Polymerization of G-actin by caldesmon

Barbara Gałązkiewicz, Małgorzata Mossakowska, Hanna Osińska and Renata Dąbrowska*

Nencki Institute of Experimental Biology, Department of Muscle Biochemistry, 3 Pasteur Str., 02-093 Warsaw, Poland

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Electron microscopy of negatively stained samples indicates that caldesmon induces polymerization of G-actin into filaments. Polymerization takes place in a very low ionic strength solution and is accompanied by an increase of intensity of fluorescence of G-actin labelled with *N*-(1-pyrenyl)iodoacetamide. The effect of caldesmon is abolished by calmodulin in the presence of Ca^{2+}.

Caldesmon *Actin polymerization* *Calmodulin*

1. INTRODUCTION

Caldesmon, a protein which has the ability to bind F-actin and calmodulin, was discovered by Sobue et al. [1] in chicken gizzard muscle. Its presence was subsequently demonstrated in other types of smooth muscle (aorta, uterus) and in non-muscle cells (platelets, absorptive epithelial cells of small intestine) [2,3].

It is known that binding of caldesmon to F-actin is abolished by Ca^{2+}-dependent binding of caldesmon to calmodulin [1]. On the other hand, it was shown by measurements of superprecipitation of actomyosin gel and actomyosin ATPase activity that binding of caldesmon to F-actin causes inhibition of actin-myosin interaction [4]. These two features make caldesmon together with calmodulin a good candidate for an actin-linked Ca^{2+}-dependent regulatory component in smooth muscle.

Binding of caldesmon to F-actin has been demonstrated by viscometry and sedimentation experiments [1]. Here, we show that caldesmon also interacts with G-actin and induces its transformation into F-actin. In the presence of calmodulin this process becomes Ca^{2+}-dependent.

A preliminary report of this work has been presented [5].

* To whom correspondence should be addressed

2. MATERIALS AND METHODS

Caldesmon was prepared from chicken gizzard muscle according to procedure [6] with the exception that the last step of purification was performed on a Affi-blue column instead of a calmodulin-Sepharose 4B column. Caldesmon was eluted from an Affi-blue column at 0.8 M NaCl and desalted on a Sephadex G-25 column.

Rabbit skeletal muscle actin was obtained as described in [7], calmodulin was prepared from bovine brain as in [8]. The purity of these proteins was checked by SDS-PAGE (polyacrylamide gel electrophoresis) (fig.1).

Labelling of actin with *N*-(1-pyrenyl)iodoacetamide was performed according to [9]. Labelled actin was ultracentrifuged at 300000 × *g* for 1.5 h. The pellet was homogenized and dialysed against 2 mM Hepes (pH 8.0), 0.1 mM $CaCl_2$, 0.2 mM ATP, and 1 mM NaN_3 to transform F-actin into its G-form and to remove the unbound dye. After dialysis the G-actin solution was clarified by centrifugation at 100000 × *g* for 45 min and filtered through a 0.45 μm millipore filter to remove nuclei [10]. The degree of labelling checked as in [9] was 0.2 mol probe per mol actin monomer.

Fluorescence measurements were performed in a temperature-controlled cuvette chamber using a Perkin-Elmer MPF-3L fluorescence spectrophotometer. The fluorescence intensity was measured at

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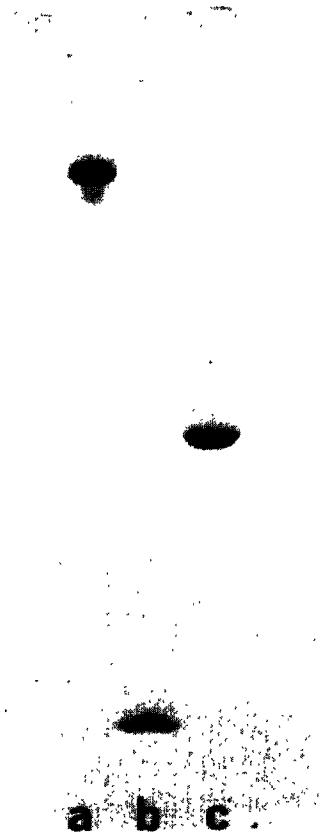


Fig.1. SDS-gel electrophoresis of purified chicken gizzard caldesmon (a), bovine brain calmodulin (b) and rabbit skeletal muscle actin (c).

