The End of a Polymerizing Actin Filament Contains Numerous ATP−Subunit Segments That Are Disconnected by ADP−Subunits Resulting from ATP Hydrolysis†

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Received November 13, 1995; Revised Manuscript Received February 1, 1996⊗

ABSTRACT: ATP hydrolysis by copolymers of ATP−actin and ADP−actin was investigated in order to analyze the effect of interfaces between ATP−subunits and ADP−subunits on hydrolysis of actin-bound ATP. Copolymers of ATP− and ADP−subunits were formed by polymerization of ATP− and ADP−actin monomers onto filaments. By changing the ratio of polymerizing ATP−actin monomers to ADP−actin monomers, the number of interfaces between ATP− and ADP−subunits and of ATP−subunits only surrounded by further ATP−subunits was varied. The rate of actin polymerization and of ATP hydrolysis was measured simultaneously on the same samples. The lag time between incorporation of actin monomers into filaments and subsequent ATP hydrolysis was found to be similar both for polymerized ATP−actin and for copolymers formed by various ratios of ATP− to ADP−actin. The experiments were performed in the presence of 1 mM $MgCl_2$, 0.05 mM $CaCl_2$, and 100 mM KCl or of 1 mM $MgCl_2$ and 0.4 mM EGTA. The type of cations was found to have no major effect on the rate of ATP hydrolysis. A quantitative evaluation of the experimental data suggests that ATP at interfaces between ATP− and ADP−subunits is hydrolyzed not more than 10 times faster than ATP of subunits surrounded by further ATP−subunits. On the basis of these results, one can conclude that an actin filament onto which ATP−actin monomers polymerize contains numerous segments of ATP−subunits that are disconnected by ADP−subunits resulting from ATP hydrolysis. The average length of the numerous ATP segments of a steadily polymerizing filament is in the range of 10 ATP−subunits or below.

In eukaryotes, the cytoplasm is organized by a system of fibrous supramolecular structures, called cytoskeleton. Actin filaments, one group of the major components of the cytoskeleton, are dynamic structures. Their polymerization and depolymerization contribute to motile activities of the cell (Wang, 1985; Westmeyer et al., 1990; Schleicher & Noegel, 1992) Actin assembly is regulated by actin-binding proteins or by polymerization-inherent factors like hydrolysis of actin-bound adenosine 5′-triphosphate (ATP)[1] (Vandekerckhove, 1990; Weeds, 1993; Wegner, 1976). The interaction of nucleotides with actin has been investigated in a number of studies. ATP has been found to have a slightly higher affinity for monomeric actin than ADP (Wanger & Wegner, 1983; Frieden & Patane, 1988). When actin monomers polymerize onto the ends of filaments, actin-bound ATP is hydrolyzed some time after polymerization. The lag between polymerization and ATP hydrolysis leads to an accumulation of ATP−actin near the polymerizing ends (ATP-cap). In the center of filaments, ADP−actin subunits prevail (Straub & Feuer, 1950; Magri et al., 1978; Pardee & Spudich, 1982; Pollard & Weeds, 1984; Carlier et al., 1994; Grazi et al., 1984).

Investigations of the mechanism of ATP hydrolysis by polymeric actin have led to different conclusions about this reaction. Initially, ATP hydrolysis was thought to occur randomly; this means ATP is hydrolyzed at each filament subunit at the same rate independently of the type of nucleotide bound to adjoining subunits (Pollard & Weeds, 1984). Later it was proposed that the rate of ATP hydrolysis at a subunit depends on the type of nucleotide bound to adjoining subunits of a filament in a cooperative manner. In this model, interactions between adjoining ATP-carrying and ADP-carrying subunits were thought to increase the rate of ATP hydrolysis compared to ATP hydrolysis at ATP−subunits only surrounded by further ATP−subunits. In a limiting case of very high cooperativity, ATP would be hydrolyzed sequentially only at interfaces between ATP− and ADP−subunits (Carlier et al., 1987).

In previous studies, the lag between actin polymerization and ATP hydrolysis has been analyzed quantitatively to obtain information about the cooperativity of ATP hydrolysis by polymeric actin (Pollard & Weeds, 1984; Carlier et al., 1987; Ohm & Wegner, 1994). This approach turned out to be not very sensitive as random ATP hydrolysis and a highly cooperative hydrolysis, in which ATP at interfaces between ATP− and ADP−subunits is hydrolyzed 1000-fold faster than ATP of subunits only surrounded by further ATP−subunits, can cause ATP to be hydrolyzed at similar rates (Ohm & Wegner, 1994). Therefore, we designed a more sensitive assay in this paper. We investigated ATP hydrolysis by copolymers of ATP−actin and ADP−actin. By changing the ratio of polymerizing ATP−actin monomers to ADP−actin monomers, the number of interfaces between

† This study was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 394).

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⊗ Abstract published in *Advance ACS Abstracts,* March 15, 1996.

