Phytochemistry, 1979, Vol. 18, pp. 1781–1783. © Pergamon Press Ltd. Printed in England. 0031-9422/79/1101-1781 $02.00/0

PARTIAL PURIFICATION OF ACTIN FROM WHEAT GERM

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(*Received* 19 *February* 1979)

Key Word Index—*Triticum aestivum*; Gramineae; wheat germ; protein purification; actin.

Abstract—A protein, one of two major components in a preparation obtained from wheat germ, has been identified as actin by its solubility properties, mobility in SDS-acrylamide gel electrophoresis, ability to react with an antiserum against turkey-gizzard actin, and ability to form needle-shaped fibrils.

INTRODUCTION

The presence of actin in cells of green plants is well supported by cytological evidence. Microfilaments of 5–8 nm diameter are visible in epidermal and parenchymal cells of many species. Heavy meromyosin (HMM), which binds specifically to actin, has been shown to decorate microfilaments in *Nitella*, *Chara*, *Haemanthus*, and *Amaryllis* (see [1, 2] for references). HMM binds to elements in phloem tissue of *Xylosma congestum* and *Vicia faba* [3, 4]. Cytochalasin B, a drug known to disrupt the structure of certain microfilaments, inhibits cytoplasmic streaming in many plant cells [1], including phloem of *Xylosma* and *Vicia* [3].

Despite the considerable cytological information available, there has been little biochemical work with extracted plant actin. Early workers reported 'actomyosin-like' preparations containing ATPase activity from *Nitella* [5], *Cucurbita* and *Nicotiana* [6]. Enrichment of an actin-like fraction from *Nitella* has been reported [2]. Only very recently, however, has a report appeared describing extraction and purification of actin from a higher plant, *Phaseolus* [7]. The lack of a reliable way to purify actin from higher plants has impeded studies on the structural and motile properties of plant cells.

We have been attempting to purify actin from higher plant cells, both to confirm its presence and to facilitate studies of plant transport and responses to stress. In this paper, we report a procedure for preparing a fibrillar protein from wheat germ, together with evidence identifying the protein as actin.

RESULTS

The procedure we used was adapted from one reported by Clarke and Spudich [8] for use with *Dictyostelium*. We chose wheat germ (or occasionally wheat embryos) as starting material, because of its low protease activity and phenol content and its high proportion of potentially meristematic cells. (Actin has been observed in mitotic spindles [9, 10].) The germ was ground in a buffer containing 60% sucrose, which promoted solubilization of actomyosin, and particulates were removed. The supernatant was then dialysed against a buffer containing 0.1 M KCl and no sucrose, in which actomyosin precipitated. The precipitate was pelleted and redissolved in a buffer containing 0.6 M KI; this dissociated the actin from myosin [8]. After further centrifugation, the cloudy solution was passed through an agarose column (Bio-Gel A-15m) and the second peak, between the cloudy, excluded material and a yellow protein, was collected. Fig. 1 shows the elution profile for one trial. Peak B represents the preparation of interest.

The following characteristics of the preparation indicate that it contains actin.

(1) SDS-acrylamide gel electrophoresis [11] revealed two major protein factors, which together constituted over 90% of the total protein. One band, containing *ca* 25–40% of the staining material, co-migrated with authentic rabbit-muscle actin. The other band, with an

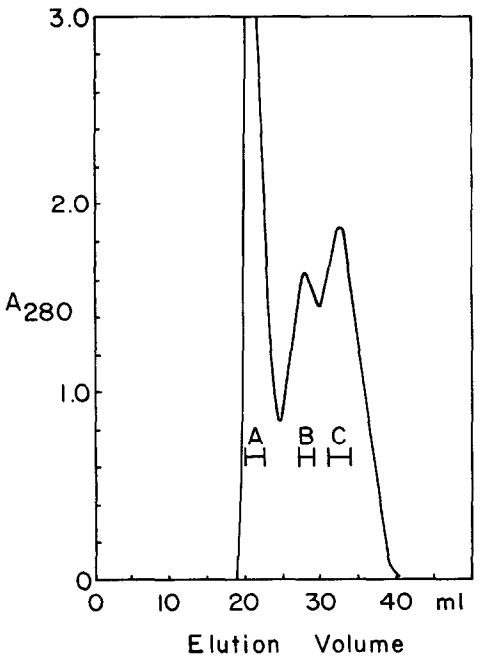

Fig. 1. Elution profile for partially purified wheat germ extract passing through agarose (Bio-Gel A-15 m). Fractions from peaks A, B and C were pooled as indicated by bars.

