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DIRECTIONAL CHANGE OF CILIARY BEAT EFFECTED WITH Mg^{2+} IN *PARAMECIUM*

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SUMMARY

Two types of Triton X-100 extracted model of *Paramecium caudatum* were prepared and effects of the ionic compositions of the reactivating medium on the swimming behaviour of the extracted models were examined. Type I, prepared by the method of Naitoh and Kaneko, did not change swimming direction depending on the concentrations of Mg^{2+} and K^+ in the reactivating medium. Type II, prepared by a different method, however, reversed the swimming direction depending on the concentrations of Mg^{2+} and K^+ in the reactivating medium, even when the concentration of Ca^{2+} was kept at an extremely low level (below 10^{-8} M). Decreasing the concentration of Mg^{2+} induced the type II model to swim backwards.

Intracellular injection of EDTA into a live paramecium cell induced reversal of the direction of ciliary beat. The concentration of Mg^{2+} at which the reversal of swimming direction occurred varied with the concentration of adenosine triphosphate (ATP) in a manner which suggested that the concentration of free ATP^{4-} might be the critical determinant of swimming direction.

INTRODUCTION

The ciliated protozoon, *Paramecium*, can sense the environmental conditions in which it resides. For example, a temporary change of temperature or ionic conditions of the medium induces the ciliate to change its swimming behaviour (Jennings, 1906). Such a change in behaviour is a consequence of a change in ciliary beating. One of the most important changes is reversal of the direction of the effective ciliary stroke which causes a backward-swimming or avoiding reaction (Jennings, 1906).

To investigate the regulatory mechanism for ciliary beating, a detergent-extracted model of paramecium cells whose motile system can be reactivated by addition of Mg and ATP is available (Naitoh & Kaneko, 1972). In the extracted models, the cell membrane is functionally disrupted so that externally applied cations affect the ciliary motile system directly. These extracted models of paramecium swim forwards at a low concentration of Ca^{2+} (below 10^{-6} M) but at a higher concentration of Ca^{2+} (above 10^{-6} M) swim backwards (Naitoh & Kaneko, 1972). These observation indicate that the direction of ciliary beat is regulated by the intracellular concentration of Ca^{2+}. This is supported by the results of intracellular injection of Ca-buffer into paramecium (Saiki & Hiramoto, 1975). It has been established by electrophysiological study that depolarization of the surface membrane accompanies the influx of Ca^{2+} (Naitoh, Eckert & Friedman, 1972). Therefore, the depolarizing excitation of the membrane causes the change in direction of ciliary beat through the increase of intracellular concentration of Ca^{2+} (Naitoh & Eckert, 1974).

In the present experiments, the extracted model was prepared by a slightly different method from that of Naitoh & Kaneko (1973), and the effects of ionic conditions in the reactivating medium on swimming behaviour were examined. The results show that not only Ca^{2+} but also Mg^{2+} affect the swimming direction of the model.

MATERIALS AND METHODS

Paramecium caudatum (mating type V, syngen 3) was reared in a hay infusion inoculated with *Aerobacter aerogenes*. Culture temperature was 25 °C. Before extraction with detergent, paramecium cells were adapted to a solution of 0·25 mM $CaCl_2$, 7·5 mM KCl and 2 mM Tris-HCl, pH 7·2, for about 3 h at 25 °C. Then the cells were collected with a low-speed centrifuge and the loose pellet was suspended in the solution for detergent extraction.

Two kinds of extracting media were used. One was identical to that used by Naitoh & Kaneko (1973). The composition of the extracting medium was 20 mM KCl, 10 mM EDTA, 0·01 % Triton X-100 and 10 mM Tris-maleate, pH 7·0. After extraction for 30 min at 0 °C, extracted cells were washed by suspending in a solution containing 50 mM KCl, 2 mM EDTA and 10 mM Tris-maleate, pH 7·0, for 15 min at 0 °C. The cells were further washed with a solution of 20 mM KCl and 10 mM Tris-HCl, pH 7·2, for 15 min at 0 °C. The extracted model prepared by this procedure is termed type I.

The composition of another extracting medium was 20 mM KCl, 5 mM $MgCl_2$, 5 mM ethyleneglycol-bis (aminoethylether) tetra-acetic acid (EGTA; neutralized with Tris), 0·01 % Triton X-100 and 10 mM Tris-HCl, pH 7·2. After extraction for 30 min at 0 °C, the cells were collected and washed by suspending in solution containing 20 mM KCl, 5 mM $MgCl_2$, 3 mM EGTA and 10 mM Tris-HCl, pH 7·2, for 15 min at 0 °C. The cells were further washed with a solution containing 20 mM KCl and 10 mM Tris-HCl, pH 7·2, for 15 min at 0 °C. The model extracted by this procedure is termed type II.

