Effect of Potassium, Calcium, and Magnesium Ions on the Protoplasmic Streaming in *Acetabularia calyculus*

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In a coenocytic marine alga, *Acetabularia calyculus*, an active and characteristic protoplasmic streaming can be seen in the stalk, until the cap is formed. The streaming is found along the fine striations of an indefinite number, running in parallel on the cortical protoplasmic gel layer. The streaming is two directional, either acropetal or basipetal, the direction being usually fixed depending on the particular striation. Thus, many streams of opposite directions are seen in the cortical region of the stalk. The rate of flow is different from one stream to another in the range of 2.5-6 μ/sec., but it is kept nearly constant at a certain locus of a stream under a certain environmental condition. In the present study, experiments were conducted to investigate the effect of concentration of K, Ca, and Mg ions in the external medium (sea water) on the protoplasmic streaming in *A. calyculus*.

There have been only a few works concerning the effect of ions on the protoplasmic streaming in plant cells. Collar studied the effect of 0.1 M solutions of various salts on the rate of protoplasmic streaming in *Chara crinita*. She showed that chlorides of Na, K, and Mg increase the rate of flow in the order of K > Na > Mg, while those of Li, Ca, and Ba retard the flow, leading eventually to a cessation. According to Gimesi and Pozsár, protoplasmic rotation in *Elodea* cells is suppressed in 0.02 M solutions of various salts of K, Na, Ca, and Al by 62% to 74% of the control rate in water.

Marine algae such as *Acetabularia* seem to be more advantageous as material for this kind of experiment than fresh water plants, because their natural medium (sea water) contains several ions in much higher concentrations. This fact makes it possible to investigate the effect of those ions not only by heightening but also by lowering their concentrations.

**Material and Methods**

*A. calyculus* was collected in the Inland Sea of Japan. It was cultivated in artificial sea water supplemented with the trace of NaNO₃ and Na₂HPO₄, at 20±1° under the illumination with fluorescent light (2500 lux) 14 hours a day. The artificial sea water used contains 2.7 g NaCl (0.462 M), 0.07 g KCl (0.01 M), 0.1 g CaCl₂ (0.009 M), 0.34 g MgCl₂·6H₂O (0.017 M), and 0.21 g MgSO₄·7H₂O (0.009 M) in 100 ml. of distilled water. In the following pages, the above artificial sea water will be simply referred to as sea water.

For experiments, young stalk of *A. calyculus* of about 2 cm long was ligated near the base, before it was freed from the shell to which the alga attached. The ligated stalk was kept in the sea water for a few hours prior to the experiment, so that the cell could recover from possible harm induced by the cutting.

The rate of protoplasmic streaming was determined by using a calibrated ocular micrometer and a stop-watch. The movement of chloroplasts served as an index of

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the rate of flow. At first, the rate of protoplasmic flow in a cell was measured in the sea water as control. The sea water was then replaced with an experimental medium whose content of K, Ca, or Mg was modified to be either higher or lower than normal. After the effect of these ions was investigated for a certain period, the experimental medium was again replaced with the original sea water to study reversibility in the effect of ions. In order to facilitate the comparison of different series of experiments, changes in the rate of flow were shown in per cent of the control.

The osmotic pressure of sea water containing modified salt concentration was made equal to that of the original artificial sea water in the following way. Since it found in preliminary experiments that changes in the concentration of Na alone in the range of 0.3–0.5 M had no appreciable effect on the flow rate, adjustment of osmotic pressure was done by varying the amount of NaCl. Namely, when an extra amount of KCl, CaCl₂, or MgCl₂ was added, the concentration of NaCl was decreased so that the osmotic pressure of the solution was kept at the level of the original sea water. On the contrary, when the concentration of one of the above salts was brought to a subnormal level or was reduced to zero, NaCl was added in the amount which is osmotically equivalent to the decreased amount of the salt in question.

Results

I. Effect of cations on the rate of streaming.

a) Potassium ion: When the KCl concentration was changed from the normal level, that is 0.01 M, the rate of flow was modified quickly. The time courses of changes in velocity caused by modification of K concentration below or above normal level are shown in Fig. 1. In the sea water containing 0.005 M KCl, which is half the normal concentration, the streaming showed the same tendency as in the K-free sea water, except that the suppression of the streaming rate was less pronounced.

