Binding of Ca Ions by *Paramecium caudatum*

YUTAKA NAITOH and IKUO YASUMASU

From the Zoological Institute, Faculty of Science, University of Tokyo, Tokyo, Japan, and the Biological Laboratory, College of Education, Waseda University, Tokyo, Japan. Dr. Naitoh's present address is the Marine Biological Laboratory, Woods Hole, Massachusetts, and the Department of Zoology, Syracuse University, Syracuse, New York

ABSTRACT Binding of ^{45}Ca by live *Paramecium caudatum* was determined under various external ionic conditions. It was found that calcium uptake was separable into at least two components, a rapid and a slow one. The rapid component was influenced by the presence of certain other ions in a manner which agrees with the law of mass action. It appears that an ion exchange system may be involved in a binding equilibrium established between *Paramecium*, Ca^{++}, and certain other ions. K^+, Rb^+, and Ba^{++} in the equilibrium medium are among those ions which inhibit calcium uptake. It is proposed that liberation of Ca^{++} from binding sites on *Paramecium* by an exchange reaction with competing ions is the first step in the mechanism of ciliary reversal in the response to external application of these ions.

INTRODUCTION

A temporary change in beat direction of cilia (ciliary reversal or reversal response of cilia) occurs in *Paramecium caudatum* and many other ciliated protozoans in response to various stimuli (1). The importance of accumulated Ca ions in the cell to the ciliary response was first emphasized by Kamada (2, 3). Jahn (4) analyzed the data of Kamada and Kinosita (5) and suggested the applicability of Gibbs-Donnan's rule to the distribution of Ca ions on the cell surface of *Paramecium*, and emphasized the importance of the surface calcium to the response. In fact, duration of the ciliary response to a K stimulation is found to be identical between different organisms equilibrated in different media respectively when the ratio of the potassium concentration to the square root of calcium concentration, $[K^+]/\sqrt{[Ca^{++}]}$, of these media was held constant, regardless of their ionic concentrations (4).[1] This strongly suggests that the response is closely correlated with the amount of Ca ions bound at the cell surface in accordance with the Gibbs-Donnan rule or the law of mass action.

Ion exchange type binding systems have been demonstrated in a number of

[1] Y. Naitoh. 1967. Ionic control of reversal response of cilia in *Paramecium:* A Ca-hypothesis. Manuscript in preparation.

cellular constituents, such as liver microsomes (6), muscle microsomes (7, 8), red cell ghost (9), and also artificial phospholipid membrane (8).

Binding of Ca^{++} by membrane fractions of various cells has been found to obey the law of mass action. Moreover, it is clear that Ca ions play an important role in membrane excitation (10).

In the present experiments, we used ^{45}Ca to examine the kinetics of Ca^{++} binding by live *Paramecium*. The results show that one kinetic component of Ca^{++} uptake, which reaches equilibrium relatively rapidly, does so in competition with certain other ions, and obeys the law of mass action.

MATERIAL AND METHODS

Specimens of *Paramecium caudatum* reared in hay infusion were washed well with a solution containing 20 mM KCl and 0.1 mM $CaCl_2$ (pH 7.2)[2] to minimize the amount of bound Ca ions in the organism[3] and reduce bacterial contamination. Then, the organisms were suspended in the solution (1-mg organisms in dry weight/ml) for 30 min or more prior to experimentation.

A 1-ml aliquot of the suspension was pipetted into a filtrating glass vessel at the bottom of which a sheet of Millipore filter was mounted (Millipore Filter Corp., Bedford, Mass.). Then, 10 ml of radioactive solution containing adequate amounts of ^{45}Ca[4] and other ions were pipetted into the vessel and mixed well with the organism suspension. After an adequate equilibration time, the mixed suspension medium in the vessel was sucked out through the Millipore filter rapidly (1 ml/sec) by a vacuum pump. Then, in order to wash out the ^{45}Ca remaining in the filter, 10 ml of an unlabeled mixture having the same ionic composition as the labeled suspension medium was poured into the vessel at a rate of about 1 ml/sec while the suction was maintained.

The Millipore filter was dried by infrared illumination, and the radioactivity of the filter due to accumulated ^{45}Ca by *Paramecium* was counted by a G-M counter. The same procedure without *Paramecium* proved that residual ^{45}Ca in the filter was almost negligible.

At least three measurements were made to determine each point presented in the figures of the present paper. Fluctuations of measured values were so small that the standard errors did not exceed the diameter of circles presenting the mean values.

All the experiments were performed at room temperatures of 19–21°C.

