Acceleration of *Paramecium* Swimming Velocity is Effected by Various Cations

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**ABSTRACT.** Acceleration of swimming velocity was induced by the transfer of *Paramecium* cells to solutions containing various multivalent cations, Fe³⁺, Ca²⁺, Mg²⁺, Tris⁺, and others. The monovalent cations, K⁺, Rb⁺, Li⁺ and Na⁺, however, suppressed the acceleration induced by the multivalent cations. Effects of Ca²⁺ and K⁺ on swimming velocity were antagonistic, as the Ca²⁺ concentration increased the K⁺ concentration acted to suppress the Ca²⁺ induced acceleration. As both the Ca²⁺ and K⁺ concentrations were low, this antagonistic relation could be represented by the concentration ratio, [K⁺]/[Ca²⁺]¹/₂.

Acceleration of the swimming velocity took place when cells were transferred to a solution with a lower concentration ratio than that of the adapting solution used. Time courses of decreasing velocity after acceleration were examined at various concentrations of Ca²⁺ and K⁺, and for various temperatures. Change in membrane potential as measured with a microelectrode, was not consistently related to the change in swimming velocity. These results are discussed in relation to the driving force for the influx of Ca²⁺.

A ciliated protozoa, *Paramecium*, senses temporal changes in the ionic composition of the surrounding medium and can change its swimming behavior (3). When *Paramecium* cells are transferred to a solution containing a high concentration of K⁺, they begin to swim backward but later return to a forward mode of swimming (5). A high concentration of K⁺ depolarizes the membrane potential and opens the channels for the influx of Ca²⁺. This influx raises the intracellular concentration of Ca²⁺ which acts on the motile system to reverse the direction of ciliary beating (1, 8). Extracellular Ca²⁺ has an antagonistic effect on K⁺ (2, 4).

When *Paramecium* are transferred to a solution containing a high concentration of Ca²⁺, their forward swimming is accelerated. A decrease in the concentration of K⁺ has a similar effect. Although many studies have been made on the mechanism of backward swimming, little attention has been paid to the acceleration of forward swimming.

We have investigated the relation between the velocity of forward swimming and the ionic composition of the medium. Our results show that *Paramecium* cells have two states of forward swimming, a fully accelerated state and a nonaccelerated state. The nonaccelerated state is more stable, and cells that have adapted always assume it. An increase in the concentration of divalent cations or a decrease in the concentration of monovalent cations induces transition to the fully accelerated state, which lasts for 10 min or more.
MATERIALS AND METHODS

The cells used were Ksy 1 of mating type V of Paramecium caudatum supplied by Professor K. Hiwatashi of Tohoku University. These cells were cultured with Aerobacter in a hay infusion containing 3 mM Tris-maleate at pH 7.0. The culture temperature was kept at 25°C. Before the experiment, the cells were suspended in a solution of 0.25 mM CaCl₂, 2 mM Tris-HCl at pH 7.0 and various concentrations of KCl then left for 2 h or more at 25°C. The cells then were concentrated to about 5 × 10⁸ cells/ml by low speed centrifugation.

Drops of the concentrated Paramecium suspension were transferred to solutions of various ionic compositions in thin glass vessels. Photographs of swimming cells were taken with 2 sec exposures at suitable times after transfer. The temperature of the vessel was kept at 25°C by circulating water under it. The swimming velocity of the cells was obtained from the tracks made in the photographs (11). The velocities of 20–30 tracks of smooth swimming cells were averaged.

Membrane potential was measured by the method of Naitoh and Eckert (7). In this experiment, Paramecium cells were placed in a small glass vessel mounted on the stage of an inverted microscope (Olympus, CK), and microelectrodes were inserted into the cells from the upper side. The recording and stimulating electrodes were filled with 1 M KCl, their resistances were between 50 and 100 MΩ. The beating frequency of the cilia was measured by the photometric monitoring method described by Naitoh and Kaneko (9).

RESULTS

Acceleration of Swimming Velocity by Various Cations. Paramecium cells adapted to a solution of 5 mM KCl and 0.25 mM CaCl₂ have a swimming velocity of about 1 mm/s. When transferred to a solution of 5 mM KCl and CaCl₂ at concentrations higher than 0.25 mM, swimming velocity increased. In 1 mM CaCl₂, the velocity was more than twice that before transfer. This high velocity was maintained for about 10 min or more, then it began to decrease.

Acceleration of swimming velocity was induced similarly by the divalent cations Ca²⁺, Mg²⁺ and Sr²⁺ (Fig. 1). The divalent cations Co²⁺, Ni²⁺ and Mn²⁺, and the trivalent cation Fe³⁺ caused acceleration at low concentrations, but were toxic so that swimming stopped after several minutes. Zn²⁺ and Cd²⁺ stopped swimming immediately.

The monovalent cations, Tris⁺ and Choline⁺ increased the swimming velocity, but concentrations higher than 30 mM were needed. Transfer from pH 7.2 to 5.5 also induced acceleration, but transfer to an alkaline pH did not. Therefore, H⁺ has an accelerating effect.

