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Nitellopsis Obtusa Internodal Cell Birefringence Change During Action Potential

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Abstract. Birefringence change during excitation was studied by using *Nitellopsis obtusa*. The velocity change of cytoplasmic streaming during an action potential was measured simultaneously by fluctuation analysis of transmitted light intensity. The origin of the retardation change was discussed by comparing optical retardation change to the time course of the action potential, the cytoplasmic streaming velocity change and the cell contraction.

By the time course analysis of retardation change, we concluded that the change of the birefringence might be the sum of the changes of cytoplasmic flow and that of the size of length and diameter of the cell. But it is still difficult to separate the change to its components.

Key words: Birefringence change – Flow birefringence – Fluctuation analysis of light intensity.

Introduction

The *Nitellopsis obtusa* internodal cell is a giant cell 500 μm in diameter and 2–10 cm in length. This cell generates action potentials upon electrical stimulation. The *Nitellopsis* internodal cell action potential has a fairly reproducible shape, and a duration of a few seconds, or about 1 000 times longer than that from nerve cells. During the action potential, cytoplasmic streaming ceases (Hill, 1941; Kishimoto and Akabori, 1959).

The plasma membrane of the internodal cell has a high water permeability and a low ion permeability. These characteristics make it an ideal semi-permeable membrane. When excited, the membrane structure changes, rendering it much less permeable to water. This low water permeability produced a hydrostatic pressure difference across the membrane, causing a small deformation of the cell (Kishimoto, 1972).

Measurements of optical retardation (birefringence) in living tissue is convenient to use in the study of cell structure (Cohen et al., 1969, 1970; Watanabe et al.,

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1973). The birefringence change during excitation was studied on the squid giant axon (Cohen et al., 1969, 1970). In these experiments, the time course of the optical retardation change was very similar to that for an action potential recorded with an intracellular microelectrode. According to Cohen et al. (1969, 1970), these phenomena originate either from a molecular relaxation process analogous to the Kerr effect, or from a change in membrane thickness due to the pressure exerted by the electrical field.

The *Nitellopsis* internodal cell has a more complicated structure than other excitable tissues, primarily because of its cytoplasmic streaming. Nevertheless, the structure change during excitation is advantageous to study in these cells because several aspects of this change have already been analyzed: the change in velocity in cytoplasmic streaming and change in cell size have been studied during the excitation process (Tazawa and Kishimoto, 1968; Kishimoto and Ohkawa, 1966). In this paper we report on the simultaneous measurements of cytoplasmic streaming velocity changes and optical retardation (birefringence) changes during excitation. The optical retardation change was shown to have two components: one due to cell volume change and another to the change in velocity of cytoplasmic streaming.

Materials and Methods

Nitellopsis obtusa internodal cells were used throughout these experiments. Cells were collected at Kawaguchi Lake in Yamanashi and stored in tap water. The cell was placed on a shallow groove in black Lucite plate in which there was a hole 10 mm in diameter through which a light beam could be passed. Two pairs of wire electrodes were mounted on both ends of the groove.

The experimental technique was basically similar to the method reported by Cohen et al. (1970). Internode birefringence was measured by a Nikon polarizing microscope (Type POP) with a 100 W Osram quartz-iodine lamp as a light source. An infrared absorbing filter was used to prevent any rise in the external temperature of the solution. A slit was inserted in the optical path to select that portion of the cell to be illuminated. Internodal cell retardation values were measured using a Babinet compensator. Two photodetectors (PIN 10) were used in these experiments. One, used for birefringence measurements, was mounted on the objective lens of the polarizing microscope. The other, used to compensate for drift in the light source, was placed in the light path of a beam reflected from a half mirror placed near the light source at 45° to the incident light. Both signals detected by the photodiodes were connected to a differential amplifier in order to balance the D.C. component. The output of the amplifier was fed either to a Yokokawa (HP) chart recorder (signal vs time) or to Nihon Koden Average Computer (ATAC). Under the optimal condition the light intensity change following a fast phenomena (less than 1 ms) could be detected. When the amplifier out-put was fed directly to the chart recorder, the response time constant to an abrupt change in light intensity was limited by the pen speed ($t \simeq 0.3$ s). A pair of platinum electrodes making contact with each of the terminal portions of the axon in the groove was used to stimulate the axon and to record propagated impulses. The action potential measured in this way was used mainly as a qualitative indicater of the condition of the cell. Intracellular recording

of the action potential was carried out using microelectrode filled with 3 M KCl solution. All experiments were carried out at room temperature, between 20 and 25° C.