RESULTS AND DISCUSSION

A. *Time Course of ^{45}Ca Binding and Effect of Coexisting K Ions*

To examine the kinetics of ^{45}Ca accumulation by *Paramecium* and the influence of coexisting K ions, eight aliquots of the *Paramecium* suspension were

[2] The pH of all the experimental media in these experiments was adjusted to 7.2 by 1 mM (in final concentration) Tris buffer.

[3] It is desirable to remove all Ca ions from the cell surface, however, washing the organisms with a Ca-free potassium solution rapidly killed the organisms.

[4] Radiocalcium solution was prepared by mixing 1 mc ^{45}Ca with 1 ml of 0.1 M $CaCl_2$ solution.

equilibrated in two kinds of radioactive media with identical Ca concentrations (1 mM in final concentration; the amount of ^{45}Ca was 10 μc/ml) but varied K concentrations (20.4 and 2.4 mM in final concentration) for periods of 1, 5, 10, and 20 min. Then, the radioactivities of each aliquot were determined.

The results shown in Fig. 1 indicate that a much greater amount of ^{45}Ca was accumulated within 1 min equilibration in the presence of low-potassium than in high-potassium concentration. This rapid uptake was followed by a rather slow phase, radioactivity reaching a plateau about 20 min after the

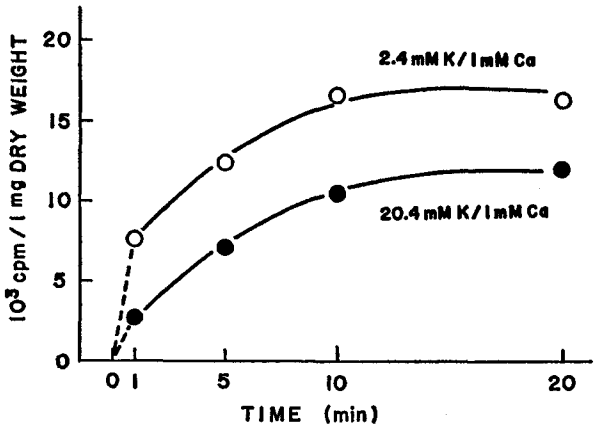

FIGURE 1. Time course of $^{45}Ca^{++}$ binding by live *Paramecium caudatum* and the effect of coexisting K ions in the equilibrium medium on the binding (open circles, 2.4 mM K^+; solid circles, 20.4 mM K^+). Ca^{++} concentration in the media was 1 mM ($^{45}Ca^{++}$, 10 μc/ml). Notice the rapid binding of $^{45}Ca^{++}$ within 1 min from the start of equilibration (dotted lines). See text for discussion.

start of the equilibration. The slow-uptake component exhibits a time course which is independent of the potassium concentration.

It is proposed that the rapid initial component of ^{45}Ca uptake is due to binding of ^{45}Ca by anionic sites on *Paramecium*, and the K ions compete for the same binding sites. The slow increase in radioactivity, which seems to be unaffected by the presence of K ions, may be due to a diffusion of ^{45}Ca into the cell, passive exchange of ^{45}Ca with preexisting Ca ions in the cell, or active Ca accumulation by the cell.

B. *The Initial Rapid Binding of ^{45}Ca and the Effect of Coexisting K Ions*

If an ion exchange type system is involved in the initial rapid binding of Ca ions by *Paramecium*, the equilibrium between the binding sites of *Paramecium*,

Ca ions, and K ions may be formulated according to the law of mass action as:

$$P\mathrm{Ca}_{\frac{1}{2}} + \mathrm{K}^{+} \rightleftharpoons P\mathrm{K} + \tfrac{1}{2}\mathrm{Ca}^{++} \qquad (1)$$

$$\frac{[P\mathrm{K}]}{[P\mathrm{Ca}_{\frac{1}{2}}]} = k\frac{[\mathrm{K}^{+}]}{\sqrt{[\mathrm{Ca}^{++}]}} \qquad (2)^{5}$$

in which P represents the binding site of *Paramecium*; $[P\mathrm{Ca}_{\frac{1}{2}}]$ represents the concentration of Ca bound by *Paramecium*; $[P\mathrm{K}]$ represents the concentration of K bound by *Paramecium*; $[\mathrm{K}^{+}]$ and $[\mathrm{Ca}^{++}]$ represent the concentrations of K and Ca ions in the equilibrium medium; and k represents the equilibrium constant.

