Sound attenuation of polymerizing actin reflects supramolecular structures: viscoelastic properties of actin gels modified by cytochalasin D, profilin and α -actinin

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Polymerization and depolymerization of cytoskeletal elements maintaining cytoplasmic stiffness are key factors in the control of cell crawling. Rheometry is a significant tool in determining the mechanical properties of the single elements *in vitro*. Viscoelasticity of gels formed by these polymers strongly depends on both the length and the associations of the filaments (e.g. entanglements, annealings and side-by-side associations). Ultrasound attenuation is related to viscosity, sound velocity and supramolecular structures in the sample. In combination with a small glass fibre (2 mm $\times$ 50 μ m), serving as a viscosity sensor, an acoustic microscope was used to measure the elasticity and acoustic attenuation of actin solutions. Changes in acoustic attenuation of polymerizing actin by far exceed the values expected from calculations based on changes in viscosity and sound velocity. During the lag-phase of actin polymerization,

attenuation slightly decreases, depending on actin concentration. After the half-maximum viscosity is accomplished and elasticity turns into steady state, attenuation distinctly rises. Changes in ultrasound attenuation depend on actin concentration, and they are modulated by the addition of α -actinin, cytochalasin D and profilin. Thus absorption and scattering of sound on the polymerization of actin is related to the packing density of the actin net, entanglements and the length of the actin filaments. Shortening of actin filaments by cytochalasin D was also confirmed by electron micrographs and falling-ball viscosimetry. In addition to viscosity and elasticity, the attenuation of sound proved to be a valuable parameter in characterizing actin polymerization and the supramolecular associations of F-actin.

Key words: compressibility, rheometry, sound velocity.

INTRODUCTION

Motility of cells is significantly regulated by spatial and temporal changes in filament assembly [1,2], which are primarily controlled by proteins capping, sequestering or cross-linking actin [3–5]. All of these molecular interactions produce a directed motive force, thus the mechanics of this interplay of molecular events is the physiologically significant parameter. Therefore many investigations have been devoted to studying the polymerization of actin. Various instruments have been constructed to obtain reliable mechanical data from the highly strain-sensitive actin gels. These instruments reveal parameters, such as viscosity (dynamic loss modulus, G'') and elasticity (dynamic storage modulus, G') [6–10]. Viscoelasticity of these cytogels depends on the length of filaments [11] and their supramolecular associations [12]. *In vivo*, highly ordered F-actin associations, e.g. fibrillar bundles, have been found to be zones of high acoustic attenuation [13]. The relationship between ultrasound attenuation and higher order structures is investigated in the present study, using a novel ultrasound-based non-destructive micro-rheometer, i.e. an oscillating rod rheometer (ORR) [14], allowing for synchronous measurements of viscoelastic properties and attenuation of ultrasound during the polymerization of actin. Viscosity is determined at minimum sensor displacements (1–50 nm) and elasticity is measured by recording ultrasound velocity. Profilin [15,16] and cytochalasin D [17–19] were used to modulate filament length, and α -actinin [20] was used to introduce F-actin associations.

Only a few investigators have determined ultrasound absorption by globular proteins [21,22], but this has never been

performed on actin during polymerization or upon its interaction with actin-binding proteins or cytochalasins. The present study reveals that during the lag-phase of actin polymerization, attenuation decreases, whereas it remains constant during the elongation phase. After the half-maximum viscosity is reached and elasticity also turns into steady state, ultrasound attenuation distinctly rises. This increase is lowest for the highest concentrations of actin. Thus it seems to depend on packing density and the homogeneity of the actin network. Furthermore, the addition of α -actinin to actin strongly modulates attenuation due to the enhancement of filament associations.

Attenuation is also related to filament length. Profilin and cytochalasin D significantly modulate the typical time course of acoustic attenuation response during actin polymerization. Cytochalasin D is known to shorten actin filaments, however, the mechanism of the severing activity of cytochalasin D remains controversial [22]. Profilin regulates actin polymerization by different mechanisms. In the presence of $MgCl_2$ profilin efficiently binds G-actin and the resulting profilin–actin complex is added to the barbed end [23,24], increasing the filament turnover rate and slightly increasing the filament length [25]. If barbed ends are capped by gelsolin (or in the absence of $MgCl_2$), profilin acts as a simple G-actin sequestering protein [26]. The present study shows that profilin from calf thymus increases the dynamic viscosity of non-muscle Mg^{2+} -ATP-actin at molar ratios of profilin:actin of ≤ 1 and lowers viscosity at higher ratios. Elasticity of non-muscle actin decreases with increasing profilin concentrations due to the reduction of free solvent molecules, by an increased demand for hydration of the proteins.

