Stepping motion of the organelle in a perfused characean cell

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Abstract The sliding motion of organelles in a perfused characean internodal cell has been investigated. A small number of myosin molecules attached to the organelle are known to slide along actin bundles lying on the inner surface of the cell. The stepping motion of the organelles was first observed using a high-speed camera with a time resolution of 1 ms. The spatial frequency of all pairwise differences in displacement of the stepping motion has been estimated to be 0.012–0.014 nm^{-1}, which implies that the organelle moves with large steps of 71–83 nm along the actin bundles in a perfused characean cell.

Key words: Stepping motion; Organelle; Actin; Step size; *Nitella*; *Chara* myosin

1. Introduction

Organelles in the protoplasm of a characean internodal cell slide along bundles of actin filaments lying on the inner surface of the cell [1–3]. The ultrastructure of the organelles reveals that small globular bodies, which are thought to be myosin molecules, are arranged on the surface of the protuberances of each organelle [4]. On the other hand, myosins have been identified from *Nitella flexilis* [5] and *Chara corallina* [6,7]. The physical shape [8] and the amino acid sequence [9] of the myosin have been elucidated, and the myosin is thought to belong to class XI. These findings have suggested that the sliding motion of the organelle is driven by the interaction between the myosin molecules attached to the organelle and the actin bundles in the cell.

The sliding motion of the organelles has been studied in intact cells [10,11] or in perfused cells [12] by tracking the organelle with a time resolution of 1/30 s (= video rate). The variance in the velocities of the organelles has been found to change in proportion to their average velocity. This result implies that the generation of motive forces in the organelle follows the Poisson process. On the basis of the assumption that the number of forces generated due to myosins follows the Poisson process, the 'step size', defined as the distance the organelle move forward as a result of a single force generated, has been estimated to be about 100 nm [10]. A crucial component of elucidating the mechanism by which organelles are driven by myosins is to estimate the step size. However, the estimated step size is not directly derived but estimated from fluctuation analysis [13]. Thus, a more direct method of estimating the step size is needed.

Assuming that the myosins driving the organelles generate forces in pulse-like fashion, the organelles move stepwise at low ATP concentrations. At low ATP concentrations, the interval between the forces generated lengthens, so the individual force generated is thought to be identified. Thus, characean cells were perfused in the present study to decrease the ATP concentration and a high-speed camera was provided to observe the organelles' motion with a time resolution of 1 ms. Applying time series analysis for detection of the stepping motion [14] to the observed organelles' motion, the current research investigated whether the stepping motion occurs and then estimated the step size directly.

2. Materials and methods

2.1. Internodal cell preparation

Internodal cells of the green alga *Nitella axilliformis* (diameter ca. 0.3–0.4 mm; length 1–4 cm) were used. The algae were grown in soil–water medium at room temperature under illumination from a fluorescent lamp during the daytime. An internodal cell was isolated from its neighbors and stored in artificial pond water (APW: 0.1 mM KCl, 0.1 mM NaCl, and 0.1 mM $MgCl_2$) prior to the experiment. For detailed observation, the chloroplasts in the region to be observed were removed via ultraviolet irradiation [15]. After ultraviolet irradiation, the cell was kept in APW for at least 1 week.

An internodal cell was perfused with a perfusion solution (5 mM EGTA, 30 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), 6 mM $MgCl_2$, 150 mM sorbitol, and 0.01 mM ATP at pH 7) in accordance with a method of intracellular perfusion [16] so as to remove vacuoles as well as the protoplasm surrounding the organelles to the degree possible. For the first few minutes after the perfusion, many organelles were suspended as clusters in the protoplasm and moved passively, but this motion of the clusters gradually ceased and individual organelle motion was observed.

Driven by the interaction between actin and myosin, motion of the individual organelle was observed in traces at least 5 min after the perfusion. All measurements were carried out at room temperature (25–27°C).

