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Reverse Motion of Organelles with Myosin Molecules along Bundles of the Actin Filaments in a Characean Internodal Cell

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We have visualized bundles of the actin filaments of a Characean internodal cell and investigated the sliding motion of organelles with myosin on the bundles. The investigation revealed that a power spectrum of the sliding velocity time series of the organelle has two remarkable peaks near 4 and 7.5 Hz. This suggests that myosin molecules attached to the organelle not independently but cooperatively produce the sliding force. Moreover, we have found that some organelles move in the opposite direction of their sliding motion for several hundred milliseconds along the bundles. The fluctuation analysis of that motion showed that a power spectrum profile of the reverse velocity time series almost agreed with that of the sliding velocity time series. This result suggests that the dynamics of the reverse motion is the same as that of the sliding motion.

A Characean internodal cell is one of the most suitable materials for studies of the sliding motion driven by an interaction between actin and myosin. For this reason, many studies of dynamics of the sliding motion using the internodal cell have been performed (1, 2, 3, 4). Advantages of the internodal cell are that bundles of actin filaments are fixed straight along surfaces of the chloroplasts settled in rows on the gel layer (5, 6) and that all of the actin filaments in the bundles have the same polarity (7, 8).

In the cell its protoplasm actively streams in the direction depending on the polarity of the filaments (7) and also small organelles in the protoplasm move along the bundles of the filaments (9, 10, 11). This motion is dependent on ATP and the organelles bond with the actin filaments in the absence of ATP by structures like a cross-bridge which are periodically arranged along the surfaces of the organelles (9). From these observations, it is reasonable that the structures like the cross-bridge are myosin molecules and the organelles' motion is driven by an interaction between actin and myosin.

Thus, in the present study, we have investigated the motion of the organelles on the bundles of the actin filaments in detail in order to characterize the dynamics of the sliding motion driven by the interaction between actin and myosin.

MATERIALS AND METHODS

In the present study, we used an internodal cell of *Chara australis*. *Chara australis* was cultivated in a bucket filled with water and whose bottom was covered with gravel at room temperature under illumination from a fluorescent lamp during the daytime.

First, we removed the chloroplasts in an observation region according to Kamitsubo's method (10), because they prevented us from observing the motion of the organelles. In his method, ultraviolet irradiation to the chloroplasts breaks them, and then they come off the gel layer together with the actin filaments present. About one week after the irradiation, only the filaments recover.

Next, a vacuole was replaced with a perfusion solution (5 mM EGTA, 30 mM PIPES, 6 mM $MgCl_2$, 150 mM sorbitol, 0.01 mM ATP at pH 7.0) by the vacuolar perfusion technique (12) for the following two reasons. One is that light scattering, etc., by the vacuole's inclusions prevent us from observing the motion of the organelles. The other is that the replacement allows us to reduce the ATP concentration in the cell so that the organelles' motion slows down. This makes it possible to observe the organelles' motion over a long period. The replacement was performed for a several minutes to remove not only the vacuole but also the protoplasm as much as possible surrounding the organelles.

Figure 1 shows the outline of our observation system. The motion of the organelles was observed under an inverted microscope (Olympus, IMT-2), which was settled on a vibration isolator (VISOLATOR, MEIRITSU SEIKI), with an ×100 objective after an ×1.5 magnification lens was inserted in the optical path. The image of the organelles' motion was recorded with a Hi-8 VTR (COMPUTER VIDEO DECK CVD-1000, SONY), which can be controlled with a computer, after we corrected the intrinsic optical unevenness of the observation system and improved the contrast of the image with a real time image quality improver (DVS-20, Hamamatsu photonics).