408 nm after excitation at 344 nm. The results were expressed as a relative fluorescence – defined as the increase in fluorescence intensity over a control sample containing only the labelled G-actin.

For electron microscopy protein samples used for fluorescence measurements were diluted to the final concentration of actin, 0.1 mg/ml, placed on grids coated with Formvar and carbon, and negatively stained with 1% aqueous uranyl acetate. They were examined in a JEM 100B electron microscope at an accelerating voltage of 80 kV.

Measurements of the fluorescence intensity as well as electron microscopic observations of actin and actin-caldesmon mixtures were performed in a solution containing 2 mM Hepes (pH 8.0), 0.1 mM $CaCl_2$, 0.2 mM ATP, and 1 mM NaN_3 with or without KCl, as indicated in the figure legends.

SDS-PAGE was performed on 7.5–15% gradient slab gels according to the method of Laemmli [11].

Protein concentration was determined using $E_{cm}^{1\%}$ at 278 nm of 2.0 for calmodulin [12] and $E_{cm}^{1\%}$ at 290 nm of 6.3 for G-actin [13]. The concentration of caldesmon was determined by the biuret method [14] except that the readings were made at 320 nm. Bovine serum albumin was used as a standard.

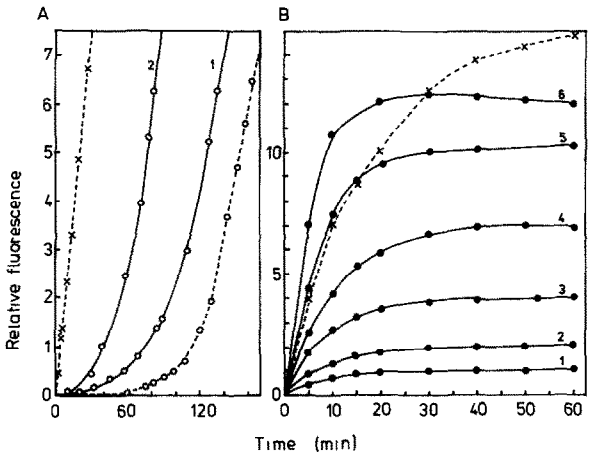
The M_r values used were 42000 for actin, 141000 for caldesmon [6] and 16600 for calmodulin [12].

3. RESULTS

Fig.2 shows the effect of varying amounts of caldesmon on polymerization of G-actin in the medium containing low KCl concentration (fig.2A) and in the presence of buffer only (fig.2B).

Polymerization was followed by measurement of the fluorescence intensity changes of *N*-(1-pyrenyl)iodoacetamide conjugated to the fast reacting cysteine of actin [9]. Addition of substoichiometric amounts of caldesmon to G-actin shortened the lag phase observed when 15 mM KCl was used to initiate polymerization of G-actin alone, and caused enhancement of the initial rate of fluorescence intensity increase. This indicates a strong nucleating effect of caldesmon on actin polymerization. Addition of caldesmon to G-actin in the absence of KCl caused increase of the fluorescence intensity of actin in a dose-dependent manner. Analysis of both emission and excitation spectra of caldesmon-actin complexes showed shapes characteristic for F-actin (fig.3). The concentration of caldesmon required to induce polymerization of actin under these latter conditions was much higher than that affecting nucleation. The minimum caldesmon amount necessary to cause G⟶F-actin transformation in the absence of salt was about 1 mol caldesmon per 40 actin monomers. A similar effect of caldesmon on G-actin was observed by measuring the viscosity (not shown).

Electron microscopic observations of samples used in the fluorescence studies (fig.2C) showed the similarity of F-actin filaments formed in the presence of either 100 mM KCl or caldesmon.



Caldesmon-induced filaments seemed to be more rigid than those formed on the influence of salt.

Polymerization of G-actin by caldesmon as monitored by measurements of fluorescence intensity (fig.4A) and viscosity (not shown) was affected by calmodulin in a Ca^{2+}-dependent manner. In the presence of calmodulin and Ca^{2+}, caldesmon lost its ability to polymerize G-actin. This property was restored by removal of Ca^{2+} by EGTA. In consequence, addition of calmodulin to the caldesmon-induced polymers of actin, assembled in the presence of Ca^{2+}, resulted in a decrease of fluorescence intensity.

