Cell Biology International, 1996, Vol. 20, No. 5, 365–373

PARTIAL CHARACTERIZATION OF A PUTATIVE 110 kDa MYOSIN FROM THE GREEN ALGA *CHARA CORALLINA* BY *IN VITRO* BINDING OF FLUORESCENT F-ACTIN

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Accepted 10 January 1996

Using the binding of heterologous, rhodamine phalloidin-labelled F-actin *in vitro*, two F-actin binding proteins were identified in protein extracts from the green alga *Chara corallina* after fractionation by anion exchange chromatography. The first protein, a putative myosin, released laterally bound F-actin at ATP-concentrations as low as 1 μM; equivalent concentrations of ADP were not effective. Binding of F-actin was inhibited by the sulfhydryl-alkylating agent N-ethylmaleimide (NEM). Binding of F-actin was also abolished by a monoclonal anti-myosin (J14) previously used for immunodetection and immunolocalization in internodal cells (Grolig *et al.*, 1988, *Eur J Cell Biol* **47:** 22–31). Immunoblotting with J14 detected a 110 kDa polypeptide only in those protein fractions that had revealed ATP-sensitive binding of F-actin. The putative myosin bound with mediocre affinity to immobilized calmodulin and free Ca^{2+}-concentration made no difference to this binding affinity. In contrast to the putative myosin, the second, less abundant protein revealed ATP-insensitive and end-wise binding to the microfilament and was not recognized by the anti-myosin antibody.

KEYWORDS: actin binding protein; calmodulin; *Chara corallina*; F-actin; monoclonal antibody; myosin; N-ethylmaleimide

INTRODUCTION

In most plant cells an extensive directional movement of cytoplasmic particles termed 'cytoplasmic streaming' occurs along dynamic and extensive tracks of actin, with myosin(s) being the putative motor(s) (reviewed by Kamiya, 1981; Williamson, 1993). Over the last few years it has become evident that myosin motor proteins are common throughout the plant kingdom: myosins are proposed to mediate a great variety of actin-based motility phenomena in plant cells.

Giant size and relatively simple organization made the internodal cells of the characean green algae *Chara* and *Nitella* favourite objects in which to study the basic mechanism and regulation of cytoplasmic streaming. In these cylindrical cells, the rotational streaming of the endoplasm, which is driven along subcortical, unipolar actin-bundles (Kersey *et al.*, 1976), ceases rapidly upon a pressure-elicited action potential that raises cytoplasmic free Ca^{2+} to micromolar levels (Williamson and Ashley, 1982). After cessation, streaming gradually returns to full velocity (up to 70 μm/s) within several minutes. Ingenious experiments that exploited the readily accessible and controllable cell models obtained by vacuolar perfusion and permeabilization of the plasma membrane, combined with the use of heterologous myosins, revealed that arrest of cytoplasmic streaming is caused by Ca^{2+}-triggered inhibition of the endogenous myosin associated with the actin bundles and endoplasmic organelles (reviewed by Shimmen, 1992; Williamson, 1992). Reversible inhibition of the myosin appears to rest on Ca^{2+}-dependent phosphorylation/dephosphorylation of

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1065–6995/96/050365+09 $18.00/0

a myosin regulatory light chain. While calcium-dependent protein kinase (CDPK) appears to be an attractive candidate for the inhibitory kinase (McCurdy and Harmon, 1992), dephosphorylation was postulated to involve a calmodulin-activated phosphatase because calmodulin inhibitors specifically block recovery of cytoplasmic streaming (Tominaga *et al.*, 1987). Calmodulin is found throughout the endoplasm (Jablonsky *et al.*, 1990), while CDPK is distributed similarly to myosin and extracted under the same perfusion conditions (McCurdy and Harmon, 1992).

Further characterization and confirmation of the proposed interactions between the assumed regulatory components requires study of the purified components in systems of even more reduced complexity. We therefore employed *in vitro* binding of fluorescently labelled F-actin to nitrocellulose-adsorbed proteins to screen, by video-enhanced microscopy, for F-actin binding proteins in protein fractions obtained by anion exchange chromatography from *Chara corallina*. Two fractions were positive: one fraction shared a number of features well known for other myosins, as ATP-dependent release of F-actin, binding to calmodulin and inhibition by NEM. This protein was recognized by a monoclonal anti-myosin antibody. The second F-actin binding activity revealed no myosin-like properties, and bound to the ends of the F-actin filaments.