To conclude, attenuation of sound is a useful parameter to

Abbreviations used: DTT, dithiothreitol; ORR, oscillating rod rheometer.

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characterize actin polymerization and to determine the density and structure of actin networks.

EXPERIMENTAL

Reagents

G-actin buffer consisted of 2 mM Tris/HCl (pH 7.4), 0.2 mM ATP, 0.5 mM dithiothreitol (DTT) and 0.2 mM CaCl_2 . Polymerization buffer consisted of 50 mM KCl and 2 mM MgCl_2 . Tris/HCl, ATP, DTT and cytochalasin D were purchased from Sigma–Aldrich (Steinheim, Germany). CaCl_2 , KCl and MgCl_2 were purchased from Merck (Darmstadt, Germany).

Proteins

G-actin and profilin were isolated from calf thymus [27,28], muscle G-actin was isolated from rabbit skeletal muscle [29], and α -actinin was prepared from chicken gizzard [30]. Protein concentrations were determined according to the method of Bradford [31].

Prior to rheological measurements, protein solutions were degassed in a vacuum for 10 min to avoid air bubbles, which otherwise strongly impair the measurements. The sample volume was 80 μl .

ORR

The ORR allows synchronous and non-destructive measurements of the viscoelastic properties of fluids and gels in microlitre (10–100 μl) volumes [14]. A glass fibre (2 mm $\times$ 50 μm) is immersed in the fluid and oscillations are stimulated via a piezomechanical bimorph actuator. These very small (1–50 nm) oscillations are detected by a phase-sensitive acoustic microscope, which allows for the synchronous determination of velocity and attenuation of longitudinal acoustic waves. Sound velocity is a function of elastic modulus.

The dynamic viscosity η (mPa s) is calculated according to the following equation [32]:

$$\eta = \left[\frac{\left(\beta + \frac{\beta'}{\tan \phi} \right) \sqrt{\frac{\omega_A \rho}{8}}}{\gamma} + \sqrt{\frac{\left(\beta + \frac{\beta'}{\tan \phi} \right) \omega_A \rho}{8\gamma^2} + \left(\frac{\omega_0^2}{\omega_A^2} - 1 \right) \frac{\omega_A}{2\gamma} - \frac{\alpha \omega_A \rho}{2\gamma}} \right]^2 \quad (1)$$

where ω_A is the excitation frequency of the rod (1–10 kHz), ϕ (ω) is the phase shift between the excitation phase and the rod oscillation at its resonance frequency, ω_0 is the resonance frequency of the rod in vacuum (approximated by measurements in air), ρ is the density (g/cm^3) of the specimen and α , β , β' and γ are geometric constants of the set-up.

The compression modulus (volume elasticity), K (GPa), corresponds to the sound velocity, c , with:

$$c = \sqrt{\frac{K + \frac{4}{3}G}{\rho}} \quad (2)$$

where G is the shear modulus and ρ is the density of the liquid. Commonly $G \ll K$, therefore the above equation is approximated by:

$$c = \sqrt{\frac{K}{\rho}} \quad (3)$$

The relationship between the elastic (Young's) modulus, E , and the compression modulus, K , is:

$$K = \frac{E}{3 \cdot (1 - 2\mu)} \quad (4)$$

where μ is the Poisson ratio (e.g. for actin filaments μ is approx. 0.4 [33]). The elastic modulus, E , is then 0.6 times the compression modulus, K .

Determination of the acoustic attenuation

Acoustic attenuation (S) is the decline of intensity of the amplitude (A) of acoustic waves between two measuring points x_1 and x_2 :

$$S = 20 \cdot \log \left(\frac{A_{x_1}}{A_{x_2}} \right) \quad (5)$$

Attenuation of pure water at 25 °C is 50 dB/mm [34].

Falling-ball assay

A falling-ball microviscosimeter (Haake, Karlsruhe, Germany) was used to compare the viscosity data derived from the ORR measurements. The ball (diameter = 3.06 mm, refined steel) of

Figure 1 Attenuation of water/glycerol mixtures and F-actin

Comparison of the attenuation (■) of a series of (A) water/glycerol mixtures and (B) F-actin using the ORR. Broken lines indicate best fits. Insets show synchronously determined viscosities (▲). Attenuation rises with increasing viscosity of (A) Newtonian liquids, but decreases with increasing viscosity of the actin polymer (B), which represents a non-Newtonian liquid. F-actin viscosity values were obtained in the final state of polymerization.

