DNase I Increases the Rate Constant of Depolymerization at the Pointed (–) End of Actin Filaments†

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ABSTRACT: We show here that DNase is distinguished from other known actin-binding proteins by its unique ability to increase the depolymerization rate constant of actin at the pointed filament end, thereby speeding up depolymerization of filaments capped at their barbed ends. This action requires relatively high DNase concentrations, 3 orders of magnitude higher than those needed to block elongation, although 10 times lower than those needed for DNase binding to the side of the filament. We propose that at high DNase concentrations, steric interference between the two DNase molecules, bound to the ends of both strands of the two-start actin helix, destabilizes actin binding to the filament.

DNase I is a high-affinity actin-sequestering protein (Lazarides & Lindberg, 1974; Mannherz et al., 1975; Hitchcock et al., 1976; Hitchcock, 1980) which has been frequently used as a tool in studies of actin. For instance, the molecular structure of actin has been determined from DNase–actin crystals [Kabsch et al., 1990; cf. Kabsch and Vandekerckhove (1992)]. DNase I binds to domains II and IV of monomeric actin (Kabsch et al., 1990) with a K_d of 1–2 × 10^{-9} M (Mannherz et al., 1980). It has been reported to bind with similar affinity to the terminal actin monomers at the pointed ends of actin filaments where these two domains are accessible (Podolski & Steck, 1988). It also binds alongside the filament (Hitchcock et al., 1976) with a much lower affinity (K_d close to 100 μM; Mannherz et al., 1980).

The major point made by this study is that DNase can act as a depolymerizing protein by increasing the depolymerization rate constant of actin at the pointed filament end. Such an action has not been observed for any of the known actin-binding proteins. For instance, when gelsolin increases the depolymerization rate, it does so by increasing the number of filament ends as the result of its filament-cutting action. Depolymerizing proteins are of interest as a possible explanation of the very rapid actin filament depolymerization rates associated with movement of either lamellipodia (Zigmond, 1993) or bacteria with actin tails, such as *Listeria monocytogenes* (Sanger et al., 1992; Theriot et al., 1992). These rates can be much faster than expected from the rate constants of depolymerization.

According to our kinetic studies, it is plausible that steric interference between two DNase molecules bound to both strands of the two-start actin helix destabilizes actin binding to the filament end.

EXPERIMENTAL PROCEDURES

Proteins. Rabbit skeletal muscle actin was prepared from an acetone powder of rabbit muscle as previously described (Murray et al., 1981) with some modifications in the chromatography step (Young et al., 1990). Pyrenyl labeling of muscle actin was carried out according to Kouyama and Mihashi (1981) with the modifications described previously (Northrop et al., 1986). Actin was stored in liquid nitrogen and defrosted as previously described (Young et al., 1990). The critical concentration of various actin preparations varied between 0.07 and 0.12 μM for uncapped filaments and between 0.5 and 0.8 μM for filaments capped at the barbed ends with gelsolin. Gelsolin, a generous gift from J. Bryan, was prepared as previously described (1988). DNase was purchased from several different sources (Sigma, Fluka, and Worthington) and used without further purification. Purity was determined by SDS gel electrophoresis and activity by its actin-sequestering activity in the presence of calcium (K_d about 1 nM). The extent of sequestration is indicated by the increase in steady-state G-actin concentration due to the sequestered actin, determined in the absence (Figure 1) or presence of a barbed-end capping protein and either in the presence of calcium (Figure 1) or in the presence of EGTA. (Actin sequestration by DNase is nearly calcium insensitive, with a K_d shift from about 1 nM in the presence of calcium to 3–5 nM on calcium removal.) The activity and the purity of the DNase preparations from Sigma and Fluka varied between 50% and 90%. None of the impurities modified the effect of DNase on depolymerization: it was the same with pure and impure preparations. Worthington DNase was of significantly greater purity with only one major band (DNase used in Figure 1). Vitamin D-binding protein which runs as a single band on SDS gels was bought from CalBiochem.

