Kinetic Evidence for a Readily Exchangeable Nucleotide at the Terminal Subunit of the Barbed Ends of Actin Filaments†

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Received September 3, 1997; Revised Manuscript Received February 2, 1998

ABSTRACT: The time course of actin depolymerization was quantitatively analyzed to obtain insight into the reactions occurring during actin disassembly. Polymeric actin was diluted, and subsequently the time course of depolymerization was measured. In the presence of 0.5 mM ATP, 100 mM KCl, and 1 mM $MgCl_2$, continuous depolymerization was observed both when the filaments were carefully diluted and when the filaments were fragmented to produce short filaments. The rates of the reactions that are known to occur during depolymerization, such as dissociation and association of ADP− and ATP−actin molecules and exchange of nucleotides bound to monomeric actin, were determined by independent experiments. When the determined rate parameters were used to calculate the time course of depolymerization, consistently in the simulations fast depolymerization of ADP−actin was followed by slower polymerization of ATP−actin that was formed from ADP−actin by nucleotide exchange. The lack of fast depolymerization and subsequent slower polymerization in the experiments suggests that our present conception about actin disassembly requires modification. Good agreement of calculated time courses with the experimentally determined continuous depolymerization was achieved if ADP bound to the terminal subunit of barbed filament ends was assumed to be readily exchangeable for ATP. Fast nucleotide exchange at terminal subunits may contribute to the stability of barbed filament ends and to their role as polymerizing ends in living cells.

In nonmuscle cells actin undergoes a continuous dynamic reorganization: actin filaments depolymerize and polymerize, and the pool of actin monomers changes. The transition between polymeric and monomeric actin is controlled indirectly by hormones and second messengers (*1*) and directly by proteins and by nucleotides and ions that bind to actin (*2*). Investigations of the effect on actin polymerization of the nucleotides ATP[1] and ADP have revealed that actin assembly is regulated by the nucleotides in a finely tuned manner. Monomeric actin can bind either ATP or ADP. ATP has a slightly higher affinity for actin monomers than ADP (*3*). When actin monomers aggregate to form helical filaments, the nucleotide is incorporated into the filament together with the actin molecules. Incorporated ATP is hydrolyzed about 100 s following binding of actin molecules to filament ends to form ADP. Thus, subunits of the core of actin filaments contain bound ADP (*4*). Monomeric actin exchanges nucleotides. The half-life of this exchange reaction is in the range of minutes and depends on the type and concentrations of cations (*5*). Filament subunits have been reported at physiologically relevant salt concentrations not to exchange nucleotides. Actin filaments are more stable in the presence of ATP than of ADP, as ATP−actin has a higher affinity for filament ends than ADP−actin (*6*−*8*).

The actin-bound nucleotides and their hydrolysis have been shown to bring about a complex mechanism of actin polymerization: Actin filaments tend to polymerize at the barbed end of the polar polymeric structures and to depolymerize at the pointed end (*9*, *10*). This "treadmilling" of actin filaments has been explained by the tendency of actin filaments to carry ATP-subunits at the barbed ends that stabilize actin filaments, and ADP-subunits at the pointed ends that destabilize actin filaments. Under conditions where filament ends with ATP-binding and ADP-binding subunits coexist, large fluctuations of filament ends that are caused by transitions between extensive depolymerization and polymerization were predicted (*11*, *12*). As the type of nucleotide bound to filament ends has a great effect on assembly and disassembly of actin filaments, we present in this paper a study on the exchangeability for ATP of ADP bound to terminal subunits based on a quantitative analysis of the time course of actin disassembly.

MATERIALS AND METHODS

Actin Preparation. Rabbit skeletal muscle actin was prepared according to the method of Rees and Young (*13*). Part of the protein was modified with *N*-ethylmaleimide at cysteine-374 and subsequently with 4-chloro-7-nitro-2,1,3-benzoxadiazole at lysine-373 to produce fluorescently labeled actin (*14*). The protein was applied to a Sephacryl S-200 column (2.5 × 70 cm) equilibrated with buffer A (0.5 mM ATP, 0.2 mM $CaCl_2$, 200 mg/L NaN_3, and 5 mM triethanolamine/HCl, pH 7.5). ADP−actin was prepared by exchange for ADP of ATP on a Sephadex G-25 Superfine column (1 × 80 cm) equilibrated with buffer B [0.5 mM ADP (<0.01% ATP), 0.01 mM $MgCl_2$, 200 mg/L NaN_3, and 5 mM triethanolamine/HCl, pH 7.5] (*15*). As ADP−

† This work was supported by the Deutsche Forschungsgemeinschaft (SFB 394).

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[1] Abbreviations: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; ϵATP, 1,N^6-ethenoadenosine 5′-triphosphate.