Figure 2 Time course of attenuation upon actin polymerization

(A) Changes in three different mechanical parameters upon the polymerization of 10 μ M non-muscle actin, synchronously measured with the ORR. (B–D) Determination of attenuation (continuous line) and viscosity (broken line) during the polymerization of (B) 5 μ M, (C) 35 μ M and (D) 130 μ M muscle actin. Typical time courses are shown. (A) The prominent increase in sound velocity during the lag phase of viscosity reflects the assembly of G-actin into aggregates (primer) with solid-like properties. Simultaneously there is a notable decrease in attenuation. During most of the elongation phase attenuation remains constant, but rises

the viscosimeter (tube volume = 300 μ l) falls through two light barriers used for switching a digital chronometer. The viscosity is calculated from:

$$\eta = k_{\text{cal}} \cdot (\rho_{\text{ball}} - \rho_s) \cdot t \quad (6)$$

where η is the viscosity (mPa s), ρ_{ball} is the density of the ball (7.8 g/cm³), ρ_s is the density of the specimen (g/cm³), t is the falling time (ms) and k_{cal} is a calibration factor eliminating geometric differences of the ball and the glass tube, as given by:

$$k_{\text{cal}} = \frac{\eta_{\text{H}_2\text{O}}}{(\rho_{\text{ball}} - \rho_{\text{H}_2\text{O}}) \cdot t} \quad (7)$$

where $\eta_{\text{H}_2\text{O}}$ is the standard value for the density of water at 25 °C (0.9 mPa s), and $\rho_{\text{H}_2\text{O}}$ corresponds to the density of the falling ball. The accuracy of the falling-ball assay for actin buffer is approx. 1%.

Electron microscopy

G-actin was mixed with polymerization buffer (and cytochalasin D, profilin or α -actinin) and droplets of 50 μ l were transferred to a humidity box. Following incubation for 40 min, copper grids with carbon glow-discharged films were dipped for 15 s on to the droplets. After removing the fluid from the grid by tipping on to a Kleenex tissue, grids were incubated with freshly prepared 1% (w/v) uranyl acetate in water [35] for 1 min. This procedure avoids breaking of the filaments by mechanical stress.

The grids were examined with a Zeiss EM 902 transmission electron microscope (Zeiss, Jena, Germany) and digital images were acquired via a CCD-Camera connected to the frame grabbing and contrast enhancement device Argus-20 (Hamamatsu Photonics, Herrsching, Germany). Further processing was performed with the HPD-CPx device control program (Hamamatsu Photonics, V.1.2).

Magnifications were calibrated using gold particles with diameters of 6 nm, which was related to the diameter of an actin filament.

RESULTS

Concentration-dependent changes of acoustic attenuation upon polymerization of actin

Interpretation of attenuation is not straightforward. It is a complex parameter, including absorption of mechanical energy, and thus is related to sound frequency, velocity and viscosity. Scattering may play the dominant role at otherwise constant conditions. This phenomenon is exemplified in Figure 1. Attenuation rises with increasing viscosity of Newtonian liquids (e.g. glycerol, Figure 1A), whereas attenuation may decrease with increasing viscosity of non-Newtonian liquids (e.g. actin solutions, Figure 1B).

During the lag phase of actin polymerization, attenuation first decreased with a concomitant increase in sound velocity (Figure 2), i.e. inelasticity (Eqns 2–4). To ensure constant temperature balance, the polymerization buffer was added ‘online’ approx. 60 s after starting the measurement (arrow in Figure 2A) and

strongly prior to the viscosity maximum (A–D). The time course of attenuation, as shown in (A) was highly reproducible and reflects a typical measurement. Arrows indicate the addition of polymerization buffer (2 mM MgCl₂/50 mM KCl). Each point on the lines represents one measurement taken at a rate of 1 Hz.

gave rise to a transient increase in sound velocity, which, after a few seconds, was followed by a steep increase in sound velocity. This significant increase in sound velocity is explained by solid-like actin nuclei propagating acoustic waves faster than monomeric proteins (compare with [14]). During the elongation phase of polymerization (represented by the viscosity increase) attenuation remained almost constant, however, during the final phase of polymerization attenuation suddenly increased. This significant increase was stronger at low actin concentrations compared with high concentrations (Figures 2B–2D). During this phase, sound velocity hardly changed (Figure 2A).

