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Diffusion Hindrance and Geometry of Filament Crossings Account for the Complex Interactions of F-Actin with α-Actinin from Chicken Gizzard†

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ABSTRACT: The interaction of α-actinin from chicken gizzard with F-actin is quite complex. The apparent dissociation constant, C, increases with the increase of actin concentration according to the following expression: $C = K_o + a[\text{actin}] - c[\text{actin}]^{5/2}$. At pH 7.5 and 37 °C, in the presence of 0.1 M KCl and 2 mM $MgCl_2$, the dissociation constant at infinite actin dilution, K_o, is 2.17 μM. The binding of α-actinin to actin is related by the term $a[\text{actin}]$ to the diffusion of actin filaments and by the term $c[\text{actin}]^{5/2}$ to the crossing number concentration of the F-actin network. Especially at low actin concentration, the binding of α-actinin to actin is increased by gelsolin, which fragments actin filaments and increases their diffusion. The different binding isotherms of α-actinin to actin filaments and to actin bundles are discussed.

Microtubules, intermediate filaments, and microfilaments are specialized structures that determine the rheologic properties of the cytosol (Janmey et al., 1991). The contribution of microfilaments is regulated by actin–severin (gelsolin) and by the actin-gelling proteins (macrophage actin binding protein, α-actinin). Overexpression of gelsolin enhances motility in NIH 3T3 fibroblasts (Casey-Cunningham et al., 1991), while human malignant melanoma cells, lacking the actin cross-linking protein, ABP, show impaired locomotion (Casey-Cunningham et al., 1992).

The viscosity of microfilaments displays a characteristic typical of an "indeterminate fluid" or of a permanently cross-linked gel: it increases indefinitely with the decrease of the shear rate (Kasai et al., 1960; Maruyama et al., 1974; Zaner & Stossel, 1982; Buxbaum et al., 1987).

Gelsolin abolishes the unusual shear rate dependence of the viscosity of F-actin. At very low shear rates, the viscosity of the F-actin–gelsolin complexes is proportional to approximately the fifth power of the filament length. Thus, gelsolin regulates the rheologic behavior of cytoplasmic actin (Janmey et al., 1988).

α-Actinin possibly regulates the mechanical properties of the cortical network of the cell. The network will behave like a solid when the deformation rate is greater than the rate of exchange of the cross-links made by α-actinin, but will deform when deformation is slow enough to permit cross-linker molecules to rearrange (Sato et al., 1987): a mechanism that, apparently, does not explain why cells do not collapse under the constant shear forces that often exist in tissues (Janmey et al., 1990).

On these premises it is clear that the binding of the actin-cross-linking proteins must be correctly understood to properly appreciate their effects on the rheology of microfilaments. Unfortunately, this is not the case. With the exception of two cases (Sato et al., 1987; Meyer & Aebi, 1990), the binding of α-actinin to F-actin has only been studied at a single F-actin concentration (Goll et al., 1972; Bennett et al., 1984; Duhaiman & Bamburg, 1984; Landon et al., 1985). The study of the interaction of α-actinin with F-actin, as a function of actin concentration, revealed that the apparent dissociation constant increased about 40 times when the actin concentration was increased from 1.2 to 48 μM. To explain this phenomenon, we proposed that the binding of α-actinin to F-actin was a function of both reagent concentration and gel structure (Grazi et al., 1990a, 1991).

We show here that the α-actinin–F-actin binding isotherm can be properly described by taking into account two parallel phenomena which occur with the increase of actin concentration: (a) the abrupt decrease of the diffusion of actin filaments and (b) the increase of the number of crossings, due to the entanglement of filaments. These phenomena display opposite effects on the bidentate binding of α-actinin to actin. The former hinders and the latter favors the formation of α-actinin cross-links between filaments.

EXPERIMENTAL PROCEDURES

G-Actin from rabbit muscle was prepared as described by Spudich and Watt (1971) and further gel-filtered through Sephadex G-150 (McLean-Fletcher & Pollard, 1980). α-Actinin from chicken gizzard was prepared according to Feramisco and Burridge (1980). Gelsolin was prepared from bovine plasma (Kurokawa et al., 1990). The absorption coefficients used were the following: actin, $A^{1\%}_{290} = 6.2$ (Gordon et al., 1976); α-actinin, $A^{1\%}_{278} = 9.7$ (Suzuki et al., 1976); gelsolin, $A^{1\%}_{280} = 15.38$ (Craig & Megerman, 1977). Molar concentrations were calculated on the basis of M_r values of 42 000 for actin (Collins & Elzinga, 1975), 200 000 for α-actinin (Suzuki et al., 1976), and 86 000 for gelsolin (Kurokawa et al., 1990).