MATERIALS AND METHODS

Plant material and preparation of extracts

Chara corallina was grown in tap water under dim light in large basins in a greenhouse (25°C).

During handling of the plants, care was taken to avoid cessation of cytoplasmic streaming. The thalli were harvested, washed in deionized water (5 min), briefly blotted to remove excess water, weighed and frozen in liquid nitrogen. After shattering in a mortar, the material was thawed in extraction buffer (0.1 M Hepes, 10 mM EGTA, 0.25 M sucrose, 20 mM KCl, 20 mM ascorbic acid, 20 mM DTT, pH 7.8) at a ratio of 0.5 ml per gram fresh weight. Cell debris was removed by centrifugation (8500 × ***g***, 10 min); for chromatography the resultant supernatant (S1) was clarified by 110,000 × ***g*** for 30 min, yielding supernatant S2. Supernatants were used immediately or frozen in liquid nitrogen and stored at − 80°C. Protein concentrations were determined according to Bradford (1976).

Anion-exchange chromatography

A Resource Q column (1 ml bed volume, capacity of 29 mg protein; Pharmacia, Freiburg, Germany), run at 4°C on a FPLC-system (Pharmacia) at a flow rate of 1 ml/min was used to fractionate the proteins of supernatant S2. After equilibration with 4 ml of start buffer (20 mM Hepes, 0.1 mM EGTA, 250 mM sucrose, 20 mM KCl, 5 mM DTT, pH 7.8), 50 ml of supernatant S2 were applied. The column was washed with 10 ml of start buffer and eluted with a linear gradient (20–500 mM KCl, 20 ml) in start buffer; fractions of 0.4 ml were collected.

Preparation of rhodamine phalloidin-labelled F-actin

G-actin (*c.* 5 mg/ml) was prepared according to Pardee and Spudich (1982) from bovine heart (Ruhlandt *et al.*, 1994) and stored at − 80°C. After dilution in buffer A (1 mg/ml in 2.5 mM imidazole, 25 mM KCl, 4 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, pH 7.4), the actin polymerized on ice overnight. For labelling with rhodamine phalloidin (RLP; Molecular Probes, Eugene, OR, U.S.A.), 47 µl of 3.3 µM methanolic stock solution of RLP were desiccated and redissolved in 1 µl of ethanol. After vigorous mixing with 145 µl buffer A, 5 µl of F-actin (1 mg/ml) were added, thoroughly mixed with the pipette and incubated on ice overnight (Kron *et al.*, 1991).

F-actin binding assay

Coverslips were cleaned overnight in 0.5 M KOH, rinsed with distilled H_2O and ethanol (96% (v/v)) and dried. After immersion in 0.1% (w/v) nitrocellulose in isoamyl acetate (stock of 2% (w/v) collodion, Electron Microscopy Sciences, Washington, PA, U.S.A.), the coverslips were dried overnight.

For use in the binding assay, RLP-labelled F-actin was diluted 1:70 in buffer A supplemented with 0.5 mg/ml BSA (buffer B, Kron *et al.*, 1991). In order to approach binding of one actin filament per immobilized protein molecule, the diluted filaments were sheared by repetitive passage (15–20 times) through a coupled pair of syringes (G 23; 0.6 mm i.d.). All samples and solutions used during the assay were stored on ice. Nitrocellulose-coated coverslips (22 mm × 22 mm) were mounted onto the slide with Vaseline in the corners to leave a gap of some 50 µl. Two × 50 µl of sample were drawn by suction symmetrically under the coverslip and washed out after 1 min with 3 × 100 µl of buffer B.

Then 2 × 50 µl of F-actin were applied symmetrically for 1 min. Surplus F-actin was removed by washing the chamber with 300 µl of buffer B. In some of the experiments the antioxidant DTT was omitted from buffers A and B to test the sensitivity of the protein's F-actin binding capacity to oxidation.

The dependence of F-actin release on the nucleotides ATP and ADP was tested by charging the chamber with 3 × 100 µl (1 min each) of the respective nucleotide concentration (stock solutions: 0.1 M in distilled H_2O).

