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Purification and Characterization of Actin from Maize Pollen[1]

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ABSTRACT

Pollen is an excellent source of actin for biochemical and physiological studies of the actomyosin system in higher plants. We have developed an efficient method to prepare relatively high levels of actin from the pollen of maize (*Zea mays L.*). The procedures of purification include acetone powder preparation, saturated ammonium sulfate fractionation, diethylaminoethyl-cellulose chromatography, a cycle of polymerization-depolymerization, and Sephacryl S-200 gel filtration. The average yield of actin is 19 milligrams per 100 grams of pollen grains extracted. This is comparable with those of *Acanthamoeba castellanii* and human platelets. The purified pollen actin is electrophoretically homogeneous and its molecular mass is 42 kilodaltons. The amino acid composition and circular dichroism spectrum of pollen actin are identical to those of muscle actin. The actin purified from pollen is able to polymerize to F-actin. The pollen F-actin activated the activity of the muscle myosin ATPase sevenfold.

It has been well established that the actomyosin system is universally distributed in higher plants (9). The actomyosin system is an important component of cytoskeleton, which plays a pivotal role in the maintenance of cell shape, cell motility, mitosis, and cytokinesis. The presence of actin and myosin in higher plants has been identified in *Amaryllis, Haemanthus,* tomato, conifer root, pollen of *Luffa,* pea tendrils, and pollen tube of lily (3, 5, 13, 17, 20, 21, 23) by biochemical and immunological methods. But the concentrations of actin in plant are lower than they are in animal cytoplasmic tissues (14). So the biochemical and physiological studies of plant actomyosin are limited, unlike the cases in other nonmuscle cells such as slime molds and *Acanthamoeba castellanii.* However, we found that pollen is a rich source of actin for purification. We have developed an efficient method to prepare sufficient quantities of pure actin for biochemical and physiological studies such as the conformational changes during the polymerization of actin and the reassembly of microfilaments *in vitro.* Here we describe this method, which includes acetone powder preparation, ammonium sulfate fractionation, DEAE-cellulose chromatography, and Sephacryl S-200 gel filtration. Furthermore, the pollen actin was characterized by mol wt determination, immunoblotting, amino acid composition, circular dichroism spectrum, and activation of myosin ATPase activities.

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MATERIALS AND METHODS

Chemicals

DEAE-cellulose and Sephacryl S-200 were obtained from Pharmacia. ATP-Na_2 salt, imidazole, SDS, acrylamide, bismethylene-acrylamide, Tris, and monoclonal antibodies against smooth-muscle α-actin were from Sigma. All other reagents used were of analytical grade.

Maize Pollen

Maize (*Zea mays* L.) plants were grown in the Experimental Station of Beijing Agricultural University in Beijing.The cultivar was Nongda No. 14. Pollen were collected in the middle of July when the plants were in pollination stage. About 1 kg fresh weight of maize pollen was collected and stored in a desiccator placed in a freezer (−70°C) for use.

Acetone Powder of Pollens

Five grams of maize pollen were put into a mortar, 14 mL of buffer A (0.2 mM $CaCl_2$, 2 mM Tris, 0.75 mM mercaptoethanol, 0.2 mM ATP, 0.005% NaN_3, pH 8.0) was added, and the mixture was ground with a pestle to a slurry. Then 6 mL of buffer A was added and the mixture was ground again. The pollen extract was made to acetone powder. The pollen extract was mixed with an approximately 20-fold volume of cold acetone (−20°C) in a beaker, and stirred in an ice bath for 1 min. After standing for a moment, the acetone was decanted. Another 20-fold volume of cold acetone was added, the mixture was stirred, and the acetone was decanted. The extract was placed into a cooled mortar and ground again with a pestle. A 10-fold volume of cold acetone was added, stirred, and centrifuged at 10,000*g* for 4 min. The precipitate was ground once more and extracted with a 10-fold volume of cold acetone and centrifuged. About 2 g of precipitate were obtained and were dried at 0°C and stored as acetone powder for further use.