2.2. Observation and displacement measurements

The experimental setup is shown in Fig. 1. The motion of organelles in the internodal cells was observed with a high-speed camera (Photron, Fastcam-Net) through an inverted microscope (Nikon, Diaphot-TMD) with a 100× objective. The microscope was placed on a vibration isolator (Meiritsu Seiki, Visolator). The images of the organelles were saved temporarily in camera memory as TIFF image files every 1 ms for several seconds (≤4367 ms). The image files were transferred from the memory to a personal computer (Gateway, G6-300) to analyze the organelles' motion. The position of organelles in individual images was estimated according to the method of Gelles

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Abbreviations: APW, artificial pond water; PDF, pairwise distance distribution function

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et al. [17]. The accuracy of the determination of the organelle position in the current experiments was ±4–7 nm.

2.3. Data analysis

First, the most probable direction in which an organelle would move was estimated for each run by fitting a straight line to the traces of the organelle. The direction estimated was the direction parallel to the actin filaments. Next, a time series of the organelle along the direction parallel to the filaments was obtained every $\tau = 1$ ms for each run with a duration of $l\tau$, where $X(i\tau)$ with $i = 0,...,l$.

The stepping motion of the organelle was analyzed according to the method of Svoboda et al. [14]. The time series were median filtered in a sliding window of 5 ms. The filtration was repeated $(l-1)/2$ times for each run [18]. The pairwise distance distribution functions (PDF) [19] were calculated by binning distance differences $x(j\tau)-x(i\tau)$ for all $j > i$ in a histogram, with bin width 0.1 pixel (= 7.25 nm). The power spectrum of the PDF $S(k)$ was calculated and normalized to unity at $k = 0$.

3. Results and discussion

An example of the time series of the organelle motion in the direction parallel to the actin bundles appears as the lower trace in Fig. 2A. A median-filtered series is also shown in the same figure (upper trace). Horizontal gridlines in the figure are drawn with spacing of 73 nm. This figure implies that the organelle moves with steps of about 73 nm. The stepping motion of the organelle was clarified via a PDF to the trace (Fig. 3A). Vertical gridlines in the figure are drawn with spacing of 73 nm. Multiple peaks with regular spacing can be identified. The spatial frequency was analyzed by Fourier transform analysis of the PDF (Fig. 4A). The estimated spatial frequency is 0.014 nm^{-1}, corresponding to a step width of 71 nm. Another example of the trace is shown in Fig. 2B. In this figure, horizontal gridlines are drawn with spacing of 83 nm. Fig. 3B shows the PDF of the time series with vertical gridlines drawn with spacing of 83 nm. The spatial frequency was estimated to be 0.012 nm^{-1} by calculating the power spectrum of the PDF (Fig. 4B). The stepping motion, like the traces shown in Fig. 2, was observed in 12 of the 107 traces. The PDF constructed from the 12 traces is shown in Fig. 5A. Vertical gridlines in the figure are drawn with spacing of 85 nm. Because the stepping motion with various steps has mingled, peaks of the PDF are not as clear as those of the PDF constructed from a single time series such as in Fig. 3A or B. The normalized power spectrum of the PDF is shown in Fig. 5B. Using this figure, the spatial frequency of peaks of the PDF shown in Fig. 5A was estimated to be 0.012–0.014 nm^{-1} (Fig. 5B, inset). Thus, the organelles are thought to move with steps of 71–83 nm.

The stepping motion of the organelle implies that the myosins attached to the organelle generate forces in pulse-like fashion, i.e. the duration of the force generated is very short,

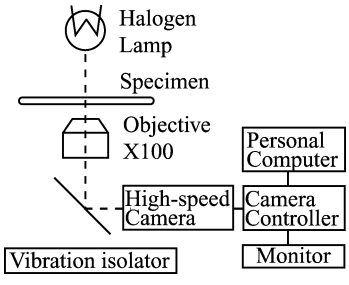

Fig. 1. The experimental setup.

