Ca^{2+} effect on protoplasmic streaming in Nitella internodal cell

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ABSTRACT Ca^{2+} ion effect on protoplasmic streaming in an internodal cell of Nitella has been investigated for various temperatures. We have found that the protoplasmic streaming at low temperature is remarkably affected by the Ca^{2+} ions in the internodal cell but larger concentrations of the Ca^{2+} ions are needed to suppress the streaming velocity at higher temperatures. These streaming behaviors of the protoplasm, furthermore, have been elucidated on the basis of the reaction equations which take into account ATP hydrolysis due to actin-myosin molecules and inactivity of the molecules due to the Ca^{2+} ions.

INTRODUCTION

Protoplasmic streaming in Nitella internodal cell is maintained by an energy released from ATP hydrolysis due to actin and myosin molecules. It is well known that the streaming suddenly stops after a membrane excitation of the cell and gradually recovers to its steady flow. Williamson showed that protoplasmic streaming in an internodal cell of Chara was suppressed by vacuolar perfusion of a solution containing Ca^{2+} ions (Williamson, 1975). The cessation of the protoplasmic streaming was also found to occur reversibly by injecting electrophoretically Ca^{2+} ions through a micro-pipette electrode into a Nitella internodal cell (Kikuyama and Tazawa, 1982). On the other hand, concentrations of Ca^{2+} ions in a Chara internodal cell, after its membrane excitation, were confirmed to increase by a measurement of photoluminescent intensity of aequorin, which is in proportion to Ca^{2+} concentrations (Williamson and Ashley, 1982; Kikuyama and Tazawa, 1983). Thus, it is clear that Ca^{2+} ions in the internodal cell decisively affect the cessation of the protoplasmic streaming.

In a previous paper, we reported streaming behaviors of the protoplasm in a Nitella internodal cell for various temperatures (Tsuchiya et al., 1991). One of the results obtained is that the streaming velocity linearly decreases with increasing inverse temperatures but its proportional coefficient changes at 10°C. It is an interesting problem what streaming mechanism causes the characteristics in its temperature dependences.

In this study, to elucidate the characteristic temperature dependencies, we have investigated Ca^{2+} ion effect on the protoplasmic streaming at various temperatures.

MATERIALS AND METHODS

Nitella axilliformis plants were cultured in soil-water medium at 20°C. An internodal cell used was isolated from its neighbors and was kept in APW (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 $MgSO_4$) before use. The internodal cell membrane was permeabilized by the methods developed by Shimmen and Tazawa (Shimmen and Tazawa, 1983). Permeabilization was performed with three Mg-EGTA media which are shown in Table 1. Mg-EGTA media with various Ca^{2+} concentrations were also obtained by adding $CaCl_2$ solutions to the No. 3 medium. The internodal cells at first were bathed with the No. 1 medium at room temperature for 20–30 min and then with ice-cooled No. 2 medium for 5–10 min. Finally, bathing the cells with the No. 2 medium at room temperature for 10 min, we obtained the permeabilized cells, where no protoplasmic streaming was observed. Putting the cell into the No. 3 medium containing ATP resulted in recovery of protoplasmic streaming.

By use of the permeabilized cells, we have investigated Ca^{2+} ion effect on the protoplasmic streaming at various temperatures by the method described previously (Tsuchiya et al., 1991).

RESULTS

We show temperature dependencies of steady velocity of the protoplasmic streaming in the permeabilized cells for various Ca^{2+} concentrations in Fig. 1, where its steady velocity in a normal internodal cell (N.C.) is also given. Logarithm of the velocity is plotted versus the inverse temperature. For Ca^{2+} ions with low concentrations above pCa = 6.8, the streaming velocities almost change on a line with the same slope above ~10°C. The slope is estimated to be ~9.9 kcal/mol, which agrees with the value obtained in the previous paper. As the inverse temperature increases, all streaming velocities for Ca^{2+} concentrations above pCa = 6.4 deviate from the line and more steeply decrease. The temperatures at which the velocity begins to deviate from the common line are seen to be lowered as pCa increases. The streaming velocity for concentration of pCa = 6.1 already somewhat deviates from the common line, and for pCa = 5.4 it changes only on a line with a larger slope even at higher temperatures. The streaming velocity in a normal internodal cell also changes on the common line and deviates at low temperature. From the deviation temperature, the Ca^{2+} concentration in the normal internodal cell is considered to be situated between pCa = 6.8 and 7.4.