S0006-2960(97)02176-4 CCC: $15.00
Published on Web 04/28/1998

actin is known to be unstable, it was used for polymerization experiments immediately following chromatography. The concentrations of ATP−actin and ADP−actin were determined photometrically at 290 nm using an absorption coefficient of 24900 M^{-1} cm^{-1} (*9*).

Fluorescence. Actin polymerization was followed by the 2.2−2.5-fold greater fluorescence intensity of polymeric actin compared to that of monomeric actin (*14*). Five percent of fluorescently labeled actin was copolymerized with unmodified actin. This low proportion of labeled actin does not significantly alter the polymerization rate or extent of assembly of unmodified actin (*16*). The excitation wavelength was 480 nm, and the fluorescence intensity was measured at 540 nm. The fluorescence intensity of monomeric and polymeric actin was calibrated by measuring the fluorescence intensities of monomeric and polymeric actin to evaluate the changes of fluorescence intensities in terms of concentrations of monomeric and polymeric actin.

The exchange of actin-bound nucleotide was measured by the increase of the fluorescence intensity on binding of ϵATP to monomeric actin. The excitation wavelength was 360 nm, and the emitted light was measured at 410 nm (*3*, *5*, *17*, *18*).

Experimental Design. Polymeric actin (10 μM) was formed by addition to monomeric ATP−actin of buffer A and of concentrated KCl and $MgCl_2$ solutions to adjust $[K^+]$ to 100 mM and $[Mg^{2+}]$ to 1 mM. Short actin filaments were formed by flow through a capillary (51 mm long, 0.15 mm diam, Hamilton syringe) (*19*). Filaments were diluted into buffer A containing 100 mM KCl and 1 mM $MgCl_2$ to yield a 0.5, 1, or 2 μM polymeric actin solution. Following dilution, the time course of depolymerization or polymerization was measured by fluorescence (*20*, *21*). In some experiments polymeric ADP−actin was formed by adjusting monomeric ADP−actin to 0.5 mM ADP, 100 mM KCl, and 1 mM $MgCl_2$ in the absence of ATP. In another set of experiments KCl was omitted from the samples. All experiments were performed at 37 °C.

RESULTS

Time Course of Depolymerization of Polymeric Actin. Actin filaments prepared by polymerization of ATP−actin were diluted into 0.5 mM ATP, 100 mM KCl, and 1 mM $MgCl_2$. Subsequently, the time course of change of the concentration of polymeric actin was measured. Continuous depolymerization was observed both when the filaments were carefully diluted (Figure 1) and when the filaments were fragmented to produce short filaments (Figure 2). A qualitative estimation shows that, according to the generally accepted mechanism and to the known rates of the reactions occurring during actin assembly, this result is unexpected: The half-life of depolymerization of the highest concentration of polymeric actin (2 μM) is about 30 s (Figures 1 and 2). Under identical conditions the half-life of exchange of actin monomer-bound ADP for ATP is about 100 s (*3*). Depolymerization of ADP−actin from filament ends is faster than exchange of actin-bound ADP for ATP. Thus, monomeric ADP−actin is expected to accumulate transiently. As the critical monomer concentration of ADP−actin is higher than that of ATP−actin, one would expect that transiently the monomer concentration reaches a maximum or the polymer concentration reaches a minimum. However, according to Figures 1 and 2, no minimum is observed in the experimentally determined time course. In another experiment ADP−actin was polymerized in the absence of ATP by addition of 100 mM KCl and 1 mM $MgCl_2$. This polymeric ADP−actin was 8-fold diluted into 0.5 mM ATP, 100 mM KCl, and 1 mM $MgCl_2$. Figure 3 shows that actin neither depolymerized nor polymerized significantly. Also in this experiment no transient accumulation of depolymerized monomeric ADP−actin and repolymerization of ATP−actin regenerated by nucleotide exchange was observed. However, it was possible to find a minimum of polymer concentration, if KCl was absent from the solution (Figure 4), in agreement with previously reported data (*21*). To explain the lack of a minimum of polymer concentration as observed in Figures