[1] Abbreviations: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate, EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

0006-2960/96/0435-4396$12.00/0

ATP− and ADP−subunits in actin filaments could be varied. If ATP at interfaces was hydrolyzed faster than ATP of subunits only surrounded by further ATP subunits, the time course of ATP hydrolysis would be expected to depend strongly on the number of interfaces introduced into filaments by copolymerization. We present results on ATP hydrolysis by copolymers of ATP− and ADP−actin and evaluate them quantitatively in terms of cooperative interactions between actin filament subunits.

MATERIALS AND METHODS

Preparation of Actin. Rabbit skeletal muscle actin was prepared according to the method of Rees and Young (1967). 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 (Detmers et al., 1981). 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 hydrochloride, pH 7.5). ATP−actin was separated from excess unbound ATP by gel chromatography on a Sephadex G-25 Superfine column (2.5 × 70 cm) equilibrated with buffer B (0.2 mM $CaCl_2$, 200 mg/L NaN_3, and 5 mM triethanolamine hydrochloride, pH 7.5). For preparation of ADP−actin, actin-bound ATP was exchanged for ADP by application to a Sephadex G-25 Superfine column (1 × 80 cm) equilibrated with buffer C [0.5 mM ADP (<0.01% ATP), 0.01 mM $MgCl_2$, 200 mg/L NaN_3, and 5 mM triethanolamine hydrochloride pH 7.5] (Neidl & Engel, 1979; Ohm & Wegner, 1994). As ADP−actin is unstable, it was used for polymerization experiments within a few hours. For some experiments, free ADP was removed by chromatography on a Sephadex G 25 Superfine column (1 × 80 cm) in buffer C (200 mg/L NaN_3, with or without 0.2 mM $CaCl_2$, 5 mM triethanolamine hydrochloride, pH 7.5). As actin in the absence of excess nucleotide is known to be unstable, it was used immediately following chromatography. The concentrations of ATP−actin and ADP−actin were determined photometrically at 290 nm using an absorption coefficient of 24 900 M^{-1} cm^{-1} (Wegner, 1976).

Fluorescence. Actin polymerization was followed by the 2.2−2.5-fold greater fluorescence intensity of polymeric actin compared to that of monomeric actin (Detmers et al., 1981). Five percent of fluorescently labeled actin was polymerized with unmodified actin. This low proportion of labeled actin does not significantly alter the polymerization rate or extent of assembly of unmodified actin (Wegner, 1982). 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.

Determination of ATP. ATP hydrolysis by actin was stopped by addition of 0.2 mL of 2 M $HClO_4$ to 0.8 mL actin samples. Following centrifugation, 2 M $KHCO_3$ was added to the supernatant to adjust the pH to a value of 7. ClO_4^- ions were precipitated as $KClO_4$ crystals by incubation for 10 min on ice. ATP concentration was determined by bioluminescence using the luciferin−luciferase system (ATP bioluminescence HS, Boehringer Mannheim). The bioluminescence intensity was calibrated by using solutions of known ATP concentrations.

Simultaneous Measurements of the Time Courses of ATP Hydrolysis and Actin Polymerization. Unbound ATP was removed from actin because determination of a relatively small decrease of ATP concentration in the presence of excess ATP may be inaccurate. Actin filaments (∼20 μM) were prepared by addition to monomeric ATP−actin of 100 mM KCl and 1 mM $MgCl_2$ or 1 mM $MgCl_2$ and 0.4 mM EGTA. According to bioluminescence measurements, more than 19 μM of the actin-bound ATP was hydrolyzed, and the polymerized actin contained less than 1 μM ATP. Copolymers of ATP−actin and ADP−actin were prepared by polymerization of a mixture of ATP−actin and ADP−actin onto 6 μM polymeric actin. The total concentration of added monomeric actin was 4 μM, and the molar contents of ADP−actin were 0%, 10%, or 50%. The experiments were performed in the presence of two different concentration of cations: In one set of experiments, the actin solutions were adjusted to 1 mM $MgCl_2$, 0.05 mM $CaCl_2$, and 100 mM KCl. In another set of experiments, the effect of Mg^{2+} ions on ATP hydrolysis in the absence of free Ca^{2+} and K^+ ions was investigated. Actin was incubated for 3 min with 0.4 mM EGTA and 50 μM $MgCl_2$ to replace actin-bound Ca^{2+} by Mg^{2+} ions (Pollard & Weeds, 1984; Carlier et al., 1987; Ohm & Wegner, 1994; Gershman et al., 1984). Polymerization was started by the addition of $MgCl_2$ (final concentration 1 mM) and polymeric actin to monomeric actin; 0.8 mL of the samples was used for measuring the time course of nucleated copolymerization by fluorescence. The rest of the nucleated copolymerization samples (∼8 mL) was used for ATP determinations initially in intervals of about 15 s and in the later phase of about 100 s. All experiments were performed at 25 °C.

