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EXAMINATION OF ACTIN POLYMERIZATION AND VISCOSITY INDUCED BY CATIONS AND IONIC STRENGTH WHEN CROSS-LINKED BY α-ACTININ

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Increasing potassium chloride concentration from 0 to 100 mM and magnesium chloride from 0 to 2 mM show a parallel rate increase in polymerizing actin, whereas increasing calcium chloride concentration from 0 to 0.2 mM decreases the rate of polymerizing actin. The presence of α-actinin has little influence on the polymerization kinetics of actin under these conditions. Viscometric measurements indicate that the presence of various mono- and divalent cations, ionic strength, and α-actinin in combination are responsible for changes in the mechanical properties of solutions containing actin. The actin filament dynamic behavior is drastically reduced under these conditions as confirmed by quasi-elastic light scattering.

KEYWORDS: α-actinin; actin polymerization; viscosity; cations; ionic strength.

INTRODUCTION

The biochemistry of α-actinin (MW ~100 kDa) has been reviewed extensively (Blanchard *et al.*, 1989; Vanderkerckhove, 1990). Agreement exists today that α-actinin (i) is a homodimer with its subunits oriented in an antiparallel fashion (Wallraff *et al.*, 1986; Schleicher *et al.*, 1988), (ii) cross-links actin filaments into a three-dimensional network (Jockusch and Isenberg, 1981, 1982), and (iii) is involved in linking the cytoskeleton to the plasma membrane (Burridge *et al.*, 1980; Fritz *et al.*, 1993; Goldmann *et al.*, 1999). The influence of α-actinin on actin polymerization, however, is still controversial (Muguruma *et al.*, 1992; Colombo *et al.*, 1993) as is its regulation by Ca^{2+} in muscle and nonmuscle tissues (Condeelis *et al.*, 1982; Duhaiman and Bamburg, 1984; Pacaud and Harricane, 1993). The viscoelastic properties of actin networks cross-linked by α-actinin have been shown to be regulated in a reversible manner by temperature through changes in association and dissociation kinetic of the actin/α-actinin system (Grazi *et al.*, 1990; Tempel *et al.*, 1996; Goldmann and Guttenberg, 1998).

The motivation of the present study is to use falling ball viscometry and quasi-elastic light scattering to examine the effects of α-actinin binding to F-actin under various ionic conditions and then compare these findings with actin polymerization assays and previously reported results.

MATERIALS AND METHODS

Protein preparations

α-Actinin was isolated from turkey gizzard as described by Craig *et al.* (1982) and further purified by a hydroxylapaptite column (http://iprotocol.mit.edu/protocol/300.htm). The purity was determined, using 10% sodium dodecylsulfate polyacrylamide gel electrophoresis according to Laemmli (1970). The protein was sterile-filtered and kept at 4°C in the dark. The protein concentration was measured with UV spectroscopy, using an extinction coefficient of 97,000 M^{-1} cm^{-1} at 278 nm.

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*This work is dedicated to Johannes Goldmann on his 10th birthday.

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Actin was prepared according to the procedure of Spudich and Watt (1971) with an additional gel filtration step as suggested by MacLean-Fletcher and Pollard (1980), using a Sephacryl S-300 column (http://iprotocol.mit.edu/protocol/299.htm). The protein concentration was determined using $E_{290\,nm}=26{,}460\ M^{-1}\ cm^{-1}$. The G-actin peak fractions at 1–2 mg/ml were sterile-filtered and stored in G-buffer: 2 mM Tris/HCl, pH 8.0, 0.2 mM $CaCl_2$, 0.2 mM DTT, 0.2 mM Na_2ATP at 4°C. Actin was labeled fluorescently with 7-chloro-4-nitrobenzeno-2-oxa-1,3-diazole (NBD) (Detmers *et al.*, 1981).