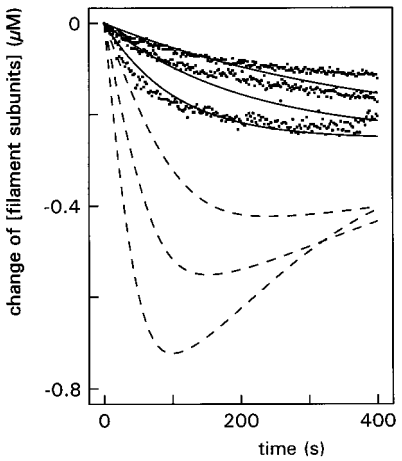

FIGURE 1: Depolymerization of actin filaments following dilution in the presence of 0.5 mM ATP, 100 mM KCl, and 1 mM $MgCl_2$. Actin filaments were formed by polymerization of ATP−actin in 0.5 mM ATP, 100 mM KCl and 1 mM $MgCl_2$. Dotted lines, time course of depolymerization. Concentrations of polymeric actin: upper curve, 0.5 μM; middle curve, 1.0 μM; lower curve, 2.0 μM. Solid lines, curves calculated for the assumption that the nucleotide of the terminal subunit is exchangeable (k_{pex} = 20 s^{-1}). Dashed lines, curves calculated for the assumption that the nucleotide of the terminal subunit is not exchangeable (k_{pex} = 0). Rate constants (see Figure 5) used for calculation of the solid and broken lines: $k_T^+ = 5 \times 10^6$ M^{-1} s^{-1}, k_T^- = 1.15 s^{-1}, $k_D^+ = 4.2 \times 10^6$ M^{-1} s^{-1}, k_D^- = 11.5 s^{-1}, kh = 0.01 s^{-1}, k_{1ex} = 0.007 s^{-1}. Concentrations of filament ends: upper curves, c_e = 0.35 nM ($k_T^+ c_e$ = 0.00175 s^{-1}); middle curves, c_e = 0.7 nM ($k_T^+ c_e$ = 0.0035 s^{-1}); lower curves, c_e = 1.4 nM ($k_T^+ c_e$ = 0.007 s^{-1}).

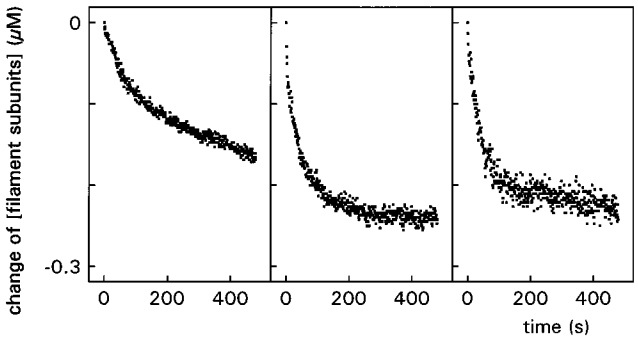

FIGURE 2: Time course of depolymerization of actin filaments following shearing and dilution in the presence of 0.5 mM ATP, 100 mM KCl, and 1 mM $MgCl_2$. Actin filaments were formed by polymerization of ATP−actin in 0.5 mM ATP, 100 mM KCl, and 1 mM $MgCl_2$. Concentrations of polymeric actin: left curve, 0.5 μM; middle curve, 1.0 μM; right curve, 2.0 μM.

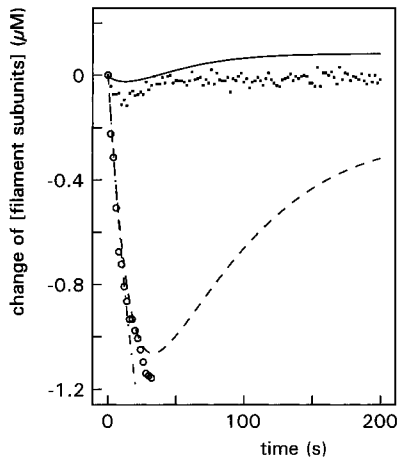

FIGURE 3: Depolymerization or polymerization following shearing and dilution of ADP−actin filaments prepared in the absence of ATP. Dotted line, time course of the filament subunit concentration following dilution into 0.5 mM ATP, 100 mM KCl, and 1 mM $MgCl_2$. Circles, time course of the filament subunit concentration following dilution into 0.5 mM ADP, 100 mM KCl, and 1 mM $MgCl_2$. Solid line, curves calculated for the assumption that the nucleotide of the terminal subunit is exchangeable ($k_{pex} = 20\ s^{-1}$). Dashed line, curves calculated for the assumption that the nucleotide of the terminal subunit is not exchangeable ($k_{pex} = 0$). Rate constants (see Figure 5) used for calculation of the continuous and broken lines: $k_T^+ = 5 \times 10^6\ M^{-1}\ s^{-1}$, $k_T^- = 1.15\ s^{-1}$, $k_D^+ = 4.2 \times 10^6\ M^{-1}\ s^{-1}$, $k_D^- = 11.5\ s^{-1}$, kh = $0.01\ s^{-1}$, $k_{1ex} = 0.007\ s^{-1}$. Dash-dotted line, calculated time course of depolymerization of ADP−actin for the purpose of estimation of the concentrations of filament ends ($c_e = 8$ nM) or of the product $k_D^+ c_e$ ($k_D^+ c_e = 0.034\ s^{-1}$).

