A MYOSIN-LIKE PROTEIN IN THE CORTICAL LAYER OF THE SEA URCHIN EGG

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INTRODUCTION

An interaction between actin- and myosin-like proteins and its implication in cellular motilities have been suggested in a variety of cells other than muscle (reviewed by Taylor, 1972, and by Rebhun, 1972). Actually, those proteins have been extracted and purified from certain kinds of cells. They may be located mainly in the cell cortex (Ishikawa et al., 1969).

In sea urchin eggs, although an actin-like protein which could interact with rabbit skeletal muscle myosin in a manner similar to muscle actin was extracted and purified from acetone powder of whole cell (Miki-Noumura and Oosawa, 1969), an actomyosin-like component has not been detected in the isolated cortex (Mabuchi and Sakai, 1972). However, extensive studies on this line have confirmed that a myosin-like component was contained in the 0.6 M KCl-extract of the isolated cortex of the sea urchin egg. It will be called "ovomyosin" in this paper.

MATERIALS AND METHODS

Eggs of the sea urchin, *Pseudocentrotus depressus*, were obtained by 0.1 M acetylcholine-induced spawning. Insemination, removal of the fertilization membrane, and isolation of egg cortices were the same as reported previously (Mabuchi and Sakai, 1972).

Temperatures between 0 and 4°C were maintained throughout the following procedure. Isolated cortices of anaphase eggs were dialyzed against 1 mM ATP–1 mM GSH–10 mM Tris-HCl (pH 7.2) for 36 h and centrifuged at 15,000 *g* for 15 min. Pelleted cortices were washed once with distilled water and homogenized after addition of 0.25 vol of 3 M KCl–50 mM

Tris-HCl (pH 8.2)–50 mM β-mercaptoethanol (ME). The suspension was allowed to stand overnight and was centrifuged at 20,000 *g* for 15 min. The pellets were once more extracted with 0.6 M KCl–10 mM Tris-HCl (pH 8.2) containing 10 mM ME (KCl-Tris-ME). To the first and the second supernates combined, were added 0.4 M ATP (pH 7.0) and 0.1 M $MgCl_2$ to final concentrations of 10 and 1 mM, respectively. This solution was then centrifuged at 260,000 *g* for 1.5 h (Hitachi 65P ultracentrifuge, RP65 rotor). The supernate was adjusted to 55% saturation with respect to ammonium sulfate. The precipitates were collected by centrifugation at 10,000 *g* for 15 min, dissolved in KCl-Tris-ME, and clarified by centrifugation. The supernate was called the "55% SAS fraction." Subfractions of the 55% SAS fraction were prepared by a stepwise dialysis for a minimum of 8 h against 3 mM Tris buffer containing successively 0.2 M KCl (pH 7.0), 0.15 M KCl (pH 7.5), and 0 M KCl (pH 7.2). The first, the second, and the third precipitates were dissolved in KCl-Tris-ME and clarified by centrifugation at 10,000 *g* for 10 min. They were called the "0.2-fraction," "0.15-fraction," and "0-fraction," respectively. The final supernate was called "0-sup."

The standard assay of ATPase activities was carried out in a total volume of 2 ml, which included 20 mM Tris-maleate (pH 7.0), 0.5 mM ATP, 5 mM $CaCl_2$ or $MgCl_2$, and 50 mM KCl. The mixture was incubated at 25°C for 30 min. Inorganic phosphate liberated was determined as reported previously (Mabuchi, 1973).

Analytical ultracentrifugation was performed on a Spinco model E ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 59,780 rpm.

Electrophoresis was carried out on polyacrylamide gels containing sodium dodecyl sulfate (SDS) according to Weber and Osborn (1969) with some modifications. The concentration of acrylamide was 7.5% containing 25 mM Tris-glycine (pH 8.5), 0.05% SDS, and 5% glycerol. Gelation was promoted with light in the presence of riboflavin. Samples were first dialyzed overnight at room temperature against a solution of 8 M urea, 0.5% SDS, 0.1 M ME, 5 mM EDTA, and 20 mM Tris-HCl (pH 8.5). Alkylation was then performed by dialysis for 2 h against a solution of 20 mM *N*-ethylmaleimide, 8 M urea, 0.5% SDS, 5 mM EDTA, and 20 mM Tris-maleate (pH 6.7), followed by further dialysis against the first dialyzing solution to stop alkylation and remove the reagent.

Rabbit skeletal muscle myosin was prepared according to Perry (1955). Actin was prepared from myosin-extracted muscle debris according to Mommaerts (1952) and stored in 0.1 M KCl-50 μM ATP-0.5 mM Tris-HCl (pH 7.4) in the F-form at 0°C. It was used within a week.