Results

1. Resting Retardation

A polarizing microscopic photograph of the Nitellopsis internodal cell is shown in Figure 1. Both cell margins appear luminous and the middle part of the cell glows slightly. A very bright area at the edge of the cell might be a sort of artifact due to edge effect. In Figure 2, the brightness distribution along the horizontal axis of the cell was shown schematically. Areas 2 and 4 have a relative brightness of 0.42, and are 3 of 0.21, compared with the brightness of areas 1 or 5.

If an object is placed in a beam of linearly polarized light, with an angle between its optical axis and the plane of polarization, the intensity of the light detected by an analyser oriented at right angles to the plane of polarization is given by Fresnel's equation

$$I = I_0 k \sin^2 2\phi \sin^2 \frac{\theta}{2}, \tag{1}$$

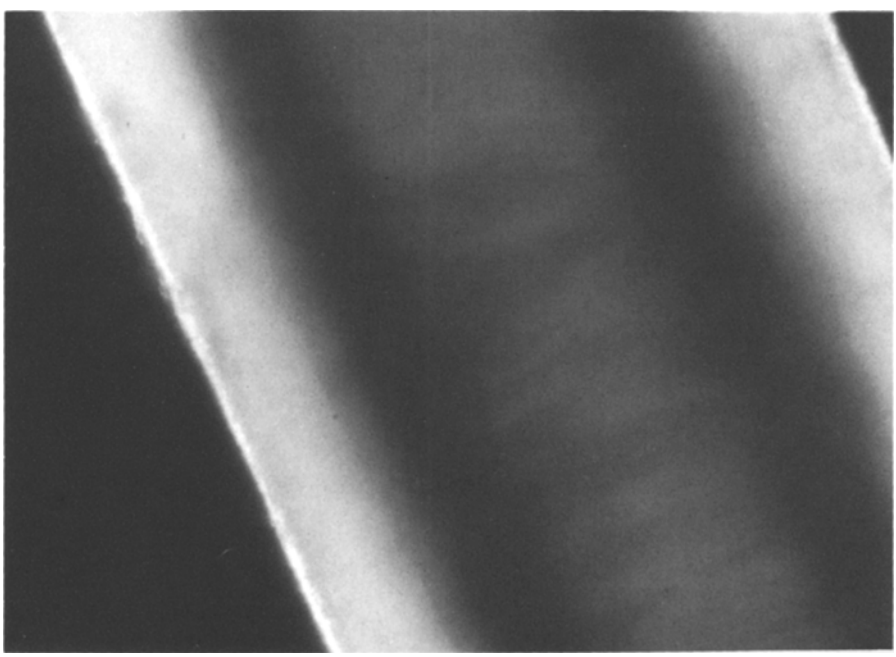

Fig. 1. *Nitellopsis obtusa* photomicrograph. The internodal cell was mounted horizontally on the stage of the Nikon polarizing microscope, at 45° to the incident light polarization plane

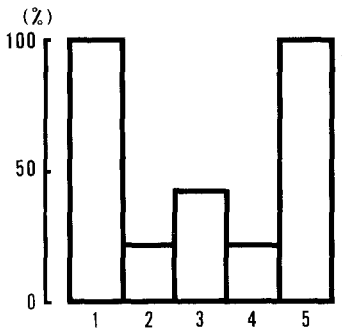

Fig. 2. Normalized *Nitellopsis obtusa* resting retardation intensity distribution. The area is divided into five blocks, numbered from 1–5