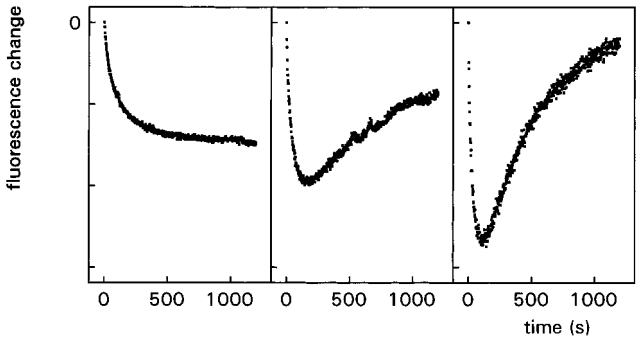

FIGURE 4: Time course of depolymerization and polymerization of actin filaments following shearing and dilution in the presence of 0.5 mM ATP and 1 mM $MgCl_2$ and in the absence of KCl, measured by the fluorescence change of the labeled actin. Actin filaments were formed by polymerization of ATP−actin in 0.5 mM ATP and 1 mM $MgCl_2$ in the absence of KCl. Concentrations of polymeric actin: left curve, 1.0 μM; middle curve, 2.0 μM; right curve, 4.0 μM.

1, 2, and 3, the results will be analyzed by a quantitative evaluation that is based on calculation of kinetic rate equations.

Theoretical Treatment of Depolymerization, Nucleotide Exchange, and Polymerization. Figure 5 shows a scheme of reactions of actin. On dilution, actin filaments are formed that carry ADP-subunits at the ends (uncapped filaments). The uncapped filaments lose ADP-subunits (rate constant k_D^-). ADP-monomers formed by depolymerization can associate with filament ends (rate constant k_D^+) to form polymeric actin or to slowly exchange ADP for ATP (rate constant k_{1ex}) to yield ATP-monomers. The ATP-monomers can associate with filament ends (rate constant k_T^+). ATP-monomers are also formed by dissociation of subunits from

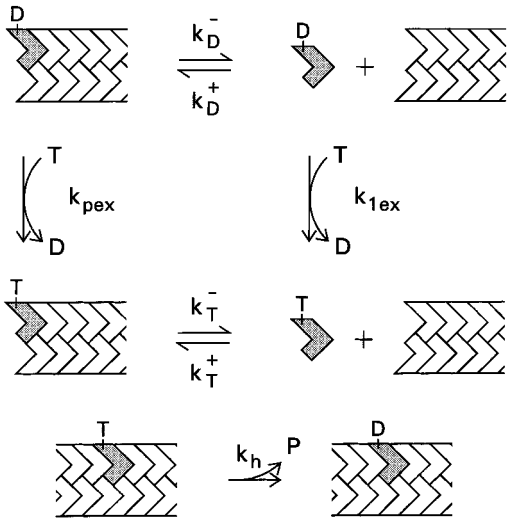

FIGURE 5: Reaction scheme of depolymerization and polymerization of ATP−actin and ADP−actin. ATP hydrolysis and exchange for ATP of ADP bound to monomeric actin or to terminal subunits of filaments are also represented.

filament ends carrying terminal ATP-subunits (capped filaments, rate constant k_T^-). A further reaction considered in this study, is the exchange of ADP bound to a terminal subunit for ATP. With the exception of the exchange at terminal subunits of ADP for ATP, all of these reactions were previously proved and documented in a number of studies (*22*). ATP hydrolysis by filament subunits is also considered (rate constant k_h). Kinetic rate equations that allow calculation of the time course of depolymerization and polymerization are derived in the appendix.

Quantitative Evaluation of the Experimental Results. The time course of depolymerization and polymerization of actin is affected by a number of rate constants (Appendix, eqs A1−A5). As far as possible, these constants were determined by independent experiments.

Rate Constant of Exchange of Nucleotides Bound to Actin Monomers, k_{1ex}. Exchange of actin-bound nucleotide was measured by the fluorescence increase on binding of the ATP analogue ϵATP to actin. Excess ϵATP was added to ADP−actin, and subsequently the time course of replacement of ADP by ϵATP was measured (Figure 6). As dissociation of ADP from actin is the rate-limiting step during nucleotide exchange (*23*, *24*), Figure 6 reflects also the time course of exchange of ADP for ATP. Under the experimental conditions the half-life of exchange was found to be 100 s ($k_{1ex} = \ln 2/(100\ s) = 0.007\ s^{-1}$).