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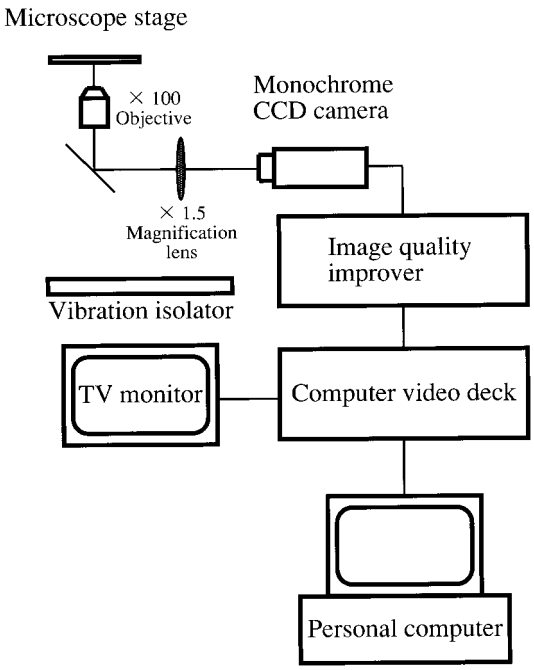

FIG. 1. The outline of our observation system.

The organelles' motion was analyzed with a personal computer. The recorded image of the organelles' motion was captured with the computer through a image grabber board (Meteor-II, Matrox). We estimated the position of some organelles in each image according to the method of Gelles et al. (13) and obtained time series of the organelles' position every 1/30 s for a few seconds.

The position of a stationary bead (Polybead polystyrene microspheres; diameter, 1.072 μm; Polysciences) on the cover glass was measured ten times in order to estimate precision in the determination of the organelles' position in our experimental design. The result was that the accuracy of the estimation of the position was $\pm$2–5 nm.

RESULTS AND DISCUSSION

At the start of the observation, not only the organelles but also the protoplasm remaining around them flows vigorously in the same direction, which is determined by the polarity of the actin filaments (7), but it slows down to a much slower speed than that of the organelles in about the first ten minutes. Thus we investigated the motion of the organelles only after ten minutes from the start of the observation, because it is reasonable that these organelles are moved not passively but by the interaction between actin and myosin.

Characteristics of the sliding motion of the organelles. We have observed the motion of about 3000 organelles which move along bundles of the actin filaments. We show the motion of one of them in Fig. 2. Most of the organelles keep moving on the bundles on which they exist, while some organelles sometimes transfer to either of the adjacent bundles and some organelles are suddenly off the bundles and in Brownian motion.

Figure 3 shows the time series of the sliding velocity of an organelle. Because the ATP concentration of the perfusion solution is 0.01 mM, the sliding velocity of the organelles is much slower than at saturated ATP