Buffer

F-buffer containing 2 mM Tris/HCl, pH 7.5, 0.5 mM Na_2ATP, 0.2 mM DTT at 20°C and 0, 50, or 100 mM KCl or 100 mM NaCl and 0, 1, or 2 mM $MgCl_2$ and 0 or 0.2 mM $CaCl_2$ was used in polymerization assays and specified in the figure legends. Throughout, 3 μM G-actin in the presence/absence of a molar ratio to α-actinin, $r_{A\alpha}=10$ was used.

Actin polymerization

Polymerization assays were carried out in a SPEX Fluorolog 1680, 0.22 double spectrophotometer following the increase in fluorescence of a mixture of 85% unlabeled actin and 15% NBD-actin. Polymerization was begun using 3 μM G-actin in the presence/absence of a molar ratio to α-actinin, $r_{A\alpha}=10$ and addition of various buffers. Fluorescence excitation and emission was set at 480 and 530 nm, respectively. The rate of polymerization monitored by the change of the fluorescence signal was calculated using the following relation (Senger and Goldmann, 1995)

$$A=K_1-K_0{*}e^{-k*t} \qquad (1)$$

where, A is the fluorescence amplitude, K_1 is the endpoint and K_0 is the starting point of the fluorescence amplitude, and k is the polymerization rate constant determined from $t\geq 300$ s.

Quasi-elastic light scattering

Light scattering was carried out in an apparatus described originally by Pieckenbrock and Sackmann (1992), with some modifications (Goldmann *et al.*, 1999). In brief, this device has 1024 parallel channels and a photomultiplier that uses a 'pseudo cross-correlation' method. Prior to QELS measurements at 488 nm and a 90° angle, all solutions were filtered and stored overnight at 4°C to achieve equilibrium of polymerization. The analysis used the following relation:

$$r_h=\frac{kT}{6\pi\eta D} \quad \text{and} \quad D=\frac{I^{\circ}}{q^2} \qquad (2)$$

where r_h=hydrodynamic radius, k=Boltzmann constant, T=temperature, D=diffusion coefficient, I°=intensity, η=viscosity, and q=scatter vector.

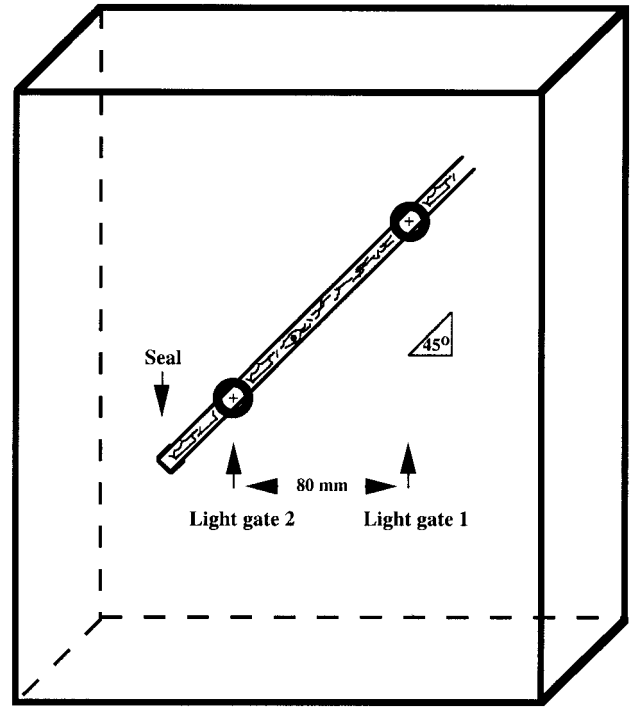

Fig. 1. The viscometer consists of an aluminum stand to hold the capillary tube (0.9 mm diameter and 137 mm length) at a 45° angle, filled with 3 μM actin in the presence/absence of α-actinin, $r_{A\alpha}=10$ and fixed to a plastic plate. The light gates are set 8 cm apart, and the time needed for a steel ball of 0.6 mm diameter and ~1 mg weight to cover this distance is measured.

