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Difference in polymerization and steady-state dynamics of free and gelsolin-capped filaments formed by α- and β-isoactins

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ABSTRACT

The polymerization of scallop β-like actin is significantly slower than that of skeletal muscle α-actin. To reveal which steps of polymerization contribute to this difference, we estimated the efficiency of nucleation of the two actins, the rates of filament elongation at spontaneous and gelsolin-nucleated polymerization and the turnover rates of the filament subunits at steady-state. Scallop actin nucleated nearly twice less efficient than rabbit actin. In actin filaments with free ends, when dynamics at the barbed ends overrides that at the pointed ends, the relative association rate constants of α- and β-actin were similar, whereas the relative dissociation rate constant of β-ATP-actin subunits was 2- to 3-fold higher than that of α-actin. The 2- to 3-fold faster polymerization of skeletal muscle versus scallop Ca-actin was preserved with gelsolin-capped actin filaments when only polymerization at the pointed end is possible. With gelsolin-induced polymerization, the rate constants of dissociation of ATP-actin subunits from the pointed ends were similar, while the association rate constant of β-actin to the pointed filament ends was twice lower than that of α-actin. This difference may be of physiological relevance for functional intracellular sorting of actin isoforms.

Actin is expressed in a variety of tissue-specific isoforms. Mammalian skeletal, cardiac and vascular smooth muscle cells contain distinct α-actins, the predominant isoform of intestinal smooth muscle is γ-actin, the actin cytoskeleton consists of cytoplasmic β- and γ-isoforms. Actin sequences are highly conserved: cardiac and smooth muscle α-actins differ from their skeletal muscle counterpart by four and eight substitutions, respectively. The sequences of cytoplasmic β- and γ-actins are almost identical, and differ from those of the muscle actins only by about 10%. Nevertheless, appearance, relative amount and localization of isoactins are strictly controlled by the cell machinery. Although at the early stages of cell differentiation expression of any actin gene is potentially possible, under normal physiological conditions, while differentiation proceeds, synthesis of specific actin isoforms is temporally regulated, and the produced proteins are spatially segregated (for reviews, see [1–4]). Pathological situations correlate either with up- and down-regulation of distinct actin genes returning to a fetal gene program, or with a failure to sort actin isoforms [5–7]. Different actin isoforms cannot substitute for each other, and changes in expression of specific actin genes are accompanied by alterations in cell structure and function [8–10]. This points to a unique physiological role of every actin isoform.

It is well documented that temporal and spatial changes in the actin isoform patterns are qualitatively similar to those of mRNA [2,11–13], and thus intracellular segregation of isoactins is determined by specific targeting of actin isoform mRNA [14–16]. However, actin isoforms are frequently distributed over a wider area than their respective mRNAs [12,17] indicating that isoform specific mRNA targeting is only a part of the mechanism for intracellular segregation of isoactins.

At the same time, the functional properties of even highly homologous isoactins are not identical. Actin isolated from developing muscles [18], smooth muscle γ-actin [19,20], scallop β-like actin [21–23] and cytoskeletal β/γ-actins [24–26] polymerize less readily and depolymerize easier than sarcomeric α-actin isoforms. Differences in the polymerization rates of cytoplasmic β- and γ-actin have also been reported [27]. Furthermore, some actin-binding proteins discriminate between cytoskeletal and muscle actin isoforms [28–30], and the preference might be influenced by specific posttranslational modifications of actin isoforms [31,32]. These observations suggest that properties of the isoactins themselves contribute to their segregation.

To address this issue we have compared skeletal muscle α-actin with scallop adductor muscle β-like actin that is correspondent to cytoplasmic β-actin [33], and the only actin species present in the

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scallop adductor muscle [34]. Scallop actin differs from skeletal muscle α-actin and cytoplasmic β-actin by 21 and 5 amino acid substitutions, respectively [33]. Most of the substitutions are located in subdomains 1 and 3. In contrast to cytoplasmic actins, scallop actin can be easily purified with high yield and homogeneity [21,22] which is indispensable for comparative studies. Previously we have shown that scallop actin polymerizes slower and depolymerizes faster than skeletal muscle α-actin. In addition, indirect evidence for a lower stability of scallop F-actin was presented [21,23]. These data suggest that kinetic parameters of actin polymerization may play a role in the isoactin's specific assembly. Therefore the aim of this work was to compare kinetics of spontaneous polymerization of the two actins, and to elucidate the effect of gelsolin on polymerization parameters.

Our results show that in the absence of gelsolin, when both ends of actin filaments are free, and the association/dissociation events at the barbed ends dominate in the overall filament dynamics, the rates for association of β-like and α-actin monomers differ very little, whereas dissociation of β-like ATP-actin subunits was about twice faster than that of α-actin. With gelsolin-induced polymerization, association of β-like ATP-actin monomers at the pointed filament ends was twice slower than that of α-ATP-actin monomers, while their rates of dissociation from the pointed ends were similar. Provided that any actin polymerization in the cell is nucleated, this difference may be of physiological relevance for functional intracellular sorting of actin isoforms.