Electrical measurement of membrane properties after extraction showed that both types of model completely lost the excitability induced by current stimulation and had no resting potentials.

The composition of the reactivating medium was ATP (neutralized with Tris), KCl, $MgCl_2$, EGTA and 10 mM Tris-HCl, pH 7·2. Concentrations of KCl, $MgCl_2$ and ATP were varied as indicated in the Results. For examination of the effect of Ca^{2+}, Ca-EGTA buffer was used. The association constant of EGTA with Ca ions at pH 7·2 was assumed to be $10^7\ M^{-1}$.

For reactivation experiments, 1 drop of a concentrated suspension of the models was added to about 1·5 ml of reactivating medium contained in a glass vessel mounted on a temperature-controlled copper plate, and then the suspension was stirred gently for a few seconds. Three to five minutes after this addition, photographs of the swimming tracks were taken by a camera mounted above the vessel with illumination from the side (Nakaoka & Oosawa, 1977). The temperature of the reactivating medium was controlled at 25 °C. Swimming velocity was obtained by averaging 30–50 tracks on the photograph.

Intracellular content of Mg^{2+} was measured by an atomic absorption method. Sample solutions for the measurement were prepared as follows; Concentrated cells of live paramecia were washed 5 times with solution of 0·25 mM $CaCl_2$, 2·5 mM KCl and 2 mM Tris-HCl, pH 7·2, then the cells were heated in a boiling waterbath for 5 min. Disrupted cells were removed by centrifugation and the supernatant was used for the measurement.

Intracellular content of ATP was measured by using the luciferin–luciferase enzyme system. The enzymes were extracted from firefly tails (Sigma Co.) by the method of Kimmich, Randles & Brand (1975). Samples for measurement of ATP were prepared by the same procedure as for measurement of Mg^{2+}. After mixing sample solution and enzyme system, the exponential decrease of the emitted light intensity was counted by a liquid scintillation counter (Hitachi Horiba Co.) and initial ATP concentration was obtained by extrapolating to the time of mixing.

Intracellular injections of chelating agents and KCl were made by the method of Koizumi (1974). For the injection, live paramecium cells were suspended in a medium containing 1 mM KCl, 1 mM EGTA, 1 % methylcellulose and 2 mM Tris-HCl, pH 7·2 (Saiki & Hiramoto, 1975). In this medium, swimming velocity was reduced and spontaneous reversal of ciliary beat was completely inhibited. Injected solutions were 20 or 100 mM EDTA, EGTA and

1 M KCl. Each solution contained 100 mM Tris-HCl, and was adjusted to pH 7·2. The volume of injected solution was one twentieth to one thirtieth of the cell volume, which was assumed to be $5{\cdot}6 \times 10^{-7}$ ml (Fortner, 1925). Observation of the direction of ciliary beat was made by phase-contrast microscopy.

RESULTS

Effects of Mg^{2+} and K^+ on the extracted type I model

When the concentration of Mg^{2+} in the reactivating medium was changed, the swimming velocity of the type I model was not changed (Fig. 1). Fig. 2 shows the effect of K^+ on the swimming velocity. When the concentration of K^+ was lower than 80 mM, the velocity was almost constant. Above this concentration the velocity decreased gradually, and no movements were observed a few min after the transfer of the models into the medium.

In all conditions examined, the extracted type I model swam in the forward direction.

