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Some Experiments on the Movement of the Myxomycete Plasmodium

Chikako Fujiki (藤木千賀子)

Department of Biology, Faculty of Science,
Ochanomizu University
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Introduction

Plasmodia of Myxomycete have various favorable characteristics for conducting experiments on protoplasmic streaming. From the plasmodia of *Physarum polycephalum*, Hatano isolated contractile proteins such as actin, myosin B and myosin A (Hatano et al., '66, '67). Hatano and Takeuchi showed the existence of ATP in the normal plasmodium at the concentration of 0.4×10^{-3} M (Hatano and Takeuchi, '60). In this connection, it is known that protoplasmic streaming was accelerated by the addition of an appropriate concentration of ATP (Kamiya et al., '57). From these facts it has been noticed that plasmodia may have a motile system similar to that of muscle.

The technique of glycerination, first developed by Szent-Györgyi (1951), is an effective method for making chemical studies of muscle physiology. It has subsequently been applied not only to muscle but also to other cells such as amoeba, spermatozoa and sea urchin eggs (Simard-Duquesne and Couillard, '62; Hoffmann-Berling, '60; Kinoshita, '66). Ohta first applied this technique on the plasmodia of *P. polycephalum* ('60). Later Kamiya developed a further technique for glycerinating plasmodia and reported that contraction took place in definite directions according to the loci. These contraction areas are confined to a zone with a moderate width along the advancing margin of the fan-like expanse and in the wall of the strand part.

In this paper, the effect of ATP on the contraction of microplasmodia has been studied with the technique of glycerination. As for microplasmodia, it is known that first there is no sign of streaming in them, but as incubation time elapses on non-nutrient substratum, they gradually begin to stream. To clarify the mechanism of transition between the immotile state and the motile one, the technique of glycerination was employed and the results were compared with electron micrographs.

Materials

Microplasmodia—The organism was grown in the form of microplasmodia in agitated submerged culture according to a slight modification of the method of Rusch (Daniel and Rusch, '61 ; Ohta et al., '63). Shapes of the organisms were round or dumb-bell (Fig. 1). Diameters of these organisms ranged from about 50 μ to 200 μ.

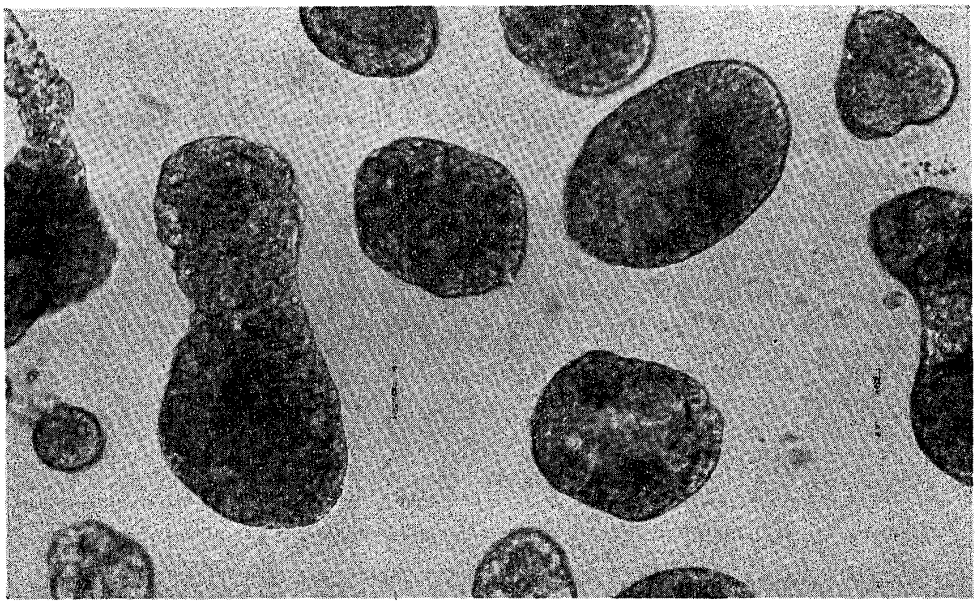

Fig. 1. Microplasmodia (×300).