Protein concentrations were calculated using for actin, E_{290} = 24.9 mM^{-1} cm^{-1}; for gelsolin, E_{280} = 150 mM^{-1} cm^{-1}; for DNase, E_{280} = 34.4 mM^{-1} cm^{-1}; and for vitamin D-binding protein, according to weight dissolved.

Fluorescence and Kinetic Measurements. Changes in actin polymerization were calculated from fluorescence measurements (excitation 366.5 nm and emission 407 nm) as previously described (Weber et al., 1987a) using a PTI photon-counting fluorimeter. Measurements were standardized against a Raman excitation peak (357 nm, emission 407 nm). Readings of different experiments are not comparable because varying slit widths were used.

All experiments were carried out at 20 °C with Mg-actin, the physiological form of actin (Weber et al., 1969; Kitasawa et al., 1982). In a KCl–Mg medium, the fluorescence increase of pyrenyl-actin starts at the maximal rate with Mg-actin but not with Ca-actin, as first observed by Selden et al. (1983). This is due to the shorter persistence of low-fluorescing ATP–

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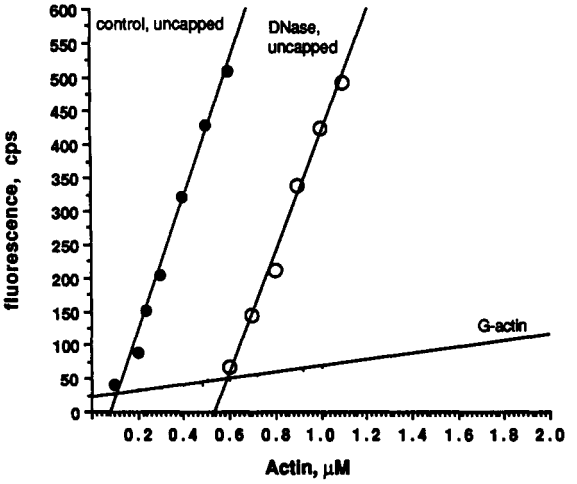

FIGURE 1: Calibration of the actin-binding capacity of DNase I. The increase in fluorescence with increasing total actin was measured in the absence of salt, lower line (G-actin), and after overnight incubation in the presence of salt, the other two lines (F-actin). The intercepts with the G-actin line indicate the total G-actin concentrations at steady state, i.e., the critical concentration in the control and the critical concentration + the DNase-sequestered actin in the presence of DNase. The difference in the actin concentrations at the intercepts, the concentration of sequestered actin, is equal to the concentration of active DNase since, with a K_d of 1 nM, DNase binds actin stoichiometrically. In this case, the active DNase was 0.5 μM out of a total DNase concentration of 0.55 μM (according to the absorption measurement); 10% pyrenyl-labeled actin.

pyrenyl-actin in polymerized Mg-actin as compared to in Ca-actin (Carlier et al., 1986). The conversion to Mg-actin was carried out as previously described (Young et al., 1990).