Ratio of Rate Constants of Depolymerization and Polymerization, k_D^-, k_D^+, k_T^-, and k_T^+. Figure 7 shows the results of a determination of the critical monomer concentrations of ADP− and ATP−actin. Various concentrations of actin monomers were added to polymeric actin, and subsequently the change of fluorescence that is brought about by dissociation of subunits or by association of actin monomers with filament ends was measured. Near the critical monomer concentration ($\bar{c}_D$, $\bar{c}_T$) where dissociation of subunits occurs at the same rate as association of monomers ($k_D^- = k_D^+ \bar{c}_D$, $k_T^- = k_T^+ \bar{c}_T$), the fluorescence does not change. According to the data summarized in Figure 7, the critical concentration of ADP−actin is 2.7 μM ($\bar{c}_D = k_D^-/k_D^+ = 2.7\ \mu$M) and that of ATP−actin is 0.23 μM ($\bar{c}_T = k_T^-/k_T^+ = 0.23\ \mu$M).

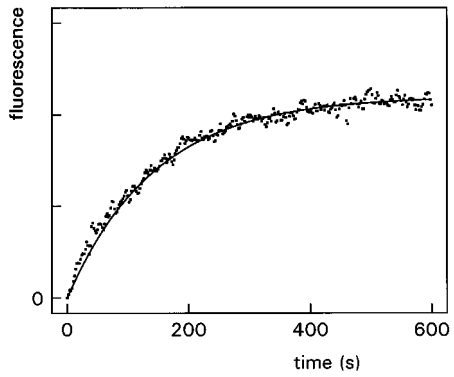

FIGURE 6: Rate of exchange of actin-bound ATP for ϵATP measured by the increase of fluorescence intensity on binding of ϵATP to actin monomers. ϵATP, 400 μM, was added to 1 μM monomeric ADP−actin in the presence of 16 μM ADP, 100 mM KCl, and 1 mM $MgCl_2$. The fitted exponential curve was calculated for a rate constant of exchange (dissociation of ADP from actin) $k_{1ex} = 0.007\ s^{-1}$.

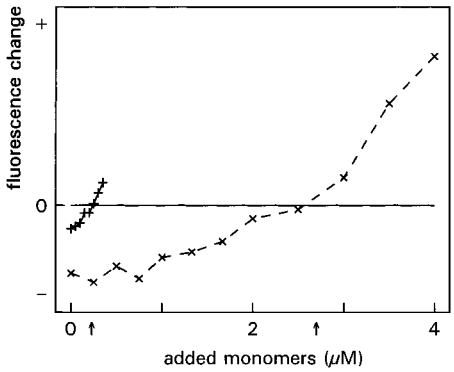

FIGURE 7: Measurement of the critical monomer concentrations of ATP−actin and ADP−actin in the presence of 100 mM KCl and 1 mM $MgCl_2$. The fluorescence change brought about by polymerization or depolymerization is plotted versus the concentration of added monomeric actin: +, 0−0.4 μM monomeric ATP−actin added to 0.25 μM polymeric actin; ×, 0−4 μM monomeric ADP−actin added to 1 μM polymeric actin. The critical concentrations of ATP−actin (0.23 μM) and ADP−actin (2.7 μM) are indicated by arrows.

Figure 8 shows the time course of polymerization of ADP− and ATP−actin monomers onto the same concentration (c_e) of filament ends. The measured time course can be approximated by exponential curves (Figure 8). The half-life of polymerization of ATP−actin was 170 s, and that of ADP−actin was 200 s. Thus, the ratio of the rate constants of association of ATP−actin (k_T^+) and of ADP−actin (k_D^+) is $k_T^+/k_D^+ = 170/200 = 0.85$.

Products of Rate Constants of Polymerization and the Concentrations of Filament Ends, $k_T^+c_e$ and $k_D^+c_e$. The half-life, $t_{1/2}$ of polymerization of actin monomers onto a constant concentration of filament ends depends on the concentration of filaments ends, c_e, in the following way (*19*):

$$k_T^+ c_e = \frac{\ln 2}{t_{1/2}} \quad \text{or} \quad k_D^+ c_e = \frac{\ln 2}{t_{1/2}} \tag{1}$$

To determine the product $k_T^+c_e$ or $k_D^+c_e$ of the depolymerization samples depicted in Figure 1, 2 μM monomeric ATP−actin was added to polymeric actin and the half-life times of polymerization were determined. The half-life times were 390 s (0.5 μM polymeric actin), 180 s (1 μM polymeric actin), and 100 s (2 μM polymeric actin, data not shown).

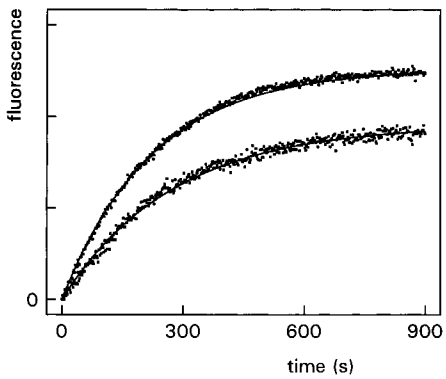

FIGURE 8: Ratio of the rate constants of association of ATP−actin monomers (k_T^+) and ADP−actin monomers (k_D^+) with filament ends in the presence of 100 mM KCl and 1 mM $MgCl_2$. ATP−actin monomers, 4 μM, (upper curve) or ADP−actin monomers, 4 μM (lower curve), were added to 1 μM polymeric ADP−actin stemming from the same sample. The half-life times of the calculated exponential curves are 170 s for ATP−actin (upper curve) and 200 s for ADP−actin (lower curve).