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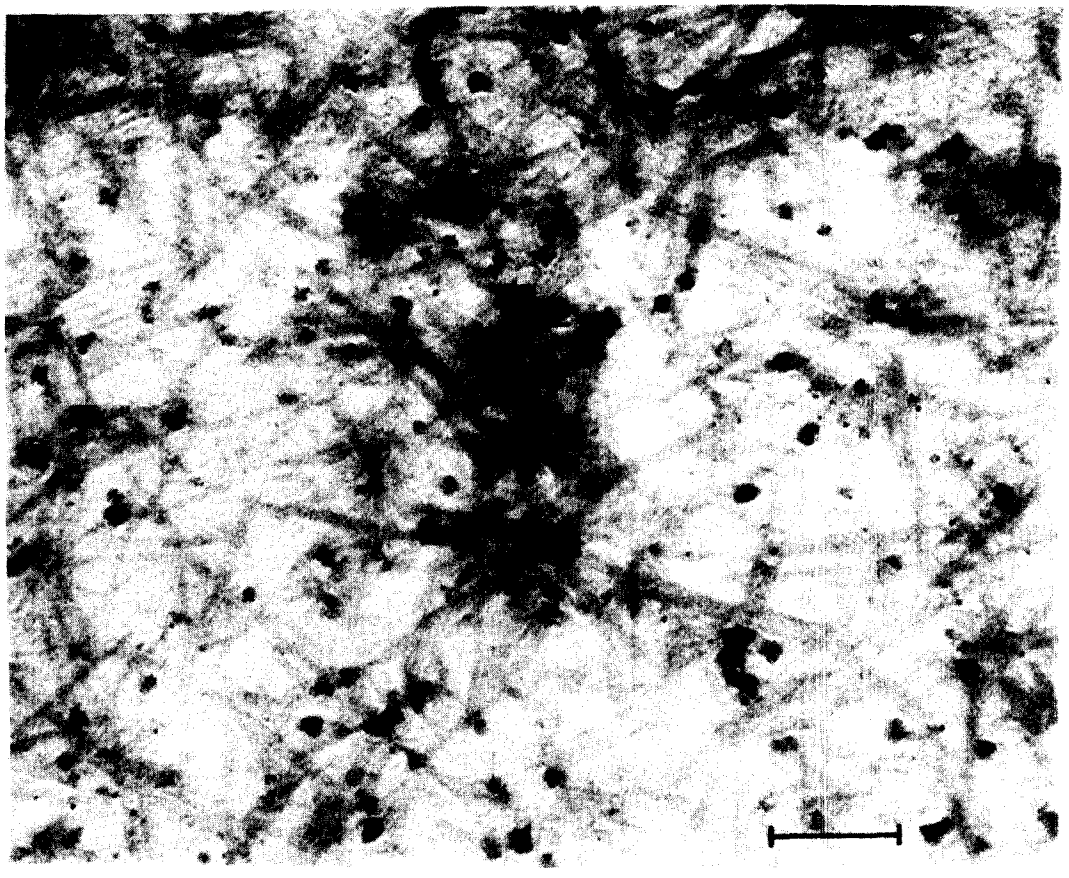

Fig. 2. Transmission electron micrograph of material from peak B (see Fig. 1), precipitated in 0.6 M KCl plus 1 mM ATP, then dialysed for 3 days against 0.1 M KCl, 2 mM ATP buffer. Bar represents 0.2 μm. The black dots are stain precipitates.

estimated MW of 58000, was similar in size to one found in the preparation of sea urchin egg actin reported by Kane [12].

(2) The preparation gave a single, strong line of precipitation when tested in agar-gel double diffusion plates against a monospecific antiserum produced in rabbit against turkey-gizzard actin. Bands A and C from the column (Fig. 1) gave no visible reaction.

(3) The preparation was treated by a procedure suggested by Kane [12]: it was dialysed against 0.6 M KCl buffer, concentrated 10-fold, and made 1 mM in ATP. The precipitate that formed overnight was fibrous and contained long filaments, but was not recognizably actin-like. This precipitate was resuspended in buffer containing 0.1 M KCl, 2 mM ATP and 2 mM $MgCl_2$, and dialysed for 3 days. The resulting birefringent precipitate had the form of needle-shaped fibrils similar to those seen with sea urchin egg actin under the same conditions [12, Fig. 5] (Fig. 2).