Because the bound RLP-labelled F-actin is found in a very narrow focal plane, low densities of bound F-actin can be easily missed during microscopical inspection. To facilitate focusing, a reference with abundant bound F-actin was prepared under the same coverslip if necessary, with reference and sample being separated by a wall of Vaseline.

Inhibition of F-actin binding

N-ethylmaleimide (NEM). After an initial binding of F-actin and subsequent release by 10 µM ATP to check the functionality of the immobilized protein, the chamber was washed by buffer A without DTT (2 × 100 µl, 1 min) prior to treatment of the immobilized protein with NEM (Sigma, Deisenhofen, Germany; 0.5 mM in buffer A without DTT; 2 × 100 µl, 2 min, 25°C); DTT was also excluded from all other steps in a second round of F-actin binding and release. Samples of the same preparation were treated with NEM in the same way but without previous ATP-dependent F-actin release, or in the presence of bound F-actin with a subsequent check for ATP-dependent F-actin release. Corresponding controls were done without NEM.

Monoclonal anti-myosin J14. After immobilization and washing with buffer B, the sample protein was incubated (20 min, 25°C) with 2 × 100 µl culture supernatant of the monoclonal antibody J14 (Parke *et al.*, 1986), diluted (10^{-1} and 10^{-4}) in buffer B. As a control, the antibody (diluted 10^{-1}) was preadsorbed with muscle myosin (0.1 mg/ml, 20 min, 25°C) prior to incubation with the sample protein. Myosin was purified from chicken breast muscle according to Hynes *et al.* (1987).

Calmodulin binding assay

Calmodulin from bovine brain (Sigma, Deisenhofen, Germany) was dissolved in buffer A (50 µg/ml) and adsorbed to nitrocellulose-coated coverslips (3 min). After blocking residual protein binding capacity with buffer B (3 min), the chamber was washed with buffer A varied in pCa (7, 5 or 3). Free Ca^{2+}-concentrations were calculated as described (Russ *et al.*, 1991). All the following steps were carried out at the respective pCa. The chamber was filled with supernatant S2 (3 min), washed with buffer A, loaded with RLP-labelled F-actin (1:70 in buffer B; 3 min) and washed with buffer A. Binding of F-actin was then inspected in the fluorescence microscope and tested for ATP-dependent release.

Video microscopy

The binding of RLP-labelled F-actin was monitored by epifluorescence (Diaplan, NPL Fluotar 40x, NA 1.32, Ploemopak N2; Leitz, Wetzlar, Germany). Images were recorded with a SIT-camera (C2400-08-C, Hamamatsu, Herrsching, Germany) onto tape (VHS, Panasonic AG-6720). After image processing (averaging and pseudo-3D; Multicon, Leitz), photographs were taken on TMax 100, using a freeze frame recorder (RGB version, Polaroid Corp., Cambridge, MA, U.S.A.) equipped with a 35 mm camera adaptor.

SDS-PAGE and immunoblotting

Proteins were separated on 7.5% gels by discontinuous SDS-PAGE (Laemmli, 1970) and silver stained (Blum *et al.*, 1987). Myosin was detected after western transfer onto nitrocellulose (Immobilon-P, Millipore, Neu-Isenburg, Germany), using monoclonal anti-myosin J14 as described (Grolig *et al.*, 1988).

RESULTS

Identification of two protein fractions with distinct F-actin binding activities

After extraction of *Chara* internodal cells, the supernatants S1 and S2 (both about 0.6 mg protein/ml) obtained by centrifugation (8500 × ***g*** for 10 min, and 110,000 × ***g*** for 30 min, respectively), contained comparable concentrations of proteins which after immobilization on nitrocellulose-coated coverslips bound fluorescently labelled F-actin. This activity survived freezing in liquid nitrogen and storage at −80°C for several months and it decreased only slowly if

the extract was kept on ice. The extract could be diluted 1:5 to 1:10, before a decrease in the density of bound F-actin was observed. Using protein fractionation by anion exchange chromatography with a linear KCl-gradient, two clearly different F-actin binding activities could be separated.

A fraction released by 230 to 270 mM KCl binds F-actin laterally

Upon elution of bound S2 proteins, the first F-actin binding protein fraction was released within the range of 230 to 270 mM KCl (Fig. 1(a)). In contrast to free F-actin, which displayed Brownian motion, the bound F-actin was stationary and images could be recorded without any blurring. In most cases the complete filaments appeared to be immobilized through lateral attachments.

