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Preferential binding of α-actinin to actin bundles

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At 37°C, the α-actinin–F-actin binding isotherm is anomalous. In 6.7% polyethylene glycol 6000, concomitantly with the formation of actin bundles, the binding isotherm becomes hyperbolic ($K_{diss.}$ = 11.3 μM). α-Actinin increases the rigidity of the networks formed by actin bundles in polyethylene glycol and by paracrystalline actin in 16 mM $MgCl_2$ but not by F-actin. It is proposed that in the cell α-actinin functions are mostly carried on by interaction with actin bundles.

α-Actinin function; Preferential binding; Actin bundle

1. INTRODUCTION

α-Actinin is a 200 kDa protein that crosslinks actin filaments and increases the rigidity of the actin gel. Both phenomena are quite complex and are not completely understood.

The binding isotherm of α-actinin with actin, as a function of actin concentration, is anomalous. The apparent association constant decreases with an increase in actin concentration [1,2]. This phenomenon was not recognized previously because the effect of actin concentration either was not tested [3–7] or was tested at relatively high actin concentrations [8].

The critical gelling concentration of α-actinin increases substantially with temperature [3], a phenomenon that led to questioning of the gelling activity of this protein in vivo [5]. It was found, however, that α-actinin is an efficient actin gelling protein, even at 37°C, provided that either the concentration of actin is low (1.2–2.4 μM) [2] or the reaction mixture is supplemented with macromolecules at a concentration equivalent to that found in the cell sap [9].

We offer evidence here that the presence of a network of actin bundles, independent of the mechanism of its formation, is a prerequisite for α-actinin functioning in vivo.

2. MATERIALS AND METHODS

G-actin from rabbit muscle was prepared according to Spudich and Watt [10] and further gel filtered through Sephadex G-150 [11]. α-Actinin from chicken gizzard was prepared according to Feramisco and Burridge [12]. The absorption coefficients used were $A^{1\%}_{290} = 6.2$ [13] for actin and $A^{1\%}_{278} = 9.7$ [14] for α-actinin. Molar concentrations were calculated on the basis of a molecular mass of 42 kDa for actin [15] and of 200 kDa for α-actinin [14]. Centrifugation was performed at 37°C in a TL100 rotor of the TL100 Beckman centrifuge.

Protein was determined by the Coomassie blue method [16] as modified by Stoscheck [17].

[^{3}H]*N*-Ethylmaleimide-labelled α-actinin was prepared and radioactivity determined as previously described [2].

The rigidity of the gels of actin was measured by the droplets method [18].

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3. RESULTS

3.1. *Effect of polyethylene glycol 6000 on the complex interactions of α-actinin with actin*

It is known that, in the presence of polyethylene glycol (PEG) 6000, F-actin undergoes massive conversion into actin bundles [19]. At 12 μM actin the boundary between filaments and bundles ranges between 6 and 7% (w/v) PEG. Addition of 0.2 μM α-actinin to the system displaces the boundary toward a lower (4–6%) PEG concentration. This shows that α-actinin favours actin bundling (Fig. 1).

The amount of α-actinin co-sedimenting with F-actin is not influenced up to 3% PEG but increases at larger PEG concentrations. The increase is concomitant with the formation of actin bundles. Under these conditions (6.7% PEG), approximately the same amount of α-actinin is sedimented by centrifugation either at 9,900 × *g* (actin bundles are collected) or at 366,000 × *g* (actin bundles plus actin filaments are collected). Thus, at this PEG concentration, α-actinin is bound almost exclusively to actin bundles (Fig. 2).

The α-actinin–F-actin binding isotherm is anomalous both in the absence [1,2] and in the presence of 3% PEG (Fig. 3). Under both these conditions actin is filamentous. The binding isotherm becomes hyperbolic concomitant with the formation of actin bundles (6.7% PEG). Double reciprocal plot analysis shows that a sin-

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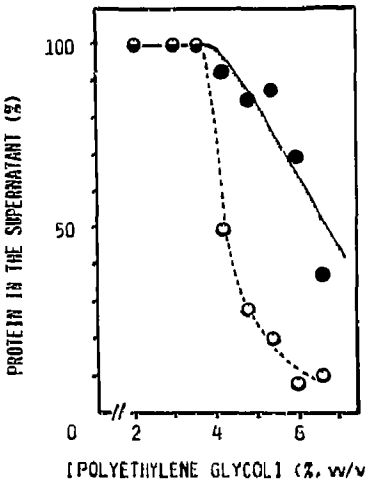