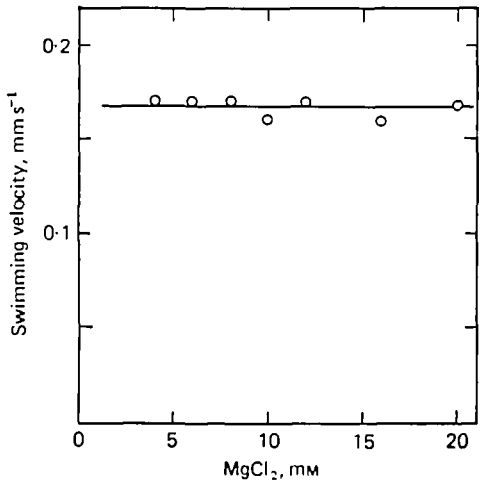

Fig. 1

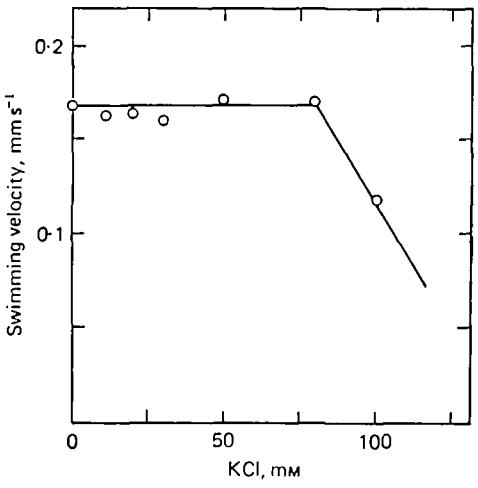

Fig. 2

Fig. 1. Effect of $MgCl_2$ on the swimming velocity of the type I model. The reactivating medium contained 20 mM KCl, 4 mM ATP, 2 mM EGTA, 10 mM Tris-HCl, at pH 7·2 and various concentrations of $MgCl_2$.

Fig. 2. Effect of KCl on the swimming velocity of the type I model. The reactivating medium contained 4 mM ATP, 4 mM $MgCl_2$, 2 mM EGTA, 10 mM Tris-HCl, at pH 7·2 and various concentrations of KCl.

Effects of Mg^{2+} on the extracted type II model

The type II extracted model changed swimming direction depending on the concentration of Mg^{2+} in the reactivating medium whose concentration of Ca^{2+} was kept extremely low by the addition of EGTA. When the concentration of Mg^{2+} was decreased, the type II model swam backwards, as shown in Fig. 3. It was confirmed that in these conditions the type I model swam in the forward direction.

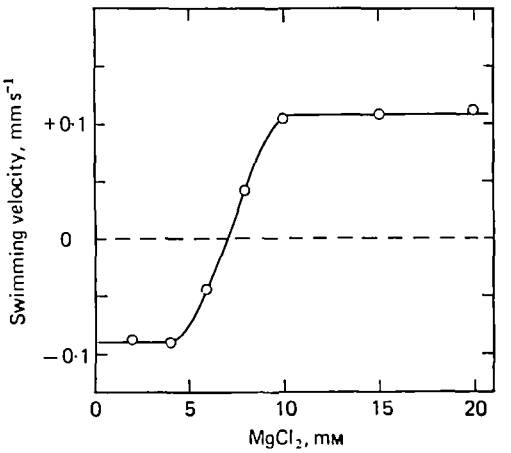

Fig. 3

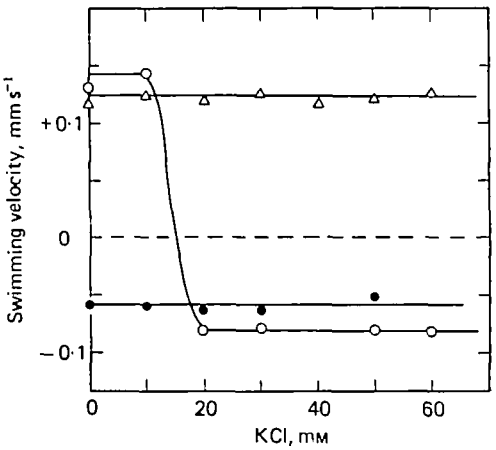

Fig. 4

Fig. 3. Effect of $MgCl_2$ on the swimming behaviour of the type II model. The reactivating medium was identical to that in Fig. 1.

Fig. 4. Effect of KCl on the swimming behaviour of the type II model. The reactivating medium contained 4 mM ATP, 2 mM EGTA, 10 mM Tris-HCl, pH 7·2, $MgCl_2$ at 4 (●), 7 (○) or 12 mM (△), and various concentrations of KCl.

The swimming velocity in the forward and backward directions was almost constant irrespective of the concentration of Mg^{2+}. Swimming direction was abruptly reversed in a narrow range of concentration of Mg^{2+}.

Microscopic observation of the swimming pattern of the model revealed that when the concentration of Mg^{2+} slightly exceeded that at which the swimming direction was reversed, almost all of the models swam in the forward direction with right-hand spiralling round the long axis of the cell body. Further increase in concentration of Mg^{2+} reversed the spiralling direction to a left forward one, which is the normal manner of swimming in the native paramecium. These observations suggest that Mg^{2+} affect the direction of spiralling. The directional change in the spiralling did not affect the swimming velocity of the model.