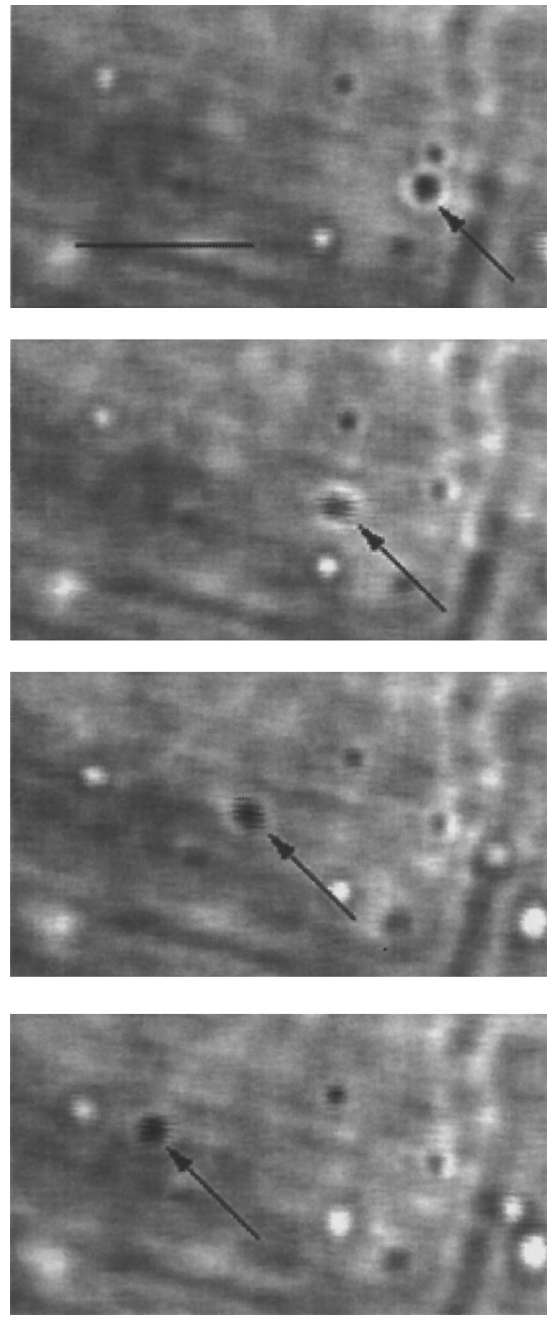

FIG. 2. The motion of an organelle along a bundle of the filaments. The scale bar indicates the length of 5 μm. In this figure, parts of the video images captured with the computer are magnified and arranged from the top in time order every 1/15 s. The arrow indicates the organelle. The organelle is running on and along a white fiber-like object. The fiber-like objects are concluded to be the bundles of the actin filaments, because many other organelles are moving along the white fiber-like objects, which is not shown in the figure.

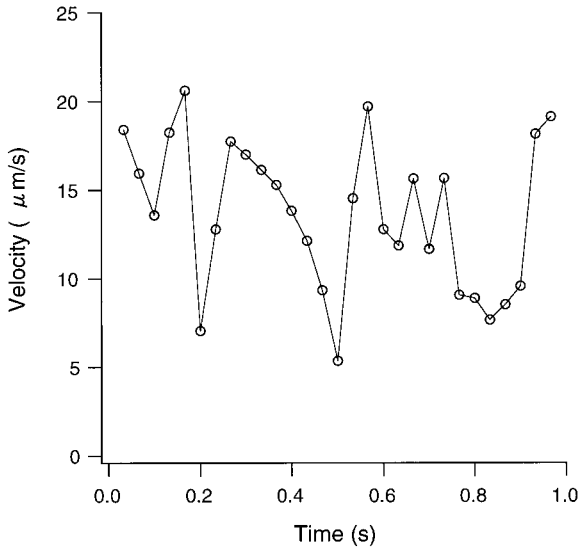

FIG. 3. Time dependence of the organelle's sliding velocity. The velocity was estimated by dividing the organelle's displacement for 1/30 s by the time interval of 1/30 s. The displacement was estimated from the acquired experimental position data for the organelle. The solid line in the figure serves to guide the reader's eye.

concentration (1 mM). This figure shows that the velocity fluctuates considerably.

Figure 4 shows the power spectrum of the velocity time series. This figure shows that there are remarkable peaks near 4 and 7.5 Hz. The spectrum suggests that myosin molecules attached to the organelle not independently but cooperatively produce the sliding force.

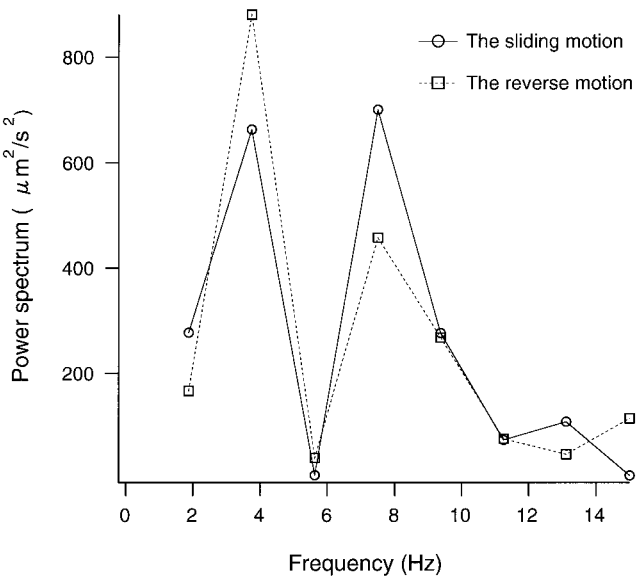

FIG. 4. Power spectrums of the time series of the organelle's sliding velocity and reverse velocity. The solid and dashed lines in the figure serve to guide the reader's eye.