Fig. 1. In polyethylene glycol 6000 α-actinin promotes actin bundling. The mixtures contained F-actin (12 μM as monomer), 0.5 mM ATP, 2 mM $MgCl_2$, 0.1 M KCl, 1 mM dithiothreitol, 10 mM Tris-HCl, PEG 6000 (w/v) as indicated in the figure, with (○) or without (●) 0.2 μM [^{3}H]*N*-ethylmaleimide-labelled α-actinin (specific activity 12,000 dpm/nmol), pH 7.5. After 60 min of incubation at 37°C, the mixtures were centrifuged for 10 min at 9,900 × *g* to sediment actin bundles, and the supernatant solutions were assed for protein.

gle dissociation constant ($K_{diss.}$ = 11.3 μM) accounts for the binding of 0.185 μM out of the total 0.2 μM α-actinin (Fig. 3b).

3.2. *The interaction of α-actinin with paracrystalline actin in 16 mM $MgCl_2$*

In the presence of 0.2 μM α-actinin (total concentration) and of 48 μM actin, more α-actinin is bound to paracrystalline actin in 16 mM $MgCl_2$ (0.091 μM, Fig. 4) than to filamentous actin in 2 mM $MgCl_2$ plus 0.1 M KCl (0.026 μM, Fig. 3). The binding of α-actinin to

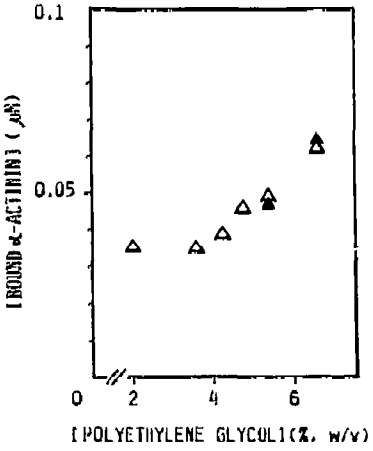

Fig. 2. Binding of α-actinin to F-actin in polyethylene glycol 6000. Experimental conditions were as described in Fig. 1. After 60 min of incubation at 37°C, the mixtures were centrifuged for 10 min either at 9,900 × *g* (▲) or at 366,000 × *g* (△). The pellets were then assayed for labelled α-actinin.

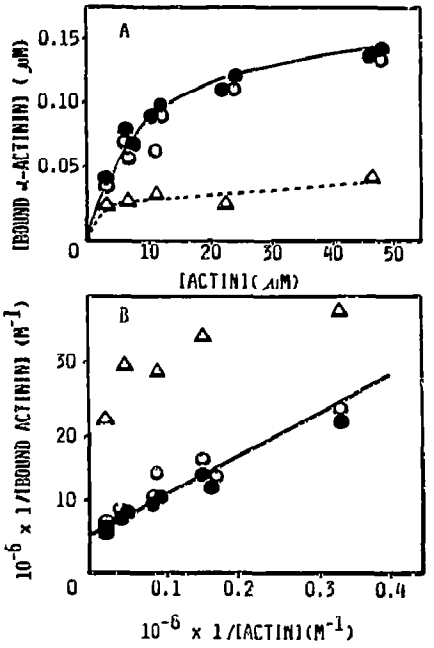

Fig. 3. Binding of α-actinin to F-actin in polyethylene glycol 6000, as a function of actin concentration. (a) Actin concentration was as indicated in the figure; PEG was either 3% (△) or 6.7% (○, ●). Other conditions were as described in Fig. 1. After 60 min of incubation at 37°C, the mixtures were centrifuged for 10 min either at 9,900 × *g* (●) or at 366,000 × *g* (○, △). The pellets were then assayed for labelled α-actinin. (b) Double reciprocal plot of the binding of α-actinin to F-actin.

paracrystalline actin, however, is not described by a simple hyperbolic function (Fig. 4).