Protein was determined by the method of Lowry et al. (1951).

RESULTS

ATPase Activity in the Subfractions of the 55% SAS Fraction

Mg^{2+} activated ATPases involved in the whole cortex, the KCl-extract, and the 55% SAS fraction more than did Ca^{2+} as reported previously (Mabuchi, 1973). This was mainly due to Mg^{2+} activation of "cortical ATPase" which had properties similar to dynein (Mabuchi, 1973). On the subfractionation of the 55% SAS fraction, this ATPase came to the 0-fraction (Table I), which was apparent from the Ca:Mg activity ratio (about 0.9), and comprised the major part of the ATPase activity of the 55% SAS fraction.

On the contrary, it was found that activation of ATPase of the 0.2-fraction was greater by Ca^{2+} than by Mg^{2+} (Table I). It was this ATPase activity in this fraction that was due to a myosin-like protein, ovomyosin, as could be confirmed later. Thus, ovomyosin was responsible for about 10% of the total Ca^{2+}-activated ATPase activity in the 55% SAS fraction, which corresponded to about 5% of the total Ca^{2+}-activated ATPase activity of the whole cortex.

Interaction between Ovomyosin and Muscle F-Actin

The viscosity of the 0.2-fraction increased on addition of rabbit skeletal muscle F-actin to a level higher than the sum of the viscosities of unmixed 0.2-fraction and F-actin (Fig. 1). A significant ATP-response was observed: the viscosity rapidly decreased on addition of ATP (final 30 μM) and then slowly recovered to some extent (Fig: 1). On the other hand, the 0.2-fraction alone did not show any viscosity change by addition of ATP. The fall and rise in viscosity could be repeated at least three times. However, full recovery of the reduced viscosity to its original level could not be attained. This was due to the fact that F-actin alone gradually reduced in viscosity when transferred from 0.1 to 0.6 M KCl (Fig. 1). The ATP-sensitivity (Portzehl et al., 1950) of the mixture lay between 40 and 64%.

To further confirm the myosin-like properties,

TABLE I

ATPase Activity of Subfractions of 55% SAS Fraction

Fraction	Total protein*	Specific activity		Total activity	
		Ca^{2+}-ATPase	Mg^{2+}-ATPase	Ca^{2+}-ATPase	Mg^{2+}-ATPase
	%	*μmol P_i/mg protein/min*		%	
0.2-fr.	7.3	0.018	0.0084	11.7	5.5
0.15-fr.	4.2	0.013	0.013	4.9	4.9
0-fr.	52.6	0.015	0.017	70.5	80.0
0-sup	35.9	0.004	0.003	12.8	9.6

* The small amount of insoluble protein which was centrifuged off to clarify each fraction (see Materials and Methods) was omitted.

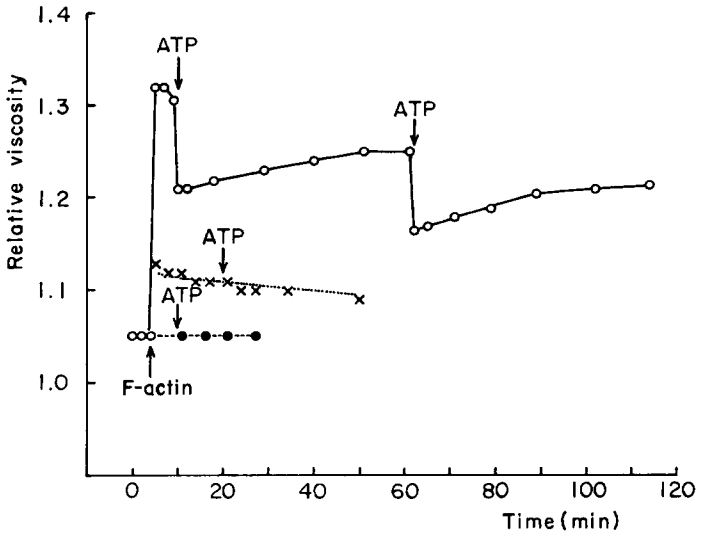

FIGURE 1 ATP-response of the mixture of 0.2-fraction and rabbit F-actin. An Ostwald-type viscometer which had an outflow time on water of 30 s and a capacity of 0.3 ml was used. Temperature was controlled at 25 ± 0.2°C. To 0.3 ml of 0.2-fraction (0.45 mg/ml in 0.6 M KCl–20 mM Tris-maleate [pH 6.8]) was added 10 μl of F-actin solution (6.1 mg/ml in 0.1 M KCl–50 μM ATP–0.5 mM Tris) at the time indicated by the arrow followed by the addition of 3 μl of 3.3 mM ATP (○—○). As a control, viscosity changes of the 0.2-fraction alone (●- - -●) and of the F-actin solution (0.3 ml of KCl-Tris-maleate plus 10 μl of F-actin) (×····×) were measured in a similar manner.

