G-ACTIN MODIFIED BY PLASMA MEMBRANE INTERACTION POLYMERIZES ONLY IN THE PRESENCE OF FILAMENTOUS MYOSIN

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1. Introduction

In non-muscle cells actin can occur in different forms depending on the immediate and local needs of the cell. This suggests the presence of regulatory mechanisms capable to allow an easy interconversion between polymerization states of actin ranging from functional microfilaments to oligomeric or even monomeric actin.

We describe here a potential regulatory mechanism mediated by the interaction of G-actin with plasma membranes. After the interaction G-actin is modified insuch a way that it does not polymerize in the presence of 2 mM $MgCl_2$ but only in the presence of myosin with the formation of contractile units.

2. Materials and methods

Plasma membranes were prepared from rat liver [1]. Rabbit muscle ATP–G-actin (myokinase-free) was prepared as in [2]; myosin as in [3] and myosin subfragment-1 as in [4].

The extinction coefficients ($E^{280}_{1\%}$) used for myosin and for the subfragment-1 were 5.43 [5] and 8.0 [6], respectively. The extinction coefficient ($E^{290}_{1\%}$) used for G-actin was 6.2 [7].

Molar concentrations were calculated on the basis of mol. wt 510 000 for myosin and 115 000 for subfragment-1 [8] and of 42 000 for G-actin [9].

To measure the phosphate organically bound to actin, the supernatant solution, obtained after sedimentation of the plasma membranes, was treated with 5% trichloroacetic acid. The protein precipitate was treated according to [10] and P_i was determined as in [11].

The inhibitory activity on DNase I was related to the concentration of actin by means of standard curves obtained by plotting known concentrations of G-actin against the corresponding inhibitory activity on a constant amount of DNase I. DNase I activity (beef pancreas DNase I from Sigma) was determined spectrophotometrically at 260 nm in a test system containing 25 mM Tris–HCl buffer (pH 7.5), 5 mM $MgCl_2$, 2 mM $CaCl_2$ and Sigma DNase type I, 40 μg/ml. Polymerization of G-actin, induced by the addition of 2 mM $MgCl_2$, was measured with an Ostwald viscosimeter maintained at 23 ± 0.1°C.

Activation of myosin by actin (5 μM as the monomer) was measured at 23°C and pH 7.0 in a test system containing: 2 mM ATP, 0.15 M KCl, 10 mM Tris–HCl buffer and myosin (0.25 mg/ml).

3. Results and discussion

We have reported [12] that the properties of both G-actin and F-actin are modified by the interaction with liver plasma membranes. These modifications are illustrated in table 1, where a typical experiment is reported.

As a result of the interaction with plasma membranes G-actin is phosphorylated, loses the ability to inhibit pancreatic DNase I and to polymerize in the presence of 2 mM $MgCl_2$ or 0.1 M KCl.

The modified G-actin, however, retains the property to activate myosin. This data, which escaped recognition in [12], is quite surprising. It was expected that the modified G-actin, which is not polymerized by 2 mM $MgCl_2$, would have not been able to activate myosin. It is in fact known that G-actin activates myosin much less than F-actin [13]. A possible expla-

Table 1
Modification of G-actin by liver plasma membranes

Sample	Time of incubation (h)	Organic phosphate (μM)	Actin retaining DNase I inhibitory activity (μM)	Relative viscosity	Myosin activation (%)
G-Actin	0	1.2	24	2.15	230
	3	1.2	22	2.10	230
G-Actin +	0	4.0	24	2.10	230
plasma membranes	3	23	2	1.07	230

The incubation mixtures contained 24 μM G-actin, 0.1 mM ATP, 0.2 mM $CaCl_2$, 2 mM Tris–HCl buffer and plasma membranes (2 mg/ml); pH 7.8; temp. 30°C. At time zero and after 3 h incubation samples were taken and actin was recovered in the supernatant solution by sedimentation of the membranes at 17 000 × g and 2°C. Organic phosphate, DNase I inhibiting activity, relative viscosity and activation of myosin by actin were determined as in section 2

nation of our data was that myosin could induce the polymerization of the modified G-actin. To test this hypothesis actomyosin threads were prepared [14] by mixing either modified or native G-actin with myosin filaments prepared as in [15]. As shown in table 2 the actomyosin threads prepared with native G-actin and those prepared with modified G-actin contracted to the same extent in the presence of 2 mM ATP.