Ammonium Sulfate Fractionation

Two grams of acetone powder were placed in a homogenizer with a small volume of buffer A and were homogenized. Additional buffer A was added to a total volume of 300 mL.

After stirring for 1 min, the mixture was centrifuged at 20,000*g* for 15 min at 4°C. The supernatant was made 50% saturated with solid ammonium sulfate. The pH was adjusted to 5.4 by the addition of 0.5 N acetic acid with a Beckman pH meter. The solution was centrifuged at 10,000*g* for 10 min at 4°C to collect the pellet. The pellet was dissolved in 20 mL of buffer B (0.1 mM $CaCl_2$, 0.75 mM mercaptoethanol, 10 mM imidazole, 0.5 mM ATP, 0.05% NaN_3, pH 7.5) and the solution was dialyzed against 1,000 mL of buffer B for 3 h.

DEAE-Cellulose and Sephacryl S-200 Chromatography

The dialysate was passed through a DEAE-cellulose 52 column (1.6 × 30 cm). The chromatography was carried out in a cold laboratory at 4°C. The column was eluted with 0 to 0.5 M KCl gradient solution. The total volume of eluate was 350 mL. The fractions of eluates were determined by SDS-PAGE to be actin, and these fractions were collected as crude actin. The actin fraction was polymerized by the addition of $MgCl_2$ to a concentration of 2 mM for 10 h at 4°C. The polymerized actin was spun at 100,000*g* for 3 h at 4°C. The pellet was dissolved in 4.5 mL of buffer C (buffer A adjusted to pH 7.5) and dialyzed against buffer C for 72 h with two changes of dialysis solution of 1,000 mL. The depolymerized actin was centrifuged at 100,000*g* for 3 h at 4°C. The solution of actin was then passed through a column of Sephacryl S-200 (1.6 × 90 cm) and monitored with an LKB UV monitor with a filter of 280 nm. The second peak of gel filtration was collected as pure G-actin of maize pollen.

SDS-PAGE was performed according to the method of Laemmli (10) in the presence of 1.0% SDS.

Determination of Protein Concentration

The concentration of protein was determined according to the method of Lowry *et al.* (12). The actin content was determined with a Shimadzu dual-wavelength TLC scanner.

Immunoblotting

Proteins separated on a 10% SDS polyacrylamide gel were electroblotted to a nitrocellulose sheet with a Bio-Rad trans-blot apparatus at 47 V for 2 h at 4°C. The nitrocellulose sheet was incubated with monoclonal antibody raised against smooth-muscle α-actin (Sigma, A9172) for 6 h at room temperature. Excess antibody was removed by four 5-min washes in PBS, and the sheet was incubated for an additional 2 h at 37°C in blocking buffer with 1:1000 diluted anti-mouse immunoglobulin G alkaline phosphatase conjugate (Bio-Rad). Finally, the sheet was visualized by the addition of nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate according to Blake *et al.* (2).

Determination of Amino Acid Composition

The amino acid composition of the pollen actin was determined by a Hitachi model 835–50 amino acid automatic analyzer. The result was compared with the actins from rabbit muscle (1) and *A. castellanii* (7).

Determination of Circular Dichroism Spectrum

The circular dichroism spectrum of pollen actin was determined by a J-500C spectropolarimeter. The scanning wavelengths were 200 to 240 nm.The result was compared with that of muscle actin (15).

Determination of the Activity of Myosin ATPase

The activities of myosin ATPase were determined according to the method of Le Bel *et al.* (11).