Falling ball viscometry

The viscosity of actin in the presence/absence of α-actinin was determined using the method outlined by Pollard (1982). Briefly, the purpose-built apparatus (Fig. 1) consisted of an aluminum stand to which a plastic plate (15 × 15 cm) was attached. Two fittings consisted each of a light gate, 8 cm apart and set at a 45° angle to hold a capillary tube of 137 mm length and 0.9 mm diameter (VWR Scientific Inc., San Francisco). The samples at different buffer conditions were loaded and

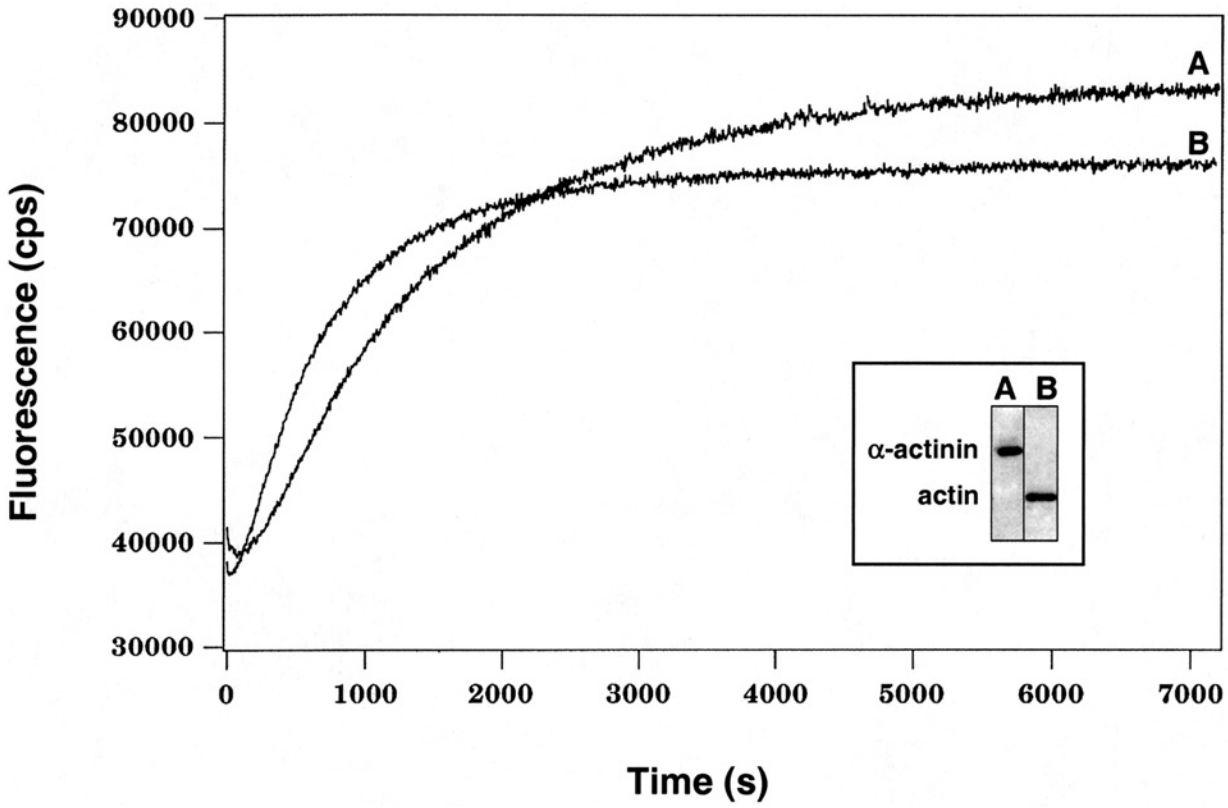

Fig. 2. Actin polymerization (3 μM) in the presence of α-actinin, $r_{A\alpha}$=10 (A) at 0.2 mM $CaCl_2$ and (B) 0 mM $CaCl_2$ and 2 mM EGTA. Buffer conditions: 2 mM Tris/HCl, pH 7.5, 0.5 mM Na_2ATP, 0.2 mM DTT, 100 mM KCl, 2 mM $MgCl_2$, at 20°C. In the absence of calcium the lag phase decreases and the polymerization rate increases. The inset shows a 10% SDS-PAGE gel of purified α-actinin (lane A) and actin (lane B).