Plasmodia—Plasmodia were cultured on rolled oats in plastic vessels (Hatano, '66).

Methods

Preparation of glycerinated models—The microplasmodia were sparsely placed on a sheet of non-nutrient 4% agar on a slide-glass with a small paint-brush. Then these microplasmodia were incubated in the humid vessel at a temperature of 23°C. After a definite incubation period, these plasmodia were covered with a sheet of cellophane membrane and thin agar sheet, and sealed with lukewarm molten agar according to Kamiya's method ('65). Then the whole block was submerged into the extraction medium containing 40% glycerol, 10 mM Tris maleate buffer (pH 7.1), and 10 mM ethylenediaminetetraacetate (EDTA). Extraction was carried out at a temperature of 0°C for one day and then these samples were transfered to new extraction medium at −15°C∼−20°C for a week.

Measurement of contraction—The glycerinated models were washed with 60 mM KCl for a few minutes at room temperature and photographed in a small amount of KCl; the amount of this KCl solution

was kept constant so as not to change the photographic magnification throughout the experiment. Then KCl was removed and test solution was added, which contains 5 mM $ATPNa_2$, 5 mM $MgCl_2$, 30 mM KCl. 2 min after the addition of test solution, the samples were photographed again. Differences of areas before and after the addition of ATP were determined from the photographs, using a planimeter. This difference was used as the index of contraction.

Measurement of ATPase activity—Microplasmodia or surface plasmodia were dropped into a sufficient amount of aceton and dried up. About 1 g of the organisms were harvested and put into ca. 200 ml of aceton with vigorous stirring, and filtered through a glass filter (No. 2). After aspiration in a desiccator to disperse the acetone, the preparation was stocked in a desiccator at room temperature. The powder weight was about one-twentieth of the fresh weight. The composition of the reaction solution used was as follows: 4 ml of Veronal acetate buffer (pH 6.0), 5 ml of solution containing 6.6×10^{-3} M $CaCl_2$ and 6×10^{-1} M KCl, and 1 ml of 50 micromoles ATP (Nakajima, '60). The reaction solution without ATP was preincubated for 5 min. About 5 mg of powder was incubated in 10 ml of reaction solution at 28°C for 15 min. Then the reaction was stopped by adding 2 ml of 20% trichloroacetic acid (TCA). In every experiment, a control experiment was run with 2 ml of 20% TCA added prior to the incubation. The precipitate was removed by centrifugation (4,000 rpm×5 min), and the supernatant was assayed by the measurement of inorganic phosphate according to the method of Fiske and Subbarow ('25).

Extraction of myosin B—All extraction procedures were carried out by Hatano's method ('67). About 70 g of surface plasmodia were used at one time. The microplasmodia used at one time were also about 70 g. As there were slime and growth medium around them, they were calculated ca. 40 g in terms of surface plasmodia.

Electron micrographs—Samples of myosin B were prepared in 0.05 M KCl solution with 10 mM Tris maleate buffer (pH 7.0). After negative staining with 1% uranyl acetate, these were investigated at a magnification of 15,000.

Protein concentration—The nitrogen content was determined by the micro-Kjeldahl method. To determine the protein concentration, a factor of 6.25 was used.

Measurement of viscosity—An Ostwald viscometer was used, of which the volume was 1.0 ml. The outflow times of solvent and protein solution were 30 sec and 31 sec, respectively. The volume of reagents added was 0.1 ml.