A fraction released by 440 mM KCl binds F-actin at ends only

The second F-actin binding protein fraction was eluted at an ionic strength of about 440 mM KCl (Fig. 1(a, b)). Its relative abundance as detected after immobilization on nitrocellulose was significantly lower than the fraction that eluted between 230 and 270 mM KCl and it bound the RLP-labelled F-actin filaments at their ends rather than laterally. The remaining free ends of such tethered microfilaments were still in Brownian motion, thus impeding proper documentation of the binding in an averaged image (Fig. 1(b)).

The activity of the lateral binding fraction is ATP sensitive

The protein fraction released within the range of 230–270 mM KCl showed ATP-sensitive binding of F-actin. The bound F-actin was readily released when the reaction chamber was charged with ATP concentrations as low as 10^{-5} M (Fig. 2(b)). At even lower ATP concentrations ($1–3 \times 10^{-6}$ M) and if the nucleotides diffused into the chamber, short range translocations and repetitive release and reattachment of the actin filaments could be observed (not shown). ADP of equivalent concentrations was not effective in the release of bound F-actin (Fig. 2(b)).

The ATP-sensitive binding of F-actin could be inhibited by application of N-ethylmaleimide (NEM) at concentrations of 0.05 to 0.5 mM (Fig. 2(c)). Inhibition of binding occurred both with or without previous ATP-dependent release of bound F-actin. In the presence of previously bound F-actin, a significantly reduced inhibitory action of NEM could be detected after a comparable incubation time (Fig. 2(c)); after treatment, the bound F-actin could still be released by ATP. Separate treatment of the F-actin with NEM had no effect on its binding to the immobilized proteins (not shown).

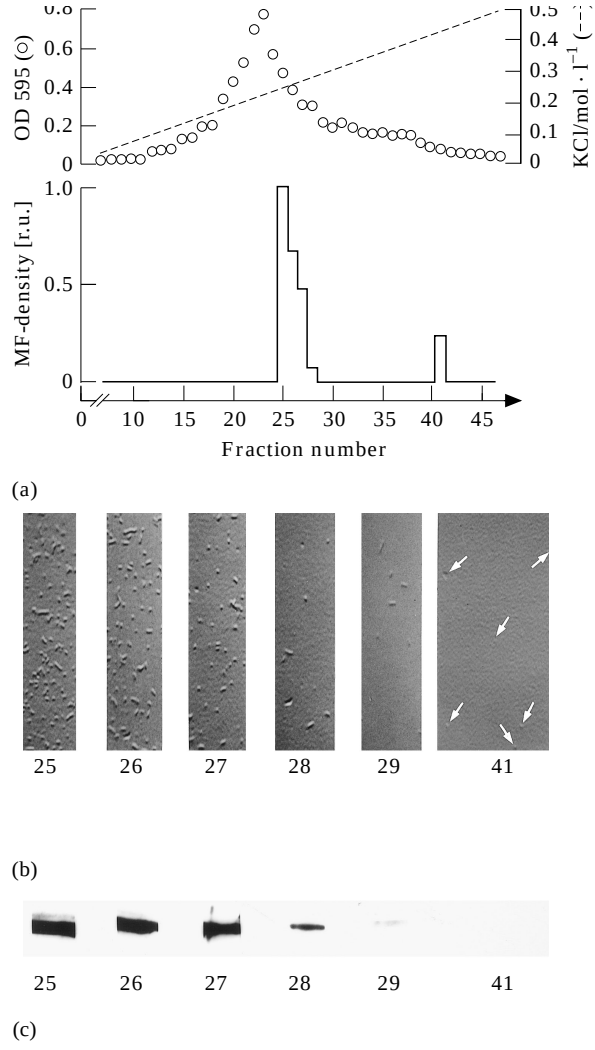

Fig. 1. Detection of two F-actin binding protein fractions after fractionation of total soluble *Chara* protein by anion exchange chromatography. (a) Upper part: profile of protein elution (○) by a linear KCl-gradient (– – –). Lower part: relative density of microfilaments (MF) attached to the nitrocellulose-coated coverslip after immobilization of the respective gradient fraction. (b) Micrographs showing the MF-densities of fractions Nos 25–29 (lateral binding of sheared F-actin) and of fraction No. 41 (end-binding of not-sheared F-actin; arrows). (c) Immunoblots of the respective fractions with monoclonal anti-myosin J14.