[^{3}H]-*N*-Ethylmaleimide-labeled α-actinin was prepared as previously described (Grazi et al., 1991). The specific radioactivity of the preparations ranged from 9600 to 706 000 dpm/nmol. Native and [^{3}H]-*N*-ethylmaleimide-labeled α-actinin were indistinguishable when assayed (1) for their capability to increase the high shear viscosity of F-actin, as measured with Ostwald viscosimeter at 2 °C; (2) to increase the low shear rate viscosity of F-actin both at 2 and at 37 °C, as measured with "falling ball" viscosimeters; and (3) to increase the rigidity of the F-actin network at 37 °C, as measured by the toluene–carbon tetrachloride droplet method (Grazi et al., 1991).

Incubation of α-actinin with F-actin was performed at 37 °C and pH 7.5 in the presence of 0.1 M KCl, 2 mM $MgCl_2$, 10 mM Tris-HCl buffer, 2 mM mercaptoethanol, and either

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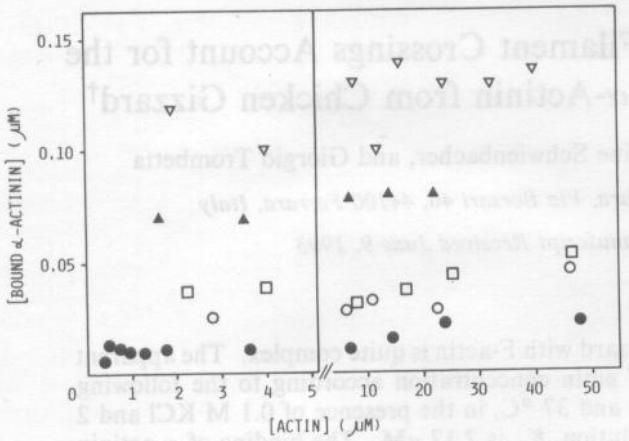

FIGURE 1: Effect of increasing concentrations of F-actin on the sedimentation of α-actinin. The mixtures contained F-actin (0.36–48 μM, as monomer) and either 90 (●), 185 (○), 200 (□), 540 (▲), or 600 nM (▽) α-actinin. The temperature was 37 °C, and the pH was 7.5. After 15–90 min of incubation, the mixtures were centrifuged, and bound α-actinin was determined as described in the Experimental Procedures section.

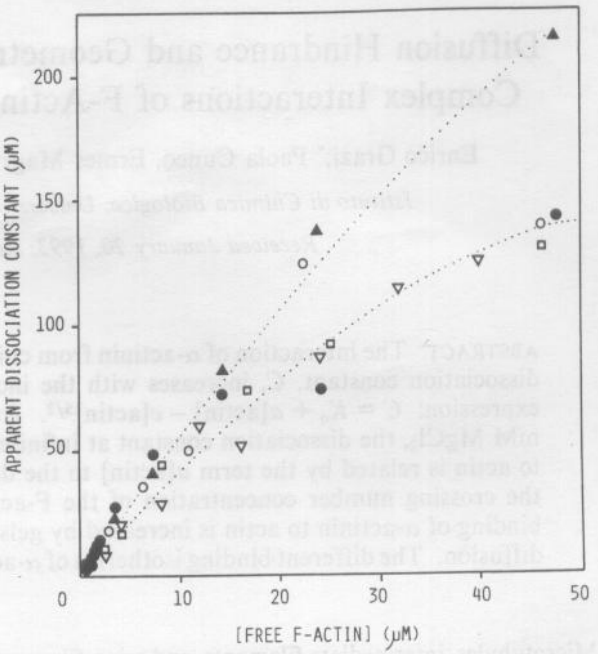

FIGURE 2: Effect of increasing concentrations of free F-actin on the apparent α-actinin–F-actin dissociation constant. The values were calculated from the data presented in Figure 1. The symbols have the same meanings given for Figure 1.