Measurements of elongation and depolymerization rates at the pointed filament end were carried out as previously described (Walsh et al., 1984; Northrop et al., 1986; Young et al., 1990) in a medium containing 10 mM imidazole buffer, pH 7.4, 0.1 M KCl, 2 mM $MgCl_2$, 1 mM azide, 1 mM dithiothreitol, 0.5 mM ATP, usually 0.2 mM $CaCl_2$, and 10 nM gelsolin–actin dimers to insure full capping of the barbed ends after dilution from the stock solution. Depolymerization measurements were carried out under conditions of virtually no back reaction because the concentration of free G-actin was less than 10 nM throughout the whole time course. Actin monomers were sequestered either by DNase (K_d for actin monomers 1 nM), or, in its absence, by vitamin D-binding protein added in excess of the total F-actin added. Vitamin D-binding protein only sequesters actin monomers and has no effect on depolymerization as indicated by control experiments, comparing the rate constant of depolymerization at low total actin concentration in the presence and absence of vitamin D-binding protein (data not shown). Known number concentrations of gelsolin-capped filaments were obtained by copolymerizing actin, in the presence of calcium, with gelsolin or gelsolin–actin dimers, strong nucleating proteins, which, according to our measurements, results in the formation of one filament per gelsolin, as has been published previously for villin (Walsh et al., 1984; Coleman & Mooseker, 1985; Northrop et al., 1986). Average filament sizes for elongation and depolymerization (average length) are given in the legends. In some of the elongation measurements, gelsolin–actin dimers were used as nuclei. Endpoints of polymerization were measured after overnight incubation of actin in the presence of a nucleating substance, since without a nucleating agent polymerization sometimes was not complete even after an overnight incubation. We used either actin–gelsolin nuclei (actin/gelsolin ratio = 25) or a small amount of polymerized actin pushed through a 10-μL Hamilton syringe at maximal speed.

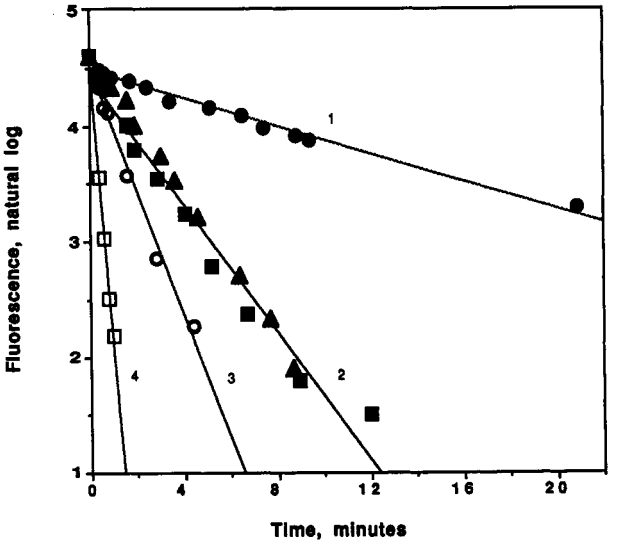

FIGURE 2: DNase increases the rate constant of depolymerization at the pointed end. Semilog plot of the time course of depolymerization, which is linear if the filament-length distribution is exponential and the rate constants do not change with time. F-actin (10 μM, 25% pyrenyl-labeled), copolymerized with either 40 nM (long actin filaments, average length 250 molecules/filament; curves 1 and 3) or 200 nM gelsolin (short filaments, average length 50 molecules/filament; curves 2 and 4; the closed triangles on curve 2 represent another control experiment to indicate the reproducibility of the data), was diluted 50-fold into the depolymerization medium containing either 250 nM vitamin D-binding protein (controls, curves 1 and 2) or 5 μM DNase (curves 3 and 4) (see the methods section) and, in addition, 10 nM gelsolin–actin complexes to maintain complete barbed-end capping after filament dilution. The extent of polymerization at each time point is indicated by the fluorescence (total – endpoint fluorescence). Slope = k_{off}[pointed ends]; [pointed ends] = [actin]/(average length); k_{off} = slope × average length. From the slopes of the control curves of –0.0001 and –0.00047 which increased to –0.0009 and –0.0042 in the presence of DNase, we calculated an increase in the rate constants from 0.023 to 0.21 s^{-1}.

RESULTS

DNase I Increases the Rate of Depolymerization of Actin Filaments Capped at Their Barbed Ends by Gelsolin. Gelsolin-capped actin filaments were diluted into a medium containing either vitamin D-binding protein or DNase in sufficient excess over the total actin to lower the concentration of free actin monomers to less than 10 nM (both monomer binding proteins have a K_d of close to 1 nM for actin; Mannherz et al., 1980; McLeod et al., 1989). In the presence of 5 μM DNase, the filaments depolymerized much faster than in the presence of vitamin D-binding protein (Figure 2) or at very low concentrations of total actin (40 nM) without any sequestering protein (data not shown).