measurements were performed after 120 min at 20°C. The time the steel ball (New England Miniature Ball Company, Norfolk CT; 0.6 mm diameter and ~1 mg weight) needed was recorded in five separate experiments. The influence of α-actinin on actin (at a constant molar ratio of 1:10) was determined from the relation

$$F_A = \frac{(t_{s1}/t_b)-1}{(t_{s2}/t_b)-1} \qquad (3)$$

where, t_{s1}=the falling ball time of a solution of F-actin in the presence of α-actinin and t_{s2} in the absence of α-actinin, and t_b=the falling ball time through the buffer. The term-specific viscosity, η_{spec} was not used in this study since actin/α-actinin are regarded as non-Newtonian samples (Cooper, 1982).

RESULTS AND DISCUSSION

Actin polymerization

The influence of α-actinin on actin polymerization was examined at various ionic conditions. Using 3 μM actin (15% NBD-actin and 85% unlabeled actin) in the presence and absence of α-actinin at $r_{A\alpha}$=10, the rate of polymerization was measured. Figure 2 shows the effect of calcium ions on actin polymerization when cross-linked to α-actinin. The rate for actin in the presence of α-actinin and calcium, using Equation (1), was $0.78 \pm 0.1{*}10^{-3}$ s^{-1} (A) compared to no calcium $1.28 \pm 0.1{*}10^{-3}$ s^{-1} (B). The rate increased in the absence of α-actinin to $1.59 \pm 0.3{*}10^{-3}$ s^{-1} with no calcium and remained the same when calcium was present ($0.72 \pm 0.1{*}10^{-3}$ s^{-1}). These results indicate that calcium ions affect the polymerization of actin but not the binding of actin to α-actinin. An opposite effect is observed when magnesium is used. At zero concentration of this divalent ion the polymerization rate drops by a factor of ~10 in the presence/absence of α-actinin compared to calcium and increases its rate with rising magnesium concentration from $0.13–0.14 \pm 0.1{*}10^{-3}$ s^{-1} at zero to $0.88–0.94 \pm 0.1–0.2{*}10^{-3}$ s^{-1} at 2 mM Mg^{2+}. These findings show that magnesium ions influence actin polymerization but not the binding to α-actinin (Fig. 3; compare rates for actin↔actin:α-actinin). A different trend is

3 μM Actin	Rates $*10^{-3}$ (s^{-1}) Actin	Actin : α-actinin (10:1)
KCl		
0 mM	0.42±0.1	0.39±0.1
50 mM	0.76±0.1	0.91±0.2
100 mM	0.64±0.1	0.88±0.1
NaCl		
100 mM	0.63±0.1	0.53±0.1
$CaCl_2$		
0 mM	1.59±0.3	1.28±0.2
0.2 mM	0.72±0.1	0.78±0.1
$MgCl_2$		
0 mM	0.13±0.1	0.14±0.1
1 mM	0.69±0.1	0.51±0.1
2 mM	0.94±0.1	0.88±0.2

Fig. 3. Determination of actin polymerization rates in the presence/absence of α-actinin, $r_{A\alpha}=10$ at various ionic conditions using static light scattering. Note: normal F-buffer composition is used: 2 mM Tris/HCl, pH 7.5, 0.5 mM Na_2ATP, 0.2 mM DTT, 100 mM KCl, 2 mM $MgCl_2$, and 0.2 mM $CaCl_2$ at 20°C and changes in ion concentration are as indicated.