Effects of filament length and entanglements on acoustic attenuation

Viscoelasticity of actin gels strongly depends on filament length [11]. Therefore cytochalasin D was used to investigate the effect of filament shortening on acoustic attenuation, and profilin was used to determine the effect of filament lengthening.

Cytochalasin D reduced both acoustic attenuation and viscosity (Figure 3A). In addition, elasticity decreased when actin polymerized in the presence of cytochalasin D (Figure 3B). The viscosity data of actin–cytochalasin D gels obtained with the ORR have been compared with the data derived from a falling-ball viscosimeter (Figures 3C and 3D). Both types of measurements confirmed that the viscosity of actin gels depends on filament length (Figure 3, compare with Figures 4A–4D). At high shear rates falling-ball viscosity was reduced, when compared with that determined with the non-destructive method (ORR) (compare Figures 3B and 3D, pure actin).

Profilin increased the viscosity of uncapped non-muscle Mg^{2+} –ATP–actin at molar ratios of profilin:actin of ≤ 1 (Figure 5A). An excess of profilin (5-fold molar excess over actin) inhibited polymerization (inset of Figure 5A). Upon polymerization of $\beta\gamma$ -actin with profilin, sound velocity slightly decreased with increasing profilin ratios (see the Discussion section).

The effect of low profilin concentrations on the viscosity of (uncapped) Mg^{2+} -actin (Figure 5A) resulted in an enhancement of the F-actin turnover rate and thus in filament lengthening [36]. Profilin acted as a simple sequestering protein only at high molar ratios (Figure 5A, inset). However, low profilin concentrations reduced acoustic attenuation upon actin polymerization (Figure 5C). In addition, profilin–actin gels at molar ratios of 0.25:1 seemed to be not as dense as profilin–actin gels at molar ratios of 1:1 (compare Figures 4E and 4F).

Effect of entanglements of F-actin on acoustic attenuation

The significant increase in acoustic attenuation prior to the viscosity maximum is a function of filament length. During this final phase of polymerization the long actin filaments entangle and thus alter the mechanical properties of the fluid [37]. Consequently, cross-linking actin filaments by α -actinin should enhance the influence of structure formation on acoustic attenuation. Indeed, acoustic attenuation increases on the addition of $1.5 \mu M$ α -actinin to $20 \mu M$ muscle actin (Figure 6). Increased entanglements and bundling of actin filaments became obvious from electron micrographs (Figures 4G and 4H). In the presence of α -actinin acoustic attenuation increased from the very beginning of the polymerization (Figure 6), and viscosity increased about twice that without α -actinin (as observed by Holmes et al. [20]), indicating the formation of a well cross-linked actin net from the very beginning of filament formation [38].

Figure 3 Effect of cytochalasin D on the mechanical properties of actin

(A) Viscosity and acoustic attenuation of actin ($18 \mu M$), polymerizing in the presence of cytochalasin D (CD; $18 \mu M$, $1.8 \mu M$ or $0.18 \mu M$). Attenuation declines considerably with increasing cytochalasin D concentrations. (B) Decrease of viscoelasticity upon the polymerization of $10 \mu M$ pure actin and $10 \mu M$ actin in the presence of equimolar cytochalasin D. (C) Steady-state viscosity of F-actin (means of three measurements) and (D) time course of the viscosity increase of polymerizing actin in the presence and absence of cytochalasin D determined with a falling-ball microviscosimeter (typical time course of single measurements).

Figure 4 Electron micrographs of negatively stained F-actin

(A) Pure 5 μ M muscle actin, (B) pure 25 μ M muscle actin, (C and D) 5 μ M muscle actin with 5 μ M cytochalasin D, (E) 5 μ M $\beta\gamma$ -actin with 5 μ M profilin, (F) 5 μ M $\beta\gamma$ -actin with 1.5 μ M profilin, and (G and H) 5 μ M muscle actin with 0.4 μ M α -actinin. Electron-microscope observations were repeated independently 2–3 times. For each condition 3–5 grids were observed. Electron micrographs were selected that represented the most typical areas on the grids.