It has been demonstrated that by an analysis of the kinetics and equilibrium of concentrations of actin monomers and filament subunits, only the ratios of rate constants of actin polymerization and depolymerization and the products of rate constants and filament end concentrations can be determined (*25*−*27*). Calculations of the time course of depolymerization according to eqs A1−A5 (Appendix) revealed that also in the refined model of depolymerization derived in this paper the time courses of depolymerization are practically indistinguishable if the product $k_T^+c_e$ or $k_D^+c_e$ is constant.

The rate constants of actin polymerization reported in the literature reveal differencies. In an electron microscopic investigation ADP−actin monomers were found to bind to barbed filament ends 3-fold slower than ATP−actin monomers (*28*), while according to this kinetic investigation in which a fluorescence label was applied, ADP− and ATP−actin monomers were found to bind to barbed filament ends at similar rates. Although different experimental conditions (concentrations of ions, temperature) may account for these discrepancies, the influence of variations of the rate constants on the calculated time courses of depolymerization and polymerization was investigated to consider the possibility that experimental errors caused by limitations of the applied methods may lead to untenable conclusions. It turned out that a minimum of the concentration of polymeric actin was always achieved if (i) depolymerization was faster than exchange of nucleotide bound to monomeric actin and if (ii) the nucleotide of the terminal subunit was unexchangeable. For instance, calculations showed that also a minimum is expected to be passed through if the two rate constants of depolymerization are identical ($k_T^- = k_D^-$) and the difference in the critical concentration is assumed to be brought about by a slow binding of ADP−actin monomers compared to that of ATP−actin monomers ($k_D^+ < k_T^+$).

The rate constant of ATP hydrolysis, k_h, has been determined previously under conditions almost identical to those of this study (*15*). The k_h value has been found to be $0.01\ s^{-1}$ in the presence of 100 mM KCl and 1 mM $MgCl_2$. Calculations according to eqs A1−A5 (Appendix) show that the magnitude of the rate of ATP hydrolysis is not critical for the time course of polymerization and depolymerization of actin.

Evaluation of the Time Course of Depolymerization and Polymerization of Actin. The time course of depolymerization and polymerization of actin was calculated according to eqs A1−A5 (Appendix), thereby using the rate parameters that have been determined by independent experiments. The concentration of filament ends (c_e) was assumed to be constant during the experiment. The type of nucleotide of the terminal subunits of filaments may be different, because some filament ends formed ATP-caps during polymerization, while other filaments may carry ADP at their terminal subunits due to fragmentation during dilution. Calculations showed that there is practically no difference between the time course of depolymerization of filaments that initially have several (e.g., 10) terminal ATP-subunits or that have only ADP-subunits. Thus, ATP-subunits that may occur at the ends of filaments before depolymerization can be assumed not to affect the interpretation of the results. The solutions contained 0.5 mM ATP and only micromolar amounts of ADP resulting from ATP hydrolysis during polymerization of actin. Therefore, exchange of actin-bound ADP for ATP was assumed to be unidirectional (*3*). Figure 1 shows that a poor agreement of calculated curves with the experimental data was achieved when all filament subunits including the terminal subunit were assumed not to exchange ADP for ATP. The calculated curves revealed a minimum of polymer concentration that can be attributed to a transient accumulation of ADP−actin monomers, which have a lower affinity for filament ends than ATP−actin. The lack of a minimum in the experiments indicates that the present model of actin polymerization is not sufficient to explain all experimental observations. A much better agreement of model calculations with experimental was reached when the terminal subunit was assumed to exchange ADP for ATP readily. Figure 1 shows that in the calculated curves the minima disappear and that the calculations are in reasonable agreement with experimental data. The postulated accelerated nucleotide exchange at the terminal subunit leads to a fast formation of ATP-capped filaments which are resistant to extensive depolymerization because of the low critical monomer concentration of ATP−actin (0.23 μM) compared to that of ADP−actin (2.7 μM).

Figure 2 shows that in the presence of 100 mM KCl and 1 mM $MgCl_2$ also sheared actin filaments depolymerize continuously. When KCl was omitted and the filaments were sheared, a minimum of polymer concentration was observed. It was not possible to calculate fitted curves because not all rate parameters could be determined sufficiently accurately. Measurements of the half-life of polymerization of actin monomers onto the ends of sheared filament were not sufficiently reproducible for determination of the product $k_T^+c_e$.

