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Actin and Myosin in Pea Tendrils[1]

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ABSTRACT

We demonstrate here the presence of actin and myosin in pea (*Pisum sativum* L.) tendrils. The molecular weight of tendril actin is 43,000, the same as rabbit skeletal muscle actin. The native molecular weight of tendril myosin is about 440,000. Tendril myosin is composed of two heavy chains of molecular weight approximately 165,000 and four (two pairs) light chains of 17,000 and 15,000. At high ionic strength, the ATPase activity of pea tendril myosin is activated by K^+-EDTA and Ca^{2+} and is inhibited by Mg^{2+}. At low ionic strength, the Mg^{2+}-ATPase activity of pea tendril myosin is activated by rabbit skeletal muscle F-actin. Superprecipitation occurred after incubation at room temperature when ATP was added to the crude actomyosin extract. It is suggested that the interaction of actin and myosin may play a role in the coiling movement of pea tendril.

Tendril coiling is a plant movement which has been studied by many authors since Darwin in 1893, but so far, the mechanism of coiling remains unsolved (18). Jaffe and Galston (6, 7) reported that the coiling movement of pea tendrils is a ATP-dependent process, and they found that an extract from pea tendrils with ATPase activity decreased in viscosity following addition of ATP, consistent with the possibility that a contractile protein may be involved (8). Yen *et al.* (26) found the presence of a contractile protein (actomyosin) in higher plants in 1965. Condeelis (2) reported the existence of F-actin in pollen tube and protoplast of *Amaryllis belladonna.* Palevitz *et al.* (15) demonstrated the presence of actin in *Nitella.* Kato and Tonomura (9) isolated and characterized myosin from *Nitella.* Biswas and Bose (1) described the presence of actomyosin from motor cells of the sensitive plant *Mimosa pudica.* Vahey *et al.* (23, 24) confirmed the presence of actin and myosin in the endocarp of tomato fruit. It is known that transvacuolar streaming is very rapid in the parenchymal cells of tomato fruit. We recently reported (13) the presence of actin and myosin in pollen tubes and indicated that they have a close relationship with cytoplasmic streaming of pollen tubes. These studies imply that actin and myosin in higher plants are responsible for the plant movements including tendril coiling (8).

In this paper we report that contractile proteins do exist in pea tendrils.

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MATERIALS AND METHODS

Plant Material

Pea (Pisum sativum L.) was grown in vermiculite in plastic boxes which were irrigated every 12 h with a diluted mineral solution (3). The plants were kept in greenhouse at about 22°C. Fresh coiling pea tendrils were harvested from 13-dy-old plants.

Rabbit Muscle Actin

Rabbit muscle actin was prepared from an acetone powder of back and leg muscle by the procedure of Spudich and Watt (22).

Extraction of Actin from Coiling Pea Tendrils

The coiling pea tendrils were mixed with a low ionic strength buffer containing 2 mM Tris-HCl (pH 8.0), 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM DTT, and homogenized in a Waring blender by seven 1-s pulses. Following incubation at 2 to 4°C for 30 min, the homogenate was filtered through cheesecloth and centrifuged at 30,000*g* for 1 h. EDTA (10 mM) was added to the supernatant, followed by ammonium sulfate to 20 to 60% saturation. The precipitate was collected by centrifugation at 30,000*g* for 15 min, and was resuspended in and dialyzed against the extraction buffer (three changes) for 24 h at 2 to 4°C. The dialyzate was polymerized to F-actin by addition of 0.1 M KCl, 2 mM $MgCl_2$, and 1 mM ATP. The polymerized actin was centrifuged at 30,000*g* for 3 h at 4°C. To increase the purity, the pelleted F-actin was suspended in fresh washing buffer (extraction buffer + 0.6 M KCl, 2 mM $MgCl_2$, 1 mM ATP) and resedimented at 30,000*g* for 3 h at 4°C.

Extraction and Purification of Myosin from Coiling Pea Tendrils

Pea tendrils were mixed with ice-cold extract buffer containing 0.3 M sucrose, 10 mM imidazole, 1 mM DTT, and 1 mM ATP (pH 7.0) and homogenized in a Waring blender by seven 1-s pulses. The homogenate was centrifuged at 30,000*g* for 1 h at 2 to 4°C and filtered through eight layers of cheesecloth to remove the floating lipid. The clear supernatant was applied directly to a DEAE-cellulose column (1.6 × 30 cm) previously equilibrated with a solution containing 20 mM imidazole, 1 mM ATP, 0.5 mM DTT, 0.2 mM $CaCl_2$ (pH 7.5). After washing the column with 400 mL of equilibrating buffer, adsorbed proteins were eluted by steps with equilibrating

buffer containing 50, 110, 150, and 230 mM KCl, respectively. During elution, the protein concentration and Ca^{2+}-ATPase activity were determined and the ATPase rich fraction was collected with an LKB fraction collector.