Fig. 2 shows Ca^{2+} concentration dependencies of the streaming velocity for various temperatures. The streaming velocity is normalized by its maximum value at each temperature. The streaming velocity is abruptly suppressed with an increase of the Ca^{2+} concentrations in the internodal cell. The streaming at 18°C almost stops for Ca^{2+} concentrations above 10^{-6} mol, which agrees with the result obtained by Tominaga et al. (Tominaga et al., 1983). The Ca^{+2} concentration for the suppression of the streaming shifts to a larger one at higher temperatures.

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TABLE 1 **Compositions of media**

Medium no.	EGTA	$MgCl_2$	PIPES	KOH	Sorbitol	ATP	$CaCl_2$
1	5	6	30	60–70	0	0	0
2	5	6	30	60–70	180	0	0
3	5	6	30	60–70	180	1	0 ~ 4

Concentrations are given in mM. All were adjusted to ~pH 7.0.

DISCUSSION

In Fig. 1, the characteristic temperature dependence of the protoplasmic streaming is seen to be definitely affected by Ca^{2+} ions in the internodal cell of Nitella. It is supposed that Ca^{2+} ions not only cause the cessation of the protoplasmic streaming but closely relate with the streaming dynamics of the protoplasm itself. Shimmen and Yano showed that the protoplasmic streaming was independent of Ca^{2+} ions in a reconstructed streaming system which is composed of a permeabilized internodal cell of Nitella and a perfusion solution containing myosin molecules isolated from rabbit skeletal muscles, where its actin molecules are sensitive to Ca^{2+} ions (Shimmen and Yano, 1986). Thus, it is considered that Ca^{2+} ions interact with myosin molecules in the protoplasm of Nitella internodal cell and inhibit ATP hydrolysis due to the actin and myosin molecules. Fig. 2 shows that higher concentrations of Ca^{2+} ions in the internodal cell are needed to stop the protoplasmic streaming at higher temperatures, that is, Ca^{2+} ions more effectively affect the streaming dynamics at lower temperatures.

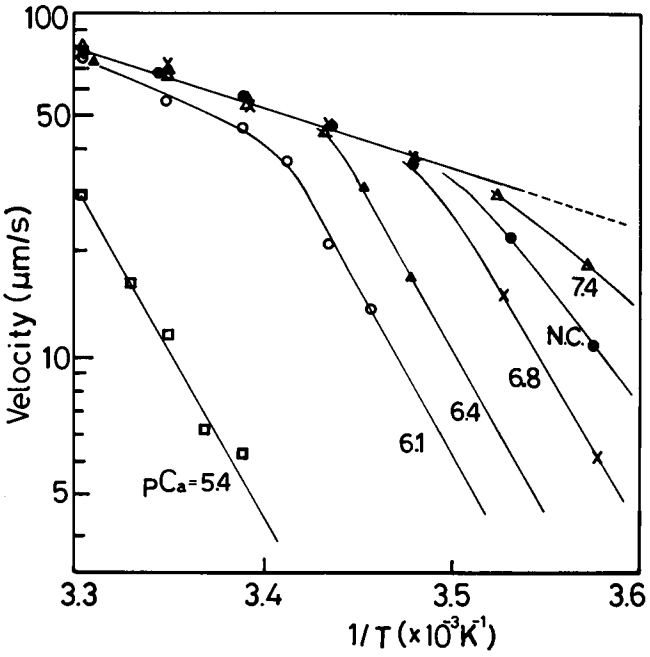

FIGURE 1 Steady velocity versus inverse temperature for permeabilized cells at various Ca^{2+} concentrations and a normal cell (N.C.).

Thus, we hypothesize that, in the generation process of the motive force for the streaming, the hydrolytic reaction of ATP due to the actin and myosin molecules competes with the inhibitory reaction of the myosin molecules due to Ca^{2+} ions.