Effect of K^+ on the extracted type II model

When sufficient Mg^{2+} were present in the reactivating medium, the extracted type II model swam in the forward direction irrespective of the concentration of K^+. However, when the concentration of Mg^{2+} slightly exceeded that at which the model began to swim in the forward direction, the initial forward swimming was reversed by an increase in concentration of K^+ in the reactivating medium, as shown in Fig. 4. Backward swimming in the presence of an insufficient amount of Mg^{2+} was not reversed by increase of K^+.

Effect of Ca^{2+} on the extracted type II model

Increasing the concentration of Ca^{2+} in the reactivating medium caused the type II model, swimming in the forward direction in the presence of a sufficient concentration of Mg^{2+}, to reverse the swimming direction to the backward one, as shown in Fig. 5. The reversal occurred at 5×10^{-7} M Ca^{2+}. A similar relation was confirmed in the type I model, as in the previous report (Naitoh & Kaneko, 1972). The type II model swimming in the backward direction at low $MgCl_2$ concentration continued to swim in the backward direction irrespective of the increase of Ca^{2+}.

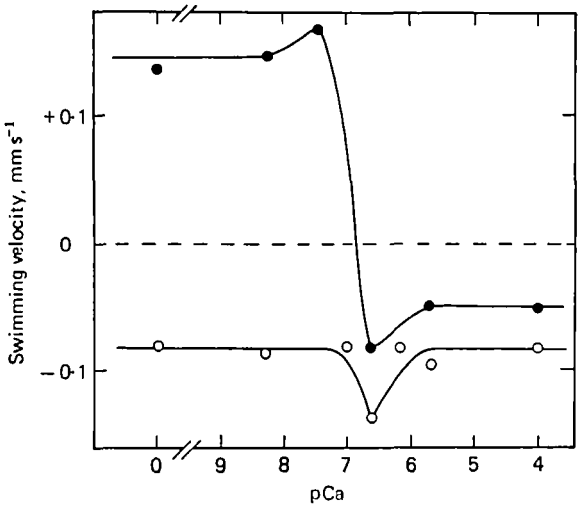

Fig. 5. Effect of the concentration of Ca^{2+} on the swimming behaviour of the type II model. The reactivating medium contained 4 mM ATP, 10 mM Tris-HCl, pH 7·2 and 4 (○) or 15 mM (●) $MgCl_2$. Concentration of Ca^{2+} was adjusted by Ca-EGTA buffer in the range between 10^{-8} and 10^{-6} M and by simple addition of $CaCl_2$ in the range above 10^{-6} M.

The velocities of forward and backward swimming were constant over almost the whole range of concentration of Ca^{2+} examined, but at around 10^{-7} M the velocity was higher than at other concentrations.

Intracellular concentrations of Mg and ATP

The Mg content of non-extracted live cells was $3{\cdot}3 \pm 0{\cdot}2$ mM (mean of 3 different cultures), on the assumption that the cell volume is $5{\cdot}6 \times 10^{-7}$ ml (Fortner, 1925).

The intracellular content of ATP was $0{\cdot}90 \pm 0{\cdot}06$ mM (mean of 3 different cultures).

It was confirmed that with these concentrations of Mg^{2+} and ATP in the reactivating medium (3·3 mM $MgCl_2$, 0·9 mM ATP, 3 mM EGTA, 20 mM KCl and 10 mM Tris-HCl, pH 7·2), the extracted type II model swam in the forward direction. Decreasing the concentration of Mg^{2+} to about 1·5 mM caused the model to swim backwards.

Intracellular injections of chelating agents and K^+

Intracellular injection of EDTA, which binds Mg^{2+} and Ca^{2+}, induced reversal of ciliary beat of non-extracted live cells. The final concentration of intracellular EDTA needed to induce ciliary reversal was 1–3 mM. Further increase of EDTA caused immediate stopping of the ciliary beat.

Intracellular injection of EGTA which preferentially binds Ca^{2+}, to a final concentration of 1–5 mM, did not induce reversal. Similar injection of KCl, to a final level of 10–30 mM, did not induce reversal.

DISCUSSION

The results of Figs. 3 and 5 show that a decrease in $MgCl_2$ concentration in the reactivating medium induces the extracted type II model to swim backwards, even when the concentration of Ca^{2+} is kept at an extremely low level. This effect of a decrease of $MgCl_2$ on the swimming direction can be explained in either of the following ways. One possibility is that the decrease of $MgCl_2$ causes the dissociation of Mg^{2+} bound to any site involved in the regulation of ciliary beat direction. The other is that the decrease of Mg^{2+} causes an increase of free ATP^{4-} in the reactivating medium and this binding induces ciliary reversal.