Figure 5 Mechanical properties of Mg^{2+} - $\beta\gamma$ -actin in the presence of profilin

Time course of (A) dynamic viscosity, (B) sound velocity and (C) acoustic attenuation upon polymerization of uncapped Mg^{2+} -ATP- $\beta\gamma$ -actin (A) with and without profilin (P), synchronously measured with the ORR. Measurements were immediately initiated after G-actin was mixed with profilin. Polymerization was induced by the 'online' addition of polymerization buffer. (A) Dynamic viscosity increases with increasing profilin concentrations, whereas excess profilin reduces viscosity (inset). (B) Sound velocity declines with rising profilin concentrations. The initial increase or decrease in sound velocity was due to temperature shifts of the sample fluid; hence, salt was added after approx. 70 s to ensure temperature equilibration. The strong increase in the first phase of polymerization was exactly in parallel at all actin and profilin concentrations. (C) At low profilin concentrations, the decrease in acoustic attenuation was prolonged. Measurements were independently repeated three to five times under the same conditions.

DISCUSSION

Supramolecular organization of F-actin as revealed by sound attenuation

ORR measurements are non-destructive and allow for synchronous determination of dynamic viscosity, sound velocity (related

Figure 6 Effect of α -actinin on the sound attenuation of polymerizing actin

Polymerization of muscle actin ($20 \mu\text{M}$) in the presence of $1.5 \mu\text{M}$ α -actinin (from chicken gizzard). Viscosity increases approx. twice that without α -actinin, indicating extensive cross-linking. Sound attenuation rises strongly at the very beginning of polymerization. The graph represents one typical time course from three independent measurements.

to the elastic modulus) and acoustic attenuation, which are related to structural arrangements. The time course of changes of these mechanical parameters during the polymerization of actin is different (see Figure 2A).

Attenuation of sound is the decrease of the amplitude of acoustic waves between two measuring points, and more generally, the decrease of the pressure exerted on the material while an acoustic wave is propagating. Attenuation is due to reflection, scattering and absorption. The first two parameters are based on the inhomogeneity of the fluid. Absorption may result from viscosity and thermal conductivity (thermal relaxation) as well. In fluids, thermal conductivity can be neglected [39]. The relationship of absorption of sound (attenuation, S) and viscosity is given by:

$$S = \frac{8\pi^3}{3\rho c^3} \cdot \eta \quad (8)$$

where η is the viscosity, ρ is the density and c is the sound velocity.

To verify whether this relationship determines the attenuation changes during the alterations of viscosity and sound velocity of actin gels, attenuation was calculated according to Eqn 8. This calculated attenuation time course (results not shown) almost matches the time course of viscosity, because c^3 is large and the small sound velocity changes during actin polymerization only marginally affect the quotient in Eqn 8 (compare with Figure 5B, profilin:actin = 1:1; sound velocity decreases, whereas the viscosity and the attenuation remain constant).

In liquids with complex molecules, ultrasound absorption is linked to intrinsic kinetic energy (vibration, rotation and translation) [39]. Thus the decrease of attenuation during the lag phase prior to the rise in viscosity, when sound velocity increases (see Figure 2A), may reflect aggregation of actin monomers forming rod-like primers. Due to this change of supramolecular arrangement, less energy is absorbed because of reduced degrees of freedom resulting in a decrease in attenuation. In the presence of profilin, molecular structures are formed that act as long-lasting primers (profilin₄-actin, [40]), therefore the attenuation decrease lasts longer (see Figure 5C).

During the elongation phase of the actin filaments, reflected by increasing viscosity, attenuation first remains constant, then

after reaching a critical filament length at $1/2\eta_{\max}$, ultrasound attenuation distinctly rises (see Figures 2, 3A and 5). Now a density of actin filaments is reached that allows them to anneal, aggregate and to entangle, thus inhomogeneous F-actin distributions appear [37,41–43]. A similar inhomogeneous structure of the actin gel is induced by cross-linking actin with α -actinin (see Figures 4G and 4H) giving rise to high attenuation (see Figure 6). This interpretation is supported by a considerable decrease in attenuation on the addition of cytochalasin D (see Figure 3A), allowing for homogeneous F-actin distributions. The assumption that attenuation reflects supramolecular associations of F-actin is also supported by the concentration dependence of F-actin on sound attenuation (see Figures 1B–1D): with increasing actin concentration the filaments become nematically orientated, resulting in a high and homogeneous packing (see Figure 4B; compare with [37]), which decreases the acoustic attenuation. The strong increase of attenuation upon the advanced elongation phase depends on the actin concentration as well: at low actin concentrations (when the actin net is isotropic, Figure 4A) attenuation increases approx. 0.4 dB/mm (during 150 s), whereas at high actin concentrations (when the actin net is nematic, Figure 4B) attenuation only increases approx. 0.07 dB/mm (during 150 s) (see Figures 2B and 2D).