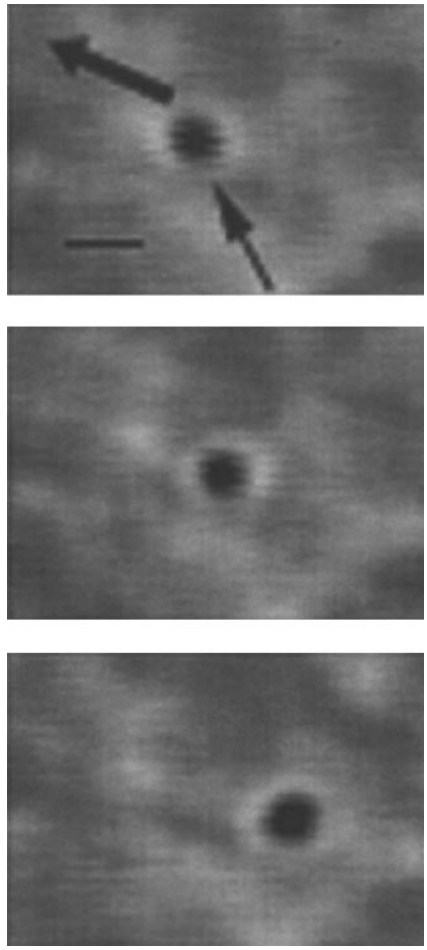

FIG. 5. Reverse motion of an organelle along a bundle of the actin filaments. The scale bar indicates the length of 1 μm. In this figure, parts of the video images captured with the computer are magnified and arranged from the top in time order every 1/6 s. The thin arrow indicates the organelle. The thick arrow indicates the original direction of the sliding motion of this organelle.

Reverse motion of the organelles along the bundles of the filaments. We have observed that some of the organelles which move along the bundles of the filaments turn back for several hundred ms. Those organelles accounted for about 0.8% of the observed organelles. Figure 5 shows the reverse motion of one of those organelles. Figure 6 shows a part of the time series of another organelle's displacement in the direction parallel to the bundle of the filaments. This figure shows the motion of the organelle only for the short period for which the reverse motion occurs. For the other long periods, the organelle moves in the direction depending on the polarity of the filaments. This figure shows that, once the reverse motion happens, the organelle repeats the backward and forward motion a few times. The amplitude of the motion is a few micrometers and the period of the motion is about one second. This comparatively ordered motion suggests

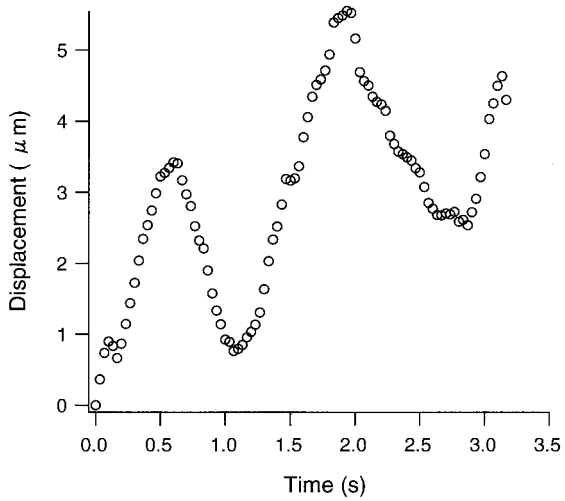

FIG. 6. Time series of the organelle's displacement parallel to the bundle. The ordinate represents the displacement in the direction of the sliding motion depending on the polarity of the filaments.

that the reverse motion is caused not by thermal fluctuation but by other causes.

Figure 7 shows the time series of velocity of the same organelle as in Fig. 6 every 1/30 s in the direction parallel to the bundle. This figure shows that the velocity fluctuates considerably.