(4) The preparation precipitated when dialysed against buffer containing 0.01 M $MgCl_2$. However, the actin in the precipitate (defined by SDS-gel electrophoresis) did not redissolve in G-buffer (low ionic strength), as did actin from *Dictyostelium* [8].

Further purification of the wheat germ actin will require techniques for keeping the protein stably active and soluble in the pure form.

DISCUSSION

Evidence presented above not only confirms the presence of actin in wheat germ, but shows that this actin has many physical similarities to actin from other organisms. These similarities include size and precipitability in 0.1 KCl and in low concentrations of Mg^{2+}, properties shared by known muscle and cytoplasmic actins [13]. In addition, the formation of fibrils in 0.6 M KCl with 2 mM ATP marks the plant actin as similar to sea urchin egg actin. These similarities, coupled with the strong immunological reaction between wheat germ actin and rabbit anti-turkey-muscle-actin and with previous cytological evidence [1, 2], mean that previous conclusions concerning the evolutionarily conservative nature of actin must apply to green plants, as well as to animals, protists and fungi [13].

Further characterization of our wheat germ preparation should include ultrastructural studies of filaments of F-actin and a demonstration of HMM binding to these filaments. We have prepared filaments from a preparation obtained by the procedure of Hartwig and Stossel [14]. The filaments are *ca* 8 nm wide. The preparation contains a protein with the same MW as that of actin, but it contains minor amounts of many other proteins as well. Thus we cannot say for certain that the filaments are made of the actin, and we have not yet tested them for HMM binding.

Success in the extraction and purification of plant actin has been slow, perhaps because of lack of information about materials and techniques. Our results suggest that wheat germ may be the material of choice for isolation of plant actin for biochemical study. We anticipate that further biochemical characterization of plant actin, and improved cytological localization of plant actin made possible by the knowledge that plant actin reacts with anti-muscle-actin antibody, will greatly aid studies of the function of actin in higher plants.

EXPERIMENTAL

Preparation of wheat germ protein. Wheat germ (commercial, untoasted), 35 g, was mixed with 150 ml 10 mM Tris buffer, pH 7.5, containing 1 mM EDTA, 0.1 mM DTT, and 60% sucrose, and the mixture was homogenized for 2 min using a Polytron (Brinkmann) homogenizer. The mixture was checked for cell breakage by microscopy. The homogenate was centrifuged for 20 min at 30000 *g* (16000 rpm) and for 1 hr at 160000 *g* (40000 rpm). The supernatant was filtered to remove lipids and dialysed at 4° against 2 changes (1 l. each) of 10 mM Tris buffer, pH 7.5, containing 1 mM EDTA, 1 mM DTT, and 0.1 M KCl. The dialysed suspension was centrifuged for 1 hr at 30000 *g*. The pellets (*ca* 1.5–2 g) were resuspended in an equal wt of 10 mM Tris buffer, pH 7.5, containing 1 mM EDTA, 10 mM $MgCl_2$, 2 mM $CaCl_2$, 1 mM DTT, 0.2 mM ATP, and 1.2 M KI. The mixture was recentrifuged for 30 min at 150000 *g* (47000 rpm), and the supernatant from this treatment was applied to an agarose column (Bio-Gel A15m, 100–200 mesh, *ca* 75 ml). The column was pretreated with the sample buffer (containing 0.6 M KI), but was eluted with buffer in which 0.6 M KCl replaced the KI, and $MgCl_2$ and $CaCl_2$ were omitted. Fractions of 3–6 ml were collected, and the A_{280} of each fraction was measured. The yield of protein in the second peak (see Fig. 1) was *ca* 20–30 mg.

Electrophoresis. Samples were heated to 100° in buffer containing 1% SDS and subjected to electrophoresis through 4% acrylamide stacking gels and 8% acrylamide separating gels prepared according to ref. [11]. Gels were stained with Coomassie brilliant blue.

Antibodies. Actin was purified from frozen turkey gizzards according to the procedure of ref. [15]. A single rabbit was injected 5× over a period of 14 days, each time with 1 mg of actin mixed with complete Freund's adjuvant; serum was collected 10 days after the last injection.

Electron microscopy. A drop of the protein suspension was added to a Formvar/carbon-coated grid. Excess moisture was removed with Kleenex. The grid was rinsed with suspension buffer, stained very briefly in alcoholic uranyl acetate and rinsed again [16].

Acknowledgements—This research was supported in part by NSF Grant PCM 76-19954. Judith Murphy provided valuable technical assistance.

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