40, 100, or 200 μM ATP. The time of incubation ranged from 15 to 90 min. Differences in the concentration of ATP and of the time of incubation did not influence the binding of α-actinin to F-actin.

F-Actin-bound [^{3}H]-*N*-ethylmaleimide-labeled α-actinin was separated from free [^{3}H]-*N*-ethylmaleimide-labeled α-actinin by centrifugation at 393000*g* for 10 min at 37 °C in the TL100 rotor of the TL100 centrifuge. In the experiments with gelsolin, centrifugation was performed for 60 min to allow sedimentation of fragmented F-actin. The pellets, containing F-actin and bound α-actinin, were dissolved in the original volume of the incubation buffer. In control experiments containing α-actinin alone, no protein was sedimented after 10 min of centrifugation. In the experiments with gelsolin, in which centrifugation was performed for 60 min, about 3% of the labeled protein was sedimented in control experiments containing only α-actinin. This was taken into account in the calculation of F-actin-bound α-actinin.

After centrifugation, radioactivity was determined in the dissolved pellet and in the supernatant solutions. To optimize radioactivity determinations (Silverman et al., 1985), the protein in the supernatant solutions and in the dissolved pellets was hydrolyzed for 4 h at 25 °C with pronase (250 μg/mL). Radioactivity determinations were performed in a Packard Tri-Carb liquid scintillation counter with 5 mL of a Packard liquid emulsifier scintillation cocktail. Protein was determined by the Coomassie Blue method (Bradford, 1976), as modified by Stoscheck (1990).

RESULTS

Effect of Increasing Concentrations of Actin on the Binding of α-Actinin to F-Actin. The binding of α-actinin to F-actin was studied as a function of F-actin concentration, at different α-actinin concentrations (90–600 nM). As is shown in Figure 1, the amount of α-actinin bound to F-actin increases sharply from zero F-actin to the lowest F-actin concentration tested in each single experiment (0.36–2 μM F-actin). The increase in α-actinin bound, however, is quite modest when F-actin is further increased from 2 to 48 μM.

This behavior is best described in Figure 2, where the apparent α-actinin–F-actin dissociation constant, *C*, is plotted as a function of free F-actin concentration. The increase in *C* is approximately linear up to 10 μM actin, while at higher concentrations it follows a more complex pattern. As will be made clear in the next section, this pattern can be described by the function:

$$C = K_o + a[F] - c[F]^{5/2} \quad (1)$$

At the basis of this equation there is a physical model. It is assumed that the binding, which should be described by the thermodynamic dissociation constant, K_o, is influenced both by the increase of crossing number concentration with actin concentration ($c[F]^{5/2}$ term) and by the increase of diffusional hindrance with the increase of actin concentration. While the term $c[F]^{5/2}$ was introduced on the basis of geometric considerations, we were unable to provide a model to correlate actin concentration with diffusional hindrance. Thus we introduced the term $a[F]$, which is the simpler possible correction. K_o is evaluated by extrapolation of the value of *C* at zero actin concentration: $K_o = 2.17 \times 10^{-6}$ M.

The second term, $a[F]$, where [F] is the concentration of free F-actin [free F-actin = (total concentration of actin) – (critical concentration (0.12 μM)) – (bound α-actinin)], accounts for the decrease in the binding of α-actinin to F-actin with the increase of actin concentration. Because of diffusional hindrance, the filaments cannot approach closely enough to each other to be cross-linked by α-actinin. As a consequence, the apparent α-actinin–F-actin dissociation constant increases. The dimensionless coefficient *a* is probably related to the average length of the actin filaments, being larger at larger average lengths.

The third term, $c[F]^{5/2}$, accounts for the bidentate binding of α-actinin at the crossings generated by the entanglement of actin filaments. At high actin concentration, filaments do overlap even though rotational and translational diffusion perpendicular to the long axis are practically forbidden. At these sites of overlap, filaments can be cross-linked by α-actinin.

The values of the coefficients *a* and *c* are selected to obtain the best fit to the experimental curve. With this procedure, the values of *C* are found to fall in a region delimited by the functions

$$C = 2.17 \times 10^{-6} + 4.2[F] - (4 \times 10^{6})[F]^{5/2}$$

$$C = 2.17 \times 10^{-6} + 5.5[F] - (3 \times 10^{6})[F]^{5/2}$$

the lower and upper curves of Figure 2, respectively.