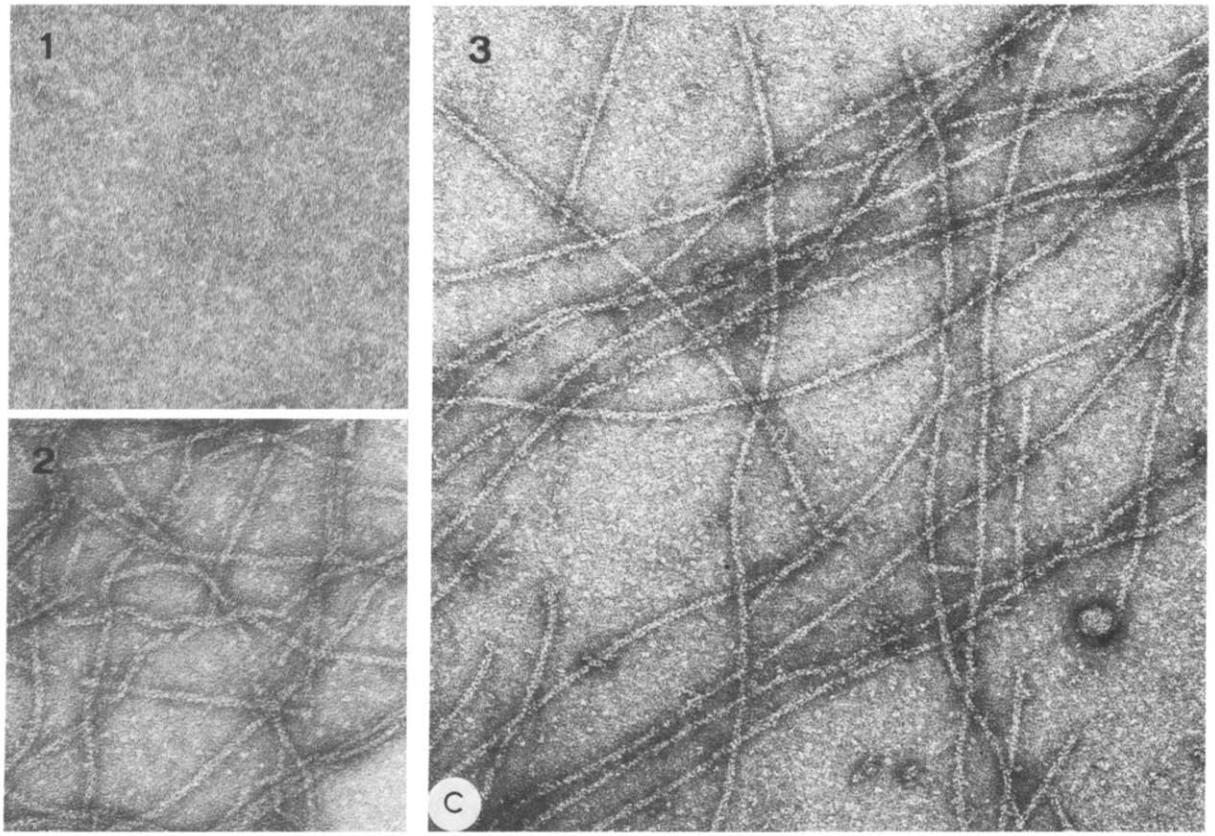


Fig.2. The effect of caldesmon on G-actin polymerization observed by fluorescence (A,B) and electron microscopy (C). (A) Samples containing 0.3 mg/ml of G-actin were preincubated with caldesmon at the molar ratio to actin monomer 1:6000 (1) or 1:2000 (2) for 10 min at 21°C. At time zero polymerization was initiated by the addition of KCl to 15 mM. Dashed lines represent G-actin polymerized either in the presence of 15 mM KCl (○) or 100 mM KCl (×). (B) Polymerization of G-actin (0.5 mg/ml) was induced by the addition at zero time of various amounts of caldesmon (solid lines) or 100 mM KCl (dashed line). The molar ratios of caldesmon to actin monomer were 1:40 (1), 1:20 (2), 1:10 (3), 1:6.5 (4), 1:5 (5), and 1:3.3 (6). (C) Electron micrographs of negatively stained samples of G-actin (1), G-actin polymerized by 100 mM KCl (2) or by caldesmon at the molar ratio to actin monomer 1:5 (3). Magnification, × 128160. For further details see section 2.