Determination of Protein Concentration

Protein concentration was determined by the method of Lowry *et al.* (12), using a cold 10% TCA precipitation of protein to remove any interfering substances such as phenolics. Twice-crystallized bovine serum albumin was used as standard. Absorbance at 290 nm, measured with a Shimadzu UV-240 spectrophotometer, was used to detect proteins in the column fractions when buffers containing ATP were used.

ATPase Activity

ATPase activity was determined according to the method of Pollard (17).

Gel Electrophoresis

Gradient polyacrylamide gel electrophoresis was carried out according to the method of Slater (21). SDS-PAGE was carried out according to the method of Laemmli (11). Myosin was identified on polyacrylamide gels by the methods of Horak and Hill (4) and Kirkeby and Moe (10).

Superprecipitation

The coiling pea tendrils were mixed with extract buffer containing 0.34 M sucrose, 10 mM imidazole, 1 mM EGTA, 1 mM DTT (pH 7.0), ground in ice bath, and centrifuged at 30,000*g* for 1 h at 2 to 4°C. To observe the superprecipitation, 1 mM ATP was added to the supernatant. The supernatant without added ATP was used as a control.

RESULTS

Actin and Myosin in Pea Tendrils

Crude actin was prepared from pea tendrils by extraction with low ionic strength buffer and fractionation with ammonium sulfate, which then was further purified by polymerizing it to F-actin. The resulting tendril actin, analyzed by SDS-PAGE, shows a mol wt that is the same as that of rabbit skeletal muscle actin (Fig. 1). Myosin from pea tendrils was partially purified by ion exchange chromatography on DEAE-cellulose (Fig. 2). At high ionic strength (0.5 mM KCl), the ATPase activity of fractions 210 to 225 is characteristic of rabbit skeletal muscle myosin, it is stimulated by K^+-EDTA and Ca^{2+}, and is inhibited by Mg^{2+} (Table I). Only these fractions exhibited a behavior characteristic of myosin. This property of pea tendril myosin is similar to those of *Nitella* (9) and tomato (24) myosin, but different with that of *Egeria densa* myosin which is not inhibited by Mg^{2+} (14). At low ionic strength buffer, the Mg^{2+}-ATPase was activated up to 5.6-fold by F-actin from rabbit skeletal muscle, as shown in Table II. We further purified pea tendril myosin using polyacrylamide gel electrophoresis. The myosin band was identified by its ATPase activity on polyacrylamide gels according to the methods of Horak and Hill (4) and Kirkeby and Moe (10). The white band on each gel which indicated the ATPase activity of myosin was cut out and extracted with buffer containing 0.09 M Tris, 0.08 M boric acid, and 0.025 M EDTA (pH 8.0). The native mol wt of myosin, determined by gradient polyacrylamide gel electrophoresis, was 440,000 (Fig. 3) which is smaller than that of rabbit skeletal muscle myosin

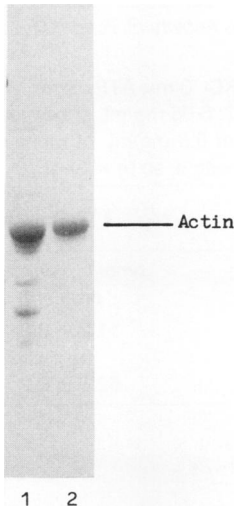

Figure 1. SDS-PAGE of actins from pea tendrils and rabbit skeletal muscle. Lane 1, Partially purified pea tendril actin; lane 2, rabbit skeletal muscle actin.