Binding of α-Actinin and the Diffusion of Actin Filaments. At infinite actin dilution, the binding of α-actinin is described by

$$[\alpha_f][F]/[\alpha_b] = K_o$$

Since $[\alpha_b]$ is given by the sum of α-actinin bound by a single bond to F-actin $[\alpha_s]$ and α-actinin cross-linking two actin filaments $[\alpha_d]$, the following relationships hold:

$$\frac{[\alpha_s] + [\alpha_d]}{[\alpha_f][F]} = \frac{[\alpha_s]}{[\alpha_f][F]} + \frac{[\alpha_d]}{[\alpha_f][F]} = 1/K_o' + 1/K_o'' = 1/K_o$$

$$K_o'K_o''/(K_o' + K_o'') = K_o$$

At finite actin concentration, the binding of α-actinin to F-actin is described by the apparent dissociation constant, C. $C = K_o'C''/(K_o' + C'')$, where C'' is the apparent dissociation constant for the bidentate binding of α-actinin to F-actin.

At finite actin concentration, three phenomena must be kept in mind: (a) the diffusion of the filaments is impaired by the increase of actin concentration (Tait & Frieden, 1982); (b) the average interfilament distance of two filaments is much larger (300 nm at 48 μM actin) (Fujime & Ishiwata, 1971) than the length of the α-actinin molecule (36 nm) (Meyer & Aebi, 1990); and (c) the diffusion of α-actinin through the actin network is not impaired (Tait & Frieden, 1982). This means that with the increase of actin concentration and the consequent immobilization of actin filaments it becomes more and more unlikely that actin filaments are cross-linked by α-actinin. As a consequence, C'' increases with the increase in actin concentration.

From the considerations referred to above, it follows that C and C'' do not describe an equilibrium. Their values are determined by the fraction of mobile F-actin, $[F_m]$, over total F-actin:

$$\frac{[\alpha_f][F]}{[\alpha_d]} = C'' \quad \text{and} \quad \frac{[\alpha_f][F_m]}{[\alpha_d]} = K_o''$$

The ratio of mobile F-actin/total F-actin is thus given by the ratio, K_o''/C'', where "mobile F-actin fraction" is an operative definition: it refers to the fraction of actin filaments still able to diffuse (Tait & Frieden, 1982). The mobile F-actin fraction is expected to decrease with the increasing concentrations of F-actin and of α-actinin.

To calculate K_o'' and C'', the value of 128 μM was taken for K_o'. This value was calculated from the binding isotherm of the 27-kDa fragment of α-actinin, a monodentate ligand, to F-actin (Mimura & Asano, 1987).

As shown in Figure 3, the fraction of mobile F-actin decreases sharply with the increase in actin concentration. The immobilization pattern of actin, polymerized in 0.1 M KCl and studied by fluorescence photobleaching recovery (Tait & Frieden, 1982), is also shown. The displacement of our curve toward lower F-actin concentrations is very likely due to the cross-linking of F-actin by α-actinin.

Binding of α-Actinin and the Rigidity of F-Actin. Assume that, at each crossing of filaments, there are n pairs of actin monomers, each one in the correct geometry to interact with the same molecule of α-actinin. The total concentration of these actin monomers, $[\beta_T]$, is thus $2n$ times the concentration of the crossings. $[\beta_f]$ of the $[\beta_T]$ monomers is the concentration