In the dilution experiment depicted in Figure 3, polymeric ADP−actin prepared in the absence of ATP was diluted into an ATP solution (buffer A) containing 100 mM KCl and 1 mM $MgCl_2$. As the critical concentration of ADP−actin is 2.7 μM, following 8-fold dilution the actin monomer concentration was about 0.33 μM, a value that is near the critical monomer concentration of ATP−actin (0.23 μM). No significant polymerization or depolymerization was observed. For quantitative evaluation of the experimental data, the product of the rate constant, k_D^- (Figure 5), and the concentration of filament ends, c_e, was estimated from the initial rate of depolymerization of polymeric ADP−actin in the absence of ATP ($k_D^-c_e = 92 \times 10^{-9}$ M s^{-1} or $k_D^+c_e = k_D^-c_e/c_D = 0.034$ s^{-1}). The later phase of depolymerization was neglected, as during the time course of depolymerization short filaments disappear so that the concentration of filament ends decreases. In Figure 3 the time courses of the filament subunit concentrations were calculated for the model lacking exchange and the model of fast exchange of ADP for ATP at the terminal subunit. Clearly, the model of fast exchange is in much better agreement with the experiments than the model lacking exchange where the polymeric actin concentration is expected to reach a pronounced transient minimum. Compared to the experiments represented in Figures 1 and 2, the initial state of filaments of the dilution experiment of Figure 3 is better defined. In the experiment of Figure 3, before dilution, all filament subunits carry ADP, while in the experiments of Figures 1 and 2, actin was polymerized in the presence of ATP so that filament ends may be capped by a number of ATP subunits. Furthermore, the accuracy of the determination of the filament end concentration, c_e, or of the product $k_D^-c_e$ is not very critical for the evaluation of the data of Figure 3, as for a great range of values of c_e or $k_D^-c_e$ (20×10^{-9} M s^{-1} $< k_D^-c_e < 200 \times 10^{-9}$ M s^{-1}) fast nucleotide exchange at terminal subunits causes neither significant depolymerization nor polymerization, while in the case of lacking nucleotide exchange the concentration of filament subunits is expected to pass through a transient, pronounced minimum.

In summary, the experiments and their quantitative evaluation show that our present model of actin polymerization is not able to explain the time course of depolymerization of actin, and that inclusion of a fast nucleotide exchange at the terminal subunit of actin filaments can account for the experimentally determined time course of actin depolymerization.

DISCUSSION

The analysis of kinetic data reported in this paper suggests that the nucleotide of the terminal subunit of actin filaments is readily exchangeable. Accelerated nucleotide exchange was concluded from the time course of depolymerization of actin. Actin filaments contain thousands of subunits, and at present there is no method available for direct observation of only one subunit out of thousands of subunits. Because of this problem one is confined to more indirect depolymerization experiments and their interpretation if one wishes to obtain a conception about the reactions occurring during actin assembly and disassembly.

Regulation of the rate of exchange of monomeric actin-bound nucleotide by actin-binding proteins has been reported. Both acceleration and retardation of exchange has been observed. Profilin accelerates the exchange of the actin-bound nucleotide (*29*), while thymosin β4 retards the nucleotide exchange (*30*). In the present study binding of an actin monomer to a filament end has been suggested to increase the rate of nucleotide exchange. In addition to regulation by association with proteins, the rate of nucleotide exchange appears to be modified also by ions. Under certain conditions, i.e., in the absence of K^+ ions and on shearing of filaments, following dilution of filaments a minimum of polymer concentration is observed that can be attributed to accumulation of ADP−actin monomers.

Exchange at the terminal filament subunit of ADP for ATP stabilizes filaments against depolymerization, as ATP−actin has a ∼10-fold higher affinity for filament ends than ADP−actin. The experimental conditions (100 mM KCl, 1 mM $MgCl_2$, 37 °C) were similar to physiological cation concentrations and temperature. As dissociation and association of actin molecules at barbed ends of actin filaments is considerably faster than at pointed ends, the measurements reflect essentially depolymerization at the barbed ends (*28*, *31*). Thus, the results and their interpretation indicate fast exchange of nucleotide at the terminal subunit of barbed ends. Fast nucleotide exchange may also contribute to the stability of barbed ends and to the role as polymerizing ends in living cells (*32*).

ACKNOWLEDGMENT

We thank Marianne Redczuk for excellent technical assistance.