If it can be assumed that all the binding sites are filled with Ca and K, the total binding capacity of *Paramecium*, Pt, can be represented as:

$$Pt = [P\mathrm{K}] + [P\mathrm{Ca}_{\frac{1}{2}}] \qquad (3)$$

and the following expression for the amount of bound calcium can be derived from Equations 2 and 3:

$$[P\mathrm{Ca}_{\frac{1}{2}}] = \frac{Pt}{kJa + 1} \qquad (4)$$

in which Ja[6] represents the ratio $[\mathrm{K}^{+}]/\sqrt{[\mathrm{Ca}^{++}]}$ in the equilibrium medium. By rearranging, Equation 4 can be put in the form of a straight line as:

$$\frac{1}{[P\mathrm{Ca}_{\frac{1}{2}}]} = \frac{k}{Pt}Ja + \frac{1}{Pt} \qquad (5)$$

Equation 4 (or Equation 5) shows that if Ja in the equilibrium medium is kept constant, the amount of Ca bound by *Paramecium* should remain constant, regardless of the ionic concentration in the equilibrium medium. In order to test this prediction, three aliquots of the *Paramecium* suspension were equilibrated for 1 min in three different radioactive media having the same Ja value (1.41) and different ionic concentrations [1, 1 mM KCl + 0.5 mM $CaCl_2$ (5 μc/ml); 2, 2 mM KCl + 2 mM $CaCl_2$ (20 μc/ml); 3, 4 mM KCl + 8 mM $CaCl_2$ (80 μc/ml) all in final concentration] respectively; then, radioactivities were determined on each aliquot.

Results shown in Fig. 2 clearly indicate that ^{45}Ca uptake by *Paramecium* during 1 min equilibration was almost identical in each of the aliquots in

[5] Activity coefficients are neglected in all the equations in the present paper because of low ionic concentrations employed in these experiments.

[6] In honor of Dr. T. L. Jahn who suggested first the importance of the ratio to the binding of Ca in *Paramecium* and its ciliary reversal. The concentrations of each ion species in the solutions employed in these experiments are all expressed in millimoles per liter.

spite of differences in the total concentration (1.5–12 mM) and the amount of ^{45}Ca (5–80 μc/ml) between three equilibrium media.

Equation 5 implies that the reciprocal value of the amount of bound Ca by *Paramecium* must have a linear relationship with *Ja* in the equilibrium medium. To ascertain this, radioactivities were determined on aliquots of the *Paramecium* suspension equilibrated in various media having various K concentrations and the same Ca concentration (1 mM, 10 μc/ml). The reciprocal values of each radioactivity plotted against the *Ja* value were found to form a straight line as shown in Fig. 3 (open circles).

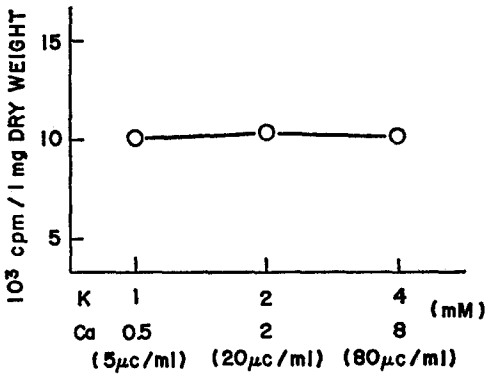

FIGURE 2. Amount of $^{45}Ca^{++}$ bound by live *P. caudatum* during 1 min of equilibration in three kinds of solutions having the same ratio, $[K^+]/\sqrt{[Ca^{++}]} = 1.41$. Concentration of K^+ and Ca^{++} and the amount of $^{45}Ca^{++}$ are represented in abscissa. The amounts of $^{45}Ca^{++}$ bound by *P. caudatum* are equal between the three ionic conditions of the equilibrium media, in spite of large differences in the ionic concentrations and amounts of $^{45}Ca^{++}$.

These observations provide strong evidence for the applicability of the law of mass action to the uptake equilibrium between *Paramecium*, Ca ions, and K ions, and saturation of the assumed binding sites of *Paramecium* with Ca and K ions, at least under the conditions employed.

C. *Concentration Effect of Various Ions on the Initial Rapid Binding of* ^{45}Ca

It is interesting to know whether ions other than K^+, for example Na^+, Rb^+, Mg^{++}, and Ba^{++}, act to compete for the same calcium-potassium binding sites on *Paramecium*, since these ions (exception of Mg^{++}) are known to induce ciliary reversal in *Paramecium* (11).[1] Aliquots of the *Paramecium* suspension were equilibrated for 1 min in several different media, each of which consisted of 1 mM $CaCl_2$ (10 μc/ml) plus one of the test ions in one of several concentrations. The radioactivities due to the bound ^{45}Ca were determined in each case, and the reciprocal values of the radioactivities were plotted against the *Ja* value of each medium. The *Ja* value, in the present cases,

were calculated as [monovalent ion]/$\sqrt{[Ca^{++}]}$ or $\sqrt{[\text{bivalent ion}]}$/$\sqrt{[Ca^{++}]}$.[7]

As is clearly shown in Fig. 3, all the data showed linear relationships with the *Ja* values, and each ion species gave a line with a different slope. All the lines crossed the ordinate at a point which corresponds to $1/Pt$, as is expected from Equation 5. These findings indicate that the law of mass action is also applicable to the equilibrium established between the proposed binding sites, Ca^{++}, and the other ions used in the present experiments.