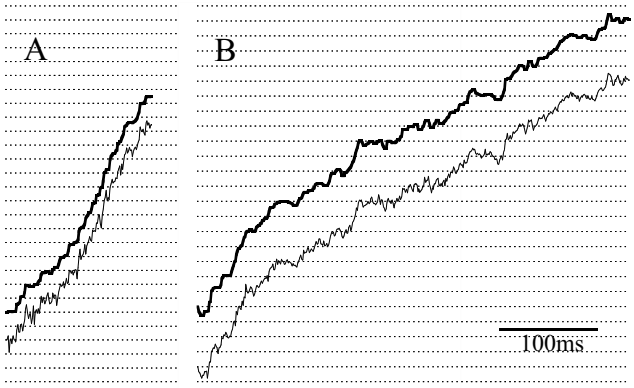

Fig. 2. Examples of the time series of the organelle motion. Positions of the organelle (vertical axis) are plotted with respect to time (horizontal axis). The lower traces are the raw data acquired at 1 kHz. The upper traces are the median-filtered data. The horizontal gridlines are drawn with spacing of 73 nm in A and 83 nm in B. The bar in B corresponds to 100 ms.

and the magnitude of the driving forces is almost constant. Moreover, thermal motion of the organelle is thought to be suppressed, otherwise the organelle diffuse about $\pm\sqrt{2Dt} \approx \pm 32$ nm within $t = 1$ ms, and thus the stepping motion cannot be detected. Where the diffusion constant of the organelle, D, is estimated to be about 5×10^2 nm^2/ms given the assumptions that the diameter of the organelle is about 1 μm [10] and the solution in which the organelles are immersed has the same viscosity as water (0.89×10^{-3} Pa s at 25°C). The suppression of the diffusion is thought to be caused by the molecular friction of the organelle on the actin bundles, which is attributed to the myosins bound weakly to the actin filaments [20,21].

Myosin must be processive like myosin V to produce stepping motion by a single myosin molecule. However, the processivity of the myosin is not necessary for the stepping motion of the organelle because several dozen myosins attach to the organelle [4]. Thus, the mechanism of the stepwise movement in the present study is considered to differ from that for stepping motion caused by the single processive motor protein.

Myosin V moves processively with 36-nm steps and this step size is attributed to the half-period of the actin filament [22,23]. Thus, the estimated step size of 71–83 nm for the stepping motion of the organelle is considered to be attributed to the full period of the actin filament (= 72 nm). The primary structure of *Chara* myosin [9] has six IQ motifs for calmodulin binding like myosin V. Thus, according to the lever arm hypothesis for myosin molecule mechanics [24], the organelle cannot readily move a distance of 72 nm by the swing of the lever arm only. The organelle may be driven not only by the swing of the lever arm but also by thermal diffusion (Fig. 6). This mechanism implies that the displacement of the system as a result of a single lever swing of the myosin (2→3 in Fig. 6) differs from that of a cycle of a single force generated (2→6 in Fig. 6). The latter is determined by the distance between the binding sites, which is equal to the integral multiple of the half-period of the actin filament (= 36 nm). When the stride of the lever swing is too short to reach the next binding site, the system is thought to move to the next binding site with the help of thermal diffusion (1→2 or 5→6 in Fig. 6). The step size of 72 nm observed in the present