APPENDIX

Kinetic Theory of Depolymerization, Polymerization, and Nucleotide Exchange at Terminal Subunits. A scheme of the considered reactions and of the rate constants is depicted in Figure 5. The rate of dissociation of subunits depends on the type of bound nucleotide (ADP or ATP); for example, a filament that has ATP bound at the terminal subunit, ADP at the second subunit, and ATP at the third subunit depolymerizes at a rate different from that of a filament that has at the corresponding positions bound ADP, ATP, and ADP. Therefore, one has to consider and to calculate the probability of the different combinations of nucleotides bound to filament subunits. The probability that a filament has a particular sequence of nucleotides is denoted as p(L, M, N, O,...) where the first index (L) represents the type of nucleotide (ATP or ADP) of the terminal subunit, the second index (M) represents the type of nucleotide bound to the second subunit, and so on. "T" and "D" represent ATP and ADP, respectively. Thus, p(T, M, N, O...) denotes the probability of a combination of nucleotides bound to filament subunits where the terminal subunit carries ATP. This probability changes according to the reactions depicted in Figure 5 (c_T and c_D are the concentrations of ATP− and ADP−actin monomers, respectively):

$$\frac{dp(\mathrm{T,M,N,O,...})}{dt} =$$

$-k_T^+ c_T p$ (T, M, N, O, ...)	association of an ATP-monomer
$+k_T^+ c_T p$ (M, N, O, ...)	
$-k_T^- p$ (T, M, N, O, ...)	dissociation of an ATP-subunit
$+k_T^- p$ (T, T, M, N, O, ...)	
$-k_D^+ c_D p$ (T, M, N, O, ...)	association of an ADP-monomer
$+k_D^- p$ (D, T, M, N, O, ...)	dissociation of an ADP-subunit
$+k_{pex} p$ (D, M, N, O, ...)	exchange of ADP for ATP at the terminal subunit
$-k_h p$ (T, M, N, O, ...)	ATP hydrolysis at filament subunits
$-k_h p$ (T, M, N, O, ...) (if M = T)	
$+k_h p$ (T, T, N, O, ...) (if M = D)	
$-k_h p$ (T, M, N, O, ...) (if N = T)	
$+k_h p$ (T, M, T, O, ...) (if N = D)	
$-k_h p$ (T, M, N, O, ...) (if O = T)	
$+k_h p$ (T, M, N, T, ...) (if O = D)	
−... + ...	(A1)

The probability of nucleotide combinations of filaments where the terminal subunit carries ADP is given by (Figure 5)

$$\frac{dp(\mathrm{D,M,N,O,...})}{dt} =$$

$-k_T^+ c_T p$ (D, M, N, O, ...)	association of an ATP-monomer
$+k_T^- p$ (T, D, M, N, O, ...)	dissociation of an ATP-subunit
$-k_D^+ c_D p$ (D, M, N, O, ...)	association of an ADP-monomer
$+k_D^+ c_D p$ (M, N, O, ...)	
$-k_D^- p$ (D, M, N, O, ...)	dissociation of an ADP-subunit
$+k_D^- p$ (D, D, M, N, O, ...)	
$-k_{pex} p$ (D, M, N, O, ...)	exchange of ADP for ATP at the terminal subunit
$-k_h p$ (D, M, N, O, ...) (if M = T)	ATP hydrolysis at filament subunits
$+k_h p$ (D, T, N, O, ...) (if M = D)	
$-k_h p$ (D, M, N, O, ...) (if N = T)	
$+k_h p$ (D, M, T, O, ...) (if N = D)	
$-k_h p$ (D, M, N, O, ...) (if O = T)	
$+k_h p$ (D, M, N, T, ...) (if O = D)	(A2)

The sum of the probabilities of all nucleotide combinations is unity:

$$\sum p(\mathrm{L, M, N, O, ...}) = 1 \qquad \text{(A3)}$$

The time course of the concentration of ATP−actin monomers c_T is given by

$$\frac{dc_T}{dt} =$$

$-k_T^+ c_T c_e$	association of ATP-monomers
$+k_T^- c_e \sum p$ (T, M, N, O, ...)	dissociation of ATP-subunits
$+k_{1ex} c_D$	exchange of ADP for ATP

(A4)

Correspondingly, the time course of the concentration of ADP−actin monomers c_D is given by

$$\frac{dc_D}{dt} =$$

$-k_D^+ c_D c_e$	association of ADP-monomers
$+k_D^- c_e \sum p$ (D, M, N, O, ...)	dissociation of ADP-subunits
$-k_{1ex} c_D$	exchange of ADP for ATP

(A5)

For reasons of limitations of computation time and memory, maximally 10 terminal subunits were considered in the calculations. Subunits located in the core of the filament were assumed to carry ADP. To test the applicability of this approximation, depolymerization and polymerization were simulated by a Monte Carlo calculation of dissociation and association at the ends of 100 filaments. Each filament was assumed to represent the 100th part of the concentration of filament ends (c_e/100) so that with each dissociation or association reaction the concentration of ATP−actin monomers or ADP−actin monomers (c_T, c_D) changes by c_e/100. Both calculations are in good agreement (calculations not shown).

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BI972176S