RESULTS

Time Course of Copolymerization and ATP Hydrolysis. The rate of actin polymerization and of ATP hydrolysis was measured simultaneously on the same samples. Figure 1 reveals the time course of polymerization and ATP hydrolysis in the presence of Mg^{2+}, Ca^{2+}, and K^+ ions. Figure 2 summarizes the results measured on Mg−actin. ATP−actin was used for the assays (upper row of Figures 1 and 2), or copolymers of ATP−actin and ADP−actin were formed by polymerization of monomeric ATP−actin and ADP−actin onto actin filaments (middle and bottom rows of Figures 1 and 2). 10% or 50% ADP−actin were copolymerized with 90% or 50% ATP−actin, respectively. In the presence of Mg^{2+}, Ca^{2+} and K^+ ions, ATP hydrolysis lagged behind polymerization by about 100 s. A similar lag time was observed both for polymerized ATP−actin (upper row, Figure 1) as well as for copolymers formed by 10% ADP− and 90% ATP−actin (middle row, Figure 1). The lag of ATP hydrolysis behind polymerization of copolymers of 50% ADP− and ATP−actin cannot be estimated directly from Figures 1 and 2 because the concentration of polymeric actin comprises a high amount of subunits, that were incorporated containing nonhydrolyzable ADP. ATP hydrolysis by polymeric Mg−actin was found to lag behind polymerization by about 50 s (Figure 2). The lag time of polymerized ATP−Mg−actin and of copolymers of 90% ATP−Mg−actin and 10% ADP−Mg−actin was similar, too (upper and middle

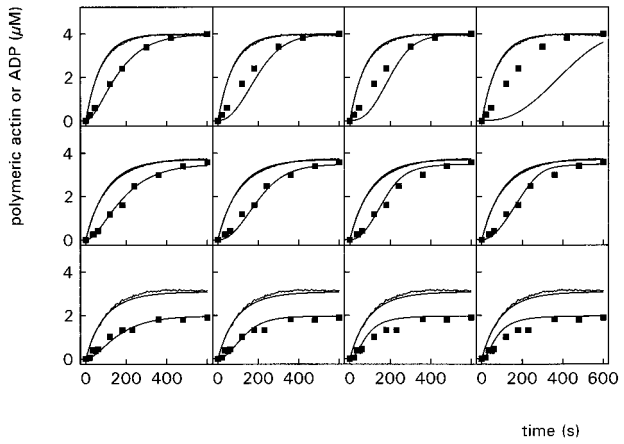

FIGURE 1: Time course of actin polymerization and of ATP hydrolysis in the presence of 1 mM $MgCl_2$, 0.05 mM $CaCl_2$, and 100 mM KCl. 4 μM monomeric actin was polymerized onto 6 μM polymeric actin. Measured time course of polymerization (continuous noisy curves): Upper row, polymerization of 100% ATP−actin; middle row, copolymerization of 10% ADP−actin and 90% ATP−actin; bottom row, copolymerization of 50% ADP−actin and 50% ATP−actin. (■) Measured time course of ADP produced by ATP hydrolysis. The time course of polymerization (upper continuous smooth lines) was calculated for the following rate constants defined in Figure 4: upper row, $k_T^+c_e = 0.0065\ s^{-1}$; middle row, $k_T^+c_e = 0.01\ s^{-1}$, $k_T^-c_e = 2.5 \times 10^{-9}$ M s^{-1}, $k_D^+c_e = 0.0028\ s^{-1}$, $k_D^-c_e = 3.6 \times 10^{-9}$ M s^{-1}; bottom row, $k_T^+c_e = 0.014\ s^{-1}$, $k_T^-c_e = 3.5 \times 10^{-9}$ M s^{-1}; $k_D^+c_e = 0.0039\ s^{-1}$, $k_D^-c_e = 5 \times 10^{-9}$ M s^{-1}, thereby c_e being the concentration of filament ends. The time course of ADP production (lower continuous lines) was calculated for the following rate constants defined in Figure 3: first column, random hydrolysis $k_i = k_a = 0.01\ s^{-1}$; second column, $k_i = 0.002\ s^{-1}$, $k_a = 10k_i = 0.02\ s^{-1}$; third column, $k_i = 0.0006\ s^{-1}$, $k_a = 100k_i = 0.06\ s^{-1}$; fourth column, $k_i = 0.00008\ s^{-1}$, $k_a = 1000k_i = 0.08\ s^{-1}$.

