brief communication

Steady and transient behaviors of protoplasmic streaming in *Nitella* internodal cell

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ABSTRACT Steady and transient behaviors of protoplasmic streaming in *Nitella* internodal cell have been investigated for various temperatures from 30°C to near 0°C. It has been found that steady velocity of the streaming linearly decreases with increasing inverse temperature but its proportionality coefficient changes at ~ 10°C. Velocity distribution, which reflects temporal fluctuations of the protoplasmic streaming, is nonGaussian and its half width becomes larger at higher temperatures. On the other hand, recovery of the protoplasmic streaming, which is observed after stopping the streaming with a current stimulus to the internodal cell, has been found to show more clear sigmoidal time courses at higher temperatures.

INTRODUCTION

Protoplasmic streaming in an internodal cell of *Nitella* is an interesting phenomena for studying motional mechanisms in biological systems. Many investigators have been attracted to the following problems: where in the internodal cell does the motive force of the streaming operate, what supplies its energy, what is the microscopic mechanism, and so on. Kamiya and Kuroda obtained the velocity profile of the protoplasmic streaming in the cell and showed that the motive force exists at the boundary between the ectoplasmic gel layer and the endoplasmic sol layer (Kamiya and Kuroda, 1956). Subcortical fibrils were observed to lie along the inner surfaces of chloroplasts located at the interface between the sol-gel layers (Kamitsubo, 1972). These fibrils were composed of bundles of thin filaments which were confirmed to be F-actin filaments (Fujime, 1980; Palevitz et al., 1974; Bradley, 1973; Nagai and Rebhun, 1966). Myosinlike molecules, on the other hand, were identified from *Nitella flexilis* (Kato and Tonomura, 1975), which are thought to be dispersed in the endoplasm or to attach to small organelles sliding along the fibrils (Williamson, 1975; Nagai and Hayama, 1979). Thus, it is clear that interactions of actin and myosin molecules participate in generation of the motive force for protoplasmic streaming.

The microscopic mechanism of chemo-mechanical energy conversion by the actin-myosin interactions has been extensively studied but remains poorly understood. In the flow dynamics of protoplasmic streaming, there still remain phenomenological problems which are important for understanding the mechanism of chemo-mechanical energy conversion.

In this study, from the phenomenological viewpoint, we have investigated the temperature dependences of protoplasmic streaming in the internodal cell of *Nitella* and its recovery process after cessation of the streaming.

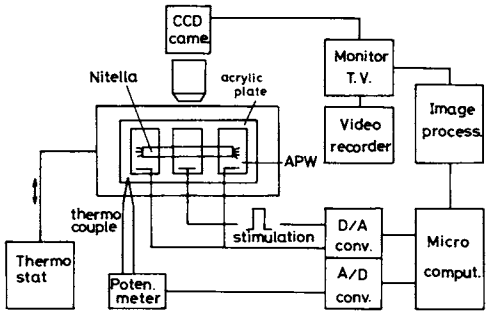

FIGURE 1 An experimental system.

MATERIALS AND METHODS

Nitella axilliformis plants were grown in a plastic basket which was filled with soil–water medium and kept at a temperature of 20°C under illumination of a fluorescent lamp during the daytime. Internodal cells of *Nitella* were 300–400 μm in diameter and 5–6 cm in length. A single internodal cell, which was isolated by cutting its neighbor cells, was placed in a vessel which was made of an acrylic plate with three pools (see Fig. 1). The side pools were used for electrical stimulation of the internodal cell and the middle pool for observations of the protoplasmic streaming. The temperature of the acrylic plate was controlled by circulation of water through a thermostatically controlled bath and its accuracy was kept within 0.1°C. The gaps between the pools were insulated electrically by vaseline. All the pools were filled with APW, which was composed of 0.05 mM KCl, 0.05 mM NaH_2PO_4, 0.2 mM NaCl, 0.05 mM $Ca(NO_3)_2$, and 0.1 mM $MgSO_4$ (pH = 7.3).

An experimental system is shown in Fig. 1. The protoplasmic streaming in the internodal cell was observed through a microscope by a TV camera and its image was recorded by a video recorder. To obtain the velocity of the protoplasmic streaming, we investigated motions of small organelles with diameters within 2–3 μm, which are dispersed in

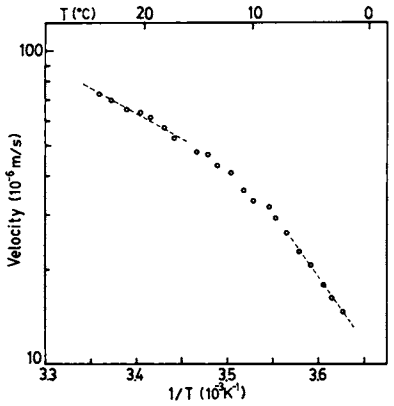

FIGURE 2 Logarithm of velocity of protoplasmic streaming versus inverse temperature.

the endoplasm and considered to reflect bulk flow due to protoplasmic streaming. By analyzing the video images, we measured transit times of the small organelles moving between two arbitrary points taken along the stream on a TV monitor. In this manner, we investigated steady velocities of protoplasmic streaming at various temperatures. When membrane excitation of the internodal cell occurs, protoplasmic streaming suddenly stops and then gradually recovers to its steady-state flow. We investigated the recovery of the protoplasmic streaming after its cessation due to the membrane excitation caused by an external electric stimulus. The recovery processes at various temperatures were recorded by the video recorder. We replayed the video images and measured transient velocities in the recovery of protoplasmic streaming.