This means, evidently, that, in the presence of filamentous myosin, filamentous actin has been formed from modified G-actin. Only filamentous myosin, however, induces the formation of filamentous actin from modified G-actin. This was shown by treating modified G-actin with myosin subfragment-1 at low ionic strength. While the addition of

Table 2
Contraction, by the action of ATP, of actomyosin threads prepared from native myosin and either native actin or actin modified by interaction with plasma membranes

Threads prepared with	% Original length
a) Myosin + native actin	57 ± 8.3 (av. 9 expt)
b) Myosin + modified actin	58 ± 10.2 (av. 12 expt)

Actomyosin was prepared by adding 1 ml of either native or modified G-actin (1.2 mg/ml), dissolved in 0.1 mM ATP, 0.2 mM $CaCl_2$ and 2 mM Tris–HCl buffer (pH 8.0); to 10 ml myosin (0.55 mg/ml, dissolved in 0.1 M KCl and 25 mM Tris–maleate buffer (pH 6.5), polymerized according to [15]. After sitting for 1 min at 2°C the mixture was kept at 22°C and after 30 min was centrifuged at 12 000 × g for 10 min. The sediment was dissolved in 0.2 ml 1 M KCl and the threads were prepared and treated according to [14]. Modified actin was prepared by the procedure in table 1

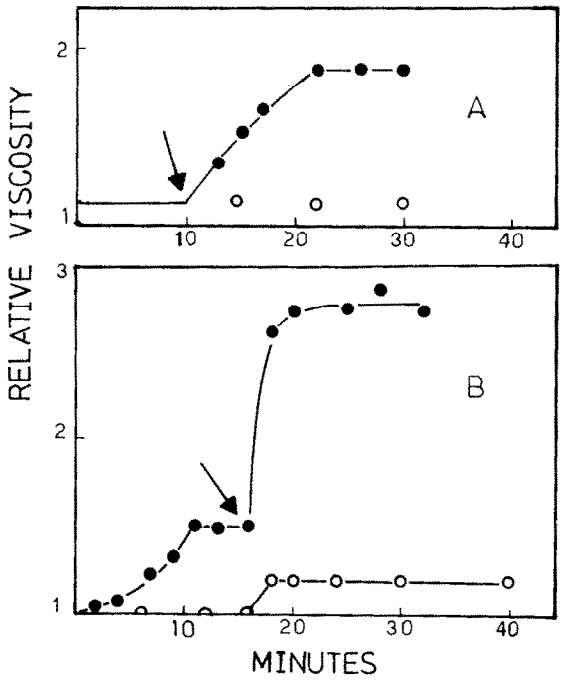

Fig.1. Effect of myosin subfragment-1 on the native and modified G-actin. (A) To a solution (1 ml) containing 10.6 μM myosin subfragment-1, 0.2 mM ATP, 0.1 mM $CaCl_2$, 1 mM mercaptoethanol and 2 mM Tris–HCl buffer (pH 7.5), at the time indicated by the arrow, 0.12 ml of either 120 μM (•) native G-actin or 120 μM (○) modified G-actin was added: temp. 22°C. Viscosity was measured as in section 2. (B) To a solution (1 ml) containing either 13 μM native G-actin (•) or 13 μM modified G-actin (○), 0.2 mM ATP, 0.1 mM $CaCl_2$, and 2 mM Tris–HCl buffer (pH 7.5); 60 mM KCl and 2 mM $MgCl_2$ were first added (time zero of the figure), followed, at the time indicated by the arrow, by 0.1 ml 130 μM myosin subfragment-1 dissolved in 2 mM Tris–HCl buffer (pH 7.5): temp. 22°C. Relative viscosity was measured as in section 2.

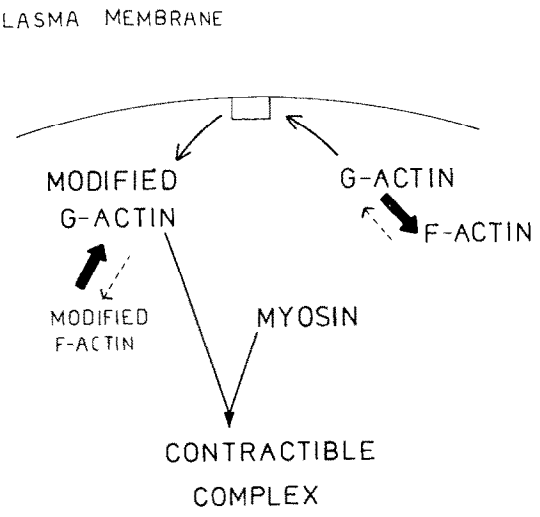

Fig.2. Effect of the interaction of actin with plasma membrane.

native G-actin to myosin subfragment-1 caused the increase of the viscosity of the solution, the addition of the modified G-actin was without effect (fig.1A).

Also without effect on the viscosity of the solution was the mixing, in the presence of 60 mM KCl and 2 mM $MgCl_2$, of modified G-actin with myosin subfragment-1 (fig.1B). Modified actin is thus kept in the cell as the monomer until filamentous myosin is available to form a contractile unit (fig.2). This feature could provide a fine regulation mechanism for the monomer–polymer conversion of actin in the non-muscle cells.

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