Materials and methods

Protein preparations

Rabbit skeletal muscle actin was purified by the procedure of Spudich and Watt [35] with an additional gel filtration step on Sephadex G150 to remove traces of actin-binding proteins. Scallop adductor muscle actin was isolated from acetone dried myofibrils prepared from the striated part of the scallop *Patinopecten jessoensis* without prior removal of myosin, and washed with 5 mM $NaHCO_3$ before acetone treatment. Actin extracted from this acetone powder is strongly contaminated with α-actinin. To remove α-actinin, the extract was clarified by centrifugation at 100,000*g* for 30 min and polymerized with 30 mM KCl overnight. Further centrifugation, depolymerization and gel filtration were performed as for skeletal muscle actin. G-actin in buffer G (0.2 mM ATP, 0.1 mM $CaCl_2$, 0.4 mM β-mercaptoethanol, 5 mM Tris–HCl, pH 8.2, 1 mM NaN_3) was stored on ice and used within a week. Homogeneity of the final actin solutions was checked by SDS–PAGE [36]. Scallop and skeletal muscle actin labeled with *N*-(1-pyrenyl)iodoacetamide at Cys374 were prepared as described [37]. Pyrenyl-labeled actin was lyophilized in the presence of 2 mM sucrose and stored at −70 °C. Before use, the lyophilized pyrenyl-actin was dissolved in buffer G, dialyzed against the same buffer overnight, and clarified by centrifugation at 100,000*g* for 3 h. In all experiments, the term "pyrenyl-labeled actin" refers to a mixture of 90% actin and 10% pyrenyl-labeled actin. Gelsolin was purified from pig stomach smooth muscle [38] and scallop adductor muscle [39]. It was stored as ammonium sulfate precipitate in liquid nitrogen. Before use, the precipitate was dissolved and dialyzed against PSAM buffer (10 mM imidazole, 0.5 mM EGTA, 0.2 mM DTT, 2 mM NaN3, pH 7.0).

Protein concentration

Concentration of G-actin was determined spectrophotometrically using an absorption coefficient of 0.63 ml mg^{-1} cm^{-1} at 290 nm. Concentration of gelsolin was determined by the biuret method.

Critical concentration

To determine critical concentrations for polymerization of rabbit and scallop actin, aliquots of pyrenyl-labeled G-actin at various concentrations in the range from 0.6 to 17 μM were polymerized with 0.1 M KCl for 12–16 h, and the intensity of pyrenyl fluorescence was measured at 25 °C.

ATP hydrolysis measurements

The rates of ATP hydrolysis during polymerization or in steady-state F-actin solutions were measured at 25 °C and 37 °C, respectively. In aliquots of the solution withdrawn after various time intervals the reaction was quenched by addition of an equal volume of 0.6 M ice-cold perchloric acid, precipitated protein was removed by centrifugation, and released Pi was determined by the Malachite Green method [40].

Determination of the relative number concentration of actin filaments

The relative number concentration of the filaments in F-actin solutions was determined by comparing their abilities to nucleate polymerization of the same G-actin species above its critical concentration [41,42]. Aliquots of 12 μM rabbit or scallop F-actin solutions were diluted 10-fold into 5 μM 10% pyrenyl-labeled skeletal muscle Ca-G-actin supplemented with 0.1 M KCl several seconds after addition of F-actin seeds. The solutions were gently mixed in fluorescence cuvettes by inversion five times (this resulted in a dead time of about 10 s), and the increase in pyrenyl fluorescence intensity associated with polymerization of the pyrenyl-labeled G-actin on the non-labeled F-actin seeds was recorded at 25 °C. The relative number concentration of filaments in F-actin solutions used as polymerization seeds was determined as the ratio of filament elongation rates derived from the slopes of the initial linear parts of the fluorescence curves.

Measurements of the relative rate constants for monomer addition to and dissociation from the filament end

Relative rate constants for monomer addition to and dissociation from the filament ends (k_+ and k_-, respectively) were determined from plots of the initial rates of filament elongation on F-actin seeds versus the monomer concentration. The measurements were performed essentially as described earlier [43,44]. The seeds (12 μM pyrenyl-labeled F-actin) were diluted into solutions of skeletal muscle or scallop pyrenyl-labeled G-actin, and the increase in pyrenyl fluorescence intensity associated with polymerization of G-actin on the seeds was recorded at 25 °C, as described above for determination of the relative number concentration of the filaments. The slopes of the initial linear parts of the fluorescence curves were plotted against the corresponding G-actin concentration. To avoid differences in the relative filament number, polymerization of different G-actins was nucleated by the same F-actin.

To convert the initial changes in pyrenyl fluorescence into changes in F-actin concentration, 12 μM rabbit and scallop G-ATP-actin was polymerized to the steady-state, and the fluorescence change corresponding to the fluorescence of 1 μM F-actin was calculated for each of the assayed actins, with F-actin concentration calculated as the difference between the total actin and the critical concentration.

Fluorescence and light-scattering measurements

All fluorescence and light-scattering measurements were performed in a Shimadzu PC 5000 spectrofluorometer. Fluorescence