Changes in the attenuation of polymerizing actin have not been measured so far. Cho et al. [21] have determined sound absorption of a series of globular proteins according to the method of Eggers and Funck [44], at operating frequencies of 1–100 MHz. In their set-up, the fluid is placed in between two quartz transducers acting as acoustic resonators. The amplitude modulation of the reflected ultrasonic wave is related to the absorption coefficient of the fluid. Using this method, Cho et al. [21] have determined the attenuation of globular proteins (e.g. myoglobin, BSA, ribonuclease A and α -chymotrypsin A), in relation to the operating frequency (1–100 MHz), temperature (4–45 °C), pH (2–12) and protein concentration. The addition of salts (e.g. < 0.2 M KCl) did not affect attenuation values, which is consistent with the ORR measurements (results not shown). The hydration of monomeric proteins did not affect attenuation. ORR measurements also revealed no change in attenuation depending on the G-actin concentration (see Figure 2, beginning of the measurements). Prior to the induction of polymerization, both sound velocity and attenuation change to a similar extent due to temperature equilibration (see Figures 5B and 5C). Subsequent to the addition of polymerization buffer, attenuation changes notably, whereas the time courses of attenuation changes and changes in sound velocity differ from each other (see Figures 5B and 5C).

Effect of filament length on acoustic attenuation

Cytochalasin D efficiently reduces sound attenuation, viscosity (see Figure 3A) and elasticity (see Figure 3B). It shortens actin filaments (compare Figures 4A–4D) and, similar to capping proteins (e.g. gelsolin, see [45]), reduces the lag phase of polymerization (see Figure 3B). The latter could only be revealed by ORR measurements, since the first viscosity value of falling-ball measurements is recorded approx. 80 s after mixing the actin solution with the polymerization buffer. In addition, the steady-state viscosities of falling-ball measurements are much lower than those revealed by ORR measurements (compare Figures 3B and 3D, pure actin). These differences are caused by the high shear rates introduced by the ball (compare with [14]) and are further visible in the decline in steady-state viscosity (see Figure 3D). This reduction is smaller after cytochalasin treatment, when only short filaments are present (see Figure 3D).

Profilin was used to enhance filament length. It increases the rate of ATP exchange of G-actin [25] and shuttles ATP–G-actin to the plus end of elongating filaments [46]. The higher viscosity values of F-actin in the presence of profilin at profilin:actin molar ratios of ≤ 1 (see Figure 5A) support the assumption of longer filaments [25,47]. Profilin reduces attenuation; the strongest effect is visible at the lowest profilin concentration (see Figure 5C). Under these conditions, the actin net is less dense than at higher molar ratios (see Figures 4E and 4F), thus the density of the actin net may be the reason for the low final sound attenuation. Sound velocity (volume elasticity) decreases with increasing profilin concentrations (see Figure 5B). This effect is supposed to be a consequence of the higher protein concentration in the sample: proteins are more compressible (smaller sound velocity) than the aqueous fluid surrounding them [14,48,49].

Although profilin also interacts with muscle actin, differences exist in the interaction of actin and actin-binding proteins derived from different sources [50]. Therefore the emphasis of the present investigation was laid on the natural partner of thymus profilin, i.e. $\beta\gamma$ -actin.

Commonly, actin polymerization is measured using fluorimetry after pyrene labelling of G-actin. Because profilin binds only weakly to pyrene-labelled G-actin [24,25,51], capped actin has been used in most experiments. Thus a potential profilin effect on the barbed ends might have been obscured by CapZ or gelsolin. In the present study, non-muscle Mg^{2+} -ATP-actin was unlabelled and uncapped. The observation that under these conditions profilin increases the dynamic viscosity (see Figure 5A) contrasts with fluorimetry measurements made by Korenbaum et al. [23], who measured a reduced fluorescence of $\beta\gamma$ -actin upon interaction with profilin. Similar dual profilin actions on actin polymerization have been reported for *Zea mays*-derived proteins. At molar profilin:actin ratios of ≤ 1 viscosity is higher, whereas at higher ratios viscosity is lower than that of pure actin [52].

Conclusions

Viscosimetry coupled to elasticity measurements via ultrasound velocity determinations allowed us to separate three phases of actin polymerization: primer formation was revealed by changes in sound velocity, the elongation phase was reflected by increasing viscosity, and a phase of supramolecular associations was revealed by changes in acoustic attenuation.

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