Results

Streaming in microplasmodia

Microplasmodia immediately after transplantation from the agitating culture onto agar do not show any streaming, but as time passes, they begin to exhibit streaming. Microplasmodia were observed at the various periods of incubation (Fig. 2). At the time when the organisms

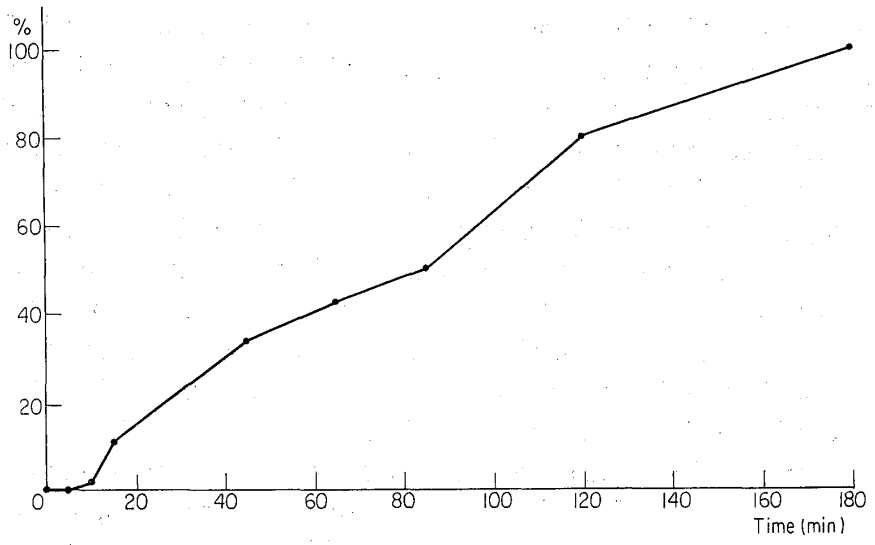

Fig. 2. Increase in number of streaming microplasmodia with time transferred to agar, abscissa: incubation time (min), ordinate: percentage of streaming microplasmodia against all microplasmodia on agar. All microplasmodia were in the immotile state at 0 min and in course of time, streaming microplasmodia increased.

were placed on agar, streaming could not be found in any microplasmodium. These organisms were composed of fine granules and these granules were used as the indication of streaming. After 15 min incubation, some organisms began to show streaming. About around 45 min on agar, streaming was found in many microplasmodia. By the end of 2 hours' incubation, almost all microplasmodia were in motile state.

Movement of microplasmodium on agar

Movement of microplasmodium on agar was studied by microphotographs and its area of every 5 min was measured. Although no distinct streaming was observed in it, microplasmodium changed its area little by little (Fig. 3). In the course of time, round microplasmodia gradually began to extend flabby pseudopodia and dumb-bell shaped ones became round. Their areas gradually increased and attained the maximum after about 15 min incubation. Then it began to decrease. At about 45 min, the area of the microplasmodium dwindled down to its original extent. This tendency of decreasing continued to 60 min. At that time, distinct rhythmic streaming was found in the organism.

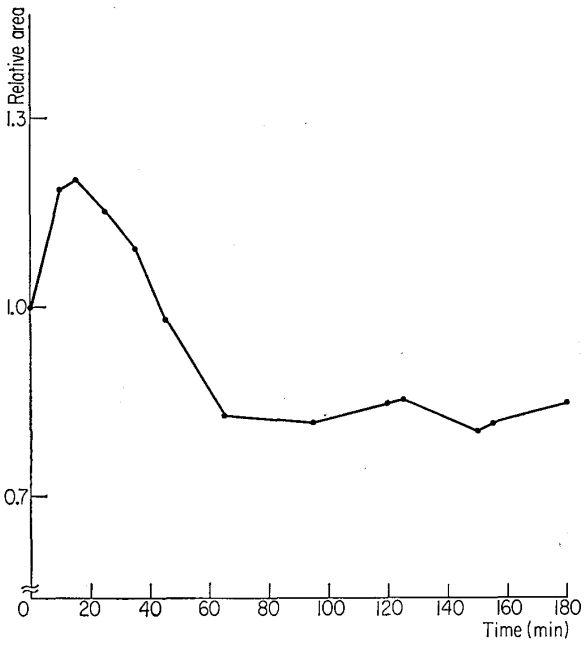

Fig. 3. Change in area of microplasmodium with time, abscissa: incubation time (min), ordinate: relative area; the original size of area was defined as 1.