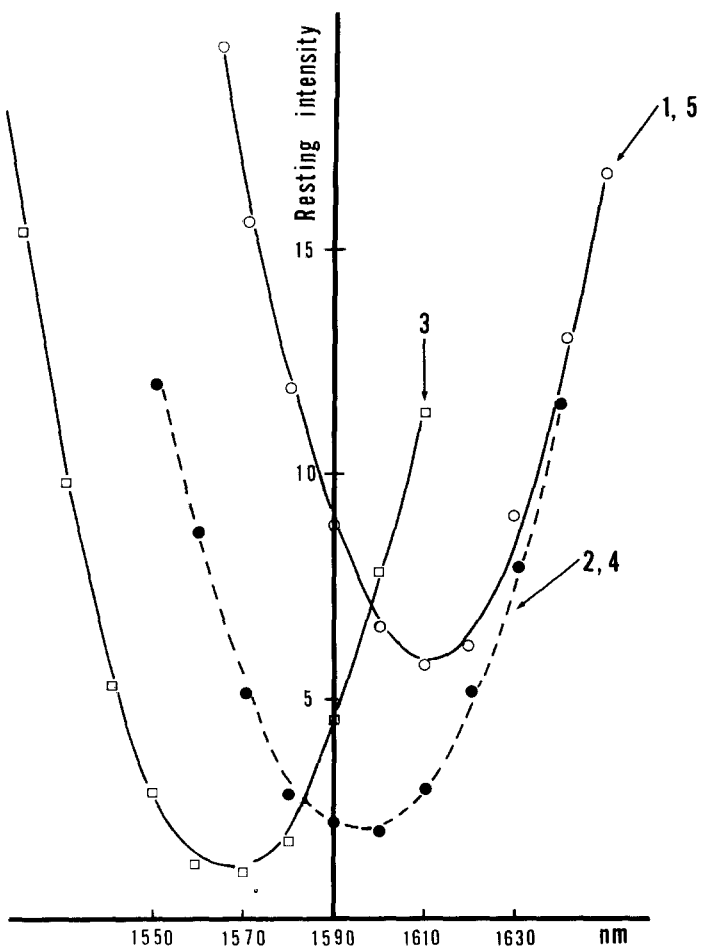

Fig. 3. Resting intensity in an internodal cell as a function of Babinet compensator readings for the five blocks defined in Figure 2

where I_0 is the intensity of the incident light, k is a constant and θ is the phase difference (in radians) between ordinary ray and extraordinary ray. By changing the angle ϕ, the maximum intensity is obtained at $\phi = 45°$. It will be shown that the light intensity I depends on ϕ in areas 1, 3, and 5, but not in areas 2 and 4.

Figure 3 shows additional retardation characteristics in each area, determined by using a Babinet compensator. Retardation is expressed by

$$R = a_\lambda(x - b)\,, \tag{2}$$

where the constant a_λ is 0.984 for white light; b is a constant determined by the compensator (1590 nm in this experiment), and x is the minimum value of the resting intensity as a function of added retardation. From Eq. (2), the retardation in each area of the cell is given by

$$R_{1 \text{ or } 5} = 0.984\ (1610\text{–}1590)\ \text{nm} = +\ 19.7\ \text{nm}\,,$$

$$R_3 = 0.984\ (1570\text{–}1590)\ \text{nm} = -\ 19.7\ \text{nm}\,.$$

The suffix indicates the number of the area defined in Figure 2. These retardation values are smaller than those of other excitable tissues: the net retardation from a squid axon is 56 nm and the average retardation of a crab leg nerve, whose diameter is 600 μm, is 32 nm (Cohen et al., 1969).

2. *Change in Optical Retardation During Excitation*

When the internodal cell was stimulated electrically, the change in birefringence light intensity in areas 1 or 5 had a similar time course to that of the action potential shown in Figure 4. The angle between the cell and the plane of polarization of the incident light was 45° for this record. With an angle of 0°, no change could be observed.

