LASER DIAGNOSIS OF NONSTATIONARY PROTOPLASM FLOW IN ALGAL CELLS ON MEMBRANE STIMULATION

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A study has been made of the effects of external conditions on the flow recovery in the protoplasm of Nitella algae after a halt produced by membrane excitation. The protoplasm flow speed has been measured with a laser Doppler spectrometer working with a US-1010 computer in real time. It has been found that under otherwise constant conditions, the recovery kinetics may be determined by the parameters of the stimulating pulse and the illumination conditions.

INTRODUCTION

Protoplasm flow occurs in virtually all plant cells [1], which is evidently based on mechanochemical conversion of ATP hydrolysis energy, as in muscles, which occurs by the interaction between the contractile proteins actin and myosin. However, the detailed mechanism has not yet been finally established. Some information can be obtained by examining the parameters of the transient response when the protoplasm flow is restored after an induced halt.

In [2] we reported our data on the recovery stages in the protoplasm flow speed after a temporary halt produced by exciting the cell membrane with a short current pulse. Here we examine the kinetics of the transient flow establishment as the parameters of the stimulating pulse (SP) are varied along with the illumination conditions. The flow speed was recorded by laser Doppler spectroscopy in real time.

SPECIMENS

We used the giant internode cells of the charaeal alga *Nitella*. The cells were isolated from the thallus of the algae several days before the experiment and directly before it were placed in cell holders filled with a culture medium. We used two-thirds of the top of a cell of length 2-3 cm and diameter 0.4-0.8 mm. A cell was taken as adapted to the illumination conditions after 40-50 min. The intensity of illumination in the plane of the cell was 1700 lux.

The cell structure is shown in Fig. 1. The two cell membranes (tonoplast and plasmalemma) are concentric cylinders bound together along two spiral lines, the so-called indifferent zones. Three channels are then formed in the cell: the central one (vacuole containing cell sap), which consists of up to 90% of the cell volume, and two lateral ones, which are filled by endoplasm. The flow directions in the side channels are opposite, and on the whole the cell shows a closed spiral stationary flow of protoplasm with a speed of 60-80 μm/sec.

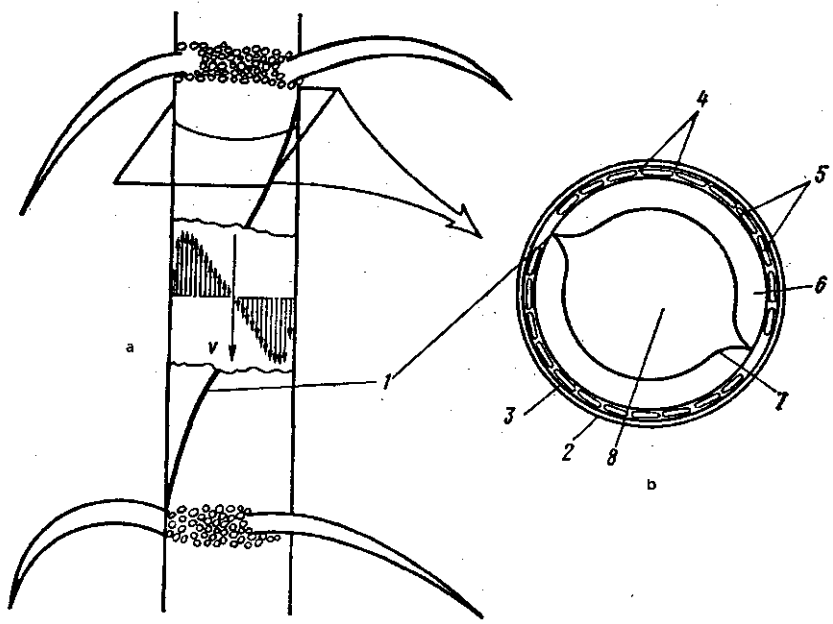

Fig. 1. General form of the alga *Nitella* (a) and structure of internode of cell (b): 1) indifferent zones; 2) cell wall; 3) plasmalemma; 4) cortex; 5) chloroplast; 6) endoplasm; 7) tonoplast; 8) vacuole containing cell sap.