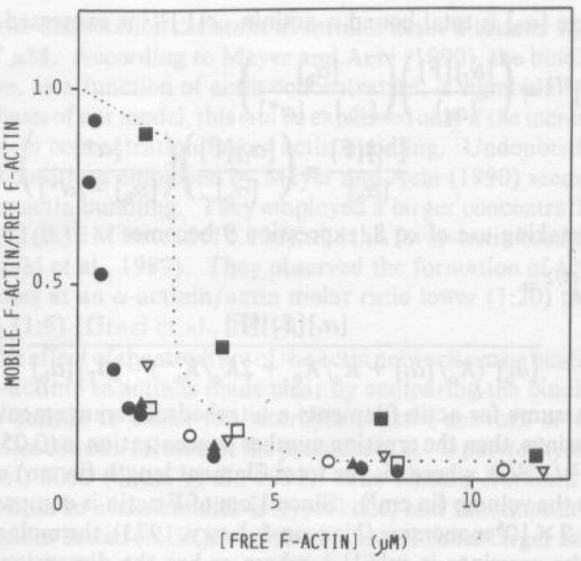

FIGURE 3: Mobile F-actin fraction as a function of F-actin concentration. Data and symbols are taken from Figures 1 and 2 for the experiments without gelsolin and from Figure 4 for the experiments performed at a gelsolin/actin molar ratio of 1:100 (■). The mobile F-actin fraction, $[F_m]/[F] = K_o''/C''$, is calculated from the following expression: $K_o''/C'' = K_o(K_o' - C)/C(K_o' - K_o)$; $K_o = 2.17$ μM and $K_o' = 128$ μM. The dotted line (actin polymerized in 0.1 M KCl) is taken from Figure 5 of Tait and Frieden (1982).

free of α-actinin, with the remaining ones binding α-actinin at the concentration $[\alpha^*]$. The interaction is described by

$$[\alpha_f][\beta_f]/[\alpha^*] = K_o \tag{2}$$

The following relationships also hold:

$$[\beta_T] = [\beta_f] + [\beta_b] \tag{3}$$

$$[\alpha^*] = [\alpha_s^*] + [\alpha_d^*] \tag{4}$$

$$[\beta_b] = [\alpha_s^*] + [2\alpha_d^*] \tag{5}$$

where $[\alpha_s^*]$ and $[\alpha_d^*]$ are the concentrations of α-actinin bound by a single bond and a double bond, respectively, and

$$[\alpha_s^*]/[\alpha_f][\beta_f] = 1/K_o' \tag{6}$$

$$[\alpha_d^*]/[\alpha_f][\beta_f] = 1/K_o'' \tag{7}$$

By making use of eqs 2–7, $[\alpha^*]$ is expressed as follows:

$$[\alpha^*] = [\beta_T]/(K_o/[\alpha_f] + K_o/K_o' + 2K_o/K_o'') \tag{8}$$

Before we proceed further, it is useful to compare the corresponding terms: $[\alpha_s]$ and $[\alpha_s^*]$; $[\alpha_d]$ and $[\alpha_d^*]$. $[\alpha_s]$ is α-actinin bound, by a single bond, to actin filaments, including filament intersections, while $[\alpha_s^*]$ is α-actinin bound, by a single bond, exclusively at the filament intersections. $[\alpha_d]$ is α-actinin that cross-links actin filaments at the intersections and along the stretches running at an interfilament distance equal to or lower than the length of the α-actinin molecule; $[\alpha_d^*]$ is α-actinin cross-linking actin filaments exclusively at the filament intersections.

Since $[\alpha^*]$ is accounted for by the term $c[F]^{5/2}$, the following relationships hold:

$$\frac{[\alpha_f][F]}{[\alpha_b]} = C$$

$$C + c[F]^{5/2} = \frac{[\alpha_f][F]}{[\alpha_b] - [\alpha^*]} = \left(\frac{[\alpha_f][F]}{[\alpha_b]}\right)\left(\frac{[\alpha_b]}{[\alpha_b] - [\alpha^*]}\right)$$

where $[\alpha_b]$ is total bound α-actinin. $c[F]^{5/2}$ is expressed by

$$c[F]^{5/2} = \left(\frac{[\alpha_f][F]}{[\alpha_b]}\right)\left(\frac{[\alpha_b]}{[\alpha_b] - [\alpha^*]}\right) - \frac{[\alpha_f][F]}{[\alpha_b]} = \left(\frac{[\alpha_f][F]}{[\alpha_b]}\right)\left(\frac{[\alpha^*]}{[\alpha_b] - [\alpha^*]}\right) \quad (9)$$

By making use of eq 8, expression 9 becomes

$$c[F]^{5/2} = \frac{[\alpha_f][\beta_T][F]}{[\alpha_b]^2(K_o/[\alpha_f] + K_o/K_o' + 2K_o/K_o'') - [\beta_T][\alpha_b]} \quad (10)$$