RESULTS

In Fig. 2, we show steady velocities of protoplasmic streaming for various temperatures, each data point is the average of 50 measurements. The abscissa and the ordinate give the inverse temperature and the logarithm of the velocity, respectively. The temperature dependence of the velocity is seen to be different above and below ~10°C. The streaming velocity is approximately fitted by Arrhenius lines with different slopes in both the temperature ranges. The activation energies are estimated to be ~10 Kcal/mol >10°C and 12 Kcal/mol <10°C. Mustacich and Ware investigated protoplasmic streaming by laser Doppler spectroscopy and found that the velocity varied linearly with temperature, with an increasing rate of 2.6 $\mu m \cdot s^{-1} \cdot °C^{-1}$ (Mustacich and Ware, 1976). The linearity was also confirmed by optical microscopy (Kamiya, 1959; Tazawa, 1968). Though our results seem to differ from theirs, plots of our data as a function of temperature also nearly fit on a straight line with an increasing rate of 2.76 $\mu m \cdot s^{-1} \cdot °C^{-1}$. This agreement is due to plotting the data in a narrow temperature range. Linear plots of velocity versus tem

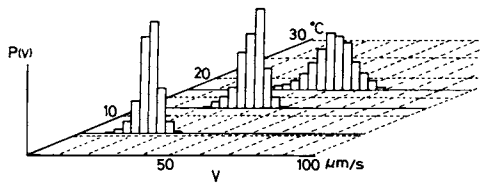

FIGURE 3 Velocity distributions of protoplasmic streaming at three temperatures. Each value of the standard deviation is 7.48, 4.58, and 3.54 μm/s, respectively.

perature predict that the flow should cease at about 2–6°C. But in our preliminary experiments, steady flow of the protoplasm has been observed even at temperatures below 0°C and it is supposed that the flow is able to continue as long as the protoplasm is not frozen. Thus, we believe that Arrhenius plots are preferable to linear plots for understanding streaming mechanism.

Fig. 3 gives histograms of the velocity of protoplasmic streaming at three temperatures, which have been obtained from 200 data and normalized for their total areas to be unity. The histograms show that the distribution of streaming velocity is a nonGaussian and nonsymmetric one which is skewed toward lower velocities. The shape of the distribution varies little with temperature but the half width which is proportional to variance of the velocity is smaller at lower temperatures. The distribution may be influenced by scattering factors which are caused by size differences of the organelles suspended in the protoplasm or structural variations of the bundles on the surfaces of the chloroplasts. But these effects should be independent of temperature and should make small contributions to the temperature-dependent broadening of the velocity distribution. Thus, the velocity distribution is thought to reflect temporal changes in protoplasmic streaming.

In Fig. 4, we show recovery of the protoplasmic

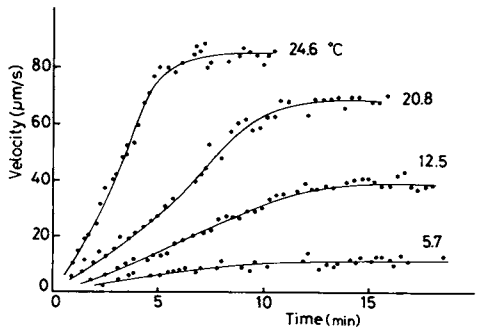

FIGURE 4 Time course of recovery of protoplasmic streaming at various temperatures. Solid curves are suitably drawn to fit to the recovery process at each temperature.

streaming at four temperatures. The horizontal axis represents the time elapsed after membrane excitation of the internodal cell by an external electric stimulus. At the low temperature of 5.7°C, the flow of the protoplasm gradually increases and reaches its steady state. The recovery processes of the protoplasmic streaming show more clear sigmoidal time courses at higher temperatures.

DISCUSSION

In the measurements of steady velocities of protoplasmic streaming, we have found that the slope of the Arrhenius plot increases below ~10°C. It is clear that protoplasmic streaming is influenced by the motive force for streaming and the viscous properties of the protoplasm (Kamiya and Kuroda, 1973; Kachar and Reese, 1988). Thus, alterations in the temperature dependences of the motive force or the viscous properties may play a role in the effects we have observed. We have also seen that protoplasmic streaming is a stochastic flow, which suggests the possibility that the motive force for the streaming fluctuates due to molecular dynamics of molecules involved in generating the motive force.

The recovery of the protoplasmic streaming shows a sigmoidal time course. The sigmoidal recovery may represent a cooperative interaction operates during the recovery process.

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