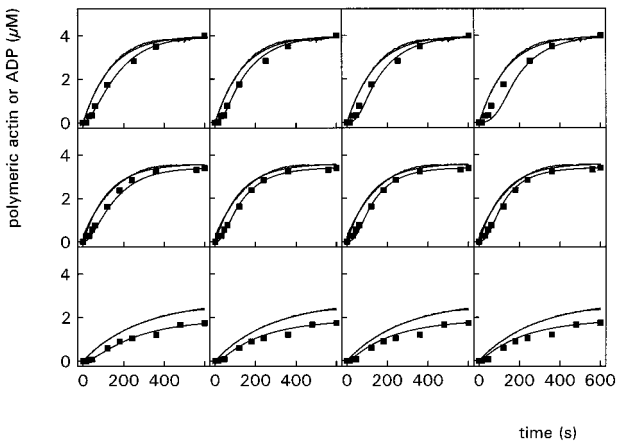

FIGURE 2: Time course of actin polymerization and of ATP hydrolysis in the presence of 1 mM $MgCl_2$ and 0.4 mM EGTA. 4 μM monomeric actin was polymerized onto 6 μM polymeric actin. Measured time course of polymerization (continuous noisy curves): Upper row, polymerization of 100% ATP−actin; middle row, copolymerization of 10% ADP−actin and 90% ATP−actin; bottom row, copolymerization of 50% ADP−actin and 50% ATP−actin. (■) Measured time course of ADP produced by ATP hydrolysis. The time course of polymerization (upper continuous smooth lines) was calculated for the following rate constants defined in Figure 4: upper row, $k_T^+c_e = 0.007\ s^{-1}$; middle row, $k_T^+c_e = 0.008\ s^{-1}$, $k_T^-c_e = 1.7 \times 10^{-9}$ M s^{-1}, $k_D^+c_e = 0.0017\ s^{-1}$, $k_D^-c_e = 2.2 \times 10^{-9}$ M s^{-1}; bottom row, $k_T^+c_e = 0.005\ s^{-1}$, $k_T^-c_e = 1 \times 10^{-9}$ M s^{-1}, $k_D^+c_e = 0.001\ s^{-1}$, $k_D^-c_e = 1.2 \times 10^{-9}$ M s^{-1}, thereby c_e being the concentration of filament ends. The time course of ADP production (lower continuous lines) was calculated for the following rate constants defined in Figure 3: first column, random hydrolysis $k_i = k_a = 0.02\ s^{-1}$; second column, $k_i = 0.007\ s^{-1}$, $k_a = 0.07\ s^{-1}$, $k_a/k_i = 10$; third column, $k_i = 0.0015\ s^{-1}$, $k_a = 0.15\ s^{-1}$, $k_a/k_i = 100$; fourth column, $k_i = 0.0003\ s^{-1}$, $k_a = 0.3\ s^{-1}$, $k_a/k_i = 1000$.

rows, Figure 2). The magnitude of the lag measured in this study is in agreement with a number of studies reported in the literature (Carlier et al., 1984; Pollard & Weeds, 1984; Ohm & Wegner, 1994) although significantly deviant results have been reported, too (Carlier et al., 1987).

Polymerized ATP−actin initially contains ATP−subunits that are surrounded by further ATP−subunits ("internal subunits", see Figure 3). In copolymers of ATP− and ADP−actin, numerous ATP−subunits are formed which are localized at interfaces between ATP-containing and ADP-containing subunits ("adjacent subunits", see Figure 3). The similar rate of ATP hydrolysis both in polymeric ATP−actin and in copolymers suggests that ATP of adjacent subunits is hydrolyzed at a similar rate as ATP of internal subunits. The experimental results do not suggest cooperative interactions between ATP− and ADP−subunits. In the following sections, the experimental data will be evaluated quantitatively in order to estimate the rates of ATP hydrolysis at adjacent and at internal subunits. Kinetic equations which permit calculation of the time course of copolymerization and ATP hydrolysis are derived in the Appendix. The rate constants of polymerization of ATP−actin and ADP−actin and of ATP hydrolysis are defined in Figure 4 and Figure 3, respectively.

Evaluation of the Experimental Data. Copolymerization of ATP−Actin and ADP−Actin. During copolymerization of ATP− and ADP−actin, adjacent and internal ATP−subunits are formed. The concentration of internal and adjacent ATP−subunits depends on the rates of incorporation of ATP−actin and ADP−actin into filaments. According to eqs 4, 5, and 7 (Appendix), the rate constants of polymerization and depolymerization (see Figure 4) multiplied by the filament end concentration, c_e ($k_T^+c_e$, $k_T^-c_e$, $k_D^+c_e$, and $k_D^-c_e$), are necessary for evaluation of copolymerization. These rate parameters were obtained in the following way: Determinations of the critical monomer concentrations (Oosawa & Kasai, 1962) of ATP−actin, $\bar{c}_{1T}$, and of ADP−actin, $\bar{c}_{1D}$, yielded ratios of the required rate parameters:

$$\bar{c}_{1T} = \frac{k_T^-}{k_T^+} = \frac{k_T^-c_e}{k_T^+c_e} \quad (1)$$

and

$$\bar{c}_{1D} = \frac{k_D^-}{k_D^+} = \frac{k_D^-c_e}{k_D^+c_e} \quad (2)$$

For determination of the critical concentrations of ATP−actin and ADP−actin, various concentrations of monomeric actin were added to polymeric actin (Wegner, 1982). Subsequently, polymerization or depolymerization was followed by fluorescence. In Figure 5, the fluorescence change versus the concentration of added actin monomers is depicted. The critical monomer concentration, where the fluorescence does not change, was found to be 0.25 μM for ATP−actin and 1.3 μM for ADP−actin in the presence of 1 mM $MgCl_2$, 0.05 mM $CaCl_2$, and 100 mM KCl. The