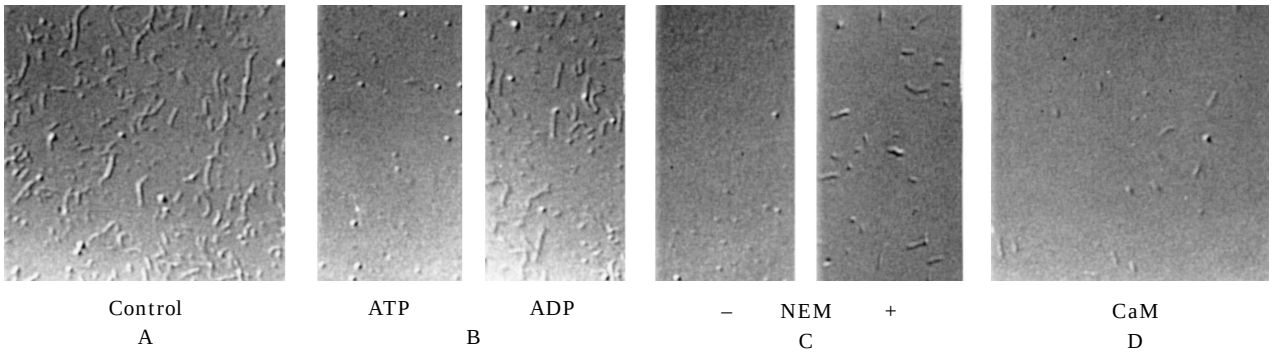

Fig. 2. The effects of ATP, ADP, N-ethylmaleimide (NEM) and calmodulin (CaM) on the F-actin binding activity of *Chara corallina* protein extract. (a) F-actin, laterally bound to immobilized *Chara* protein (control). (b) Complete release of the F-actin by 10^{-5} mol/l ATP. No release of F-actin after application of the same concentration of ADP. (c) Inhibition of F-actin binding after treatment of immobilized *Chara* protein with 0.5 mM NEM for 3 min in the absence (−) of F-actin; reduced inhibition in the presence (+) of bound F-actin. (d) F-actin as bound to *Chara* protein which has been immobilized indirectly via calmodulin adsorbed to the nitrocellulose-coated coverslip.

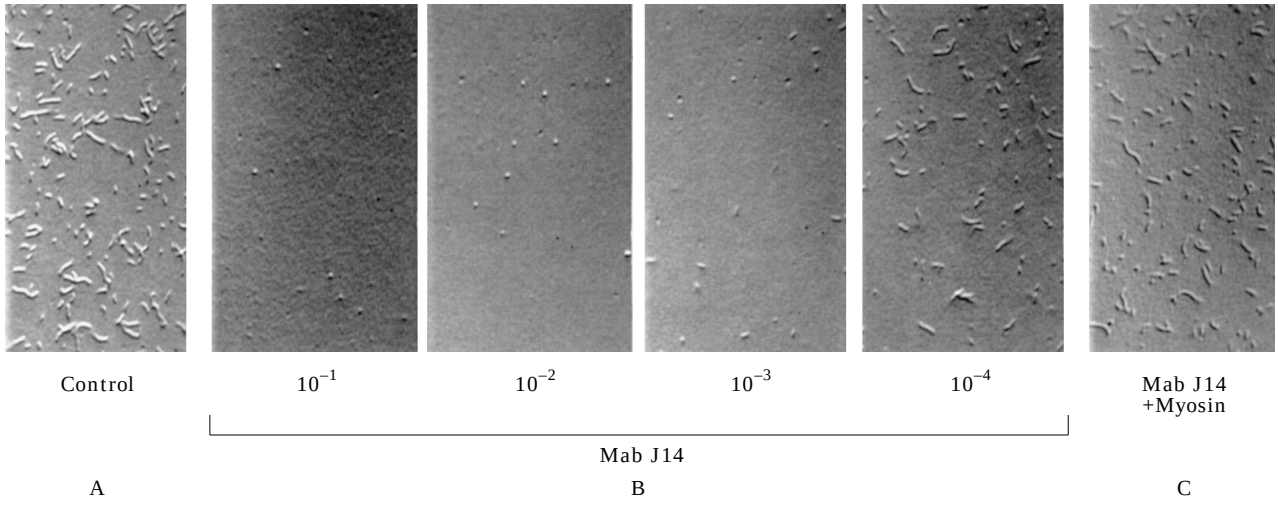

Fig. 3. Blocking of ATP-sensitive binding of F-actin by monoclonal anti-myosin J14. (a) Control; (b) increase of F-actin binding with increasing dilution (10^{-1}–10^{-4}) of the antibody; (c) preadsorption of the antibody with purified chicken muscle myosin abolishes its capacity to block the binding of F-actin.