Figure 4 shows the power spectrum of the velocity time series of the reverse motion from about 2 s to about 3 s shown in Fig. 7. This figure shows that remarkable peaks occur in the neighborhood of 4 and 7.5 Hz. This characteristic of the spectrum profile is

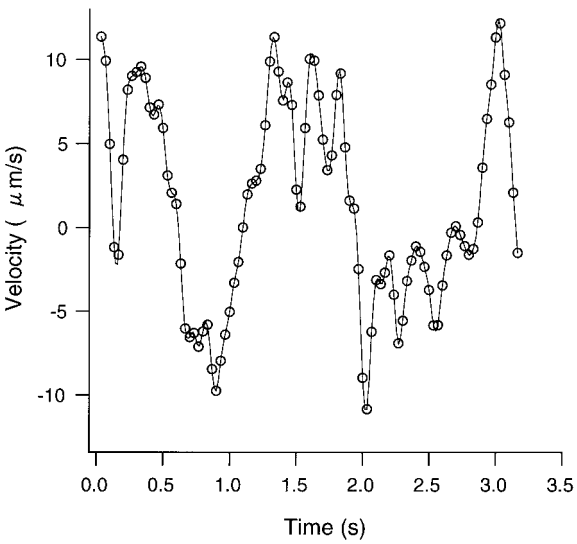

FIG. 7. Time dependence of the organelle's velocity. The ordinate represents the velocity in the direction of the sliding motion depending on the polarity of the filaments. The velocity was estimated in the same way as in Fig. 3. The solid line in the figure serves to guide the reader's eye.

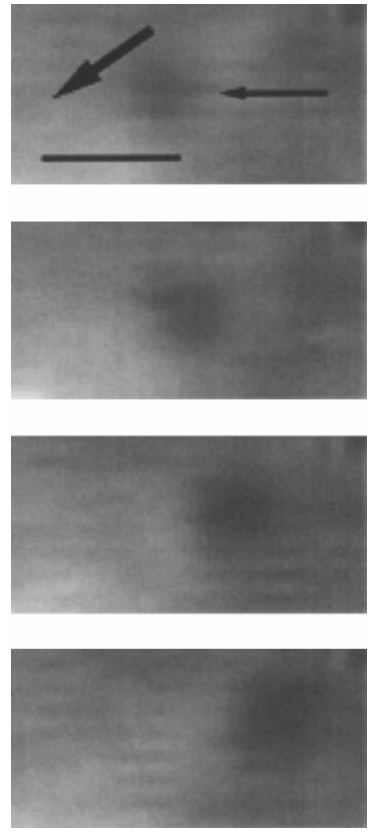

FIG. 8. Reverse motion of an organelle along a bundle of the actin filaments in the intact cell. The scale bar indicates the length of 1 μm. The thin arrow indicates the organelle. The thick arrow indicates the direction of the sliding motion depending on the polarity of the filaments. In this figure, parts of the captured video images are enhanced and showed in the same way as in Fig. 5. The reason why quality of the images is poor that the background of these images is a chloroplast and the reason why the left corners of the images are bright that there is a narrow chloroplast-free space between the chloroplast and its adjacent one.

very similar to that of the spectrum profile of the normal sliding velocity. This means that the dynamics of the reverse motion is the same as that of the sliding motion. Therefore, there are some possibilities that the reverse motion is also driven by actin and myosin.

One of the possibilities is that some of the recovered filaments in a bundle have converse polarity and the organelle, after a half rotation on its own axis, moves on those filaments due to the normal interaction between actin and myosin. Thus, in order to ascertain this possibility, we have examined whether the same phenomenon occurs or not in an intact cell, in which all the filaments in a bundle have the same polarity (7, 8). The experiment was performed in almost the same method as the prepared cell by the ultraviolet irradiation except the use of the intact cell. The result was that the reverse motion occurred. Figure 8 shows the reverse motion of an organelle in the intact cell.

From the above, we can see that the reverse motion does not necessarily occur due to the polarity inversion. Therefore, there is a possibility that the reverse motion may be closely related to a new aspect of the generation process of motion by the interaction between actin and myosin.

In the present study, we have visualized the bundles of the actin filaments and investigated the organelles' motion on the bundles in detail. In this investigation we have characterized the power spectrum profile of the sliding velocity time series, found the reverse motion of the organelle along the bundle of the actin filaments and observed that its dynamics is the same as that of the sliding motion. An explanation for the dynamics of the reverse and the sliding motion observed in the present study needs further study.

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