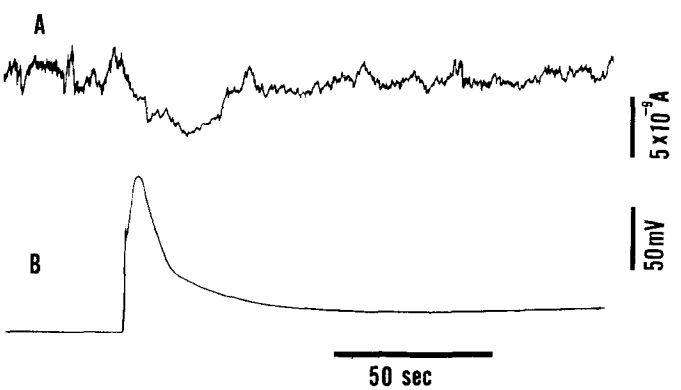

Fig. 4. A Intensity change during excitation without using compensator. As the sign of this change is negative, the intensity is decreased during excitation. Note that intensity fluctuation is apparently decreased during that time. **B** Time course of action potential recorded intracellularly

The intensity change ($\Delta I/I$) is a good parameter in describing the retardation change during excitation; ΔI is the intensity change during stimulation and I is the value for the resting retardation. In Figure 4, $\Delta I/I$ is of the order of 5×10^{-3}, significantly larger than that from squid giant axons, which are of the order of 10^{-5} (Cohen et al., 1970). The order of magnitude of the intensity change was varied linearly with respect to the added retardation from a Babinet compensator; the direction of the retardation change was also determined by the use of a Babinet compensator. The fluctuation of I is due to cytoplasmic streaming and decreased when the streaming stopped (Fig. 6). In this case action potential was recorded intracellularly using microelectrode.

In order to obtain further evidence on the retardation change, accumulation test was carried out by using ATAC averaging computer. If there is the retardation change associated with the excitation of the cell, signal to noise ratio of the change must be improved. The number of signal addition was limited less than 4, because the size of action potential and the recovery of cytoplasmic flow become poorer when the number of electric stimulation exceed 4. In Figure 5, the time course of retardation changes are shown against number of addition. In this case, action potential of the cell was measured extracellularly and optical signals were recorded in the middle of the cell. Hence the optical change must be occurred in advance of the potential change. The time course of retardation change, however, showed slower rising time constant than that of action potential. Frequently, retardation change showed two components but sometimes not.

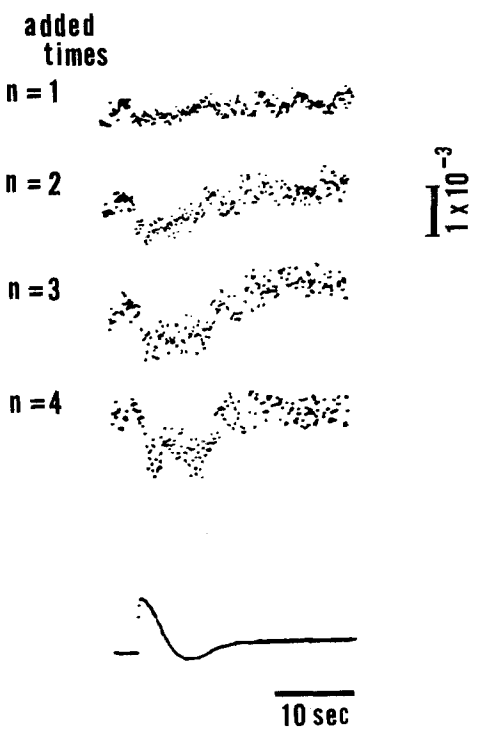

Fig. 5. Intensity changes in *Nitellopsis obtusa* during electric stimulation. Number of sweeps averaged is presented by *n*. Action potential was recorded extracellularly

3. Cytoplasmic Streaming Velocity Changes During an Action Potential

Cytoplasmic streaming in the Nitellopsis internodal cell is a typical example of circular streaming, with a velocity of 60–70 μm per second at room temperature. This streaming ceases abruptly when an action potential is generated, then recovers gradually. The relation between the action potential and the streaming cessation was previously reported by Tazawa and Kishimoto (1968). According to their finding, the cytoplasmic streaming stops at the peak of the action potential and recovers at the same rate as the motive force. Furthermore, the viscosity of the cytoplasm remains almost unchanged during the entire excitation period, observations were made by analyzing the Brownian motion of the granules in the cytoplasm (Kishimoto and Ohkawa, 1966).