The algae were harvested from lakes in the Lithuanian SSR and were cultured in aged tap water at room temperature with natural illumination.

METHOD

The protoplasm flow speed was measured with an automatic system consisting of a laser Doppler spectrometer and US-1010 computer [3]. The laser Doppler spectroscopy method involves recording the frequency shift occurring on scattering at a moving particle. The Doppler shift is isolated by nonlinear mixing of the radiation scattered by organelles and inclusions moving with the protoplasm and radiation scattered by the immobile cell walls. Figure 2 shows the scheme. See [2] for the algorithm for determining the flow speed from the signal spectra. Basic characteristics: speed measurement range from 10 μm/sec to 1 cm/sec (accuracy about 5%) and duration of one measurement τ = 1-3 sec.

In some experiments, we not only measured the protoplasm flow speed but also the potential difference between the vacuole and the environment by means of standard microelectrode techniques [4]. The flow was halted by an SP of length τ_0 = 0.5 sec applied from a standard source.

RESULTS

The membranes in chareal algal cells are excitable, i.e., they can generate an action potential (AP) on applying a depolarizing current pulse exceeding a certain threshold. The passage of the AP is accompanied by virtually instantaneous flow halt [5-7]. We have previously shown [2] that in some cases the flow recovery does not involve a simple monotone speed increase to the initial value v_0. Instead, the speed may attain $V = (1.1\text{-}1.4)v_0$ and return to the initial level

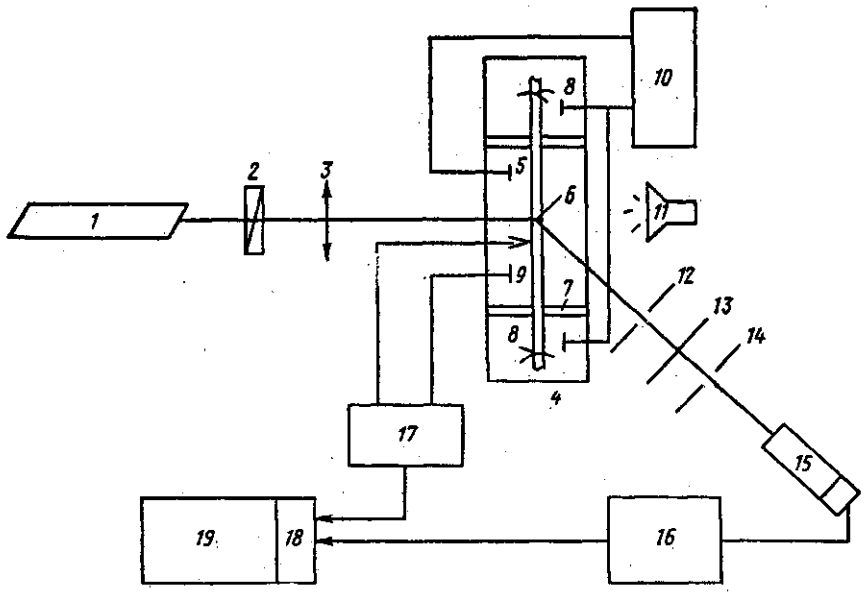

Fig. 2. Scheme for the laser Doppler spectrometer working with US-1010 computer: 1) He-Ne laser; 2) filter; 3 and 13) lenses; 4) liquid cell; 5) stimulating electrode; 6) measurement volume; 7) cell holders; 8) biological cell; 9) electrodes; 10) standard power supply; 11) illuminator; 12 and 14) stops; 15) photomultiplier; 16) amplifier performing analog filtration; 17) pH meter used as electrometer amplifier; 18) analog-digital converter; 19) US-1010 computer with plotter, graphics VDU, printer, and tape punch.

and after a series of slowly decaying oscillations.

