PHOSPHORYLATION OF ACTIN AND REMOVAL OF ITS INHIBITORY ACTIVITY ON PANCREATIC DNAase I BY LIVER PLASMA MEMBRANES

Enrico GRAZI and Ermes MAGRI
Istituto di Chimica Biologica dell'Università di Ferrara, Ferrara, Italy

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1. Introduction

It was recently reported that the 1:1 actin–DNAase I complex is dissociated by 5′-nucleotidase and the DNAase activity is restored [1]; the same result is obtained by incubating the actin–DNAase I complex with liver plasma membranes [2]. The assumption, in this case, is that actin is bound to the 5′-nucleotidase embedded in the plasma membranes and DNAase I is released free in the solution.

We have confirmed the results of these experiments and describe here a new, independent mechanism of protection of DNAase I against the inactivation by G-actin. Both G-actin and F-actin, when incubated with liver plasma membranes, are phosphorylated and lose their inhibitory activity on DNAase I. The inactivated G-actin does not polymerize. The inactivated F-actin still activates myosin.

2. Materials and methods

Plasma membranes were prepared from rat liver [3] and actin from rabbit muscle [4]. Actin concentration was measured from the absorbance at 290 nm considering that the absorbance of 1 mg of pure actin/ml (light path 1 cm) is 0.62 [5]. Molar concentration of G-actin was calculated on the basis of a molecular weight of 48 000 [6]. To measure the phosphate organically bound to actin the supernatant solution, obtained after sedimentation of the plasma membranes, was treated with 5% trichloroacetic acid, treated as in [7] and inorganic phosphate was determined as in [8].

The inhibitory activity on DNAase I was related to the concentration of actin by means of standard curves obtained by plotting known concentrations of either G- or F-actin against the corresponding inhibitory activity on a constant amount of DNAase I. DNAase activity (beef pancreas DNAase 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 DNA 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 F-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

3.1. *Modification of G-ATP actin by rat liver plasma membranes*

We have found that, by incubation with rat liver plasma membranes, G-ATP actin is phosphorylated and gradually loses the inhibitory activity on DNAase I. The loss of the inhibitory activity, apparently, parallels the loss of polymerizability (fig.1A,B).
In the absence of plasma membranes actin is completely stable when incubated under the conditions described in fig.1. The actin modifying activity of the plasma membranes differs from preparation to preparation. In some experiments complete loss of the DNAase I inhibitory activity was obtained after only 1 h incubation.

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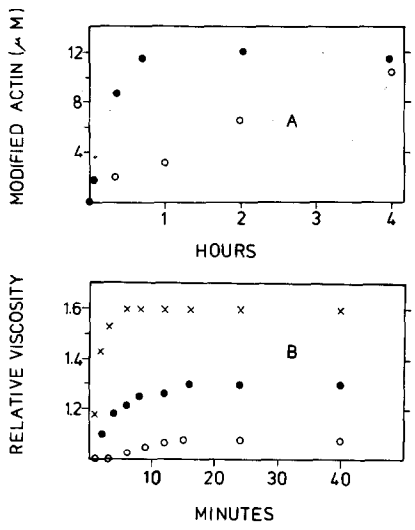

Fig.1. Modification of G-ATP actin by liver plasma membranes. The interaction between actin and plasma membranes was studied at 30°C and pH 7.2 in a system containing: plasma membranes (3 mg/ml), 12 μM G-ATP actin, 0.2 mM ATP, 0.1 mM $CaCl_2$, 1 mM mercaptoethanol and 2 mM Tris–HCl buffer. At timed intervals samples of the incubation mixtures were taken and actin was recovered in the supernatant solution by sedimentation of the membranes at 17 000 × *g* and 2°C. The phosphate organically bound to actin, the inhibitory activity on DNAase I and the polymerizability of G-actin were measured as described in section 2. (A) Determination of the phosphate organically bound to actin (•) and of the concentration of actin deprived of the inhibitory activity on DNAase I (○). (B) Determination of the polymerizability of G-ATP actin by 2 mM $MgCl_2$. G-ATP actin incubated 4 h without plasma membranes (×) or 2 h (•) and 4 h (○) with plasma membranes.

3.2. *Modification of F-actin by rat liver plasma membranes*

The effects of the interaction of F-actin with plasma membranes were similar to those of the interaction with G-actin. F-actin was phosphorylated (approximately 1 mol of phosphate was bound per mole of actin) and the DNAase I inhibitory activity was lost. The modified actin, however, fully retained the property to activate myosin. Of interest is that the relative viscosity of F-actin (12 μM as the monomer) was decreased from 1.55 to 1.35 after the interaction with plasma membranes. Apparently the modified actin has a lower tendency to exist in the polymeric form (table 1).

Acknowledgement

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Table 1
Modification of F-actin by liver plasma membranes

Sample	Time of incubation (min)	Organic phosphate (μM)	Actin retaining the DNAse I inhibitory activity (μM)	Relative viscosity	Myosin activation (%)
F-actin	0	0.8	12	1.55	250
	60	0.8	12	1.55	250
F-actin + plasma membranes	0	5.3	12	1.55	250
	60	18.1	2	1.35	250

Conditions were the same as those of the experiment of fig.1 except that 0.1 M KCl was also added. F-actin was 12 μM as the monomer. Organic phosphate, DNAase I inhibiting activity, viscosity and the activation of myosin by actin were determined as described in section 2

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