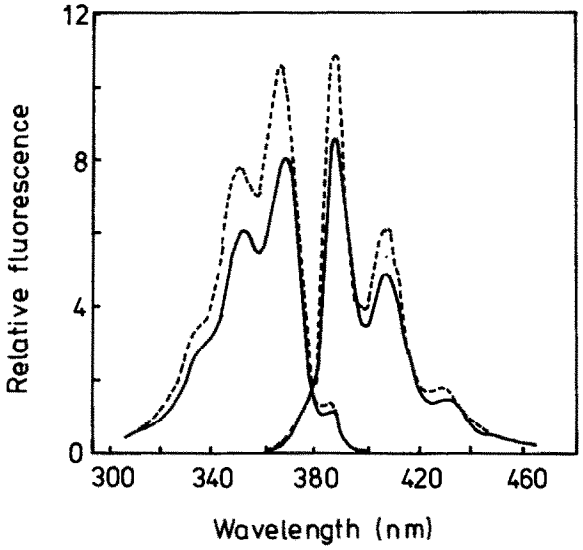


Fig.3. Fluorescence spectra of *N*-(1-pyrenyl)iodoacetamide-labelled G-actin polymerized by caldesmon (solid lines) or by 100 mM KCl (dashed lines). The excitation spectra (left) were observed at 408 nm and the emission spectra (right) were obtained after excitation at 344 nm. Concentration of actin was 0.5 mg/ml, the molar ratio of caldesmon to actin monomer 1:5.

Electron microscopy of samples used in the fluorescence measurements showed that during 3 h after addition of calmodulin to the caldesmon-induced actin polymers in the presence of Ca^{2+}, when the fluorescence intensity decreased by 74%, the number of filaments and their average length was dramatically reduced (fig.4B).

4. DISCUSSION

The studies of Sobue et al. [1] have shown that caldesmon interacts with F-actin in the presence of calmodulin in a Ca^{2+}-dependent manner. This interaction does not alter the state of either actin filaments or polymerization-depolymerization equilibrium of actin [15]. Our preliminary results [5] indicated that caldesmon can cross-link actin filaments and form F-actin bundles which partially dissociate in the presence of calmodulin and Ca^{2+}. This paper presents evidence that caldesmon also interacts with monomeric actin. It accelerates nucleation of actin monomers in the presence of salt and induces polymerization under conditions at which G-actin does not polymerize spontaneously. The latter effect requires a much higher concentration of caldesmon.

It is known that a soluble fragment of myosin, heavy meromyosin, can induce polymerization of G-actin [16,17]. Moreover, poly-L-lysine and polyamines, such as spermine and spermidine, also cause G⟶F-actin transformation [18,19]. Hence, it is suggested that the process of actin polymerization involves non-specific electrostatic interactions of polycations with negatively charged actin which, in consequence, abolishes repulsions between actin monomers [20]. Large protein molecules, like caldesmon, presumably contain highly positively charged regions which may interact with actin in a way similar to that of polycations. It is plausible that F-actin formation in the absence of salt occurs through a condensation of oligomers stabilized by caldesmon.

Caldesmon-induced actin polymers resemble those formed in the presence of salt, although they seem to be more rigid and, at higher caldesmon to actin ratios, show a tendency to form a network.

In the absence of calmodulin caldesmon induces polymerization of G-actin independent of Ca^{2+} concentration. In the presence of calmodulin polymerization of G-actin by caldesmon takes place in the absence but not in the presence of Ca^{2+} when caldesmon strongly binds to calmodulin. The question arises whether the Ca^{2+}-dependent regulation of G⟶F-actin transformation by caldesmon observed in vitro has any relevance to the situation in living cells.

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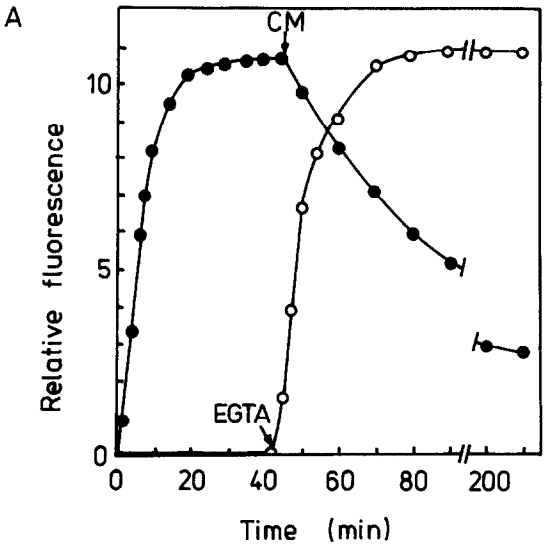