The rate of depolymerization is determined by the concentration of the filament ends and by the rate constant of depolymerization, k_{off}. Thus, DNase may have either increased the number of pointed ends by cutting actin filaments or increased k_{off} by binding to the pointed ends. These two possibilities can be distinguished by comparing the effect of DNase on long and short actin filaments, since cutting would have a much greater proportional effect on long than short filaments. For instance, 10 cuts administered to F-actin assembled in two long filaments would increase the filament end concentration 6-fold as compared to a 2-fold increase if the 10 cuts were administered to 10 short filaments.

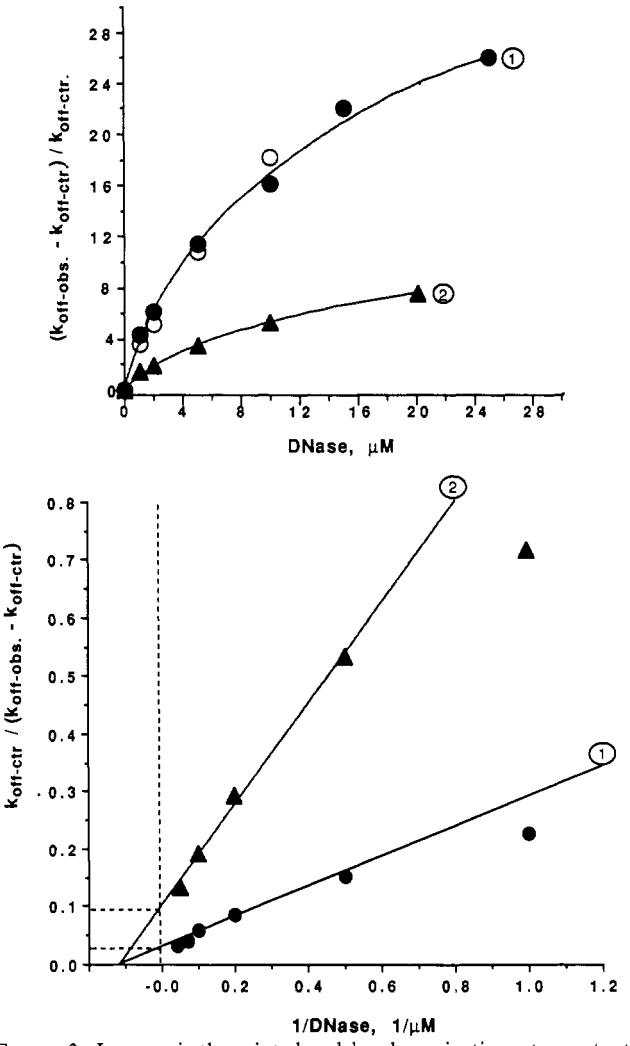

FIGURE 3: Increase in the pointed-end depolymerization rate constant with increasing DNase concentrations comparing actin preparations with low- and high-intrinsic rate constants. (Top) Ordinate, factor by which DNase increased the rate constant over the control value $(k_{off\text{-}observed} - k_{off\text{-}control})/k_{off\text{-}control}$. The observed and control rate constants were obtained as explained in the legend for Figure 2. The control rate constants (slope × average length) are 0.025 s^{-1}, curve 1 (low rate constants), and 0.11 s^{-1}, curve 2 (high rate constants). Curve 1: open circles, short filaments, 25 monomers/gelsolin; closed circles, long filaments, 200 monomers/gelsolin; 25% labeled pyrenyl-actin, 0.2 μM F-actin, 2.0 μM vitamin D-binding protein in the controls. Curve 2: 40 monomers/filament; 20% labeled pyrenyl-actin, 0.2 μM F-actin, 0.25 μM vitamin D-binding protein in the controls. (Bottom) Double-reciprocal plot of the data in Figure 3, top; same curve numbers. The abscissa intercepts show the K_d values for curves 1 and 2 to be 9 and 8 μM, respectively; the ordinate intercepts show that at saturation (at infinite DNase concentration) the rate constants were increased by a factor of 33 for the slow actin to 0.83 s^{-1}, curve 1, and by a factor of 9 to 1.1 s^{-1} for the fast actin, curve 2.