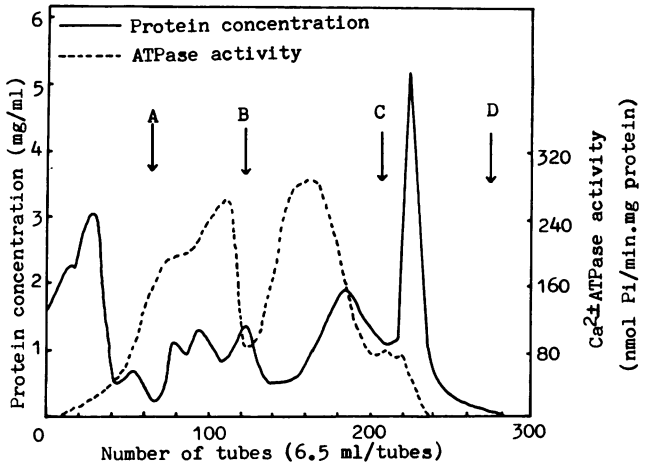

Figure 2. Fractionation of crude myosin extract from pea tendrils by DEAE-cellulose chromatography. A, 50 mM KCl; B, 110 mM KCl; C, 150 mM KCl; D, 230 mM KCl.

Table I. *ATPase Activity of Pea Tendril Myosin Purified by DEAE-cellulose Chromatography*

ATPase assays were carried out at 37°C in 0.5 M KCl, 10 mM ATP, 10 mM imidazole (pH 7.0), and either 2 mM K^+-EDTA, 10 mM $CaCl_2$, or 10 mM $MgCl_2$. Values given are the mean ± SD (n = 3).

K^+-EDTA	Ca^{2+}	Mg^{2+}	Control
nmol Pi/min·mg protein			
73.6 ± 1.1	58.2 ± 0.8	44.0 ± 0.5	56.1 ± 1.2

Table II. *Mg^{2+}-ATPase Activity of Tendril Myosin Activated by Rabbit Muscle F-actin*

Conditions: 0.03 M KCl, 3 mM ATP, 5 mM $MgCl_2$, 10 mM imidazole buffer (pH 7.0), at 37°C; 0.05 mg/mL of pea tendril myosin and in the presence or absence of 0.5 mg/mL of rabbit skeletal muscle actin. Values given are the mean ± SD (n = 3).

	Mg^{2+}-ATPase activity	Relative Activity
	nmol Pi/min·mg protein	
Pea tendril myosin	11.2 ± 0.3	1
Pea tendril myosin + rabbit F-actin	62.7 ± 0.2	5.6

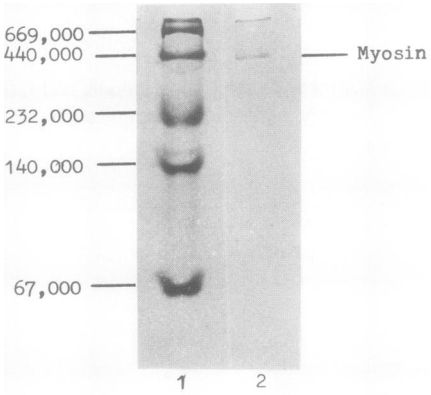

Figure 3. Gradient polyacrylamide gel electrophoresis of pea tendril myosin. Lane 1, Standard proteins: thyroglobin (669 kD), ferritin (440 kD), catalase (232 kD), lactate dehydrogenase (140 kD), and bovine serum albumin (67 kD). Lane 2, Pea tendril myosin.

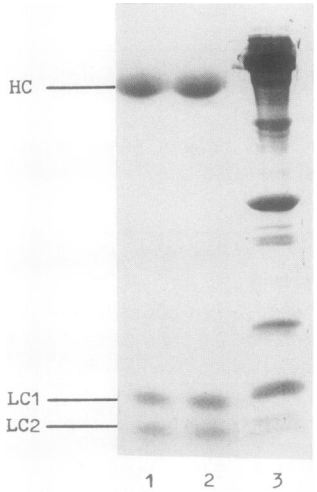

Figure 4. SDS-PAGE of pea tendril myosin. Lanes 1 and 2, Pea tendril myosin; lane 3, rabbit skeletal muscle myosin. HC, heavy chain; LC1, light chain 1, LC2, light chain 2.

(460,000). The subunit composition of pea tendril myosin and the mol wt of heavy and light chains were determined using 10% and 4 to 30% gradient SDS-PAGE, respectively. The results (Figs. 4 and 5) show that pea tendril myosin is