A myosin-specific antibody inhibits F-actin binding and recognizes a 110 kDa protein from the laterally binding fraction

ATP-sensitive binding of F-actin to the immobilized *Chara* proteins also decreased if the sample was preincubated with increasing concentrations of the monoclonal anti-myosin J14 (Fig. 3(a, b). Preadsorption of the antibody with purified chicken muscle myosin completely impeded the capacity of the antibody to block the F-actin binding (Fig. 3(c)). In the Western blotting analysis, the monoclonal anti-myosin antibody J14 detected a 110 kDa protein in decreasing amounts (Fig. 1(c)) in the respective gradient fractions with correspondingly decreasing ATP-sensitive binding to F-actin (Fig. 1(b)).

Calmodulin interacts with the laterally binding fraction

To test the calmodulin binding activity of the laterally binding fraction, calmodulin from bovine brain was bound to the nitrocellulose-coated coverslip, residual protein binding capacity blocked by surplus BSA, and the chamber was then perfused with the respective *Chara* protein fractions (Fig. 2(d)). When F-actin was applied to the chamber, ATP-sensitive binding was observed. No such binding was found in the control, where no calmodulin had been adsorbed. In contrast to the firm, lateral attachment of F-actin to directly immobilized *Chara* extract protein, however, the laterally bound F-actin appeared less strictly immobilized and was gradually washed out upon

perfusion of the chamber with ATP-free solutions. No major differences in the amount of bound F-actin were observed with respect to the pCA prevailing during calmodulin adsorption or during subsequent steps of the assay (not shown).

The end-wise F-actin binding protein is ATP-insensitive and is not recognized by an anti-myosin

In the case of the fraction that was released by 440 mM KCl, F-actin binding persisted in the presence of ATP, and no protein was recognized by the antibody J14 (Fig. 1(c)). In contrast to the fractions containing the ATP-sensitive, lateral F-actin binding activity, the fraction with the F-actin end-binding activity contained only very few proteins as revealed by silver staining (not shown).

DISCUSSION

Our use of fluorescently labelled microfilaments to probe for F-actin binding proteins from *Chara corallina* has proven to be a very suitable method for the typically small amounts of protein obtainable from plant extracts. The ability to probe rapidly partially purified protein fractions, and the adsorption of the sample protein to nitrocellulose reduced the risk of proteolytic degradation. In contrast to F-actin affinity chromatography or cosedimentation (Miller and Alberts, 1989; Lange, 1992), our use of a layer of immobilized sample protein minimized the chance of non-specific, indirect interactions of aggregates with the actin polymer. Using the assay to screen protein fractions obtained by anion exchange chromatography, we succeeded in detecting two proteins from *Chara* that bound F-actin in different ways: one bound the polymer laterally, the other at its end.

The first protein could be partially characterized as a putative myosin by monitoring the effects of various agents, including ATP, NEM, a myosin-specific antibody and calmodulin, on its capability to bind F-actin. F-actin, even in the case of longer filaments, mostly bound very firmly over the entire length, was selectively released by ATP but not ADP, with ATP being effective at μmolar concentrations.