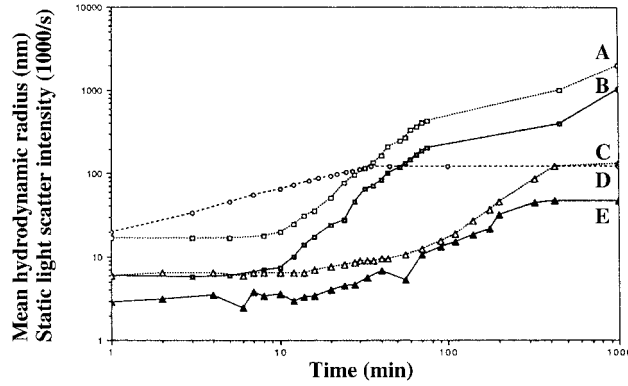

Fig. 4. Actin polymerization in the presence/absence of α-actinin at a molar ratio of 10:1 and in the presence of gelsolin of 100:1 using quasi-elastic light scattering to measure the hydrodynamic radius, r_h (nm) and intensity, I (1000/s). (A) and (B) show I and r_h of 3 μM actin in presence (□), and (D) and (E) in the absence (△) of α-actinin at 10:1, respectively, and (C) I in the presence of gelsolin (○) at a molar ratio of 100:1. Normal F-Buffer conditions are used: 2 mM Tris/HCl, pH 7.5, 0.5 mM Na_2ATP, 0.2 mM DTT, 100 mM KCl, 2 mM $MgCl_2$, and 0.2 mM $CaCl_2$ at 20°C.

observed when using monovalent ions like K^+ and Na^+. Rising potassium concentration from (0→50 mM) in the presence/absence of α-actinin shows an increase in polymerization rate; however, further increases to 100 and 200 mM K^+ and Na^+ (data not shown) reduce the rate, indicating that (i) K^+ and probably Na^+ have a saturable effect on polymerizing actin and (ii) they do not affect the binding of α-actinin to actin significantly at these concentrations (Fig. 3; compare actin↔actin:α-actinin).

Quasi-elastic light scattering

To test how the actin filament dynamic changes in the presence of α-actinin, quasi-elastic light scattering at 3 μM actin in the presence/absence of α-actinin at $r_{A\alpha}=10$ was used. Figure 4 plots the static light scatter intensity, I, and the hydrodynamic radius, r_h, in relation to the polymerization time of actin. For comparison, traces with actin are shown in the presence/absence of α-actinin and gelsolin at a molar ratio of 1:10 and 1:100, respectively. Actin in the presence of α-actinin shows initially higher values for I and r_h (A+B) but similar polymerization behavior for actin in the absence of α-actinin (D+E). After 10 min a more dramatic increase in I and r_h is observed for actin in the presence of α-actinin. A plateau value is reached after 300–400 min for both actin in the presence/absence of α-actinin, and the value for I and r_h is approximately six times higher for actin in the presence than in the absence of α-actinin. At that time the actin polymerization is completed and only bundle/cluster formation of actin/α-actinin further increases I and r_h. In the presence of gelsolin (C), however, actin shows an initially higher rate and intensity (due to an increased number of nucleators) but plateaus after 30 min.

Falling ball viscosity

A prerequisite for applying the falling ball method is that the yield strength of the solution be low enough. The first experiment was, therefore, directed at determining the optimal actin concentration. Prior to measuring the falling ball time, the capillary tube was filled with various G-actin concentrations in F-buffer, sealed at one end, and left at a 45° angle for 120 min and 20°C. Figure 5A shows that the falling ball time increases exponentially between 1–10 μM actin. To measure the effect of various ions and ionic strength as well as the addition of α-actinin on the falling ball time, subsequent experiments were performed at 3 μM actin in the presence/absence α-actinin at a molar ratio, $r_{A\alpha}=10$.

