Dynamic Organization of ATP and Birefringent Fibrils during Free Locomotion and Galvanotaxis in the Plasmodium of

Physarum polycephalum

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Abstract. Directed migration by a cell is a good phenomenon for studying intracellular coordination. Dynamic organization of both ATP and birefringent fibrils throughout the cell was studied in the multinuclear ameboid cell of the Physarum plasmodium during free locomotion and galvanotaxis. In a directionally migrating plasmodium, waves of ATP as well as thickness oscillations propagated from just inside the advancing front to the rear, and ATP concentration was high at the front on the average. In a DC electric field, locomotion was inhibited more strongly, ATP concentration decreased more, and birefringent fibrils were formed more abundantly at the anodal than at the cathodal side. Inside the cell there were a few undulations in the distributions of ATP and birefringent fibrils. In short, birefringent fibrils become abundant where ATP concentration decreases. The possible mechanism of the coordination in the directed migration and the implications of the scaling law are discussed.

Materials and Methods

Organism

The plasmodium of Physarum polycephalum (HU195 × HU200) was fed with oatflakes on a 1% agar gel. The organisms were allowed to move on 1% plain agar overnight in 22 x 33 cm² troughs, giving a large migrating plasmodium.

Measurement of Stationary Locomotion Velocity in a DC Electric Field

The frontal part of the large plasmodium as above was cut into several pieces, and a few pieces were placed in the middle of a narrow trough (2 x 20 cm²). A few hours later the plasmodium extended in both directions. This bipolar plasmodium was useful for studying asymmetrical effects of a DC electric field. When a unidirectionally extending plasmodium was needed, one side of the agar was blocked with a hydrophobic polymer sheet (Saran Wrap, Asahikasei Co., Tokyo, Japan) thus allowing the plasmodium to migrate in one direction. A DC current was supplied through a pair of Ag/
AgCl electrodes placed at both ends of the trough. The position of the leading front advanced linearly with time, and the stationary locomotion velocity was determined as a function of the applied current intensity. The experiments were repeated five times at a given DC field, and results were averaged.

Measurement of Thickness Oscillations in a Directionally Migrating Plasmodium

By observing the organism through a transmitted light, the brightness level $I(x, t)$ at position $x$ and at time $t$ reflected the thickness of the protoplasm there, and was monitored by microcomputer image processing (11). The brightness level oscillated, and the phases of the oscillations differed from position to position. But when the organism was migrating directionally as above, the phases of the oscillations were laterally synchronous, and so the phase relationship from one end to the other in the plasmodium was studied as a one-dimensional case.

Propagation of the thickness oscillations before and after an application of a DC electric field was determined as follows. The time courses of the brightness level $I(x, t)$ at various positions along the plasmodium are shown in Fig. 4. With the approximation that a propagating wave does not change its form during propagation, the oscillation at point $x$ and time $t$ with a frequency $w$ is given by $I(x, t) = I(k \times x - wt)$, where $k$ is the phase gradient vector indicating the wave propagation. The phase gradient vector $k$ is then calculated as $k/w = -\text{grad}(I)/(dl/dt)$. Based on this notion, we evaluated the $k$ vector graphically. Curves were drawn that connected the equal phases of the oscillations in the neighboring points (Fig. 4, dotted lines), and spatial gradients of these curves were determined at various positions. The experiments were repeated at least 10 times.
Measurement of Intracellular ATP Distribution

The organism was allowed to extend uni- or bidirectionally either on wet filter paper or on a plain agar in a narrow space as above. For measuring the two-dimensional distribution of ATP concentration, the whole plasmodium was frozen in liquid N₂, and cut into ~40 pieces. Each piece was put into a hot buffer (95°C, 10 mM Tris-HCl, pH 7.1) for 10 min to extract ATP. The organism was cut at appropriate time intervals into "~,40 pieces, and each piece was subjected to ATP and protein assays as above.

Distributions of ATP concentration in the plasmodium were laterally uniform (see Fig. 5). This lateral synchrony was used to demonstrate chemical waves in the plasmodium by measuring time courses of the one-dimensional distribution of ATP. The organism was cut at appropriate time intervals into strips together with filter paper (~5 mm wide and 5 cm long) and each strip was frozen immediately in liquid N₂. The strip was then cut into ~10 pieces, and each piece was subjected to ATP and protein assays as above. The ATP and protein assays were repeated three times and independently, respectively, for each sample, and the experiments were repeated four times.