Assume for actin filaments a tetrahedral arrangement of crossings, then the crossing number concentration is $(0.052)(l^{3/2})(V^{-3/2})$, where l is the total filament length (in cm) and V is the volume (in cm³). Since 1 cm of F-actin is composed of 3.7×10^6 monomers (Hanson & Lowy, 1973), the molarity of the crossings is $m[F]^{3/2}$, where m has the dimension of [concentration]$^{-1/2}$ and equals 0.178. It follows that $[\beta_T] = 2n(0.178)[F]^{3/2}$. By substituting this expression for $[\beta_T]$ in eq 10, we have

$$c[F]^{5/2} = \frac{0.356n[\alpha_f][F]^{5/2}}{[\alpha_b]^2(K_o/[\alpha_f] + K_o/K_o' + 2K_o/K_o'') - 0.356n[\alpha_b][F]^{3/2}}$$

and

$$c = \frac{0.356n[\alpha_f]}{[\alpha_b]^2(K_o/[\alpha_f] + K_o/K_o' + 2K_o/K_o'') - 0.356n[\alpha_f][F]^{3/2}}$$

The two curves of Figure 2 are described by values of c equal to 4×10^6 (lower curve) and 3×10^6 M$^{-3/2}$ (upper curve). The corresponding values of n are 2.05 and 1.04, respectively. Our model thus predicts that subtle differences in the geometry of the filament crossings influence the binding of α-actinin to F-actin.

Effect of Gelsolin on the Binding of α-Actinin to F-Actin. The binding of α-actinin to F-actin was also studied in the presence of gelsolin. As is shown in Figure 4, at a gelsolin/actin molar ratio of 1:100, the concentration of bound α-actinin reaches a maximum (0.24 μM) at 2 μM F-actin, decreases up to 20 μM F-actin, and then remains practically constant up to 40 μM F-actin. At a gelsolin/actin molar ratio of 1:50, the concentration of bound α-actinin reaches 0.21 μM at 2 μM F-actin and then remains practically constant up to 40 μM F-actin.

In the absence of gelsolin, the concentration of bound α-actinin increases sharply up to 2 μM F-actin (0.12 μM bound α-actinin) and then increases slightly up to 40 μM F-actin. At 20 μM F-actin, the curves representing bound α-actinin in the presence of gelsolin (1:100 gelsolin/actin molar ratio) and in its absence are practically superimposable.

In the absence of gelsolin and at the gelsolin/actin molar ratios of 1:100 and 1:50, the values of C are 7.04, 2.46, and 3.1 μM, respectively, at 2 μM F-actin and 86, 78, and 47 μM, respectively, at 24 μM F-actin. In the absence of gelsolin and at the gelsolin/actin molar ratios of 1:100 and 1:50, the values of the apparent dissociation constant of the bidentate binding of α-actinin, C'', are 7.45, 2.5, and 3.16 μM, respectively, at 2 μM F-actin and 261, 199, and 74.7 μM, respectively, at 24 μM F-actin.

At a gelsolin/actin molar ratio of 1:100, the value of the F-actin mobile fraction is 0.04 at 11.7 μM F-actin (Figure 3), a value that is much lower than the value of 0.7 at 24 μM F-actin calculated by Doi and Frieden (1984) from experiments on fluorescence photobleaching recovery in the absence of α-actinin. Again, this discrepancy must be largely due to the cross-linking of F-actin by α-actinin. The value of the mobile F-actin fraction, at the gelsolin/actin molar ratio of 1:50, is higher than that found at a gelsolin/actin molar ratio of 1:100, only at F-actin concentrations larger than 24 μM. Apparently fragmentation of actin filaments by gelsolin favors cross-linking of F-actin by α-actinin (C'' decreases).

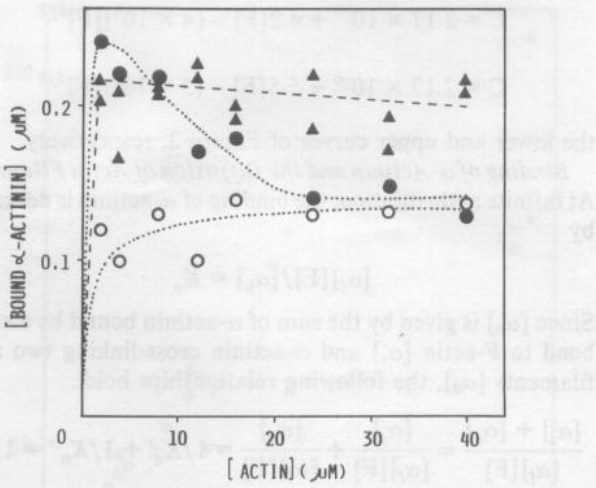

FIGURE 4: Effect of gelsolin on the binding of α-actinin to F-actin. The incubation mixtures contained 0.6 μM α-actinin (specific radioactivity 64 000 dpm/nmol) and F-actin as indicated, with gelsolin at 1:100 (●) and 1:50 (▲) gelsolin/actin molar ratios or without (○) gelsolin. The pH was 7.5. After 15 min of incubation at 37 °C, the mixtures were centrifuged for 60 min at 37 °C. The amount of α-actinin sedimented was determined as described in the Experimental Procedures section. Bound α-actinin = α-actinin sedimented in the presence of F-actin–α-actinin sedimented in the absence of F-actin (0.021 μM).