It is noteworthy that when the KCl concentration was raised above the level of normal sea water, the rate of flow also tended to drop. In the medium containing

Fig. 1. Effect of the concentration of K ion on the protoplasmic streaming of *Acetabularia calyculus*. Experimental solutions: Sea water containing (a) 0.047 M, (b) 0.023 M, (c) 0.013 M, (d) 0.005 M and (e) no K. Vertical lines indicate the time of medium replacement.
0.047 M KCl, which is about five times as high as the K concentration of the normal sea water, the flow was retarded conspicuously, which was soon followed by complete cessation. When replaced with the sea water, however, streaming was restored to the control level as seen in Fig. 1. Thus, the effect is perfectly reversible. It is concluded from these results that the rate of flow decreases, no matter whether the concentration of K is raised or lowered from the normal level of sea water. In other words, the K concentration of the normal sea water is optimal for the protoplasmic streaming.

b) Calcium ion: Varying the concentration of bivalent cations also showed conspicuous effect on the protoplasmic streaming. When Ca was removed partially or entirely from the sea water, the rate of flow decreased invariably (Fig. 2). In the sea water, whose Ca concentration was half that of the control, the rate of flow was dropped slowly.

At the concentration of 0.045 M, which was about five times as much as the normal Ca content, the velocity of flow increased immediately up to 150% of the control after 4 minutes. At the concentration of 0.023 M, i.e., 2.5 times of the normal Ca level, the rate also increased to an extent as great as 185% of the control after 5 minutes. Here again the effect is perfectly reversible, since the initial rate was soon restored after the cell was brought back to the original sea water, regardless of the previous Ca concentration in the experimental medium (Fig. 2).

c) Magnesium ion: Contrary to the case of Ca, lowering of Mg concentration causes acceleration of the streaming (Fig. 3). The flow was activated to about 160% of the normal rate in 4 minutes, after Mg concentration was lowered to 0.013 M, or half the concentration in the sea water. An extra amount of Mg (MgCl₂ + MgSO₄), however, gradually retarded the streaming. In the sea water containing 0.126 M Mg which is five times as high as the normal concentration, the streaming came to a complete stop within 5 minutes. At the concentration of 0.063 M, or 2.5 times as
high as the normal concentration, the streaming could occur slowly with the speed of 20% of the normal rate. The effect is thus quite opposite to that of Ca.

II. Morphological changes.

Ca and Mg ions brought forth also conspicuous morphological changes in cytoplasm, which were again quite opposite to each other. In order to facilitate observation, the stalk of Acetabularia was centrifuged at 600 g for 10 minutes in the sea water so that the majority of chloroplasts, which hinder the detailed observation of the cytoplasm, was collected at the centrifugal end of the cell. Under
Fig. 5. Effect of the concentration of Ca on morphological changes of the cytoplasm of the stalk of *A. calyculus* after the centrifugation (600 g, 10 minutes) in sea water. Dark field.

a: A centrifuged stalk after 10 minutes of incubation in the sea water containing 0.045 M CaCl₂ (five times as high as the normal concentration). Thin streams united one another to form thicker, but fewer number of streams. C: cluster of chloroplasts ×550.
b: A centrifuged stalk after 50 minutes of incubation in the same medium as above. The united streams formed large balls (B) by incorporating clusters of chloroplasts. ×270.
c: A centrifuged stalk after 10 minutes of incubation in Ca-free sea water. Vacuolization (arrow) took place in plasmol. ×550.
such a condition, protoplasmic streaming was observed to occur along many well-
distinguished striations running in parallel to the longitudinal axis of the cell (Fig.
4). When the concentration of Ca was made higher than the normal, however, several
thin streams united one another to form thicker, but fewer number of streams (Fig.
5a). Those streams finally formed aggregates by incorporating clusters of chloro-
plasts still remaining after centrifugation (Fig. 5b).

Ca-free sea water, on the other hand, gave rise to vacuolization in plasmasol
which had been streaming along parallel striations. After 45 minutes, plasmasol
completely stopped moving. In this case chloroplasts remained scattered without
forming aggregates (Fig. 5c).