Although no inhibitors are currently available that specifically block the interaction of myosin with F-actin, the sulfhydryl-alkylating agent N-ethylmaleimide (NEM) has been employed to modify covalently and inhibit efficiently enzymes such as myosins with essential SH-groups. Collecting the endoplasm from the *Chara* internodal cell by centrifugation and treating it with NEM, Chen and Kamiya (1975) showed that NEM acts on the endoplasm and not on the cortical actin bundles. As shown in this study, the immobilized putative *Chara* myosin, functionally tested by previous ATP-dependent release of bound F-actin, was no longer able to bind F-actin *in vitro* after treatment with 0.5 mM NEM for 3 min. Experiments without previous ATP-dependent F-actin release but with the same sample of protein confirmed that inhibition was not caused by residual ATP. In contrast to the putative *Chara* myosin, NEM-treatment of purified muscle heavy meromyosin leads to irreversible binding of F-actin by blocking myosin ATPase-activity (Meeusen and Cande, 1979). ATP-reversibility of the binding of muscle myosin to F-actin is very sensitive to oxidation (T. Q. Uyeda, pers. comm.; our own experience with myosin purified from chicken breast muscle), whereas the putative *Chara* myosin of the current study retained this ability even after treatment with buffers lacking any antioxidant. Together with the extraordinary translocation velocities of more than 70 μm/s *in vivo*, these observations hint to major functional differences between the *Chara* putative myosin and conventional muscle myosin.

The monoclonal antibody J14 recognizes a common structural feature or epitope of myosins from muscle, non-muscle and plant cells (Parke *et al.*, 1986). Therefore, the observed concentration-dependent inhibition of F-actin binding of the immobilized *Chara* protein by the large (IgM) antibody probably is due to sterical blocking of the F-actin binding site, which is close to the ATP-binding site (Reedy, 1993). Abolishment of this inhibitory effect by preadsorption of the antibody with purified chicken muscle myosin proves that, in the case of the immobilized *Chara* protein, the antibody has interacted with a myosin-specific epitope. Immunodetection of decreasing amounts of a 110 kDa protein (Fig. 1(c)) precisely in the gradient fractions of anion exchange chromatography that had correspondingly decreasing ATP-sensitive binding to F-actin (Fig. 1(b)) indicates that this protein carries the ATP-sensitive F-actin binding activity. Complete blocking of the F-actin binding capacity by either NEM or the antibody J14 shows that the same protein is the target for both agents.

Combining the tested features of the 110 kDa polypeptide, we conclude that it represents the more abundant of two proteins previously detected by immunoblotting with anti-myosin J14 in crude extracts of *Chara* and localized by indirect

immunofluorescence of organelles of the streaming endoplasm and along the subcortical actin bundles (Grolig *et al.*, 1988). The second, much less abundant protein of $M_r \approx 205$ kDa could not be detected as separate in the fractions of anion exchange chromatography; it may coelute with the 110 kDa protein. In view of immunodetection after rapid extraction into 20% (v/v) trichloroacetic acid (Grolig *et al.*, 1988), it appears unlikely that the 110 kDa protein is a product of proteolytic degradation of the 205 kDa protein. Recently, a myosin sequence coding for a polypeptide of about 100 kDa has been reported for the green alga *Acetabularia* (Vugrek and Menzel, 1994), supporting the occurence of small molecular weight myosins in lower plants. Detection of the 110 kDa myosin-like protein of *Chara* in the $10^5 \times g$ supernatant indicates that the organelle-associated fraction which was previously detected by immunofluorescence in perfusion-fixed cells (Grolig *et al.*, 1988) is complemented by a soluble pool.

All known unconventional myosins can bind calmodulin- or calmodulin-related light chains via multiple myosin IQ motifs positioned at the myosin neck domain (Hasson and Mooseker, 1995; Wolenski, 1995). This binding of calmodulin to myosin is unlike that of most target enzymes that are activated by high affinity binding to Ca^{2+}-calmodulin (Allan and Hepler, 1989). Recovery of the Ca^{2+}-inhibited cytoplasmic streaming in *Chara* is specifically blocked by calmodulin inhibitors, suggesting that a calmodulin-activated phosphatase is involved in dephosphorylation of a regulatory light chain (Tominaga *et al.*, 1987). We were therefore prompted to test the binding of calmodulin to the putative myosin. Calmodulin is found in abundance throughout the endoplasm (Jablonsky *et al.*, 1990), so it might easily associate with this putative myosin.

After adsorption of calmodulin to the nitrocellulose-coated coverslip, binding of ATP-releasable F-actin was observed only if *Chara* protein fractions containing the putative myosin were perfused through the reaction chamber before introduction of fluorescent actin filaments. The sandwich-like, indirect mode of binding was obvious because, unlike the very firm attachment when F-actin was bound to directly immobilized *Chara* protein, increased movement of the bound actin filaments was detected. Because an increasing proportion of bound F-actin disappeared upon chamber perfusion, the binding of the putative myosin to the immobilized calmodulin appears to be considerably weaker than to the F-actin. No significant difference in the amount of bound F-actin was observed if the assay was performed at different pCa (3, 5 or 7). However, the majority of characterized myosins show a higher affinity to calmodulin in the absence of Ca^{2+} (Wolenski, 1995), the chicken brain class-V myosin also binds calmodulin in a calcium-independent manner (Espreaficio *et al.*, 1992).