Binding of α-Actinin and the Rigidity of F-Actin. Assume that gelsolin (gelsolin/actin molar ratio 1:100) is essentially all bound to F-actin, even at the lowest concentration of actin employed (2 μM): the dissociation constant for the gelsolin–F-actin complexes is lower than 10^{-9} M (Doi & Frieden, 1984). If this is the case, the average filament is composed of 100 monomers and the filament concentration is one-hundreth the concentration of actin. The concentration of α-actinin cross-linking the filaments $[\alpha_d]$ can be calculated from the known values of C and K_o'. As is shown in Figure 5, $[\alpha_d]$ decreases with the increase in actin concentration: it is 234 and 55 nM at 2 and 24 μM actin, respectively, with the total concentration of α-actinin being 600 nM. The ratio $[\alpha_d]$/[filament] is 12.5 and 0.23 at 2 and 24 μM actin, respectively. This explains why α-actinin increases the rigidity of the actin network, even at 37 °C, provided that the concentration of F-actin is low (Grazi et al., 1990a, 1991).

In the absence of gelsolin, the effect of α-actinin on the rigidity of F-actin is displayed at nanomolar concentrations. At 37 °C, 10 nM α-actinin doubles the rigidity of the network formed by 2.4 μM F-actin (Grazi et al., 1990a). By way of an example, assume that the average filament length is 4 μm and that the change of C, as a function of actin concentration, is expressed by $C = K_o + 5.5[F] - (3 \times 10^6)[F]^{5/2}$. From the values of $K_o = 2.17$ μM and $K_o' = 128$ μM, it is found that, at 2.4 μM F-actin and 10 nM total α-actinin, the ratio $[\alpha_d]$/[filament] is 0.8; thus, on the average, each filament is connected to 1.6 filaments. This explains the effect of α-actinin on the rigidity of F-actin.

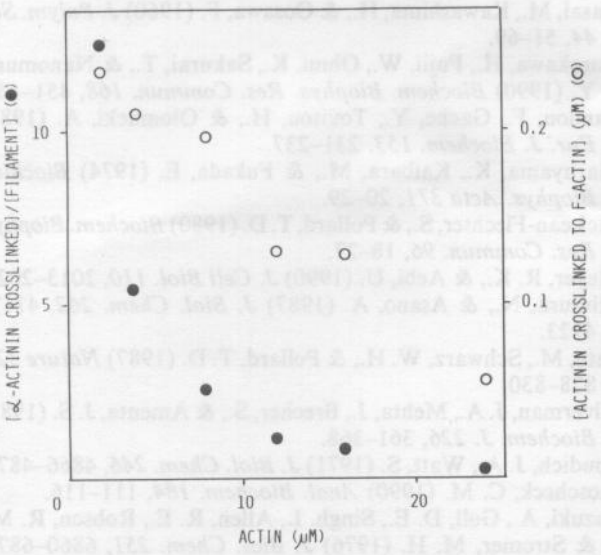

FIGURE 5: Cross-linked α-actinin concentration as a function of actin concentration. The concentration of α-actinin cross-linked to F-actin, $[\alpha_d]$, was calculated from the expression: $[\alpha_d] = [\alpha_f][F]-(K_o' - C)/K_o'C$. The values of $[F]$,$[\alpha_f]$, and C were calculated from the experiment in Figure 4 (samples with gelsolin). $K_o' = 128$ μM. Actin filament concentration was calculated by assuming that the average filament was composed of 100 monomers.

DISCUSSION

The α-actinin–F-actin binding isotherm is anomalous: the amount of α-actinin bound to F-actin does not increase hyperbolically as a function of the F-actin concentration (Grazi et al., 1990a, 1991). This behavior is explained by taking into account the properties of the actin network.