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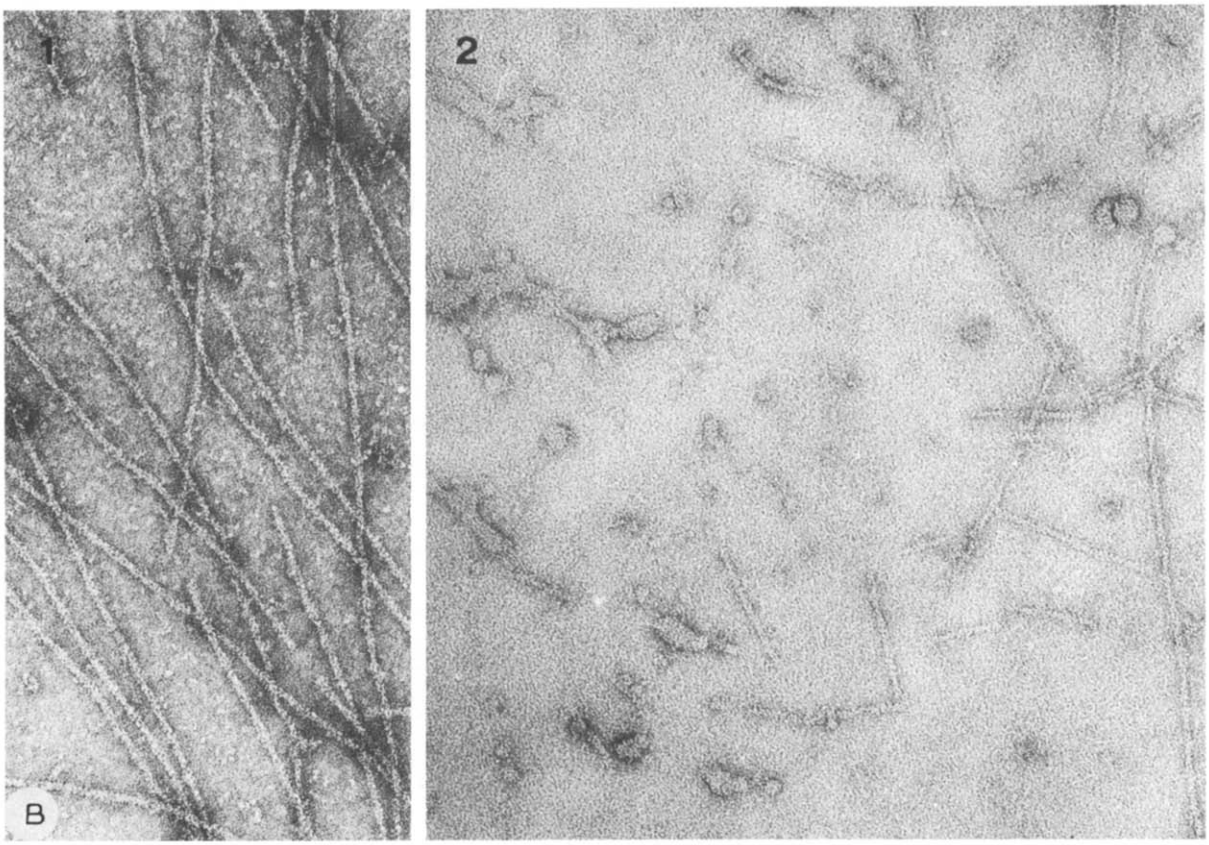

Fig.4. The effect of calmodulin on caldesmon-induced polymerization of G-actin observed by fluorescence (A) and electron microscopy (B). (A) Caldesmon (●) or caldesmon + calmodulin (○) were added to G-actin in the buffer (see section 2) containing 0.1 mM $CaCl_2$ at zero time. Arrows show addition of either 0.1 mM EGTA or calmodulin (CM). Concentration of G-actin was 0.5 mg/ml, the molar ratio of caldesmon to actin monomer 1:5 and calmodulin to caldesmon 5:1. (B) Electron micrographs of negatively stained samples of G-actin polymerized by caldesmon (1), the same 3 h after addition of calmodulin (2). Magnification, × 129600.

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