In vitro motility studies of Kachar (1985) with cell fractions containing actin bundles and their associated organelles revealed a bimodal distribution of measured organelle velocities, predicting that two different myosins exist in *Chara corallina*. Recently, an actin-based motor protein of about 230 kDa was purified by Yamamoto *et al.* (1994) from endoplasm squeezed out from previously perfused internodal cells of *Chara corallina*. This myosin was eluted from a DEAE-anion exchange column at about 0.25 M KCl, similar to the ionic strength needed to elute the putative myosin detected in our preparations. In contrast to our 110 kDa protein, the 230 kDa myosin was not recognized by the monoclonal anti-panmyosin (Amersham, N 1169; Yamamoto *et al.*, 1994), which is identical to the antibody J14. Conversely, a polyclonal antibody raised against the 230 kDa *Chara* myosin did not detect the 110 kDa protein described here (Yamamoto *et al.*, 1994). Moreover, this polyclonal antibody also did not recognize smooth muscle myosin or the myosin recently purified from lily pollen tubes (Kohno *et al.*, 1992; Yakota and Shimmen, 1994). A polyclonal antibody raised against myosin from lily pollen tube, however, recognizes a 170 kDa myosin in dicots and monocots (Yakota *et al.*, 1995). Although the antibody J14 did not recognize the 230 kDa *Chara* myosin after Western transfer (Yamamoto *et al.*, 1994), it remains possible that J14 might block the binding of F-actin of the 230 kDa *Chara* myosin if it were immobilized on a nitrocellulose-coated coverslip where it probably retains a more native conformation.

In contrast to non-plant cells (Pollard and Cooper, 1986; Hartwig and Kwiathkowski, 1991), only very limited information is available on F-actin binding proteins as regulatory modules of the actin cytoskeleton in plants cells (Shibaoka and Nagai, 1994). Interestingly, with respect to the as yet completely unknown interaction of the plant actin cytoskeleton with intracellular anchorage sites, a second protein was capable to bind the RLP-labelled actin filament at its end. Persistence of the F-actin binding in the presence of ATP and no interaction with the anti-myosin antibody indicate that the protein is not related to myosin. In non-plant cells, so far mostly end-binding to

proteins to the fast-growing plus-end of F-actin has been described (Pollard and Cooper, 1986; Hartwig and Kwiathkowski, 1991), the respective proteins exhibiting nucleating and/or capping activity *in vitro*. Such a capping protein has recently been shown to influence actin assembly and cell motility profoundly in *Dictyostelium in vivo* (Hug *et al.*, 1995). As in other eukaryotic cells, end-binding proteins in plant cells also presumably mediate the anchorage, outgrowth and length distribution of actin filaments that are crucial for cytoskeletal dynamics and reorganizations occurring during the cell cycle (Traas *et al.*, 1987; Schmit and Lambert, 1990; Sawitzky and Grolig, 1995) or as being elicited by environmental stimuli (Grolig and Wagner, 1988; Nagai, 1993; Mineyuki *et al.*, 1995).

ACKNOWLEDGEMENTS

F.G. thanks Dr Geoffrey O. Wasteneys (Australian National University, Canberra, Australia) for proof reading of the manuscript, Dr Taro Q. Uyeda (Stanford University, CA, U.S.A.) for valuable advice on the purification of myosin, Michael Heidecker (Max-Planck-Institut für Biochemie, Martinsried, Germany) for a practical introduction to the F-actin binding assay, Prof T. Wieland (Max-Planck-Institut für medizinische Forschung, Heidelberg, Germany) for a generous gift of rhodamine phalloidin, Prof B. H. Anderton (St George's Hospital Medical School, London, U.K.) for kindly providing MAb J14, and Prof R. Schnetter for sharing the SIT-camera. Financial support of the Deutsche Forschungsgemeinschaft is gratefully acknowledged (Gr 910/4-1).

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