When a constant amount of polymerized actin in the form of either short or long gelsolin-capped filaments was diluted, the long filaments depolymerized more slowly than the short ones because of the lower concentration of filament ends (Figure 2). The effect of DNase was independent of filament length; it increased the slope of both depolymerization curves (k_{off} [pointed ends]) about 9-fold. This rules out filament cutting by DNase and shows that DNase increased the rate constant of depolymerization from the pointed filament end.

The depolymerizing action of DNase required high concentrations and was not saturated even at 27 μM DNase (Figure 3, top). Measurements with increasing DNase concentrations using different preparations of DNase and actin show that the increase in the rate constant for depolymerization is half-maximal at 8–9 μM Dnase (Figure 3, bottom, abscissa intercept). At saturation, DNase raised the depolymerization rate constant, k_{off}, to a value close to 1 s^{-1} (Figure 3, bottom, ordinate intercept)[1] for different actin preparations whose initial k_{off} values differed over a 5–7-fold range.[2]

DNase even increased the depolymerization rate constant of phalloidin actin (a single experiment, data not shown). In this case, the absolute value of k_{off} remained very low because of the cross-linking of actin monomers by phalloidin, but the relative increase in k_{off} was similar for phalloidin and for native actin.

Inhibition of Elongation by DNase. DNase binding responsible for the increase in the rate constant of depolymerization would not be expected to occur at the same site as that responsible for inhibition of elongation at the pointed end (Podolski & Steck, 1988), since the former requires much higher DNase concentrations. Similarly, the K_d for DNase binding to the sites alongside the actin filaments (Hitchcock et al., 1976) is more than 10 times higher (Mannherz et al., 1980) than the $K_{50\%}$ for the depolymerizing activity. However, there is a DNase-binding site at the pointed end that may remain unoccupied at low DNase concentrations, since elongation can be blocked by binding of DNase to only one of the two strands of the two-start actin helix (Figure 6, filament c). It is possible that the depolymerization rate constant is increased when both strands bind a DNase molecule.

We looked for kinetic evidence indicating whether one or two DNase molecules bind to the pointed end during inhibition of elongation. Blocking of elongation by free DNase cannot be determined since the free G-actin needed to measure elongation binds DNase stoichiometrically. (In the presence of 2 μM G-actin in excess over total DNase (Figure 4), the concentration of free DNase is 0.5 nM as compared to concentrations of the DNase–G-actin complex (DNase–actin) between 200 and 5000 nM (Figure 4); 0.5 nM free DNase would bind to the pointed end with a half-time of 140 s as compared to 3 s for 200 nM DNase–actin.) However, the K_d of DNase for the pointed end can be calculated if the K_d of DNase–actin for the pointed end is known (see Discussion). Therefore, we measured blocking of elongation at the pointed filament end by DNase–actin. Short gelsolin-capped actin filaments or gelsolin–actin dimers were added as nuclei for elongation to a mixture of G-actin (constant concentration) and increasing concentrations of DNase–actin (which had been titrated to stoichiometry as explained in the legend for Figure 4). Elongation was inhibited from the start, and the extent of inhibition did not increase with time after the first reading (about 10–15 s after mixing). Inhibition was half-maximal at about 0.2 μM DNase–G-actin (Figure 4), and a double-reciprocal plot of DNase concentration versus inhibition of elongation gave a straight line (Figure 4, inset). A straight line would not be expected if each of the two strands of the actin filament could bind DNase–actin with equal affinity; instead, the line would slope downwards with increasing DNase concentration (Figure 4, inset) according

[1] It should be noted that at the pointed end, the value of k_{off} measured at virtually zero G-actin is lower than the value that determines the critical concentration (Weber et al., 1987b).