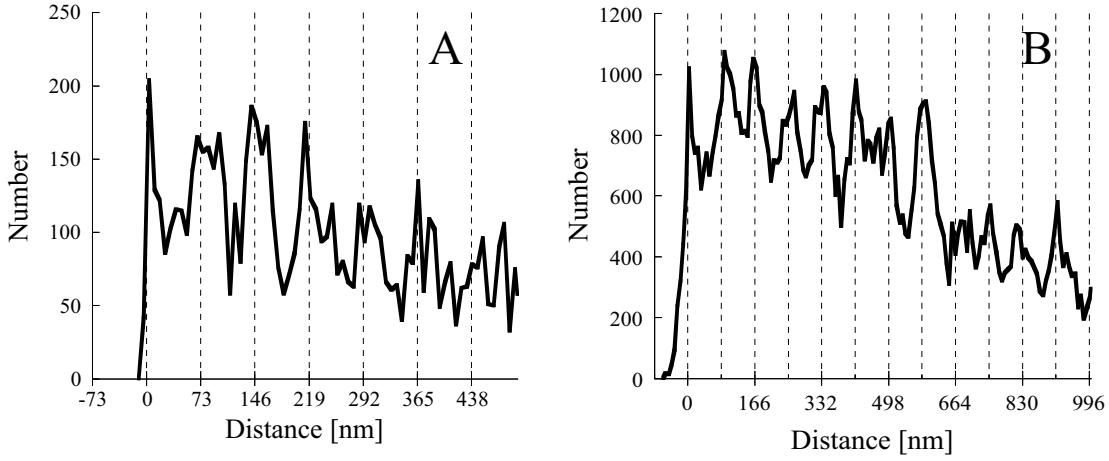

Fig. 3. The PDF for a single time series. A: PDF for the median-filtered time series in Fig. 2A. Multiple peaks with regular spacing of 73 nm can be identified. The vertical gridlines are drawn with spacing of 73 nm. B: PDF for the median-filtered time series in Fig. 2B. The vertical gridlines are drawn with spacing of 83 nm.

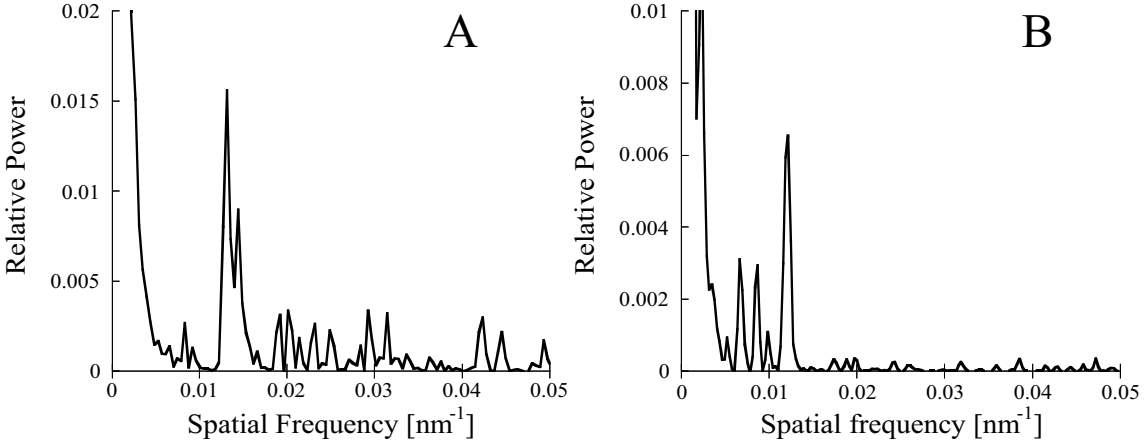

Fig. 4. The normalized power spectrum of the PDF. A: The power spectrum of the data in Fig. 3A. A prominent peak is shown at 0.014 nm^{-1}. B: The power spectrum of the data in Fig. 3B. A prominent peak is shown at 0.012 nm^{-1}.