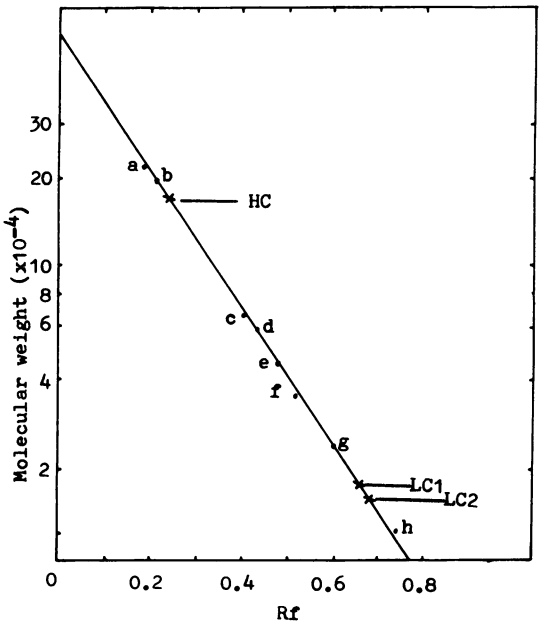

Figure 5. Determination of mol wt of subunits of myosin from pea tendrils using SDS-PAGE. Protein standard used was: a, ferritin (220 kD); b, rabbit skeletal muscle myosin heavy chain (200 kD); c, bovine serum albumin (67 kD); d, catalase (60 kD); e, rabbit skeletal muscle actin (43 kD); f, lactate dehydrogenase (36 kD); g, trypsinogen (24 kD): and h, Cyt c (12.5 kD). HC, heavy chain; LC1, light chain 1; LC2, light chain 2.

composed of a single type of heavy chain of mol wt approximately 165,000 and two types of light chains of 17,000 and 15,000. The mol wt of the heavy chain of pea tendril myosin is lower than that of skeletal muscle myosin (200,000) but is similar to that of *Acanthamoeba castellanii* myosin II (175,000) (16).

The mol wt of the myosin heavy chains from various higher plants appear to differ. In 1980, Vahey and Scordilis (23) isolated myosin from endocarp of tomato fruit and found a mol wt for the heavy chain of 220,000. In 1982, they again reported (24) the isolation of myosin from endocarp of tomato fruit and found that the mol wt of the heavy chain was only 100,000. We isolated myosin from plant pollens and found the mol wt of the myosin heavy chain from pollens of Chinese cabbage and *Luffa cylindrica* was 220,000 (27) and 165,000 (13), respectively. The mol wt of the heavy chain of pea tendril myosin is the same as the heavy chain of *L. cylindrica* myosin. This indicates that the mol wt of myosin heavy chains from various higher plants may differ, but it remains to be determined whether this difference is due to proteolysis or to the presence of different myosins in higher plants.

Superprecipitation

Superprecipitation occurred after 1 h incubation at room temperature when 1 mM ATP was added to the crude actomyosin extract from pea tendrils. This phenomenon is due to the contraction of actomyosin in the presence of ATP. In the absence of ATP, no superprecipitation occurred.

DISCUSSION

The coiling movement of pea tendrils has two component reactions, an initial contraction followed by different elongation of the dorsal and ventral sides (6). During contraction, the ventral side of tendril contracts, while the dorsal side elongates. ATP is the native source of chemical energy for the coiling movement of pea tendrils (7). In fact, the primary effect of ATP is on contraction (7). So, the coiling movement may result at least in part from the contraction of the ventral surface of the tendril. ATP has been shown to be the energy source in various types of contractile systems. Thus, it is possible that the contraction of the ventral surface of the tendril is controlled by a similar mechanism. In this paper, the presence of the contractile proteins. *i.e.* actin and myosin, in pea tendrils has been demonstrated. When ATP is added to the crude actomyosin extract, superprecipitation occurs. Superprecipitation implies that actomyosin contracts using ATP as energy source. These results suggest a possible role for these contractile proteins in the coiling movement of pea tendrils.

Our recent study on the cytoplasmic streaming of pollen tubes using cytochalasin B and other drugs as inhibitor shows that the motive force of the growth of pollen tubes is the cytoplasmic streaming and the motive force of cytoplasmic streaming is the interaction of actin and myosin (13). The interaction of actin and myosin probably plays a role in the mechanism of coiling movement also. Sheetz *et al.* (19, 20) proved that myosin molecules can walk on the F-actin cables in Nitella cells and the interaction of actin and myosin can be visualized directly with a fluorescence microscope. Wang and Yen (25) recently reported the presence of spectrin α, β, ankyrin, band 4.2 and actin in the plasma membrane of *Vicia faba* leaf cells. It is suggested that F-actin may be anchored on the membrane skeleton of plasma membrane, just as the F-actins of animal nonerythroid cells are anchored on their membrane skeletons. It is well known that the cytoskeleton plays an important role in cell motility (5). So, the actin and myosin which are the main components of the cytoskeleton may be responsible for the coiling movements of pea tendrils.

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