[2] For instance, actin that has been kept for more than 1 year in liquid nitrogen often has 5-fold lower rate constants, both for association and dissociation, than actin freshly extracted from acetone powder. Nevertheless, DNase raised the rate constants to similar values (0.8 and 1.1 s^{-1}, respectively).

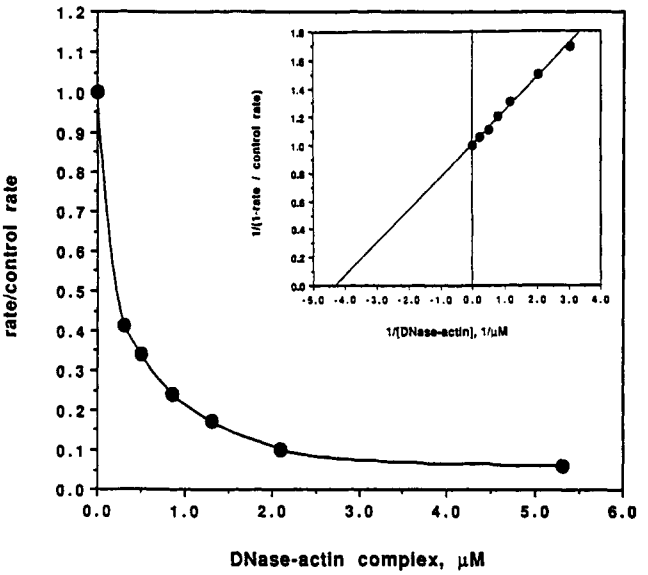

FIGURE 4: Inhibition of pointed-end elongation with increasing concentrations of DNase–actin. Elongation was started by the simultaneous addition of polymerizing salts and 10 μM F-actin copolymerized with 0.4 μM gelsolin to a medium containing 2.0 μM G-actin and increasing concentrations of DNase–actin. Stoichiometry of the mixture of DNase and G-actin was assessed by comparing the final extent of polymerization of actin in the presence and absence of DNase–actin: excess actin results in an increase and excess DNase results in a decrease of the endpoint of polymerization as compared to the control. After correcting the stoichiometry by the addition of either DNase or actin to the mixture, DNase–actin was used for the experiment. Any remaining deviations from stoichiometry at the time of the experiment (second set of controls) were taken into account in the calculations. Control rate = 0.35 μM^{-1} s^{-1}. Inset: Double-reciprocal plot of the data in the figure.

to the expression (see Appendix for the derivation; the equilibrium constants are defined by Figure 6):

$$\frac{1}{1-\frac{\text{rate}}{\text{control rate}}} = 1 + \frac{K_1}{[\text{DNase–actin}]\left(1 + \frac{[\text{DNase–actin}]}{K_1} + \frac{[\text{G-actin}]}{K_2}\right)} \quad (1)$$

It is possible that DNase increases the depolymerization rate constant by decreasing the binding constant of actin for the pointed end. Actually, the relatively low concentration of 0.2 μM for 50% blocking by DNase–G-actin as compared to a K_d of free G-actin for the pointed end of 0.5–0.7 μM might suggest the opposite, a 2–3-fold DNase-induced increase in actin binding to the filament end. However, this concentration does not necessarily represent the K_d. It would do so only if binding of DNase–G-actin to the first available site on one actin strand (Figure 6, filament b) could block the addition of another actin molecule to the second strand.

