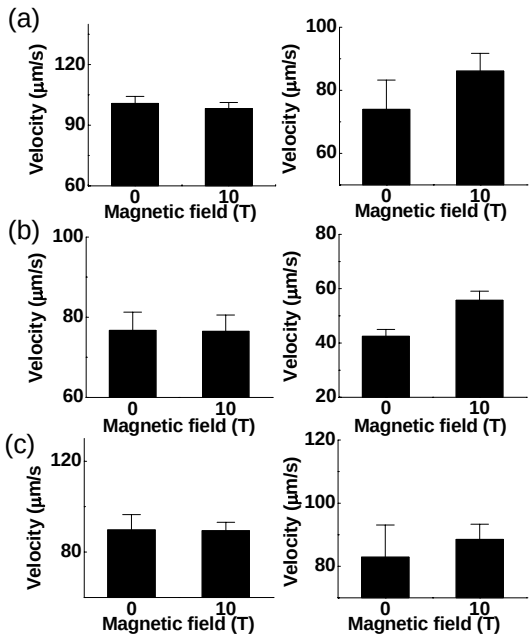

(a)
Velocity (μm/s)
100
50
0
0
40
80
120
Time (s)
(b)
Velocity (μm/s)
80
40
0
0
40
80
120
Time (s)

Fig.3


(a)
Velocity (μm/s)
120
90
60
0
10
Magnetic field (T)
Velocity (μm/s)
100
80
60
0
10
Magnetic field (T)
(b)
Velocity (μm/s)
100
80
60
0
10
Magnetic field (T)
Velocity (μm/s)
80
60
40
20
0
10
Magnetic field (T)
(c)
Velocity (μm/s)
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80
0
10
Magnetic field (T)
Velocity (μm/s)
120
100
80
0
10
Magnetic field (T)

Fig.4

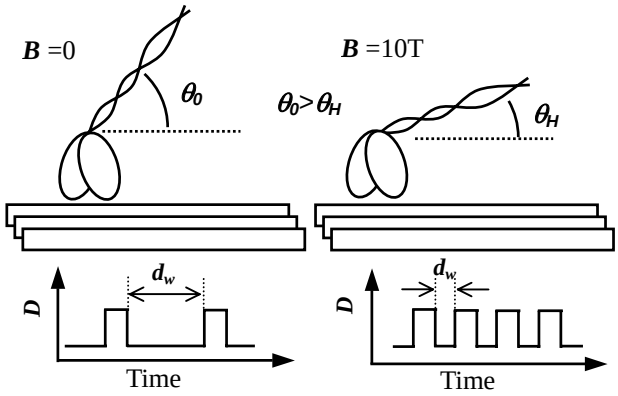

Fig.5