Competitive Effects of Divalent and Monovalent Cations. Most monovalent cations were antagonistic to the effects of divalent cations. Paramecium cells adapted to a solution of 5 mM KCl and 0.25 mM CaCl₂ were transferred to a solution of 0.25 mM CaCl₂, and the concentration of KCl was varied. When the concentration of KCl was lower than 5 mM, the swimming velocity increased (Fig. 2). When it was higher than 5 mM, the velocity was not changed by the transfer. Therefore, a decrease in the concentration of KCl had the same effect as an increase in the concentration of CaCl₂. When cells were transferred to a solution of 0.25 mM CaCl₂ containing varied concentrations of the monovalent cations Rb⁺, Li⁺ and Na⁺, low concentrations increased the swimming velocity. Concentrations higher than 5 mM suppressed acceleration.

If the concentration of Ca²⁺ in the solution to which cells had been transferred was
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Fig. 1. Cations that induce acceleration of swimming velocity. *Paramecium* cells adapted to a solution of 0.25 mM CaCl$_2$ and 5 mM KCl were transferred to solutions containing 5 mM KCl and varied concentrations of Cl-salt cations. Cation species: Fe$^{3+}$, Ca$^{2+}$, Mg$^{2+}$, Sr$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Tris$^+$ and Choline$^+$. Swimming velocity was measured 5 to 7 sec after transfer. Bars represent standard deviations.

Fig. 2. Inhibition of the acceleration of velocity by monovalent cations. *Paramecium* cells adapted to 0.25 mM CaCl$_2$ and 5 mM KCl were transferred to solutions containing 0.25 mM CaCl$_2$ and varied concentrations of Cl-salt cations. Cation species: K$^+$, Rb$^+$, Li$^+$ and Na$^+$. Velocity was measured 8 to 10 sec after transfer. Bars represent standard deviations.
Fig. 3. (a) KCl (mM) vs. Velocity (mm/sec)

Fig. 3. (b) $[K^+] / [Ca^{2+}]^{1/2}$ vs. Velocity (mm/sec)
increased, a higher concentration of K$^+$ was required to suppress acceleration (Fig. 3a).
In other words, when the concentration of K$^+$ was increased, a higher concentration of Ca$^{2+}$ was required to induce acceleration. Fig. 3a also shows that there are two swimming states for *Paramecium*, the fully accelerated and nonaccelerated states. The swimming velocities in each state is constant and independent of the concentration of Ca$^{2+}$ or K$^+$ used to induce or suppress acceleration, but transition between the two states is gradual with a change in ionic composition. The velocity in the fully accelerated state is about 2.5 times that in the nonaccelerated state.

When the concentration of Ca$^{2+}$ was below 1 mM the data given in Fig. 3a could be shown as a single relation (Fig. 3b) by the ratio of the concentration of K$^+$ to the square root of the concentration of Ca$^{2+}$. Small values for this ratio induced acceleration.

**Acceleration of Cells Adapted to Different Ionic Compositions.** When *Paramecium* cells that had been adapted to solutions of 0.25 mM CaCl$_2$ and varied concentrations...
of KCl were transferred to a solution containing 0.25 mM CaCl₂ and a concentration of KCl lower than that in the solution used for adaptation, swimming velocity increased (Fig. 4). In summary, if the transfer caused a decrease in the concentration of K⁺, an increase in the concentration of Ca²⁺, or both, acceleration always took place.

Decrease in Swimming Velocity after Acceleration. The swimming velocity that was increased by a transfer to a solution containing a higher concentration of Ca²⁺, or a lower concentration of K⁺, began to decrease about 10 min after transfer and eventually reached the velocity before transfer.

Cells adapted to solutions of 0.25 mM CaCl₂ and varied concentrations of KCl were transferred to a solution of 0.25 mM CaCl₂ and 1 mM KCl (Fig. 5). The decrease in the concentration of K⁺ produced full acceleration. Thereafter, the velocity gradually decreased and had the same time course, which was independent of the concentration of K⁺, as before transfer. Distributions of the swimming velocities of many cells changed (inset, Fig. 5). During the transition from the fully accelerated state to the nonaccelerated state, distribution was not split but broadened. The transition ended in about 40 min, the time for half transition being about 17 min.

When cells were transferred to solutions of 0.25 mM CaCl₂ and varied concentra-

Fig. 5. Time courses of decreasing velocity after acceleration. Paramecium cells adapted to solutions of 0.25 mM CaCl₂ and 2.5 mM (○), 5 mM (●), 7.5 mM (▲) or 10 mM (□) KCl were transferred to a solution of 0.25 mM CaCl₂ and 1 mM KCl, after which swimming velocity was measured. Inset: distribution of swimming velocities after acceleration. Distributions were obtained from 150–200 cells adapted to 5 mM KCl. The ordinate shows the fraction of cells with the same velocity. Figures in the distributions are the times in minutes after transfer.
tions of KCl (lower than the concentration before transfer) full acceleration was induc-
ed, then the velocity decreased as shown in Fig. 5. When cells were transferred to
solutions with varied concentrations of CaCl₂ (higher than the concentration before
transfer) the decrease in velocity after full acceleration took place later, and the time
transition to the nonaccelerated state was lengthened with increasing concentrations
of Ca²⁺.