RESULTS

Purification and Yield of Pollen Actin

The G-actin of maize pollen was purified by the procedures described in "Materials and Methods." The extract of pollen was made to acetone powder with the same method used for extraction of actin from skeletal muscle (19). Most of the actin could be recovered from the acetone powder of maize pollen, which was a rich source of plant actin so far isolated from plants. After ammonium sulfate fractionation of the extract, it was purified by DEAE-cellulose chromatography, a cycle of polymerization-depolymerization, and Sephacryl S-200 gel filtration. The actin obtained was electrophoretically homogeneous: only one band appeared on the slab gel (Fig. 1) with a mol wt of 42,000. The yield of pollen actin purified by the above method is higher than any of the present methods of purification of actin from higher plants. We have obtained 19 mg of purified actin from 100 g of maize pollen, an amount comparable with the yield of actin from human platelets (18). Table I shows the yield of actin during the purification procedures.

Immunoblotting of Pollen Actin

The actins from maize pollen and rabbit muscle were electrophoresed on an SDS polyacrylamide gel simultane-

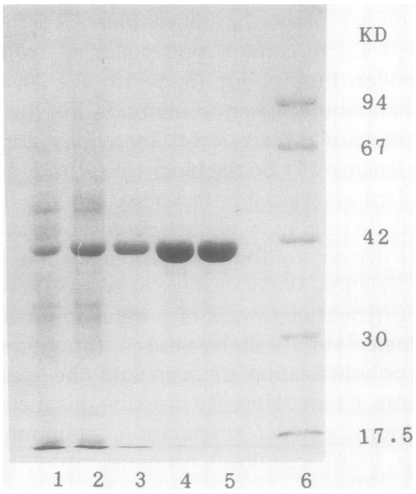

Figure 1. SDS-PAGE of actin from pollen at different stages of the purification procedures. Lane 1, Acetone powder; lane 2, 50% ammonium sulfate; lane 3, DEAE-cellulose; lane 4, polymerization-depolymerization; lane 5, Sephacryl S-200; lane 6, standard proteins.

Table I. *Purification of Actin from Maize Pollen*

Step	Volume	Protein	Actin	
	mL	*mg*	*mg*	%
Crude extract[a] (acetone powder)	858	1097.0	211.8	19.3
50% Ammonium sulfate	63	172.3	51.4	29.6
DEAE-cellulose	46	96.4	24.5	53.1
Polymerization-depolymerization	4.5	11.9	9.0	75.0
Sephacryl S-200	10	7.1	6.5	91.5

[a] Thirty grams of maize pollen were extracted.

ously and were transferred to a nitrocellulose sheet. The proteins separated were visualized either by Coomassie blue staining or by immunostaining (see "Materials and Methods"). The results showed that the pollen actin is immunochemically identical to the smooth-muscle α-actin (Fig. 2).

Amino Acid Composition

The amino acid composition of pollen actin was determined by a Hitachi automatic amino acid analyzer. The result is shown in Table II, which includes the amino acid composition of actins from rabbit skeletal muscle (1) and *A. castellanii* (6). Comparing the amino acid composition of pollen actin with those of muscle and *A. castellanii,* they are similar to each other except for a little difference that may be due to experimental errors. It has been reported that there are some variations in amino acid composition and sequence of actin among the three kinds of organisms, *i.e.* plants, animals, and protists (14).

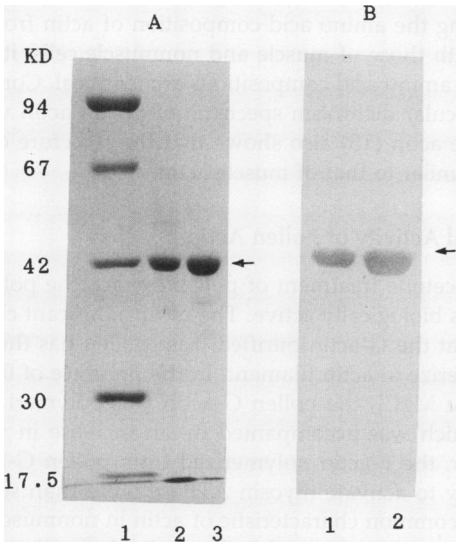

Figure 2. SDS-PAGE and immunoblot of pollen actin in comparison with muscle actin. The proteins were visualized either by Coomassie blue staining (A) or by immunostaining after transfer onto a nitrocellulose sheet (B). A, Lane 1, Molecular weight marker (values in kilodaltons on left); lane 2, partially purified actin from maize pollen after DEAE-cellulose chromatography; lane 3, purified actin from rabbit muscle. B, Lane 1, Partially purified actin from maize pollen; lane 2, purified actin from rabbit muscle.