Below, we derive simple model equations for the protoplasmic streaming and discuss Ca^{2+} ion effect on the streaming. Though it is sure that the ATPase activity of actin-myosin molecules occurs through many intermediate processes (Lymn and Taylor, 1971; Inoue et al., 1977), here, we consider a simplified reaction process of ATP hydrolysis due to the actin-myosin molecules as:

$$\mathrm{AM} + \mathrm{ATP} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \mathrm{AM}*\mathrm{ATP} \xrightarrow{k_{\mathrm{AM}}} \mathrm{AM} + \mathrm{ADP} + \mathrm{Pi} + \Delta E, \tag{1}$$

where $k_{+1,-1}$ and k_{AM} are reaction coefficients in each process, respectively. A rate equation for concentrations of the intermediate complex AM*ATP is easily obtained from the reaction (Eq. 1) as:

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{AM}*\mathrm{ATP}] = k_{+1}[\mathrm{AM}][\mathrm{ATP}] - (k_{-1} + k_{\mathrm{AM}})[\mathrm{AM}*\mathrm{ATP}]. \tag{2}$$

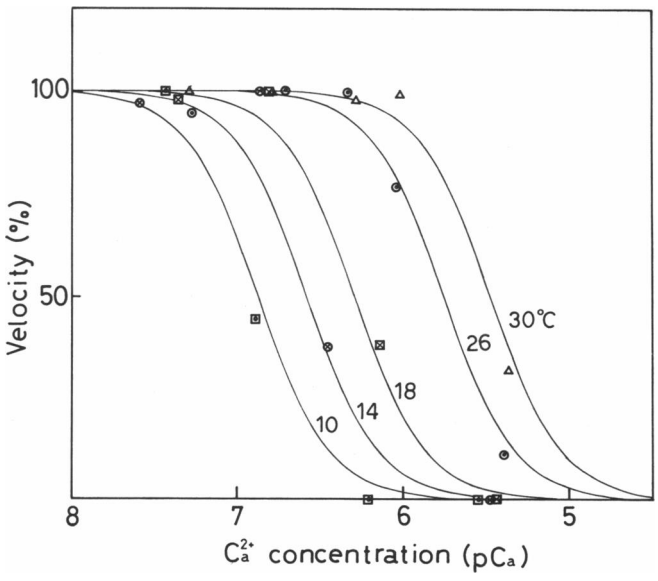

FIGURE 2 Ca^{2+} dependencies of the protoplasmic streaming at some temperatures. Solid curves shows theoretical ones obtained from Eq. 17.

It is supposed that total concentration $[AM]_0$ of the actin-myosin molecule is constant in an internodal cell and also the concentration of ATP molecules is kept constant in the reaction process with the actin-myosin molecules because of high concentration of the ATP molecule in the protoplasm (Shimmen, 1978). Thus, taking into account the following relations:

$$[AM] = [AM]_0 - [AM*ATP], \quad [ATP] = [ATP]_0, \quad (3)$$

where $[AM]_0$, $[ATP]_0$ are the total concentrations of actin-myosin molecules and the constant concentration of ATP molecules in the internodal cell, respectively. We can get steady concentration of the intermediate complex AM*ATP from Eq. 2 as:

$$[AM*ATP]_{st} = [AM]_0[ATP]_0/([ATP]_0 + K_1), \quad (4)$$

here

$$K_1 = (k_{-1} + k_{AM})/k_{+1}.$$

Next, we suppose the motive force for the protoplasmic streaming is proportional to the generation rate of the energy released from the ATP hydrolysis. Thus, the motive force F is given as:

$$F = c\frac{d}{dt}\Delta E = ck_{AM}[AM*ATP]_{st}, \quad (5)$$

where c is a proportionality coefficient. Hence, we can represent a motional equation for the protoplasmic streaming as follows:

$$\frac{d}{dt}V = F - \gamma V, \quad (6)$$

where V is the velocity of the streaming and γ is a resistive coefficient due to viscous property of the protoplasm. Using the relations 4, 5, and Eq. 6, we get the steady velocity of the protoplasmic streaming as:

$$V_{st} = (c/\gamma)k_{AM}[AM]_0[ATP]_0/([ATP]_0 + K_1). \quad (7)$$

Last, we consider Ca^{2+} ions effect on the protoplasmic streaming by taking into account the following reaction equation:

$$AM + nCa^{2+} \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} A*MCa_n. \quad (8)$$