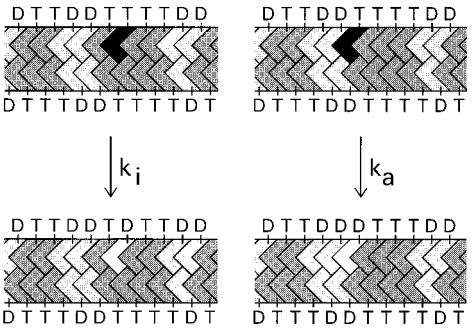

FIGURE 3: Reaction scheme of hydrolysis of ATP by actin. Left, hydrolysis of ATP at an "internal" ATP−subunit that is surrounded by further ATP-carrying subunits (rate constant k_i); right, hydrolysis of ATP at an "adjacent" ATP−subunit that is localized at an interface between ATP-carrying and ADP-carrying subunits (rate constant k_a). T and D stand for ATP or ADP, respectively.

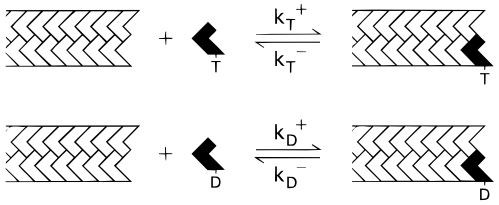

FIGURE 4: Reaction scheme of polymerization and depolymerization of ATP−actin (rate constants k_T^+, k_T^-) and ADP−actin (rate constants k_D^+, k_D^+). ATP and ADP are abbreviated by T or D, respectively.

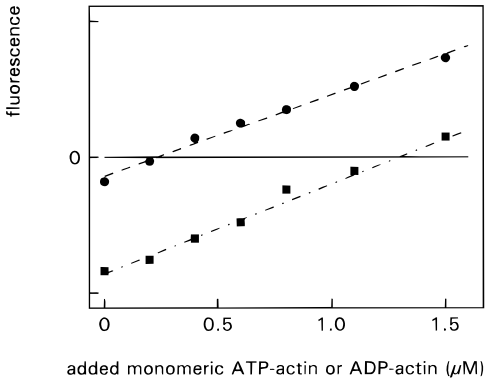

FIGURE 5: Determination of the critical monomer concentrations of ATP−actin and ADP−actin. Plot of the change of fluorescence, that is brought about by polymerization or depolymerization, versus the concentration of added monomeric actin. At the critical monomer concentration, the fluorescence change is zero. (●) Various concentrations of monomeric ATP−actin were added to 0.2 μM polymeric actin. (■) Various concentrations of monomeric ADP−actin were added to 0.2 μM polymeric ADP−actin. The critical monomer concentration of ATP−actin or ADP−actin is found to be 0.24 or 1.3 μM, respectively. Experimental conditions: 1 mM $MgCl_2$, 0.05 mM $CaCl_2$, 100 mM KCl, pH 7.5, 25 °C.

corresponding values obtained in the presence of 1 mM $MgCl_2$ and 0.4 mM EGTA were 0.21 μM and 1.28 μM.

If pure ATP−actin or pure ADP−actin is polymerized, the rate parameters $k_T^+ c_e$ and $k_D^+ c_e$ can be extracted straightforward from the time course of polymerization. The rate parameters $k_T^+ c_e$ and $k_D^+ c_e$ have been shown to depend on the half-lifetime of polymerization, $t_{1/2}$, in the following way (Ditsch & Wegner, 1994):

$$t_{1/2} = \frac{\ln 2}{k_T^+ c_e} \text{ or } = \frac{\ln 2}{k_D^+ c_e} \qquad (3)$$

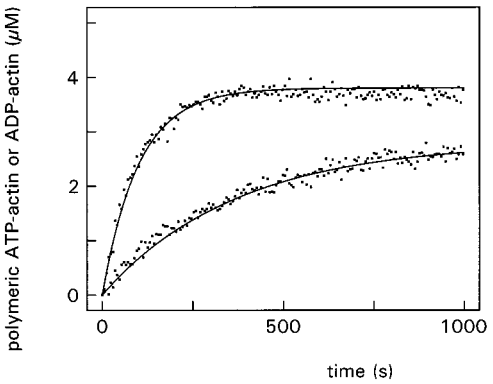

FIGURE 6: Determination of the ratio of the rate constants of association of ATP−actin (k_T^+) and ADP−actin (k_D^+) with a filament end. 4 μM monomeric ATP−actin (upper curve) or 4 μM ADP−actin (lower curve) was added to 6 μM polymeric ADP−actin stemming from the same polymeric actin sample. The half-lifetimes of the calculated exponential curves are 70 s or 255 s, respectively.