The falling ball time was examined at various experimental conditions using the relation in Equation (3). In the first set of experiments increasing potassium concentrations from zero to 100 mM were used. At zero potassium, the falling ball time for actin in the absence of α-actinin was longer than in the presence of α-actinin, indicating that

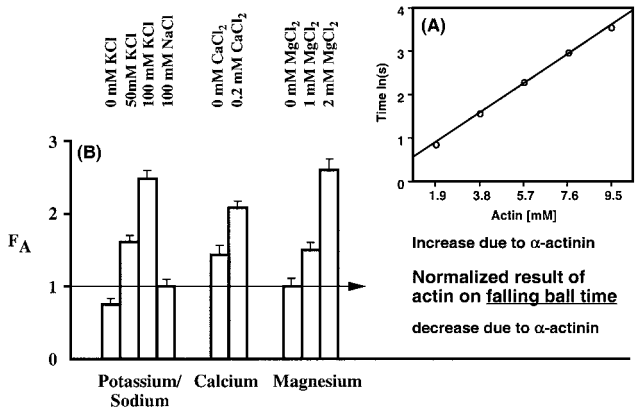

Fig. 5. (A) shows a linear relation of falling ball time, ln (s), and actin concentration after 20 min incubation in F-buffer. (B) indicates the relation F_A determined from the falling ball time using Equation (1) at various ions and ionic strength for 3 μM actin in the presence/absence of α-actinin at $r_{A\alpha}=10$. Normal F-Buffer conditions are used (2 mM Tris/HCl, pH 7.5, 0.5 mM Na_2ATP, 0.2 mM DTT, 100 mM KCl, 2 mM $MgCl_2$, and 0.2 mM $CaCl_2$ at 20°C) and changes in ion concentration are as indicated.

α-actinin lost its cross-linking activity; thus at 50 mM the time increased significantly and rose even further at 100 mM potassium (Fig. 5B). Comparing the times reveals a maximum for actin and actin/α-actinin at 50 mM and 100 mM, respectively, and a minimum at 200 mM potassium for both species (data not shown). Replacing potassium with sodium at 100 mM, no time difference between actin and actin/α-actinin was observed, indicating that cross-linking did not occur.

Measuring the falling ball time of actin and actin/α-actinin in the presence/absence of divalent ions revealed a different behavior. α-Actinin in the absence of calcium cross-links actin and its effect becomes more apparent at 0.2 mM calcium. However, using zero magnesium at 3 μM actin in the presence/absence of α-actinin, $r_{A\alpha}=10$ shows no influence in cross-linking activity due to α-actinin, and only when the magnesium concentration was increased to 2 mM the effect due to α-actinin is noticeable (Fig. 5B). Interestingly, when the actin concentration was increased to 8 μM at $r_{A\alpha}=10$ in the absence of magnesium, normal cross-linking of α-actinin occurred (data not shown).

This study shows the dependence of ionic conditions on the rate of actin polymerization and actin viscosity. The analysis of the effects that $CaCl_2$, $MgCl_2$, and KCl have on actin confirms previous observations that Ca^{2+} inhibits not only actin nucleation (Rouayrenc and Travers, 1981; Tobacman and Korn, 1982) but also actin polymerization in the presence/absence of α-actinin. The presence of Mg^{2+} and K^+ stimulates actin polymerization and each has an additive effect in the presence/absence of α-actinin. However, increasing K^+ to >100 mM or substituting K^+ for Na^+ at 100 mM slows down polymerization in both species significantly. Several researchers have explained these differences as conformational changes induced in monomeric actin that have implications on the rate of filament growth (Reichenstein and Korn, 1979). The binding of α-actinin to actin under all these ionic conditions had little to no influence on the actin polymerization. Examining the viscosity of actin shows with increasing mono-and divalent ion concentration a higher ratio of F_A in the presence of α-actinin, except for Na^+ where no cross-linking due to α-actinin was observed. At zero K^+, a small reduction in the viscous ratio occurs, which is also reflected in a decrease in the rate of actin polymerization. The opposing effect observed for Ca^{2+} with regard to actin viscosity and polymerization rate can be explained as a stabilizing effect on monomeric actin (Kabsch *et al.*, 1990), which is counter productive for polymerizing actin and reflected in the lag phase (Goldmann and Isenberg, 1993; Senger and Goldmann, 1995; Senger *et al.*, 1995). In conclusion, quasi-elastic light scattering confirmed that the presence of α-actinin as well as increasing ionic strength reduced actin filament dynamic behavior.

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