The equation represents the hypothesis that n Ca^{2+} ions bind to an acto-myosin molecule to inhibit the ATP hydrolysis due to the actin-myosin molecules. As the result, Eqs. 1 and 8 are in a competitive state. A rate equation for the complex with n inactive myosin molecules is given as:

$$\frac{d}{dt}[A*MCa_n] = k_{+3}[AM][Ca^{2+}]^n - k_{-3}[A*MCa_n]. \quad (9)$$

In this case, the concentration of the actin-myosin molecules in the internodal cell are revised as:

$$[AM] = [AM]_0 - [AM*ATP] - [A*MCa_n]. \quad (10)$$

Taking into account relation 10, from rate Eqs. 2 and 9, we get the steady concentrations of the intermediate complex as:

$$[AM*ATP]_{st} = [AM]_0[ATP]_0(1 - B)/\{[ATP]_0(1 - B) + K_1\}, \quad (11)$$

where $B = [Ca^{2+}]^n/(K_I + [Ca^{2+}]^n)$ and $K_I = k_{-3}/k_{+3}$. Therefore, the velocity of the protoplasm is given as a function of Ca^{2+} concentrations as follows:

$$\begin{aligned} V_{st}([Ca^{2+}]) &= (c/\gamma)k_{AM}[AM]_0[ATP]_0(1 - B)/\{[ATP]_0(1 - B) + K_1\} \\ &= V_{st}(1 - B)/\{1 - B[ATP]_0/([ATP]_0 + K_1)\} \\ &= V_{st}/\{1 + [Ca^{2+}]^n(K_1/K_I)/([ATP]_0 + K_1)\} \\ &= V_{st}/(1 + K[Ca^{2+}]^n), \end{aligned} \quad (12)$$

where

$$K = (K_1/K_I)/([ATP]_0 + K_1). \quad (13)$$

Below, we discuss our experimental results on the basis of Eqs. 7 and 12 derived above.

First, the change of the streaming velocity on the common line in Fig. 1 is considered to reflect that the hydrolysis reaction of ATP due to the actin-myosin molecules is not still affected from Ca^{2+} ions at higher temperatures. Thus, taking into account the reaction coefficient k_{AM} in Eq. 7, we can approximately express Ca^{2+}-independent streaming velocity as:

$$V_{st} \propto k_{AM} \propto \exp(-E_1/kT)$$

or

$$\ln V_{st} = A_1 - B_1/T. \quad (14)$$

Fitting Eq. 14 to the common curve in Fig. 1, we get the constants as:

$$A_1 = 9.0 \pm 0.4$$

and

$$B_1 = 2.2 \pm 0.1 \times 10^3 \quad (E_1 = 9.9 \text{ Kcal/mol}).$$

Next, we rewrite Eq. 12 as:

$$\ln K = \ln\{(V_{st} - V_{st}([Ca^{2+}]))/V_{st}([Ca^{2+}])\} + n\text{pCa}, \quad (15)$$

where $\text{pCa} = -\ln [Ca^{2+}]$. The left hand side of Eq. 15 depends only on temperature and the concentration of ATP molecules because the constant value K, as is seen in the Eq. 13, is given by the reaction coefficients as K_1

and K_I, which are generally functions of temperature. Therefore, the right hand side of Eq. 15 should take a constant value depending only on temperature, even though the concentration of Ca^{2+} varies. Fig. 3 shows the inverse temperature versus the r.h.s. of Eq. 15, which is able to be estimated from only experimental data. Where a suitable value of n is taken to be 2 and Eq. 14 is used for the Ca^{2+}-independent streaming velocity V_{st}. As is expected, the r.h.s. of Eq. 15 is nearly independent on the Ca^{2+} concentrations and depends only on temperature. It is, furthermore, important that plots of the r.h.s. approximately fits on a line. Therefore, a temperature dependence of K in the Eq. 15 is estimated to be represented as:

$$\ln K = A_2 + B_2/T, \qquad (16)$$

and each constant

$$A_2 = -31.0 \pm 0.8$$

and

$$B_2 = 1.26 \pm 0.24 \times 10^4,$$

respectively.