The time taken for the decrease in velocity was effected by the temperature of the
solution to which the cells had been transferred. Raising the temperature from 20 to
30°C shortened the time to about half that at taken 20°C.

**Beating Frequency of Cilia and Membrane Potential.** The beating frequency of the
cilia of *Paramecium* cells stuck to a glass slide was measured by photometry (9). An
increase in the concentration of Ca²⁺ or a decrease in the concentration of K⁺ induced
accelerated beating. In the solution to which the cells had been adapted the beating
frequency was between 15–20 Hz; after acceleration it was between 40–50 Hz. A
transitional change in frequency occurred, which corresponded to the change in
velocity of freely swimming cells.

As previously reported, an increase in the concentration of Ca²⁺ or K⁺ depolarizes
the membrane potential (6), and the depolarized potential became more negative
within 1–2 h. The cations Ca²⁺ and K⁺ had opposite effects on the swimming velocity,
but produced similar changes in the membrane potential.

**DISCUSSION**

An increase in the concentration of Ca²⁺ induces acceleration of the swimming
velocity of *Paramecium* cells. An increase in the concentration of K⁺ suppresses the
acceleration by Ca²⁺ and a decrease in the K⁺ concentration induces acceleration.
For example, when *Paramecium* cells are adapted to a medium containing 0.25 mM
CaCl₂, a few millimoles of KCl and a buffer, the membrane current is carried mainly
by Ca²⁺ and K⁺, and the membrane potential is determined by the counterbalance of
the influx of Ca²⁺ and the efflux of K⁺.

If, under these conditions, the concentration of Ca²⁺ is increased, the influx of Ca²⁺
also increases. By contrast, an increase in the concentration of K⁺ results in a decrease
in the influx of Ca²⁺. In other words, an increase in the concentration of Ca²⁺ or a
decline in the concentration of K⁺ both cause an increased influx of Ca²⁺. Therefore,
the increased influx of Ca²⁺ apparently is correlated directly with acceleration of the
swimming velocity. The driving force for the influx of Ca²⁺ and the efflux of K⁺ is
mainly controlled by the concentration ratio, [K⁺]/[Ca²⁺]¹/₂.

Using Triton-extracted models of *Paramecium* cells, we recently found that raising
the concentration of intracellular Ca²⁺ to about 2–4 × 10⁻⁷ M an increase in the
frequency of ciliary beating (Nakaoka *et al.*, in preparation). Although intracellular
Ca²⁺ at more than 10⁻⁶ M reverses the direction of ciliary beating (8), a concentration
of Ca²⁺ below this value accelerates ciliary beating, and the frequency is nearly doubl-
ed. Therefore, the acceleration induced by an increase in extracellular Ca²⁺ or a
decrease in extracellular K⁺ probably is due to an increased concentration of intrac-
cellular Ca²⁺ brought about by an increased influx of Ca²⁺. The increase in the con-
centration of intracellular Ca²⁺ is not so large that reversal of ciliary beating is induc-
ed. A large increase in intracellular Ca²⁺, enough for reversal, requires the opening of
voltage sensitive Ca²⁺ channels.
The divalent cations Mg$^{2+}$, Sr$^{2+}$ and Mn$^{2+}$ also induce acceleration. Mg$^{2+}$, however, does not directly affect ciliary beating in the Triton-extracted models. It has been reported that all divalent cations are bound to anionic sites on the membrane of the *Paramecium* cell (10) and that bound cations must increase the effective potential difference across the membrane. Possibly, Mg$^{2+}$ may release membrane-bound Ca$^{2+}$ to flow into the intracellular space. Monovalent cations also may release membrane-bound Ca$^{2+}$, but in this case the potential difference is decreased.

The acceleration caused by transfer to high concentrations of Ca$^{2+}$ or to low concentrations of K$^+$ continued for 10 min or more, then the velocity decreased to the value before transfer. To decrease the velocity, the concentration of intracellular Ca$^{2+}$ must be reduced in some way, probably by the pumping out of Ca$^{2+}$ and the inhibition of the influx of Ca$^{2+}$. Actually, the time required for the decrease in velocity is long when the concentration of extracellular Ca$^{2+}$ is high and when the temperature is low.

We note again that there are two discrete states of forward swimming; the fully accelerated and nonaccelerated states. Cilia seem to have not only a switching system to reverse the direction of beating, but one to accelerate forward swimming as well. Both may be regulated by Ca$^{2+}$. Further investigations on living cells and on Triton-extracted models are needed to determine if two such systems do exist.

**REFERENCES**


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