The changes in the velocity of cytoplasmic streaming exhibits a type of flow birefringence and therefore is a major component of retardation change measurements. In order to estimate the contributions of that component, streaming velocity measurements and optical retardation change measurements were carried out simultaneously. Since cytoplasm contains a large number of granules, streaming could easily be observed through a microscope; the light intensity transmitted through an internodal cell fluctuated rapidly by moving granules transversed across a visual field of the microscope. In order to detect the light intensity fluctuation selectively, the angle between the cell and the plane of polarization of the incident light adjusted 0°. When streaming stopped, small fluctuations were observed (Fig. 6). Thus, these fluctuations might present a good measure of cytoplasmic streaming velocity.

Since granules in the cytoplasmic flow is homogeneous and the length of visual field of optical microscope is constant, we can estimate the cytoplasmic streaming speed by counting the number of intensity fluctuation peaks at a certain interval (an interval of 10 s in this case). The streaming velocity in the resting state was found to be 70 μm per second by the conventional method (Tazawa and Kishimoto, 1968), as is shown in Figure 7.

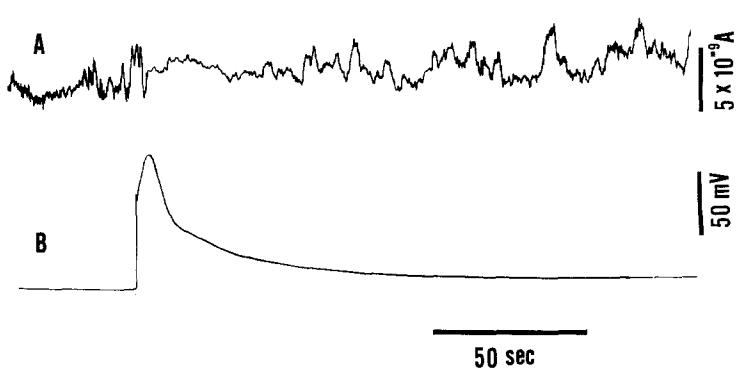

Fig. 6. **A** Intensity fluctuation was recorded during excitation. When the angle between the axon and the plane of polarization of incident light was 0°. **B** Time course of action potential was recorded intracellularly. A clear decrease of the fluctuation is observed at the top of the action potential

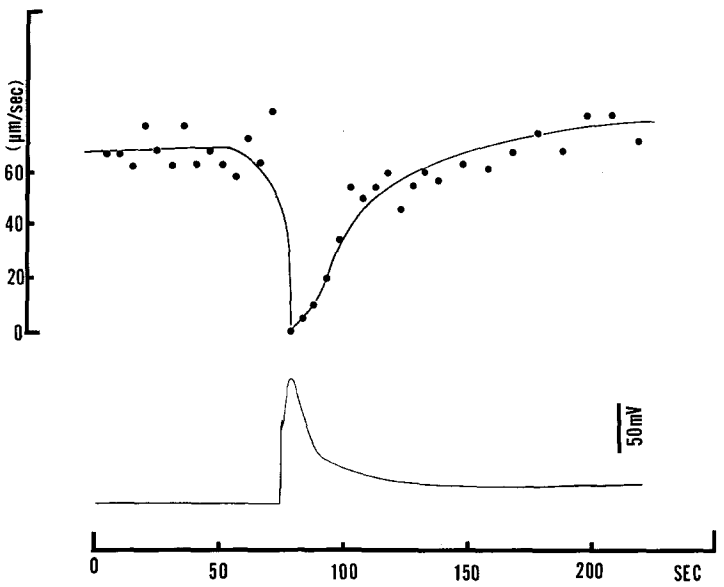

Fig. 7. Cytoplasmic streaming velocity change during excitation, plotted by counting intensity fluctuation peaks in a unit of time (above), and intracellular action potential is shown (below)

Discussion

1. Retardation Change

The direction of the retardation change was determined experimentally using Babinet compensator and found to be opposite in area 3 and area 1 or 5. When the retardation was added, the observed intensity change during excitation was varied linearly as a function of the amount of added retardation (not shown). From these observations, the following conclusions are reached; the changes in light intensity are not due to changes in light scattering, optical rotation or linear dichroism, and thus must be due to optical retardation.