For evaluation of the copolymerization kinetics, the ratio of $k_T^+ c_e / k_T^- c_e$ was determined by polymerization of pure ATP−actin or pure ADP−actin onto polymerized ADP−actin taken from the same sample in order to be sure that the concentrations of filament ends c_e are identical in both polymerization experiments. Figure 6 shows the results obtained in the presence of 1 mM $MgCl_2$, 0.05 mM $CaCl_2$, and 100 mM KCl. Under the applied experimental conditions, the half-lifetime of polymerization of ATP−actin was 70 s, and that of ADP−actin was 255 s (Figure 6). Thus, ATP−actin monomers associate with a filament end 3.6-fold faster than an ADP−actin monomer ($k_T^+ c_e / k_D^+ c_e = 255/70$). In the presence of 1 mM $MgCl_2$ and 0.4 mM EGTA, association of ATP−actin with a filament end was 5-fold faster than that of ADP−actin.

The rate parameters $k_T^+ c_e$, $k_T^- c_e$, $k_D^+ c_e$, and $k_D^- c_e$ were fitted to the experimentally measured time courses of copolymerization, thereby using sets of rate parameters which are in agreement with the determined critical concentrations and the ratios of polymerization rates of ATP−actin and ADP−actin. Fitted time courses of copolymerization which were calculated by using eqs 4−9 (Appendix) are depicted in Figures 1 and 2. The rate parameters differed from experiment to experiment (see legends to Figures 1 and 2). This may be explained by different concentrations of filament ends, c_e, contained in different preparations of polymeric actin.

ATP Hydrolysis. In Figures 1 and 2, fits of calculated time courses of ATP hydrolysis to experimental results are depicted. The left columns of Figures 1 and 2 show curves calculated for random hydrolysis ($k_i = k_a$, Figure 3). The computed curves are in good agreement with the measured time course of ATP hydrolysis. We also computed the time courses of cooperative ATP hydrolysis. In these calculations, ATP hydrolysis in adjacent subunits was assumed to be 10-fold ($k_a/k_i = 10$, second column, Figures 1 and 2), 100-fold ($k_a/k_i = 100$, third column), or 1000-fold ($k_a/k_i = 1000$, right column) faster than ATP hydrolysis by internal subunits. It was not possible to approximate all experimental data by a highly cooperative model of ATP hydrolysis. In Figures 1 and 2, the time course of highly cooperative ATP hydrolysis ($k_a/k_i = 100$ or 1000) of copolymers formed from 90% ATP−actin and 10% ADP−actin was approximated by optimally

fitted curves. The time course of ATP hydrolysis calculated for ATP homopolymers revealed a pronounced initial lag phase that was not observed in the experiments (top row, third column of Figures 1 and 2, although less significantly in Figure 2; right column, Figures 1 and 2). The calculated time course of ATP hydrolysis of filaments formed by polymerization of 50% ATP−actin and 50% ADP−actin tended to be faster than the experimental results (bottom row, third column and right column of Figures 1 and 2). A reasonable agreement of calculations with measured time courses of ATP hydrolysis was obtained for low cooperativity. If ATP of adjacent subunits was assumed to be hydrolyzed 10-fold faster than ATP of internal subunits, the measured time courses could be fitted satisfactorily (second column). Thus, quantitative evaluation of the experimental data suggests that ATP in adjacent subunits is hydrolyzed at the same rate or not very much faster than ATP of internal subunits. ATP appears to be hydrolyzed randomly or in a slightly cooperative manner. Cations such as Mg^{2+}, K^+, or Ca^{2+} were found to have no major effect on the interaction between ATP− and ADP−subunits.

DISCUSSION

In this paper, we investigated ATP hydrolysis by copolymers of ATP− and ADP−actin. By changing the ratio of polymerizing ATP− to ADP−actin monomers, the number of adjacent and internal ATP−actin incorporated into filaments was varied. If due to cooperative interactions between actin filament subunits ATP of adjacent and internal ATP− subunits was hydrolyzed at different rates, the measured time course of ATP hydrolysis would be expected to depend strongly on the number of adjacent and internal ATP− subunits produced by copolymerization. In previous studies, the lag between polymerization of ATP−actin and ATP hydrolysis has been analyzed quantitatively in terms of the cooperativity of ATP hydrolysis by polymeric actin (Carlier et al., 1987; Ohm & Wegner, 1994). This approach turned out to be not very sensitive as random and cooperative hydrolysis can cause ATP to be hydrolyzed at similar rates (Ohm & Wegner, 1994). The investigations on copolymers are more sensitive to cooperativity of ATP hydrolysis than previous results obtained from polymerized ATP−actin.