Circular Dichroism Spectrum of Pollen Actin

The circular dichroism of muscle actin had been determined by Murphy (15). Pardee and Ramburg (16) reported that the circular dichroism spectrum of chick brain actin was very similar to that of muscle actin. So far no information on the circular dichroism spectrum of plant actin has been reported. Here we described the circular dichroism spectrum of pollen actin, which was determined by a Hitachi J-500C spectropolarimeter. The result shows that the circular dichroism spectrum of pollen is very similar to those of muscle and brain actins. The comparison of circular dichroism spectra of pollen actin with that of muscle actin is shown in Figure 3.

Activation of Myosin ATPase by Pollen F-Actin

Another characteristic of the actin is that its fibrous state can activate the activity of myosin ATPase. We determined the myosin ATPase activities at different concentrations of F-actin from maize pollen. The result shows that the activities of the muscle myosin ATPase in the presence of F-actin from maize pollen is sevenfold higher than the activity of muscle myosin ATPase in the absence of F-actin. The result is shown

Table II. *Comparison of the Amino Acid Compositions of Actins from Maize Pollen, A. castellanii, and Rabbit Muscle*

Amino Acid	Maize Pollen	*A. castellanii*[a]	Rabbit Muscle[b]
Lys	22	21	21
His	9	10	11
Arg	22	22	21
Asp	32	32	34
Thr	27	28	27
Ser	25	25	25
Glu	40	43	44
Pro	21	15	18
Gly	30	31	30
Ala	30	31	30
Cys	5	4	5
Val	19	22	18
Met	18	13	12
Ile	21	22	23
Leu	26	32	26
Tyr	14	18	18
Phe	13	15	13
Trp	N.D.	N.D.	N.D.

[a] Taken from Gorden *et al.* (6).
[b] Taken from Adelstein *et al.* (1).

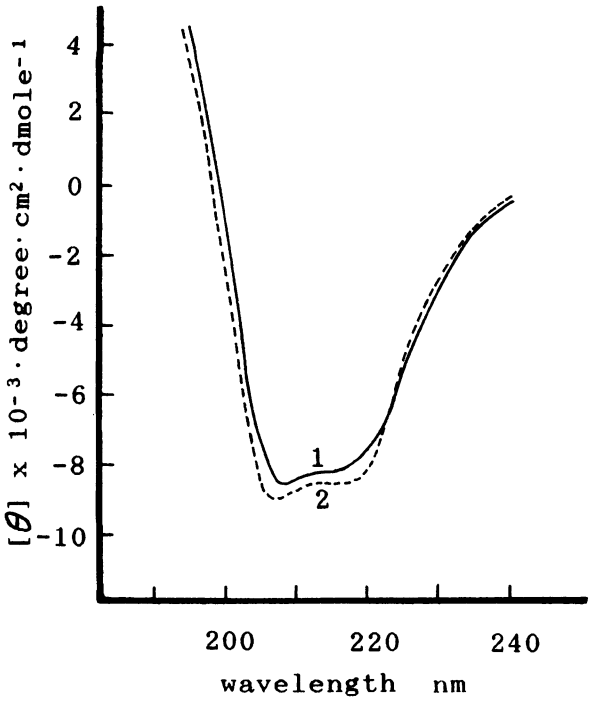

Figure 3. Circular dichroism of pollen actin in comparison with that of muscle actin. Solid line, pollen actin; broken line, muscle actin.