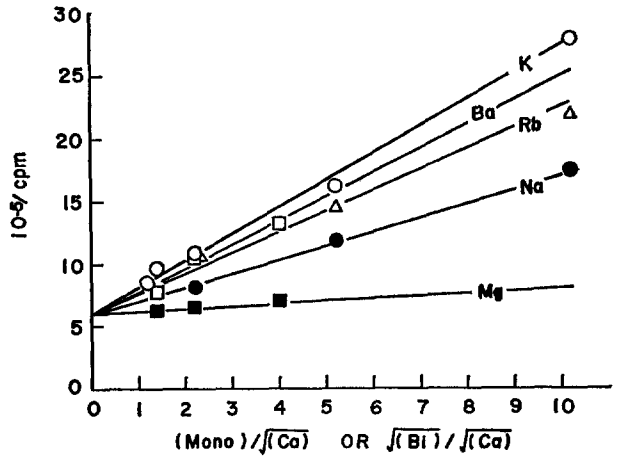

FIGURE 3. Effects of various coexisting ions in the equilibrium medium (○, K^+; △, Rb^+; ●, Na^+; □, Ba^{++}; ■, Mg^{++}) on the binding of $^{45}Ca^{++}$ by live *P. caudatum*. Abscissa represents the ratio, [monovalent ion]/$\sqrt{[Ca^{++}]}$ or $\sqrt{[\text{bivalent ion}]}$/$\sqrt{[Ca^{++}]}$. Ordinate represents the reciprocal value of the counts per minute from the $^{45}Ca^{++}$ bound by 1 mg dry weight of *Paramecium*. See text for discussion.

From the value of $1/Pt$ and the slope of each line, the value of the equilibrium constant, k, for each ion was calculated, and listed in Table I. The equilibrium constant represents the ability of the given ion to prevent the binding of Ca^{++} to the sites. In other words, those ions having the large equilibrium constants as K^+, Rb^+, and Ba^{++} bind comparatively easily to the sites in exchange with Ca ions, whereas those with small constants as the Mg ion have a low affinity for the site.

[7] The equilibrium between the binding sites, Ca ions, and bivalent ions can be formulated as:

$$P\text{Ca}_{\frac{1}{2}} + \tfrac{1}{2}B^{++} \rightleftharpoons PB_{\frac{1}{2}} + \tfrac{1}{2}\text{Ca}^{++} \qquad (1')$$

$$\frac{[PB_{\frac{1}{2}}]}{[P\text{Ca}_{\frac{1}{2}}]} = k\frac{\sqrt{[B^{++}]}}{\sqrt{[\text{Ca}^{++}]}} \qquad (2')$$

in which B^{++} represents bivalent ion and $[PB_{\frac{1}{2}}]$ represents the amount of bound bivalent ions by *Paramecium*. Thus, in the case of bivalent ions, $Ja = \sqrt{[B^{++}]}/\sqrt{[\text{Ca}^{++}]}$.

Reversal response of cilia in *Paramecium* can be elicited by the external application of certain monovalent (K^+, Rb^+, Na^+) and certain bivalent (Ba^{++}) ions. Magnesium ions, on the other hand, do not induce reversal (11).[1] The effectiveness of ions in eliciting the reversal response of cilia seems to be correlated with their equilibrium constant of binding on *Paramecium*. That is, those ions having relatively large constants such as K^+, Rb^+, and Ba^{++} induce prolonged responses, while the response to Na^+ is significantly shorter in duration than that to K^+. Magnesium, which has a very small constant, does not induce reversal.[1] These correlations suggest that the liberation of bound Ca^{++} from the binding sites of *Paramecium* by exchange with other ions is directly concerned with the reversal response of cilia.

TABLE I

CALCULATED EQUILIBRIUM CONSTANTS IN THE EQUILIBRIUM BETWEEN BINDING SITES OF *PARAMECIUM*, CA^{++}, AND OTHER IONS

The equilibrium between binding sites, Ca^{++}, and other ions is formulated in accordance with the law of mass action as in Equations 1, 1′, 2, and 2′. Calculation was made from the data in Fig. 3.