The number of ATP molecules occurring in the terminal subunits of a filament depends on the rate and duration of polymerization. Calculations show that a single filament can contain even thousands of ATP−subunits if it polymerizes steadily in the presence of micromolar ATP−actin monomer concentrations. For instance, if the average lifetime of an ATP molecule in a polymer is 100 s and if at a concentration of 10 μM actin monomers about 50 actin molecules are incorporated into a filament [$k_T^+ c_T \approx 5\ \mu M^{-1}\ s^{-1} \times 10\ \mu M = 50\ s^{-1}$ (Pollard, 1986)], the number of ATP−subunits near the filament end can be calculated to be 5000 (100 s × 50 s^{-1} = 5000).

Cooperativity affects the length of segments of contiguous ATP−subunits. High cooperativity leads to formation of long ATP segments because existing ATP segments are disconnected by ATP hydrolysis of internal subunits only very rarely (Pantaloni et al., 1985). Calculations show that the average length of ATP segments of steadily polymerizing filaments essentially depends on the ratio of the rates (k_a/k_i) of ATP hydrolysis at adjacent and internal subunits (eqs 11−15 in Appendix). According to calculations, a single interface between ATP− and ADP−subunits that migrates toward the end of a filament, due to sequential hydrolysis of ATP at the interface, would occur if ATP at the interface was hydrolyzed more than 10^6-fold faster than internal ATP ($k_a/k_i > 10^6$; Ohm & Wegner, 1994). If ATP is hydrolyzed randomly ($k_a = k_i$), in polymerizing filaments numerous ATP segments occur which contain two subunits on the average. If due to cooperative interactions ATP of adjacent subunits is hydrolyzed 10-fold or 100-fold faster than at internal subunits (k_a/k_i = 10 or 100), ATP segments of steadily polymerizing filaments contain on the average 4 or 16 subunits, respectively. Thus, cooperative interactions between filament subunits, if they exist at all, have only a moderate effect on the average length of ATP segments. As the quantitative evaluation of the experimental data suggests that ATP in adjacent subunits is hydrolyzed less than 100-fold faster than ATP of internal subunits or even perhaps at the same rate, one can conclude that ATP segments of polymerizing filaments contain on the average not more than 10 contiguous ATP−subunits.

ATP hydrolysis has been shown to bring about a difference between the critical concentrations of the two ends of actin filaments. The barbed ends have a higher apparent affinity for actin monomers than the pointed ends. Therefore, actin filaments "treadmill" slowly; that is to say they polymerize at the pointed ends and depolymerize simultaneously at the barbed ends (Wegner, 1976; Pollard & Mooseker, 1981; Wegner & Isenberg, 1983; Selve & Wegner, 1986). It is still an open question, that has to be solved in future, how treadmilling of actin filaments can be explained on the basis of the determined rates of polymerization and depolymerization of ATP−actin and ADP−actin and of the mechanism and rate of ATP hydrolysis by polymeric actin.

ACKNOWLEDGMENT

Our special thanks go to Dr. E. Kinne (Max Planck Institute of Molecular Physiology, Dortmund) for valuable advice and good cooperation. We thank Marianne Redczuk for excellent technical assistance.

APPENDIX

Kinetic Equations for Copolymerization of ATP−Actin and ADP−Actin. For calculation of the time course of ATP hydrolysis in copolymers, it is necessary to know the length distribution of the segments of ATP−subunits created by copolymerization. ATP−actin and ADP−actin have been shown to copolymerize in a random manner; that is to say, ATP−actin associates with an ATP-capped filament end in the same manner as with an ADP-containing filament end (Ohm & Wegner, 1991). Thus, the rate of polymerization, that is expressed by the rate of incorporation of ATP−actin monomers or ADP−actin monomers into filaments, is given by

$$\frac{dc_T}{dt} = -k_T^+ c_T c_e + k_T^- c_e p_{T1} \quad (4)$$

$$\frac{dc_D}{dt} = -k_D^+ c_D c_e + k_D^- c_e p_{D1} \quad (5)$$

$$p_{T1} + p_{D1} = 1 \quad (6)$$

The rate constants k_T^+, k_T^-, k_D^+, and k_D^- are defined in

Figure 4. c_T and c_D are the concentrations of monomeric ATP− or ADP−actin, respectively. c_e is the concentration of filament ends. p_{T1} or p_{D1} are the probabilities that the terminal subunit of a filament carries an ATP−subunit or ADP−subunit, respectively.