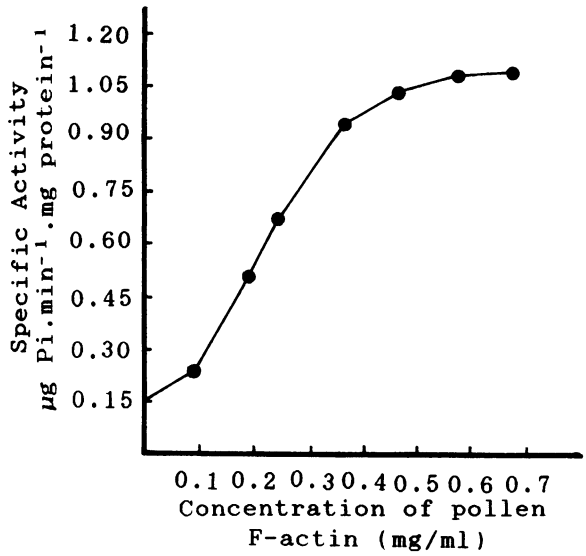

Figure 4. Activation of myosin ATPase from muscle by F-actin from maize pollen in various concentrations. The specific activity of myosin ATPase from rabbit muscle in the absence of pollen F-actin was 0.15 μg of Pi min^{-1} mg^{-1} protein.

in Figure 4. It implies that the F-actin polymerized from the purified pollen actin is able to interact with myosin to do biological processes.

DISCUSSION

Purity and Yield of Plant Actin

Since the discovery of actin in *Physarum polycephalum* by Hatano and Oosawa (7), many authors have identified actin in various plants (9). Meagher and McLean (14) recently pointed out that the typical methods for purifying actin from animals and protists, such as the cycle of polymerization and depolymerization and DNase affinity chromatography, had not yet proven quantitative in plant systems. However, it is quantitative for the purification of actin from pollen when the acetone powder method was applied.

The acetone powder of maize pollen contains most of the actin in the pollen cells. After chromatography on DEAE-cellulose and Sephacryl S-200 columns, very pure actin can be obtained. The purity of actin isolated by the combination of acetone powder preparation and Sephacryl S-200 gel filtration is better than that purified by Sepharose 4B. On the SDS polyacrylamide gel only one sharp band of actin appears whose mol wt is 42,000. Moreover, the yield by our method is higher than those of other methods (22). It is comparable with the yield of actin isolated from *A. castellanii* (6) and human platelets (18). Cresti *et al.*(4) and Heslop-Harrison *et al.*(8) had described that in the ungerminated pollen grains of many species of angiosperm there were crystalline-fibrillar structures that might be a storage form of F-actin. They indicated that actin is an abundant and universal constituent of pollen. Our result is consistent with their finding that actin is indeed an abundant component in pollen grains.

Characterization of Actin from Pollen

The biochemical characteristics of pollen are identical or similar to those of actins from muscle and *A. castellanii.* Comparing the amino acid composition of actin from maize pollen with those of muscle and nonmuscle cells, it appears that their amino acid compositions are identical. Comparison of the circular dichroism spectrum of pollen actin with that of muscle actin (15) also shows that the structure of pollen actin is similar to that of muscle actin.

Biological Activity of Pollen Actin

After acetone treatment of pollen extract, the pollen actin isolated is biologically active. The most significant characteristic is that the G-actin purified from pollen has the activity to polymerize to actin filament. In the presence of 0.1 M KCl and 2 mM $MgCl_2$ the pollen G-actin was polymerized to F-actin, which was accompanied by an increase in viscosity. Moreover, the F-actin polymerized from pollen G-actin has the ability to activate myosin ATPase more than sevenfold. This is a common characteristic of actin in nonmuscle cells.

The above discussion of pollen actin shows that pollen is an excellent source of actin for the study of microfilament cytoskeleton in higher plants.