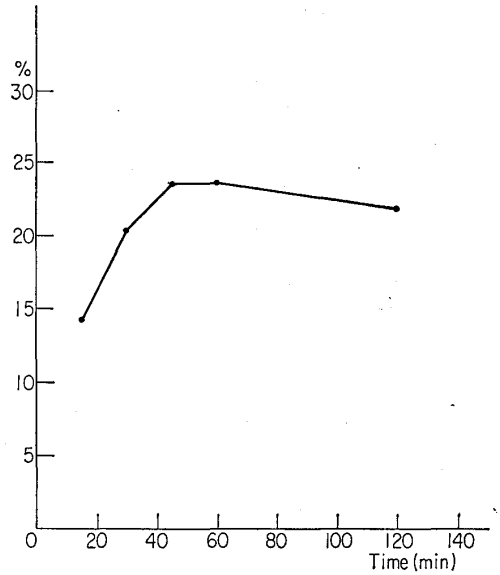

Fig. 4. Rate of contraction with increasing incubation time, abscissa: incubation time (min), ordinate: rate of contraction.

Contraction of glycerinated models

From above results, distinct changes in microplasmodial area were found at 0 min, 15 min, 45 min and 120 min after incubation. Therefore, the organisms were glycerinated at the above-mentioned intervals. At 0 min, however, the microplasmodia could not adhere to the agar, so that it was difficult to measure their contraction. Fig. 4 shows the contraction of glycerinated models induced by 5 mM ATP solution. It can be said that the contraction rate rose with time of incubation. AMP or inorganic pyrophosphate had little effect on the contraction of these models.

ATPase activity in microplasmodia and surface plasmodia

It has been established that contractile proteins in plasmodium have ATPase activity. As has been mentioned, microplasmodia showed no significant movements, but the surface plasmodia showed vigorous streaming. Therefore the difference of ATPase between the two were examined. Results are shown in Table 1. Pi values liberated by ATPase in microplasmodia are much higher than that of surface plasmodia. In parallel with this experiment, histochemical analyses of ATPase localization in a microplasmodium were carried out by Usui (Fig. 5). In this micrograph, Pi splitting by ATPase appeared mainly in mitochondria, on the nuclear membrane and on the protoplasmic membrane. Therefore, it is likely that Pi splitting is mainly due to

Table 1. ATPase activities of microplasmodia and surface plasmodia.

Exp.	Pi(γ)/0.5 mgN	
	Microplasmodia	Surface plasmodia
1	3.34	1.53
2	4.02	1.53
3	4.75	1.23