The rigidity of the system (7.1 μM actin as monomer) increases from 2.6 to 6.7 dyn/cm^2 in the transition from

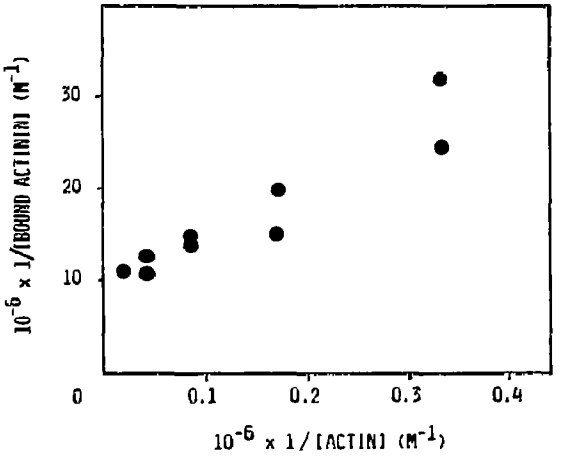

Fig. 4. Double reciprocal plot of the binding of α-actinin to actin paracrystals in 16 mM $MgCl_2$. The mixtures contained F-actin as indicated in the figure, 0.2 μM [^{3}H]*N*-ethylmaleimide-labelled α-actinin (specific activity 12,000 dpm/nmol), 0.5 mM ATP, 16 mM $MgCl_2$, 1 mM dithiothreitol, 10 mM Tris-HCl, pH 7.5. After 60 min of incubation at 37°C, the mixtures were centrifuged for 10 min at 366,000 × *g*. The pellets were then assayed for labelled α-actinin.

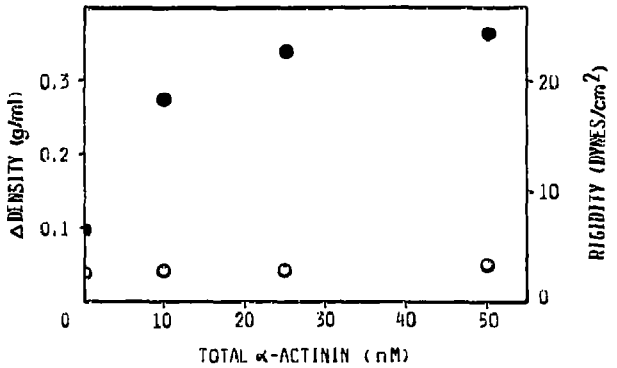

Fig. 5. Estimate of the effect of increasing α-actinin concentrations on the rigidity of the network formed by paracrystalline actin. The mixtures contained F-actin (7.1 μM as monomer), 0.5 mM ATP, 1 mM dithiothreitol, 10 mM Tris-HCl and either 16 mM $MgCl_2$ (● or 2 mM $MgCl_2$ plus 0.1 M KCl (○). α-Actinin concentration was as indicated in the figure. After 60 min of incubation at 37°C and pH 7.5, rigidity measurements were performed. Δ density represents the difference between the density at which the droplets remained stationary in the complete system and in the salt solution without protein.

F-actin to paracrystalline actin. The rigidity is further increased to 24.3 dyn/cm² when paracrystalline actin is supplemented with 0.05 μM α-actinin (Fig. 5).

4. DISCUSSION

In 6.7% PEG 6000, the binding of α-actinin to actin bundles is described by a single dissociation constant of 11.3 μM. This contrasts with the anomalous behaviour displayed by F-actin and characterized by the apparent decrease of the binding constant to α-actinin, as a function of the increase of F-actin concentration.

The parallel arrays of actin filaments, formed either in 6.7% PEG 6000 or in 16 mM $MgCl_2$, bind α-actinin tighter than does F-actin. This is in keeping with the observation that, in the cell, α-actinin is mostly associated with actin fibers [20,21]. It is likely that the arrays of filaments offer an ordered matrix of actin, which favours by bidentate binding of α-actinin. The crosslinking by α-actinin prevents the filaments from sliding in actin bundles. As a result, since the network of actin bundles is largely anastomosed, the rigidity of the system is increased by α-actinin, even at 37°C. In F-actin, at least at 37°C, the monodentate binding of α-actinin prevails. This is indicated by the total lack of effect of α-actinin on the rigidity of the network formed by F-actin.

These observations support the view that, in the cell, α-actinin functions are mostly carried on by interaction with actin bundles.

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