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LITERATURE CITED

1. **Adelstein RS, Kuehl WH** (1970) Structural studies on rabbit skeletal actin. I. Isolation and characterization of the peptides produced by cyanogen bromide cleavage. Biochemistry **9:** 1355–1364
2. **Blake MS, Johnston KH, Russell-Jones GJ, Gotschlich EC** (1984) A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. Anal Biochem **136:** 175–179
3. **Condeelis JS** (1974) The identification of F-actin in the pollen tube and protoplast of *Amaryllis belladonna.* Exp Cell Res **88:** 435–439
4. **Cresti M, Hepler PK, Tiezzi A, Ciampolini F** (1986) Fibrillar structures in *Nicotiana* pollen: changes in ultrastructure during pollen activation and tube emissin. *In* DL Mulcahy, G Bergamini Mulcahy, E Ottaviano, eds, Biotechnology and Ecology of Pollen. Springer-Verlag, New York, pp 283–288
5. **Forer A, Jackson WT** (1975) Actin in the higher plant *Haemanthus katherinae* Baker. Cytobiologie **10:** 217–226
6. **Gordon DJ, Eisenberg E, Korn ED** (1976) Characterization of cytoplasmic actin isolated from *Acanthamoeba castellanii* by a new method. J Biol Chem **251:** 4778–4786
7. **Hatano S, Oosawa F** (1966) Isolation and characterization of *Physarum* actin. Biochim Biophys Acta **127:** 488–498
8. **Heslop-Harrison J, Heslop-Harrison Y, Cresti M, Tiezzi A, Ciampolini F** (1986) Actin during pollen germination. J Cell Sci **86:** 1–8
9. **Jackson WT** (1982) Actomyosin. *In* CW Lloyd, ed, The Cytoskeleton in Plant Growth and Development. London, Academic Press, pp 3–29
10. **Laemmli UK** (1970) Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature **227:** 680–685
11. **Le Bel D, Poirier GG, Beaudoin AR** (1978) A convenient method of ATPase assay. Anal Biochem **85:** 86–89
12. **Lowry OH, Rosebrough HJ, Farr AL, Randall RJ** (1951) Protein measurement with Folin phenol reagent. J Biol Chem **193:** 265–275
13. **Ma YZ, Yen LF** (1989) Actin and myosin in pea tendril. Plant Physiol **89:** 586–589
14. **Meager RB, McLean M** (1990) Diversity of plant actins. Cell Motil Cytoskeleton **16:** 164–166
15. **Murphy JA** (1971) Circular dichroism of the adenine and 6-mercaptopurine nucleotide complexes of actin. Biochemistry **10:** 3723–3728
16. **Pardee JK, Ramburg JR** (1979) Actin from embryonic chick brain. Isolation in high yield and comparison of biochemical properties with chicken muscle actin. Biochemistry **18:** 2245–2252
17. **Pesacreta TC, Carley WW, Webb WW, Partharathy MV** (1982) F-actin in conifer roots. Proc Natl Acad Sci USA **79:** 2898–2901
18. **Rosenberg S, Stracher A, Lucus RC** (1981) Isolation and characterization of actin and actin-binding protein from human platelets. J Cell Biol **91:** 201–211
19. **Spudich JA, Watt S** (1971) The regulation of rabbit muscle contraction. I. Biochemical studies of the interaction of tropomyosin-troponin complex with actin and proteolytic fragments of myosin. J Biol Chem **246:** 4866–4871
20. **Tang XJ, Lancelle SA, Hepler PK** (1989) Fluorescence microscopic localization of actin in pollen tubes: comparison of actin antibody and phalloidin staining. Cell Motil Cytoskeleton **12:** 216–224
21. **Vahey M, Titus M, Trautwein R, Scordilis S** (1982) Tomato actin and myosin: contractile proteins from a higher land plant. Cell Motil **2:** 131–147
22. **Villanueva MA, Ho SC, Wang JL** (1990) Isolation and characterization of one isoform actin from cultured soybean cells. Arch Biochem Biophys **277:** 35–41
23. **Yen LF, Wang XZ, Teng XY, Ma YZ, Liu GQ** (1986) Actin and myosin in pollens and their role in the growth of pollen tubes. Chin Sci Bull (Kexue Tongbao) **31:** 267–272