We examined the way the recovery curve altered for cells adapted to light and darkness as influenced by the SP with speed recording in one section of the cell or simultaneously in two. We also made simultaneous measurements of the flow speed and the vacuole-environment potential difference. The SP was varied from the threshold value $U = U_0$ to $U = (8\text{-}10)U_0$. The time intervals between successive SP were 40-60 min. The scheme for supplying the SP is shown in Fig. 2.

The data show that the speed oscillations are not due to pathological states in the cell. After 3-10 periods (the exact number is dependent on the initial amplitude), the oscillations are damped out and the cell returns to its normal physiological state, as is evident from the reproducibility of the results obtained with a single cell over several days.

Under othewise equal conditions, the recovery curve is determined by U and the illumination conditions: 1) for $U < U_0$, the flow speed is unaltered; 2) for $U = U_0$, the length of the first phase in the recovery T_0 (Fig. 3) is minimal and is as follows: for cells adapted to light 2-6 min, and for ones adapted to darkness 8-11 min; then the second phase (oscillation) is absent; and 3) for $U > U_0$,

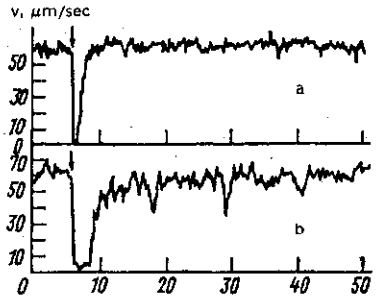

Fig. 3

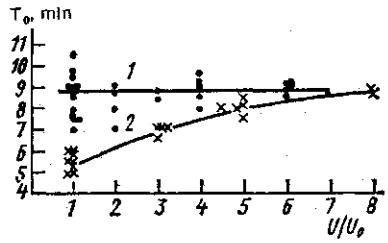

Fig. 4

Fig. 3. Protoplasm flow speed patterns on varying U. The instant of stimulation is shown by the arrow: a) $U = U_0$; b) $U = 5U_0$, where U_0 is the threshold.

Fig. 4. Dependence of the length of the first phase T_0 in the recovery on the ratio of U to U_0 for cells adapted to darkness (1) and to light (2). Measurements made on six cells.

T_0 is increased for cells adapted to light (Fig. 4) and one gets slowly damped oscillations; the oscillation period is independent of U, while the amplitude is proportional to U/U_0 (Fig. 3); there is no dependence of T_0 on U/U_0 for cells adapted to darkness (Fig. 4).

The speed curves (Fig. 3) show appreciable fluctuations before and after stimulation, which have several causes. Firstly, there is error in determining the speed from the spectrum shape, particularly because the averaging time τ is limited [3] in deriving a single spectrum. The upper limit to τ was chosen from the condition $\tau \ll T_0$. Secondly, there is a substantial spread in the speeds of the particles [6].

We used five cells in two-point measurements, with both measurement volumes between greased cell holders at a distance of 1.0-1.5 cm apart. The results were as follows: 1) the flow halted simultaneously in the two sections within the accuracy τ; 2) if U/U_0 was sufficient, oscillations occurred either in one section or both simultaneously; and 3) the oscillation periods in the two sections were virtually identical, but there was a certain phase difference between them.

We also obtained the following results in recording the flow and vacuole-environment potential difference simultaneously: 1) the potential recovery time after the AP was $\tau \ll T_0$, and 2) in some cases, the speed oscillations were accompanied by ones in the potential (Fig. 5): the period in the potential was 11.1 ± ± 3.0 min, amplitude 13.6 ± 4.1 mV, while the period in the velocity was 5.6 ± ± 1.4 min. On average, the period in the potential was twice that in the velocity. However, we considered that the current data do not enable one to draw an unambiguous conclusion on the correlation between the oscillations in the flow speed and membrane potential.