Ion species	Equilibrium constant
Sodium	0.19
Potassium	0.35
Rubidium	0.28
Magnesium	0.033
Barium	0.32

Liberation of Ca^{++} from the sarcoplasmic reticulum is thought to be concerned with the control of contraction in striated muscle fibers (12). Displacement of bound calcium has also been proposed as a step underlying permeability changes during membrane excitation (10). In the glycerol-extracted *Paramecium* model, ciliary reversal occurs only in the presence of Ca^{++} and ATP.[8] All present evidence indicates that ciliary reversal is dependent on calcium.[1]

It is proposed, on the basis of these observations, that Ca^{++} liberated from cellular binding sites activates, directly or indirectly, a contractile system which is energized by ATP. Contraction of this proposed contractile component in turn results in the reversal of effective beat direction of the cilia. In this model ciliary reversal is closely analogous in fundamental mechanism and its control to contraction of muscle.

The locality of the binding sites is still unknown. However, it is highly probable that the sites may be on the surface or at least, near the surface of

[8] Y. Naitoh. 1967. Reversal response of cilia in glycerol-extracted model of *Paramecium*. In preparation.

the organism because Ca binding reaches an equilibrium relatively rapidly and because the bound Ca is intimately concerned with the behavior of the cilia, which, of course, are located at the cell surface.

Ciliary reversal of *Paramecium* is closely associated with the depolarization of the membrane, regardless of whether depolarization is the result of applied current, application of KCl (13, 14), or spontaneous (13, 14, 15). It will be of great interest to our understanding of ciliary reversal to determine in subsequent studies the relationship between depolarization and the release of Ca^{++} from binding sites on *Paramecium*.

We are grateful to Dr. R. Eckert of Syracuse University and Dr. H. Sanui of the University of California, Berkeley, for their critical reading of the manuscript.

Received for publication 8 August 1966.

REFERENCES

1. JENNINGS, H. S. 1906. Behavior of the Lower Organism. Columbia University Press, New York.
2. KAMADA, T. 1938. Intracellular calcium and ciliary reversal in *Paramecium*. *Proc. Imp. Acad. Tokyo.* **14**:260.
3. KAMADA, T. 1940. Ciliary reversal of *Paramecium*. *Proc. Imp. Acad. Tokyo.* **16**:241.
4. JAHN, T. L. 1962. The mechanism of ciliary movement II. Ciliary reversal and ion antagonism. *J. Cellular Physiol.* **60**:217.
5. KAMADA, T., and H. KINOSITA. 1940. Calcium-potassium factor in ciliary reversal of *Paramecium*. *Proc. Imp. Acad. Tokyo.* **16**:125.
6. CARVALHO, A. P., H. SANUI, and N. PACE. 1963. Calcium and magnesium binding properties of cell membrane materials. *J. Cellular Physiol.* **62**:311.
7. KOKETSU, K., R. KITAMURA, and R. TANAKA. 1964. Binding of calcium ions to cell membrane isolated from bullfrog skeletal muscle. *Am. J. Physiol.* **207**:509.
8. MIKULECKY, D. C., and J. M. TOBIAS. 1964. Phospholipid-cholesterol membrane model. *J. Cellular Physiol.* **64**:151.
9. SANUI, H., A. P. CARVALHO, and N. PACE. 1962. Relationship of hydrogen ion binding to sodium and potassium binding by rat liver cell microsomes and human erythrocyte ghosts. *J. Cellular Physiol.* **59**:241.
10. TOBIAS, J. M. 1964. A chemically specified molecular mechanism underlying excitation in nerve: A hypothesis. *Nature.* **203**:13.
11. OLIPHANT, J. F. 1942. Reversal of ciliary action in *Paramecium* induced by chemicals. *Physiol. Zool.* **15**:443.
12. HUXLEY, A. F. 1964. Muscle. *Ann. Rev. Physiol.* **24**:131.
13. KINOSITA, H., S. DRYL, and Y. NAITOH. 1964. Change in membrane potential and the response to stimuli in *Paramecium*. *J. Fac. Sci., Univ. Tokyo, Sect. IV.* **10**:291.
14. NAITOH, Y. 1966. The reversal response elicited in nonbeating cilia of *Paramecium* by membrane depolarization. *Science.* **154**:660.
15. KINOSITA, H., A. MURAKAMI, and MOMOKO, YASUDA. 1965. Interval between membrane potential change and ciliary reversal in *Paramecium* immersed in Ba-Ca mixture. *J. Fac. Sci., Univ. Tokyo, Sect. IV.* **10**:421.