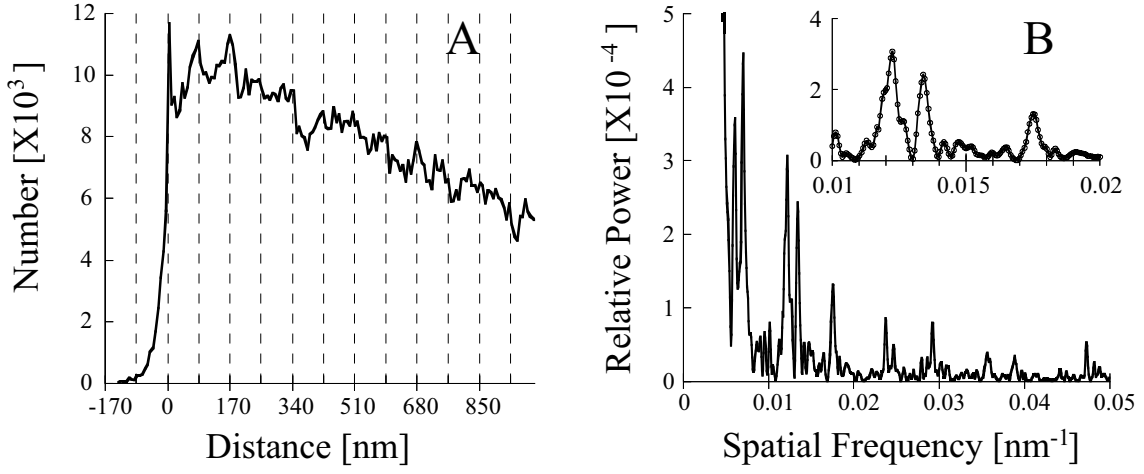

Fig. 5. A: The PDF for all of the observed stepping motion of the organelle constructed from 12 independent time series. Multiple peaks with regular spacing of ~85 nm can be identified. The vertical gridlines are drawn with spacing of 85 nm. B: The normalized power spectrum of the PDF. Prominent peaks are shown around ~0.013 nm^{-1} corresponding to a step width of ~80 nm. Inset, the same figure with a range of 0.01–0.02 nm^{-1}.

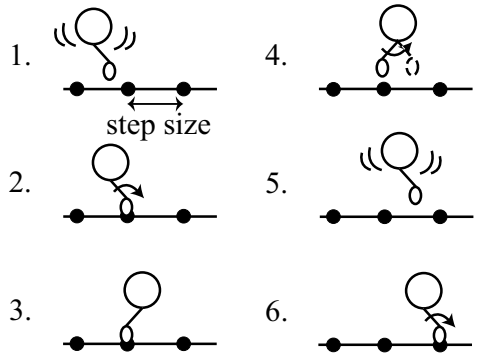

Fig. 6. A possible mechanism for the stepping motion of the organelle. Large circles represent the organelles, horizontal lines the actin filaments, small closed circles on the horizontal lines the binding sites for the myosins, ovals the motor domains, and straight lines from the ovals the lever arms of the myosin. (1) Thermal diffusion of the organelle. The distance between the binding sites corresponds to the step size. (2) The organelle reaches the binding site. The myosin swings its lever arm. (3) After the lever swing. (4) ATP binding to the myosin causes the myosin to detach from the actin and change its structure (swinging back of the lever). (5) Same as 1. (6) Same as 2. See text for details.

study suggests that the binding sites for the organelle are arranged at every full period of the actin filament and the organelle moves with steps of 72 nm.

It is also possible that the 72-nm step consists of two successive 36-nm substeps, like the 4-nm substeps within the 8-nm step of the kinesin [25]. To detect these substeps, the resolution time in the observed system must be shorter than the interval between the two successive steps. Since there are no prominent peaks around 0.028 nm^{-1} corresponding to 36-nm steps in the power spectrum of the PDFs (Figs. 4 and 5), the interval of the two successive substeps must be shorter than the resolution time of the present experimental system (1 ms), if the substeps exist.

In the present observation, the stepping motion was observed in 12 of 107 runs. In the other 95 runs, the organelles moved without showing steps. The authors have postulated that in these 95 runs, the intracellular perfusion was insufficient in removing the protoplasm in the cell. Thus, the stepwise displacement was thought to be smoothed out by the viscosity of the protoplasm remaining around the organelles.

In conclusion, results have indicated that the organelle moves with 71–83-nm steps in a perfused characean cell. The large step size has been attributed to the full period of the actin filament, and possible mechanisms for the stepping motion have been discussed. Further investigation of the organelle motion with higher temporal resolution is expected to elucidate the molecular mechanism of the motion.

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