Table 1. *The concentrations of Mg and ATP in the reactivating medium at which the swimming direction of the type II model was reversed.*

	Added		Calculated		
Expt. no.	total Mg^{2+}, mM	total ATP^{4-}, mM	free Mg^{2+}, mM	free ATP^{4-}, mM	Mg-ATP^{2-}, mM
1	1·5	0·9	0·68	0·08	0·82
2	4·0	2·5	1·61	0·11	2·39
3	6·5	4·0	2·60	0·10	3·90
4	8·0	5·0	3·11	0·11	4·89

Conditions for the experiments were 20 mM KCl, 2 mM EGTA and 10 mM Tris-HCl, pH 7·2, 25 °C. For calculations of the concentrations of free Mg^{2+}, free ATP^{4-} and Mg-ATP^{2-} complex, the association constant of ATP with Mg^{2+} was assumed to be $14{\cdot}5 \times 10^3\ M^{-1}$ (Nanninga, 1961; Smith & Alberty, 1954). The concentration of the association of EGTA with Mg^{2+} at pH 7·2 are small and were neglected in the calculation.

In Table 1 the concentrations of ATP^{4-} amd Mg^{2+} in the reactivating medium at which the swimming direction of the type II model was reversed are summarized. Using these values, the respective concentrations of free ATP^{4-}, free Mg^{2+} and Mg-ATP^{2-} complex at the reversal of swimming direction can be calculated and are summarized in the same Table. These calculations show that the concentration of free Mg^{2+} needed to induce the reversal increases in proportion to the increase in

concentration of the Mg-ATP^{2-} complex. In contrast to this, the concentration of free ATP^{4-} needed to induce reversal is almost constant, irrespective of the change of Mg^{2+} and ATP^{4-} concentrations. That is, when the concentration of free ATP^{4-} exceeds about 0·1 mM with decreasing concentrations of Mg^{2+} in the reactivating medium, the extracted type II model begins to swim backwards. Therefore, the concentration of free ATP^{4-} in the reactivating medium determines whether the model swims in the forward or backward direction. Hayashi (1974) has reported that free ATP^{4-} acts to inhibit the axoneme and dynein ATPase from sea-urchin spermatozoa. This might be related to the present results.

The effect of K^+ on the swimming direction shown in Fig. 4 can be explained in the above way. On increasing the concentration of K^+ in the reactivating medium, the association constant of ATP with Mg^{2+} must decrease and this causes an increase of free ATP^{4-}, accompanied by an increase of Mg^{2+}. Calculating the concentration of free ATP^{4-} (Nanninga, 1961; Smith & Alberty, 1956) at the reversal of the swimming direction in Fig. 4, this increases from 0·08 to 0·09 mM on increasing K^+ from 10 to 20 mM.

Such effect of Mg^{2+} on type II models is supported by the results of intracellular injection of EDTA into a native paramecium cell. However, intracellular injection of KCl did not induce the reversal, whereas the type II model swam in the backward direction on increasing the concentration of KCl in the reactivating medium. This probably suggests that the intracellular concentration of Mg^{2+} is sufficiently high, so that the increase of KCl could not induce reversal.

Kamada's (1938) report that intracellular injection of citrate or oxalate induced ciliary reversal can be interpreted by the effect of Mg^{2+}. As the association constant of citrate or oxalate with Mg^{2+} is about $2{\cdot}5 \times 10^3\ M^{-1}$ (Campi, Ostacoli, Meirone & Saini, 1964), which is almost the same value as that with Ca^{2+}, injection of these reagents must decrease the intracellular concentration of Mg^{2+} and this induces ciliary reversal.

The different effect of Mg^{2+} on the type I and type II models probably originates in the differences in the conditions for extraction by detergent. The type II model might retain some substances which can sense the concentration of free ATP, whereas the type I loses these substances.

One more remarkable point in the present results is that the swimming velocity of the extracted model was the same for forward or backward swimming with changes in the concentrations of Mg^{2+} and K^+ in the reactivating medium. It may be presumed that changes in the intracellular concentrations of Mg^{2+} and K^+ do not affect the swimming velocity. Ca^{2+}, however, might affect the swimming velocity, especially at a concentration around 10^{-7} M. At this concentration, the swimming velocity of the extracted model increased, particularly in the backward direction. This might be related to the fact that in the native paramecium cell increased frequency of ciliary beat accompanies the reversal of ciliary beat upon membrane depolarization (Kinoshita, Murakami & Yasuda, 1965; Machemer, 1974).

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