In Mg-rich sea water, the flowing plasmasol was vacuolized and eventually ceased
to flow, just as in Ca-free sea water. The behavior and the morphological changes
of the cytoplasm in Mg-free sea water were almost indistinguishable from those in
Ca-rich sea water.

Thus, it was demonstrated that Ca and Mg ions exert exactly opposite effects on
the visible morphological changes of the cytoplasm, as was seen in their effect on
the rate of streaming.

Discussion

The foregoing experiments show clearly that the rate of protoplasmic streaming
in the stalk of Acetabularia always drops down, no matter whether the external K
concentration is raised above or lowered below the normal level. The effect is per-
fectedly reversible as the initial rate was restored in a few minutes after the material was
brought back to the original sea water of the standard constitution. Thus, the author
knows from the above observation that the optimum concentration of K for the stream-
ing in A. calyculus is around 0.01 M, which is the K level in the sea water. As has
been mentioned before, it is to be noted in this connection that varying Na concen-
tration from 0.3 to 0.5 M did not show any effect on the rate of streaming.

Bivalent cations exert also conspicuous effect on the streaming. In Ca-rich sea
water the streaming is accelerated, whereas in Mg-rich sea water it is retarded. In
Ca-poor sea water, on the other hand, the streaming is retarded, but in Mg-poor sea
water it is accelerated. These facts suggest that the importance of balance between
Ca and Mg ions for the streaming in this organism. As described in the foregoing
section, the opposite effects of these two ions are manifested also in morphological
changes in the cytoplasm. Namely, in both Ca-rich and Mg-poor sea water, fewer
but thicker streams are formed from numerous thin streams, and further the thicker
streams tend to fuse locally with each other, finally giving rise to large balls by in-
corporating chloroplasts. In contrast to the above cases, when the streaming is
retarded under the influence of Mg-rich or Ca-poor sea water, distinct streams of the
normal cytoplasm become gradually obscure. Later vacuolization takes place and
streaming comes to a complete stop.

On the basis of some of his experiments on Acetabularia, Takata3, 4 suggested
the presence in this plant of ATP-sensitive protein which constitutes the mechno-
chemical system responsible for protoplasmic streaming. If we assume, from this
standpoint, that the protoplasmic streaming is caused in the last analysis by the
interaction between ATP and the ATP-sensitive protein, the opposite effects of Ca
and Mg ions on the streaming may be explained either by their opposite effects on
ATPase activity of the ATP-sensitive protein, or by their effects on the physical
nature of the protein molecules *per se*.

**Summary**

1) The protoplasmic streaming in the stalk of *Acetabularia calyculus* was influenced markedly by changes in the concentration of K, Ca, and Mg ions in the external medium (sea water).

2) The optimum concentration of K for the streaming was found to be around 0.01 M. The rate of flow decreased at higher as well as lower concentrations.

3) An additional amount of Ca ion in the external medium increased the rate of streaming, whereas subnormal Ca concentration retarded the streaming.

4) Contrary to Ca, higher concentration of Mg ion caused a decrease in the rate of flow, while lowering the Mg concentration below the normal level accelerated the streaming.

5) It was found that Ca and Mg ions exert exactly opposite effects on morphological changes of the cytoplasm as they did on the rate of streaming.

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**References**


**摘要**

佐藤妙子：ホソケガサ *Acetabularia calyculus* の原形質流動におよぼすカリウム、カルシウム、およびマグネシウムイオンの影響

1) ホソケガサの原形質流動は、外液の K, Ca, Mg イオンの濃度の変化によって著しく影響される。
2) 流動にたいする K イオンの最適濃度は、正常海水中の K イオン濃度、すなわち 0.01 M 付近にある。濃度がそれより高くなるも、あるいは低くなるも、流速は減少する。
3) 外液の Ca イオン濃度を正常海水より高くすると、流運動は促進され、低くすると抑制される。
4) Ca イオンとは反対に、Mg イオンは、その濃度を正常海水よりも高くすると、流速は減少し、低くすると増加する。
5) Ca イオンと Mg イオンは、流速にたいしてのみならず、細胞質の形態的変化にたいしても、相互にあい交する影響をおよぼす。（大阪大学理学部生物学教室）