tests for the superprecipitation were carried out. Muscle F-actin and the 0.2-fraction were dialyzed against 0.6 M KCl-10 mM Tris-maleate (pH 7.0). To the former (6.0 mg/ml) was added 3.3 vol of the latter (5.0 mg/ml) followed by dilution with 1 mM $MgCl_2$ ten times. When ATP was added to the mixture to a final concentration of 0.1 mM, an apparent superprecipitation occurred. When the changes in turbidity were measured 15 min after the addition of ATP at 25°C, increases in $OD_{660\ nm}$ were around 20% compared with the control.

Molecular Weight and Sedimentation Constant of Ovomyosin

Fig. 2 shows the electrophoretic patterns on SDS-polyacrylamide gels of whole cortices, 0.2-fraction, and rabbit skeletal muscle myosin. The mobility of the main band of the 0.2-fraction was identical with that of the heavy chain of skeletal muscle myosin, the molecular weight of which was 200,000 (reviewed by Taylor, 1972). Because of the incomplete purity of the present ovomyosin fraction, it is not evident at present whether

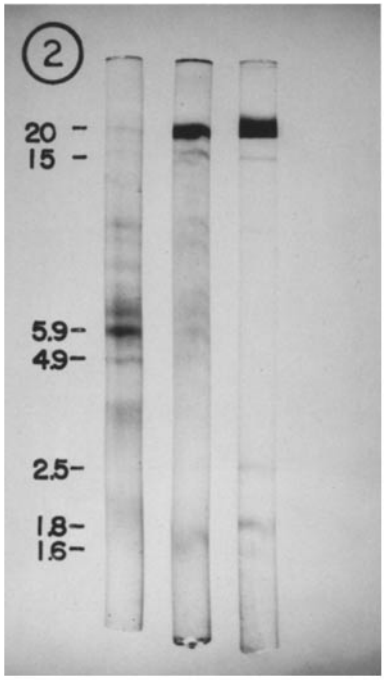

FIGURE 2 Electrophoretic pattern of ovomyosin. Gels were stained with a solution of 0.001% Coomassie brilliant blue, 10% acetic acid, and 40% methanol. *Left:* whole isolated cortices; *middle:* 0.2-fraction; *right:* rabbit skeletal muscle myosin. Numbers at the left of the gels indicate the molecular weight $\times 10^{-4}$.

ovomyosin has moieties that correspond to the light chains of skeletal muscle myosin, although an L_2-like band appeared (mol wt, 18,000). An actin-like band was not observed in the 0.2-fraction at all. However, the actin-like band (mol wt, 49,000) did appear with the whole cortex in addition to the myosin-like band. From the densitometric trace of the gel, the amount of ovomyosin heavy chain was calculated to correspond to about 1% of total protein of the cortex. The main component (mol wt, 59,000) in the whole cortex may be tubulin. The electrophoreses of 0.15-, 0-, and 0-sup fractions showed the absence of the myosin-like band.

Analytical ultracentrifugation of the 0.2-fraction revealed a single, hyper-sharp sedimentation boundary which is characteristic among muscle myosin. The sedimentation coefficient ($s_{20,w}$) was 6.3S at 1.8 mg protein/ml. Shoulders appeared on both sides of the peak, indicating some contaminants of both heavier and lighter components in this fraction.

Aggregates of Ovomyosin

The 0.2-fraction was dialyzed against 0.2 M KCl-3 mM Tris-HCl (pH 7.7) to obtain precipitates; these were sufficiently dispersed by pipetting and were observed with an electron microscope through negative staining. Typical bipolar aggregates for myosin (Huxley, 1963; Kaminer, 1969) were predominant as shown in Figure 3 *a*. A bare central zone and gross projections at the ends were clearly observed (Fig. 3 *a–d*). The length of the aggregates was 3,700 ± 200 Å and the width of the bare central zone was 170 ± 20 Å. Unit filaments could be seen in the bare zone, the diameter of which was 15–20 Å (Fig. 3 *b–d*). In some preparations, the loose aggregates were formed as shown in Figure 3 *e*, where unit filaments each having a head (arrow) could be seen.