The probability p_{D1} decreases by association of an ATP−actin monomer with a filament end that contains ADP at the terminal subunit, or by dissociation of an ADP−subunit from a filament end that has bound ATP at the penultimate subunit. p_{D1} increases by association of an ADP−actin monomer with a filament end that contains ATP at the terminal subunit, or by dissociation of an ATP−subunit from a filament end that has bound ADP at the penultimate subunit. Thus, the time course of p_{D1} is given by (Keiser et al., 1986):

$$\frac{dp_{D1}}{dt}c_e = p_{T1}k_D^{+}c_e c_D - p_{D1}k_T^{+}c_e c_T + p_{D2}p_{T1}k_T^{-}c_e - p_{T2}p_{D1}k_D^{-}c_e \quad (7)$$

p_{D2} and p_{T2} are the probabilities that the penultimate subunit contains ADP or ATP, respectively. In a system of polymerizing actin filaments, in which during association of several actin monomers with filaments ends the actin concentration remains practically constant, steady-state assumptions can be made to express the probability p_{D1} in terms of the rate constants and concentrations:

$$\frac{dp_{D1}}{dt}c_e = 0 \quad (8)$$

$$p_{D2} = p_{D1} \quad (9)$$

The probability p_{D1} can be extracted from experimental data by using eqs 4−9. The required determination of the rate parameters $k_T^{+}c_e$, $k_T^{-}c_e$, $k_D^{+}c_e$, and $k_D^{-}c_e$ is described under Results (evaluation of copolymerization of ATP− and ADP−actin).

Length Distribution of ATP Segments Created by Copolymerization. By means of the probability p_{D1}, the length distribution of ATP segments created by copolymerization can be calculated: The probability that any incorporated subunit contains ATP is equal to $1 - p_{D1}$. Accordingly, the concentration, $c_{(n+1)i}$, of segments containing $n+1$ subunits can be expressed in terms of the concentration, c_{ni}, of segments containing n subunits:

$$c_{(n+1)i} = c_{ni}(1 - p_{D1}) \quad (10)$$

Thus, the length distribution of ATP segments created by random copolymerization is exponential. The concentrations of ATP segments created by copolymerization are marked with the subscript "i" in order to emphasize that the calculated length distribution is initial. This length distribution is subsequently changed by loss of ATP due to hydrolysis. The total concentration of initial ATP segments, c_i, is given by

$$c_i = \sum_{n=1}^{\infty} c_{ni} = \sum_{n=1}^{\infty} c_{1i}(1 - p_{D1})^{n-1} = \frac{1}{p_{D1}}c_{1i} \quad (11)$$

A second equation required for calculation of c_i and c_{1i} can be derived by consideration of the concentration of ATP−actin, $-dc_T$, incorporated into filaments during a time interval dt (see eq 4). This concentration is equal to the concentration of ATP−subunits being contained in ATP segments, of course:

$$-dc_T = \sum_{n=1}^{\infty} nc_{ni} = \sum_{n=1}^{\infty} nc_{1i}(1 - p_{D1})^{n-1} = c_{1i}\frac{1}{{p_{D1}}^2} \quad (12)$$

Equations 11 and 12 permit calculation of c_i and c_{1i} in terms of p_{D1} and the concentration of ATP−actin, $-dc_T$, incorporated into filaments during a time interval dt.

ATP Hydrolysis. A reaction scheme of ATP hydrolysis by actin filaments is depicted in Figure 3. If the ATP hydrolysis is cooperative, two rate constants of ATP hydrolysis have to be introduced: (i) rate constant k_i of ATP hydrolysis of a subunit surrounded by ATP-containing subunits (internal subunit); (ii) rate constant k_a of ATP hydrolysis of a subunit at an interface between ADP−subunits and ATP−subunits (adjacent subunits).

Kinetic equations for the time course of ATP hydrolysis have been derived previously (Ohm & Wegner, 1994): The time course of hydrolysis of ATP being bound in subunits (concentration $[ATP]_b$) is given by:

$$\frac{d[ATP]_b}{dt} = -k_a(2c_s - c_{s1}) - k_i[[ATP]_b - (2c_s - c_{s1})] \quad (13)$$

c_s is the concentration of ATP segments, and c_{s1} is the concentration of ATP segments containing only one ATP−subunit. Equation 13 contains three variable concentrations ($[ATP]_b$, c_s, and c_{s1}). Two additional equations, which are necessary for calculation of the time course of ATP−subunits, were derived previously (Ohm & Wegner, 1994):

$$\frac{dc_s}{dt} = -k_a c_{s1} + k_i[[ATP]_b - (2c_s - c_{s1})] \quad (14)$$

and

$$c_{s1} = \frac{{c_s}^2}{[ATP]_b} \quad (15)$$

For calculation of the time course of hydrolyzed ATP which is depicted in Figures 1 and 2, the concentrations of ATP incorporated into filaments within time intervals of 1 s were computed by using eqs 4−9. As ATP bound to subunits is created by copolymerization, the initial concentration of ATP ($[ATP]_b$) incorporated into filaments during a time interval dt is equal to $-dc_T$ (eq 4). Correspondingly, the initial concentrations of ATP segments (c_s) and of ATP segments containing only one subunit (c_{s1}) are equal to c_i and c_{1i}, respectively (eqs 11 and 12). The time courses of ATP hydrolysis were calculated by using eqs 13−15. The total concentration of hydrolyzed ATP was obtained by summing up the contributions of all filament subunits, thereby taking into account that the subunits were incorporated into filaments at different time. The resulting curves are depicted in Figures 1 and 2.

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BI9527045