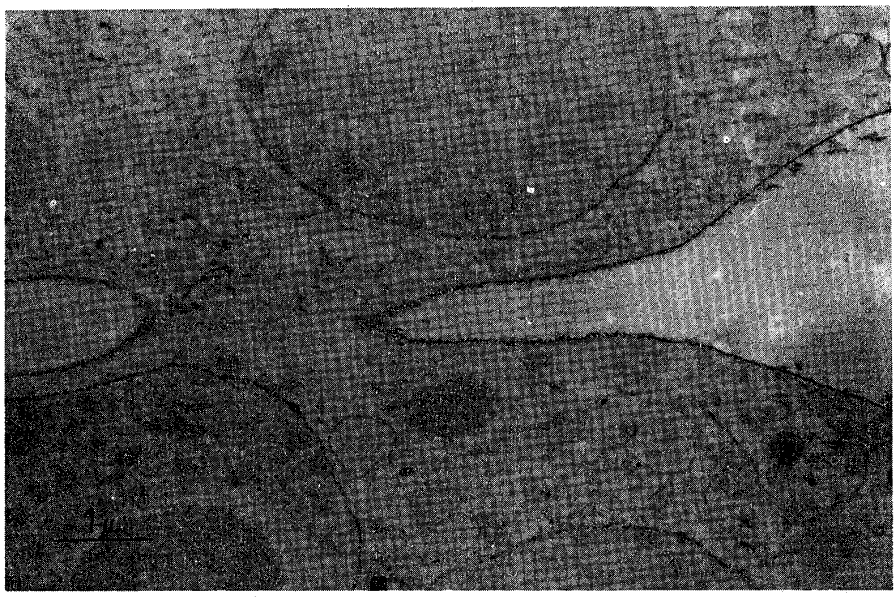

Fig. 5a. Cytochemical demonstration of ATPase activity in an immotile microplasmodium (0 min microplasmodium). The electron opaque deposites are the enzyme reaction products. (Courtesy of Miss N. Usui)

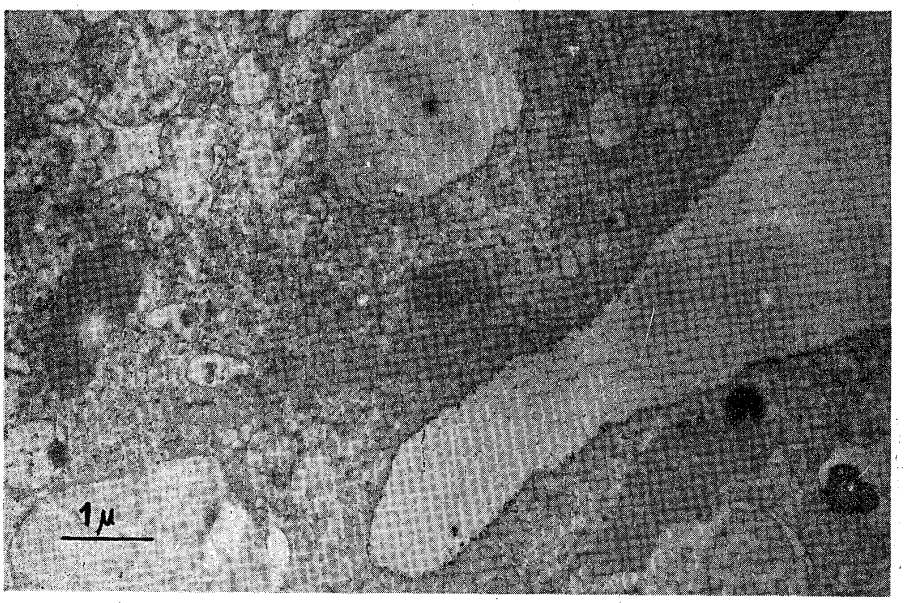

Fig. 5b. ATPase activity in a microplasmodium after 45 min incubation. The fibers also can be observed along the periphery. (Courtesy of Miss N. Usui)

membranous structures and not due to the ATPase of fibers. This point will be discussed later.

Myosin B content in microplasmodia and surface plasmodia

Out of 72 g of frozen surface plasmodia, ca. 21 mg of protein expected as myosin B were collected. The protein in 0.5 M KCl solution had ATPase activity of ca. 60% (protein concentration, 0.56 mg/ml, final con. of ATP, 0.9 mM). This value is not so high as the myosin B isolated by Hatano. As far as the micrographs revealed, however, the present myosin B was associated with some granular components (Fig. 6). Although these granules have not yet been identified, they are likely to be some fragments of mitochondria or other organelles.