DISCUSSION

A myosin-like protein, ovomyosin, found in the egg cortex of the sea urchin, is identical with rabbit skeletal muscle myosin with respect to sedimentation coefficient (6.3S) and molecular weight of its heavy chain (200,000). Moreover, ovomyosin forms bipolar aggregates at low ionic strength, as does muscle myosin. The protein reacts with rabbit skeletal muscle actin in a manner similar to muscle myosin, although the interaction seems less strong (low ATP-sensitivity, low degree of superprecipitation). This may be due to the impurity of the fraction as well as the difference in protein sources. The low reactivity of the present ovomyosin is consistent with the fact that ovoactin (also found in the sea urchin egg) shows low reactivity with rabbit skeletal muscle myosin with respect to ATP-sensitivity and Mg^{2+}-dependent activation of ATPase (Miki-Noumura and Oosawa, 1969).

Specific activity of ovomyosin is considerably lower than skeletal muscle myosin. It is not likely that the enzyme activity was lost by auto-oxidation during its preparation because it was constantly protected by thiols. A possible explanation is that the enzyme of the embryonic cell has lower specific activity than vertebrate skeletal muscle myosin. The Ca:Mg activity ratio of the ovomyosin fraction is smaller than that of muscle myosin or other myosin-like proteins. In the latter cases, the ratio is very high while it was only about 2 with the 0.2-fraction; this seems to indicate contamination by the cortical ATPase in the fraction.

Evidence has recently been presented demon-

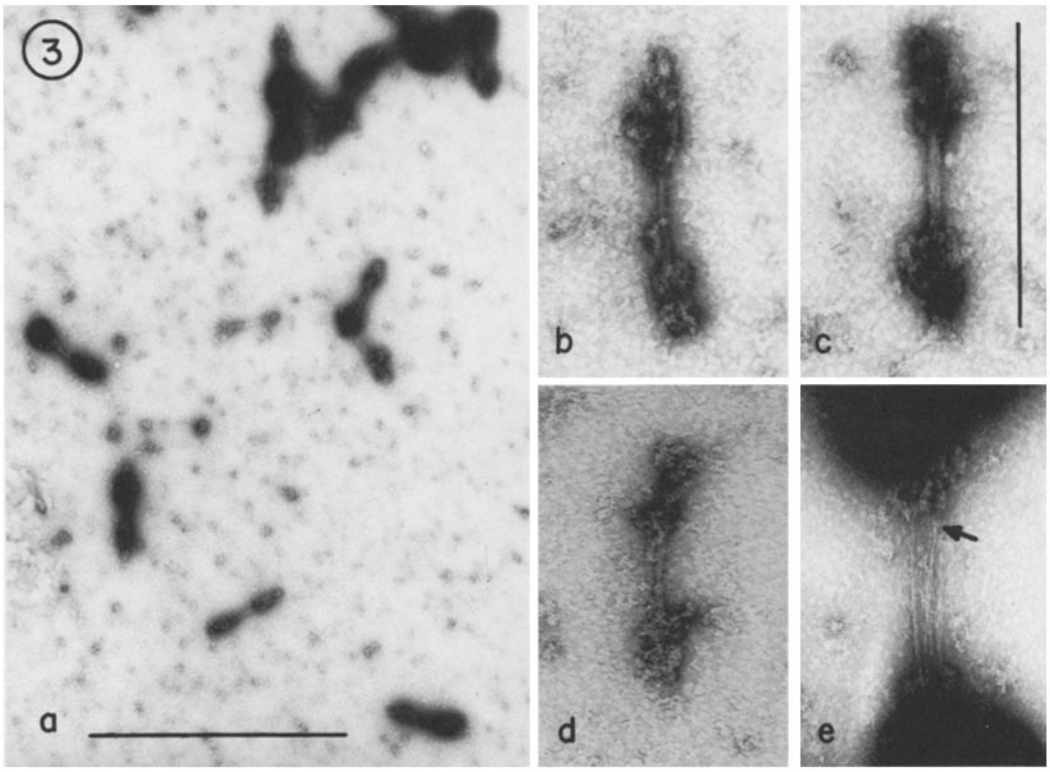

FIGURE 3 Aggregates of ovomyosin. Negative staining was performed with 1% uranyl acetate. 0.2-fraction was dialyzed against 0.2 M KCl–3 mM Tris-HCl (pH 7.7). *a-d*: typical bipolar aggregates. *e*: a loose aggregate. (*a*) × 34,000. Scale, 1 μm. (*b-e*) × 100,000. Scale, 400 nm. The arrow indicates a head portion of an unit filament.

strating that bundles of microfilaments run parallel to the equatorial cortical layer of dividing cells (reviewed by Rappaport, 1971); these have been considered to be actin-like filaments (Perry et al., 1971). Although the localization of ovomyosin in the cytoplasm cannot be denied, it is reasonable to conclude that it exists mostly in the cortical gel layer. In addition, existence of ovomyosin in isolated cortices of metaphase starfish eggs has also been confirmed (Mabuchi, unpublished). Further analyses are required for the demonstration of an interaction between the microfilaments and ovomyosin.

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