The minimum value of the curve for areas 1 and 5 in Figure 3, however, does not lie close to zero, as predicted by Eq. (1). This can be attributed to partial depolarization of the incident light by scattering and partially to the lack of optical homogeneity of the internodal cell.

Birefringence difference between discrete cell areas from 1–5 reflects mainly the complex structure of cell wall of *Nitellopsis obtusa*. The contribution of membrane orientation and cytoplasmic flow to total birefringence of the cell might be small, because the thickness of membrane is negligible small and cytoplasmic flow is fairly slow.

In the resting state, a large electric field exists across the membrane and the cytoplasm streams at a constant speed. These two factors may consist of a major part of birefringence changes of the cell. When a cell is stimulated electrically, the electric field disappears and the flow ceases. Consequently, since two components of the birefringence are removed, the total birefringence decreases.

2. *Contribution of Electrical Field Change*

The rise time of the action potential of the internodal cell was about 1 s and its decay time was several tens of seconds, as shown in Figures 4 and 5. If the birefringence change is to be reduced to the Kerr effect alone, then the time course of the birefringence change should exactly parallel that of the action potential. Comparing the time course of optical retardation change with that of action potential, the parallelism between the two was considerably disturbed through the whole time course. Therefore, an alternative explanation must be considered.

3. *Flow Birefringence*

Flow birefringence occurs when soluble molecules in the cytoplasm become oriented parallel to the direction of cytoplasmic flow. When the flow stops, the orientation of the molecules becomes random and birefringence decreases:

$$R_{\text{flow}} = R_{\text{flow}}\, e^{-6\textcircled{H}\mathrm{D}t}, \tag{3}$$

$\textcircled{H}_D$ is a rotary diffusion coefficient for the molecules and R_{flow} is considered to be a function of the flow speed v. From general flow birefringence theory (Broersma, 1960), the magnitude of birefringence (R_{flow}) is proportional to flow velocity. Thus, the flow birefringence change follows Eq. (3) when the flow is suddenly stopped, and it recovers gradually in relation to the recovery of flow speed. These changes might correspond to the optical retardation change shown in Figure 5.

4. *Contribution of the Deformation Process*

Changes in length and radius have been observed during excitation in an internodal cell (Kishimoto and Ohkawa, 1966; Sandlin et al., 1968) and expected it may contribute to the total optical retardation changes, too. Kishimoto and Ohkawa (1966) found that a 5 cm long cell decreased by 0.1 μm in length during excitation. Sandlin et al. observed that a 3–4 cm long internodal cell with a diameter of 350 μm decreased 0.2–0.3 μm in length and 0.1–0.3 μm in diameter during excitation. Thus, in those studies, the ratio of length changed to total cell length was 10^{-5}, and was 10^{-3} for the ratio of the diameter changed in total cell diameter. The time course of these changes was very similar to that of the retardation change shown in Figure 4. So that the similar time courses of optical retardation change and volume change suggest that the retardation change may be partly due to the deformation caused by the cell volume change. According to Kishimoto and Ohkawa, the decrease in cell volume is made by a decrease in hydrostatic pressure inside the cell. If the decrease in cell volume were to occur without wrinkling the cell wall, membrane, or tonoplast, the cell would have to twist slightly along its long axis.

Thus, it can be concluded that the total optical retardation change will be the sum of flow birefringence and shape (deform) birefringence. Only way to resolve these two components is to stimulate the cell with maintaining cytoplasmic flow

stops. In order to perform these experiments, the repetitive electric stimulation was applied to the cell every 1 min. Under these conditions, action potential was produced without the flow of cytoplasm. The shape of the action potential, however, became poorer, so that the retardation change could not accumulate as was shown in Figure 5. Furthermore, the reproducibility of the optical change was very poor. From the fact described above, we may conclude that the birefringence of the *Nitellopsis obtusa* internodal cell was varied by the change of cytoplasmic flow and that of the size of length and diameter of it. But we can not dissolve the variation to each components at present.

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