Analysis of Spatio-Temporal Distribution of Birefringent Fibrils with Use of Microcomputer Image Processing

Tiny plasmodia ~1 mm wide were allowed to migrate overnight in a narrow gap between two sheets of agar gel (3). The birefringent fibrils in this thin-spread plasmodium were observed with a polarizing microscope (Optiphot; Nikon Kogaku Co., Tokyo, Japan), appearing dark where longitudinal and bright where lateral as seen in Fig. 1 a. The video images were averaged to decrease the noise level (Image Sigma II; Nippon Avionics Co., Tokyo, Japan) and taken to a microcomputer as 256 x 256 pixels with 256 bright-levels through a video digitizer (ALT 256-8-40MA; Altec System Co., Osaka, Japan). The fibrous structures A(i, j) were extracted by operating a weighing matrix P or its transposed matrix iP to a pixel of the original frame G(i, j) at a point (i, j):

\[ A(i, j) = \sum_{k} G(i + k, j + l) \times P(k, l) \]

where P was chosen as a 7 x 7 matrix whose elements are shown in Fig. 1. The above operation gives lateral or horizontal fibers (Fig. 1, b and c). The amount of birefringent fibers in a given area was obtained by adding the brightness in each pixel in these processed images. The image was divided into 16 x 16 parts, and the density of birefringent fibers in each part was measured at 5-s intervals (see Fig. 7). The spatial density distribution of birefringent fibers was obtained by adding the birefringence in each part for 2 min (a period of the oscillations), and depicted as a smooth line by using a Spline function (see Fig. 8). Weak DC currents (Power Supply KS7510; Marysol Sangyo Co., Tokyo, Japan) were passed through Ag/AgCl electrodes placed at both ends of a thin agar gel sufficiently apart (3 cm) from the cell to avoid effects of electrode deposits. The experiments were repeated more than five times at a 0.4-mA current, where the strongest asymmetric effects were observed.

All experiments were performed at 23 ± 1°C. All results reported are typical excepting locomotion experiments.

Results

Propagation of ATP Waves in a Directionally Migrating Plasmodium

A series of one-dimensional distributions of ATP at 15-s intervals is shown in Fig. 2, where the organism is migrating unidirectionally leftward in the figure. ATP concentration is high on the average at the frontal region, and undulates over the whole plasmodium. These undulations in the ATP distribution propagate from just inside of the front toward the middle, as shown by dotted lines in the figure. The propagation velocity reaches ~1 cm/min.

Asymmetric Inhibition of the Locomotion in the Plasmodium by the DC Electric Field

Electric current inhibits the locomotion of the Physarum plasmodium at both anodal and cathodal sides (Fig. 3). As the electric current increases, the locomotion velocity (v)
Figure 5. Typical two-dimensional distribution of ATP concentration in a bipolar plasmodium. (a) A plasmodium extending evenly to two directions. (b) A plasmodium 1 h after the application of a DC electric current (0.4 mA), showing galvanotaxis toward a cathode (−). Bars indicate SD as in Fig. 2.

4. Before the stimulation (time zero), the oscillation propagates from two ends toward the inside, the propagation velocity being higher near the ends (≈1 cm/min). This feature coincides with that of the ATP wave (Fig. 2). After the stimulation, the thickness decreases at the anodal side, and the oscillation weakens and prolongs at the cathodal side. Overall, the waves propagate unidirectionally from anodal to cathodal side.

Changing Patterns of ATP Distribution with a DC Electric Current

Changes in two-dimensional distribution of ATP before and after an application of a DC electric field to bipolar plasmodia are shown in Fig. 5. In the control (a) ATP distribution is symmetric longitudinally, being high at both ends and undulating inside. After a long exposure to the DC field (b), ATP distribution turns into asymmetric; ATP becomes high near the anodal end, while ATP lowers near the cathodal.