DISCUSSION

To interpret the results, we give some evidence on the supposed mechanism

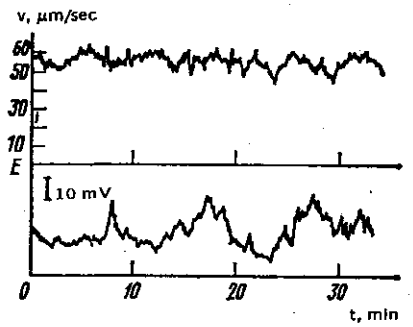

Fig. 5. Protoplasm flow-speed curves and vacuole-environment potential differences for 1 of 14 cells.

driving the protoplasm and on the ion fluxes through the membrane on excitation. One hypothesis is that the driving force is produced by interaction between endoplasmatic actin filaments, with one end of each attached to the cortex, and myosin dissolved in the endoplasm [6]. It has been confirmed by experiment [8] that a homogeneous and stationary flow can exist in such a system by reference to muscle proteins, but the detailed mechanisms are not clear. There is also evidence that the cytpolasm contains unpolymerized G actin and actin-binding proteins [9].

When the membrane is depolarized by more than a certain amount, calcium channels are activated in a short time [10], which raise the Ca^{2+} concentration in the cytpolasm from the normal 10^{-1} M to 10^{-4}-10^{-5} M at the AP peak [7]. It is assumed [11] that the Ca^{2+} pumping time down to the normal level for light-adapted cells is less than that for cells kept in darkness, which is responsible for increasing T_0 for dark-adapted cells. Further, there is evidence that not all the Ca^{2+} channels operate in the light, although they do in darkness, and that the number of open channels varies with the SP magnitude [12]. This would explain the difference between the $T_0 = f(U/U_0)$ curves for cells kept in light and in darkness.

One therefore assumes that the form of the flow response to an external stimulus is determined by the amount of Ca^{2+} entering the cytoplasm. It is possible that the halt is caused by rapid polymerization of the G actin and the actin-binding proteins, with the active actomyosin system frozen in a dense passive network. That halt mechanism would explain the observed rise in protoplasm viscosity immediately after the AP [5,6]. When the excess Ca^{2+} has been pumped away, the network collapses, and the mobility apparatus recovers. Other evidence that the Ca^{2+} halts the flow is that the membrane remains excitable in a calcium-free medium, but AP generation is not accompanied by flow halt [5].

The following suggestions may be made on the origin of the flow fluctuations in the second phase.

1. The oscillations are caused by periodic change in the driving force, and one reason for this may be periodic change in the ionic composition of the intracellular medium due to fluctuations in membrane potential.

In particular, that situation is characteristic of the plasmodium *Physarum*

[13]. The strands of this fungus show reciprocating motion in the protoplasm due to contraction waves propagating along the walls. There are oscillations in the membrane potential and intracellular Ca^{2+} concentration, whose period coincides with that of the flow-speed oscillations. One of the existing hypotheses indicates that Ca^{2+} concentration oscillations represent the process controlling the rhythm of the autowave activity in these strands. Nevertheless, there is no final agreement on the relation between the transmembrane potential and the protoplasm mobility for plant cells or plasmodium, which is due in particular to our lack of knowledge on the properties of the actomysin isolated from these cells.

However, in our case the amplitude of the potential oscillations is insufficient to activate the Ca^{2+} channels [10]. Therefore, if we assume that there is a relation between the oscillations in the potential and velocity, the control ions must be ones whose permeability determines the resting potential. There is then necessarily a nonlinear relation between changes in potential and speed. However, the data do not rule out the oscillations in the membrane and cytoplasm being coupled only at the time of AP excitation, their subsequent progress being independent. This is the subject of further research.

2. Another reason for speed oscillations may be periodic change in the channel width. As the scatterer concentration in the endoplasm is substantially higher than that in the cell sap, and the speed determined from the spectrum shape corresponds to the most probable speed in the measurement volume, any changes in the recorded speed evidently reflect mainly changes in the speeds of particles in the endoplasm. In that case, one should consider changes in the thickness δ of the endoplasm flow channel, which may be due to deformation of the boundary between the cortex and the endoplasm or, more likely, tonoplast deformation. A change in δ (which is usually 10-20 μm) of 20% would cause a flow-speed change of 30%.

As there is a certain phase difference between the speed oscillations in two sections of a cell, any process responsible for the oscillations must be of autowave type.

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