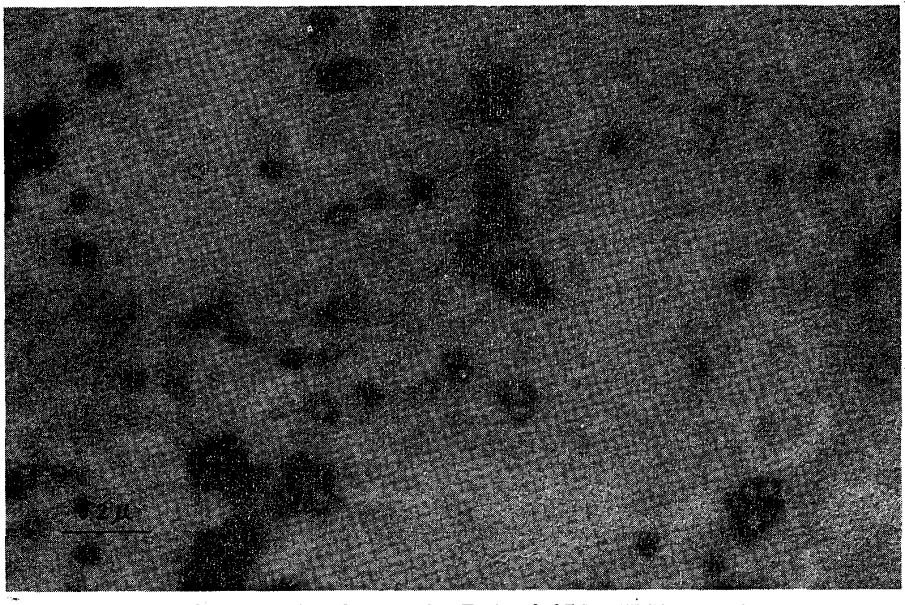

Fig. 6. Electronmicrograph of myosin B in 0.05 M KCl solution with 10 mM Tris-maleate buffer (pH 7.0).

Then a trial of myosin B isolation from microplasmodia was carried out. About 75 g of microplasmodia gathered by centrifugation (4,000 rpm $\times$5 min) were used at one time. Nevertheless, no myosin B was isolated from microplasmodia, although the same isolation procedures were applied. The absence of myosin B fibers was also confirmed by micrographs.

The proteins in questions have been observed as fibers in surface plasmodia by electron micrographs (Wohlfarth-Bottermann, '64). In microplasmodia, however, fibers could not be found. But in the case of microplasmodia after 45 min incubation, fibers existed along the protoplasmic membrane (Fig. 5a, b).

Discussion

The present experiment indicated that the contraction rate rose higher with time of incubation and attained a plateau by the end of 45 min incubation. Microscopically, it was also observed that a feeble streaming developed into vigorous one with time. Therefore these two phenomena seem to have a close relation. It has been also shown that microplasmodial area grew large from the beginning to the end of 15 min incubation and since then, it began to diminish to the original extent. This observation suggests that a reconstitution of the inner structure may occur in the protoplasm. All these facts indicate the reorganization or remaking of the contractile systems which are believed to cause the streaming. Microplasmodia showed much higher ATPase activity than surface plasmodia. The proportion of membrane against the volume may be much higher in microplasmodium than surface plasmodium, so that this higher activity may be mainly due to the membranes.

From microplasmodia, proteins could not be obtained as myosin B. Moreover, in electron micrographs of microplasmodium, fibrous structures were not found at the beginning of incubation on agar, but after 45 min incubation, bundles of fibers clearly appeared (Usui, unpublished). From these evidences it seems probable that such fibers were broken down in microplasmodium in shaking culture and in course of time on agar they became reorganized.

The transition from an immotile state to a motile one has been reported in other organisms such as *Nitella* or *Chara* (Yotsuyanagi, '53). This phenomenon also seems to be explained from the standpoint of fiber reorganization. A further proof of this assumption remains in future.

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Summary

1. Glycerinated models of microplasmodia of the Myxomycete, *Physarum polycephalum*, were prepared and contraction induced by ATP was investigated.

2. The contraction rate rose with time of incubation of microplasmodia.

3. Microplasmodia changed their areas and adhered actively onto agar by the end of 15 min incubation.

4. Number of streaming microplasmodia increased with time on agar.

5. Myosin B could not be isolated from microplasmodia.

6. It has been suggested that contractile systems were reorganized in microplasmodia with time.

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