Transients of ATP distribution are shown in Fig. 6. Shortly after the application of a DC field, ATP decreases more in the anodal side than in the cathodal, resulting in an overall gradient of ATP concentration with a few humps inside (b). As the time passes, ATP concentrations at the cathodal end and just inside the anodal decrease, and two large and broad peaks appear inside (c and d), leading to the asymmetric ATP distributions (Fig. 5).

Changing Patterns of Birefringent Fibrils with a DC Electric Current

When the cell does not show polarity, the birefringent fibrils appear and disappear periodically and synchronously throughout the cell (Fig. 7) (3). The DC field causes the amount of the fibrils at the anodal side to increase, while the oscillation of the fibrils becomes weak at the cathodal.

Changing patterns in the distribution of the fibrils after applying a DC field are shown in Fig. 8. At first, birefringent fibrils increase both just inside the anodal front and at the extreme end of the cathodal (b). At a later stage, birefringent fibrils increase at two particular positions inside, and yet there is on the average a gradient in the amount of birefringent fibrils, being more abundant toward the anodal (c and d).

Discussion

Our present and previous results indicate that ATP concent-
tation in the plasmodium oscillates, propagates as waves, and distributes nonuniformly in such a manner that the different distribution patterns correspond to different cell behaviors. General features of these phenomena are explained theoretically in terms of the self-organization in chemical systems far away from equilibrium (16). Here the nonlinear chemical kinetics coupled with diffusion, for example, self-organize chemical patterns that depend on kinetic parameters and boundary conditions, and hence the size of the system. But qualitative features may not depend on the particular system, because the kinetic equations with different parameters can often be reduced to the same equations by scaling. This scaling law may hold well in the plasmodium, because ATP distributions are similar in small (\( \sim 2 \)-cm) and large (\( \sim 40 \)-cm) plasmodia (24).

On the assumption that scaling is valid in the plasmodium, we can compare changes in ATP distribution (Fig. 6) with those in birefringent fibrils (Fig. 8). The ATP concentration decreases most just inside the anodal end where the birefringent fibrils increase most. Both ATP and birefringent fibrils distribute with gradients throughout the plasmodium with a few undulations, although detailed distributions of undulations are somewhat different between the two. So we may conclude that on the first approximation the more ATP decreases, the more abundantly birefringent fibrils are formed. Does ATP regulate the cytoskeletal organization directly? Some evidence supports this: ATP causes contraction in a reconstituted actomyosin thread (12), and high ATP dissolves actin.

Our results also provide a new look at the mechanism of galvanotaxis in ameboid cells. Previous studies seem chiefly to try to identify the location of active sites in the cell: either the preferential migration toward the cathode in the Physarum plasmodium is explained by the selective suppression of the pseudopodial extension at the anodal side (1); or the protoplasm moves toward the cathodal because the protoplasm at the anodal side contracts and pushes the protoplasmic sol toward the cathodal side (7, 22). Our results are consistent with these theories based on local mechanism but, more importantly, showed the protoplasm react as a whole to the electric field and self-organize new distributions of intracellular ATP and cytoskeletons (Figs. 5, 6, and 8).

Furthermore, our results and theory described above give a mechanism of why the cell is able to behave coordinately as an integrated unity. If the cytoskeletons are controlled by chemical patterns such as ATP, cellular coordination is automatically satisfied because the chemical patterns themselves are formed by taking into consideration the kinetic parameters and boundary conditions. An alternative approach for cellular coordination in directed migration is trying to correlate the particular arrangements of organelles such as a microtubule-organizing center with cell polarity (21, 28). This approach seems awkward in multinucleated cells such as the Physarum plasmodium, and also fails to answer what brings about a polar arrangement of organelles in terms of physical chemistry. Our theory answers why the cell polarity originates in terms of physical chemistry if the scaling law is applicable.

The next step of the research will be to make clear what enzymatic processes are responsible for the organization of chemical patterns in the Physarum plasmodium. Until now mitochondria have been shown to be involved in keeping the oscillatory contraction (20). Glycolytic system (14) and mitochondria (2) should be two potent candidates, because oscillations occur autonomously, chemical patterns are formed, and waves propagate in these systems. It remains unclear how these phenomena in cell-free systems are related to cellular functions.

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