Using these (constants) values, we can get the temperature dependencies of the protoplasmic streaming for various Ca^{+2} concentrations from the following equation:

$$\ln V_{st}([Ca^{2+}]) = \ln V_{st} - \ln (1 + K[Ca^{2+}]^n), \qquad (17)$$

which is rewritten from the Eq. 12. The calculations are shown in Fig. 4. Experimental data nearly agree with theoretical curves for various Ca^{2+} concentrations. We can also show Ca^{2+} dependencies of the streaming velocity for a parameter of temperature; those are shown with solid curves in Fig. 2.

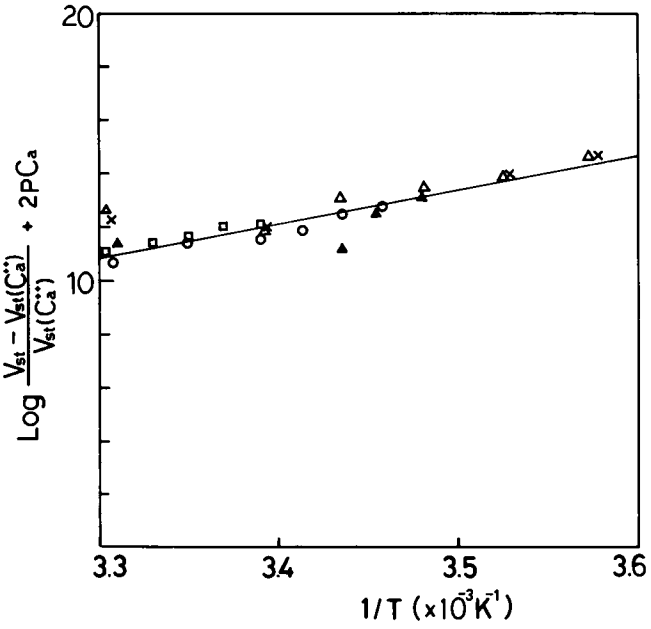

FIGURE 3 Inverse temperature versus values of the r.h.s. in Eq. 14, which are estimated from experimental data.

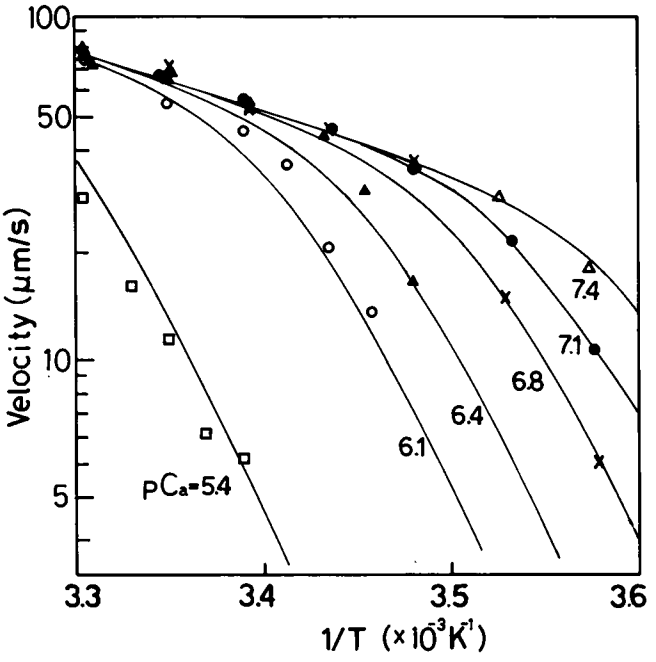

FIGURE 4 The temperature dependencies of the protoplasmic streaming for various Ca^{2+} concentrations. Solid curves are theoretically obtained from Eq. 17.

In conclusion, we have found that the characteristic temperature dependencies of the streaming velocity are subjected to the Ca^{2+} concentrations in the internodal cell. Taking into account ATP hydrolysis due to the actin-myosin molecules, which is more effectively inhibited by Ca^{2+} ions at lower temperatures, we have been able to elucidate the characteristic temperature dependencies of the protoplasmic streaming in Nitella internodal cell.

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