α-Actinin diffuses freely through the actin network (Fujime & Ishiwata, 1971; Tait & Frieden, 1982); consequently, it undergoes a "normal" monodentate interaction with the monomers of actin filaments. The same is not true for the bidentate interaction. At very low actin concentrations, actin filaments diffuse freely and approach each other closely enough to be cross-linked by α-actinin. At these very low actin concentration, the concentration of cross-links formed by α-actinin is related hyperbolically to the concentrations of α-actinin and actin. As the actin concentration increases, the diffusion of actin filaments decreases abruptly and a larger and larger fraction of actin monomers of the filaments becomes unavailable for cross-linking by α-actinin. The increase in the concentration of actin, however, is accompanied by an increase in the entanglement of the filaments, with a consequent increase in the number of cross-links between filaments, i.e., the number of sites available for the bidentate binding of α-actinin to actin. When these two phenomena are taken into consideration, the binding of α-actinin to F-actin is described quite faithfully.

Sato et al. (1987) studied the binding of α-actinin (1.6 μM) to F-actin (10–180 μM) and calculated an apparent dissociation constant of 26 μM. They did not detect the anomaly of the binding isotherm since they did not explore actin concentrations between 0 and 5 μM. This is made clear by their own data. The intecept of the straight line on the vertical axis [Figure 3 of Sato et al. (1987)] should yield the reciprocal of the total concentration of α-actinin, 1.6 μM. It yields instead a value of only 0.72 μM.

Meyer and Aebi (1990) explored the binding at lower actin concentrations than Sato et al. (1987), and as expected from our model, they found a much lower (1.2 μM) apparent dissociation constant, close to the value found in this article for the dissociation constant at infinite actin dilution: K_o = 2.17 μM. According to Meyer and Aebi (1990), the binding curve, as a function of actin concentration, is sigmoidal. On the basis of our model, this can be explained only if the increase in actin concentration favors actin bundling. Undoubtedly, the conditions employed by Meyer and Aebi (1990) seem to favor actin bundling. They employed a larger concentration of KCl (0.15 M instead of 0.1 M), and this favors actin bundling (Suzuki et al., 1989). They observed the formation of actin bundles at an α-actinin/actin molar ratio lower (1:20) than ours (1:6) (Grazi et al., 1991).

The effect of the structure of the actin network on the binding of α-actinin to actin is made clear by comparing the binding of α-actinin to either the isotropic F-actin network or the bundles of actin formed in the presence of 6.7% poly(ethylene glycol) 6000 (Grazi et al., 1992). The binding isotherm of α-actinin to actin bundles is hyperbolic, and the amount of α-actinin bound (to 50 μM actin) is about 3 times larger than that bound to the isotropic F-actin network; the α-actinin–actin dissociation constant (11.6 μM) is about 6 times larger than the dissociation constant at infinite actin dilution, as determined in this article. The parallel arrays of filaments in the bundles offer a homogeneous and ordered matrix of actin monomers; thus, the number of sites available for cross-linking by α-actinin is much larger than that in F-actin (this explains the larger amount of α-actinin bound). Cross-linking by α-actinin does not require filament diffusion (the filaments are already ordered); thus, the correction terms, $a[F]$ and $c[F]^{5/2}$ of eq 1, vanish and the binding isotherm becomes hyperbolic. The geometry of the binding, however, seems to be less favorable than that with F-actin since the dissociation constant increases.

α-Actinin (30 nM at 37 °C) increases the rigidity of the network formed by actin bundles (12 μM as monomer) by 2.3 times in 6% poly(ethylene glycol) (Grazi et al., 1990b). A similar effect on the network formed by F-actin is detectable only at low (2 μM) actin concentrations. In this case, an adequate number of α-actinin cross-links per filament is reached only if filaments are free to diffuse, i.e., if the actin concentration is low. With actin bundles, on the contrary, α-actinin most likely cross-links filaments of the same bundle, thus making the sliding of filaments along each other more difficult. As a consequence, since actin bundles are largely interconnected, the rigidity of the network increases.

We have proposed a model that accommodates most of the data available on the α-actinin–actin interaction and the effect of α-actinin on the rigidity of the gel formed by actin at 37 °C. The model stresses the relationship between the average filament length, the diffusion of actin filaments, the formation of actin bundles, and the cross-linking by α-actinin.

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