This is not the case. The data in Figure 5 show that the extent of blocking by DNase–G-actin increases with increasing concentrations of free G-actin (in spite of the associated decrease in the concentration of free DNase). As can be seen from Figure 6, filament c, if DNase does not completely block access, there is room on the second strand of the actin helix for another actin molecule before blocking is complete (actin cannot be bound to the other side of DNase). Actin cannot elongate the filament at position C (Figure 6, filament e) where it would only be attached by a dimer bond since such a bond, with a K_d between 0.1 and 1.0 M [cf. Pollard and Cooper (1986)], is much too weak [cf. Oosawa and Asakura (1975)].

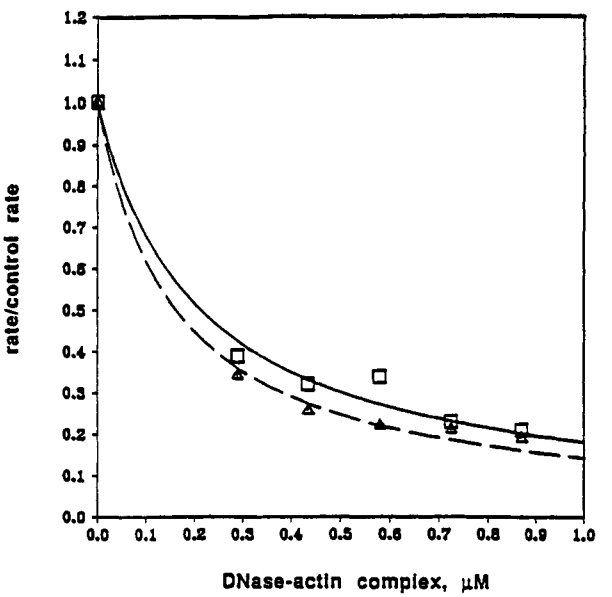

FIGURE 5: Effect of the G-actin concentration on the inhibition of elongation by DNase–actin: 10% labeled pyrenyl-G-actin, 1.3 μM, squares, and 3.9 μM, triangles; nuclei, 5 nM gelsolin–actin dimers. The solid (1.3 μM G-actin) and dashed (3.9 μM G-actin) lines were calculated using eq 1 in Results with K_1 = 0.26 μM and K_2 = 6.7 μM.

The concentration of DNase–actin for 50% blocking of elongation goes down with increasing G-actin concentration, as indicated by eq 2, because the DNase–actin complex is bound more tightly after one more actin molecule has been added to the second strand of the actin filament (Figure 6, compare filament b with c). The K_d for DNase–actin binding to the pointed end (K_1) and the K_d of G-actin for subsequent binding to the second strand (K_2) were obtained by data fitting (Table 1) using the expression (cf. Appendix; the equilibrium constants are defined by Figure 6):

$$\frac{\text{rate}_{\text{DNase–actin}}}{\text{rate}_{\text{control}}} = \frac{1}{1 + \frac{[\text{DNase–actin}]}{K_1}\left(1 + \frac{[\text{G-actin}]}{K_2}\right)} \quad (2)$$

The values of the K_d of DNase–actin for the pointed end (K_1) varied between 0.3 and 0.7 μM and of the K_d of actin subsequently bound to the second strand (K_2) between 1 and 7 μM. The increase in the K_d for monomer binding to the filament in the presence of DNase suggests that DNase partially inhibits access of monomer to the second strand. If it had blocked access completely, there would have been no effect of G-actin on the inhibition of elongation.

DISCUSSION

High-Affinity DNase Binding at the Pointed End

K_d of DNase–G-Actin and DNase I for the Pointed Filament End. Blocking of elongation of gelsolin-capped filaments of skeletal muscle actin by very low DNase concentrations (about 300 nM) was first shown by Podolski and Steck (1988). However, these experiments were carried out in the presence of phalloidin which is known to greatly strengthen actin–actin bonds (Wieland et al., 1975; Dancker et al., 1975; Coluccio & Tilney, 1984; Kabsch et al., 1990), lowering the critical concentration at the pointed ends to the nanomolar range (Faulstich et al., 1977; Cano et al., 1992) and reducing

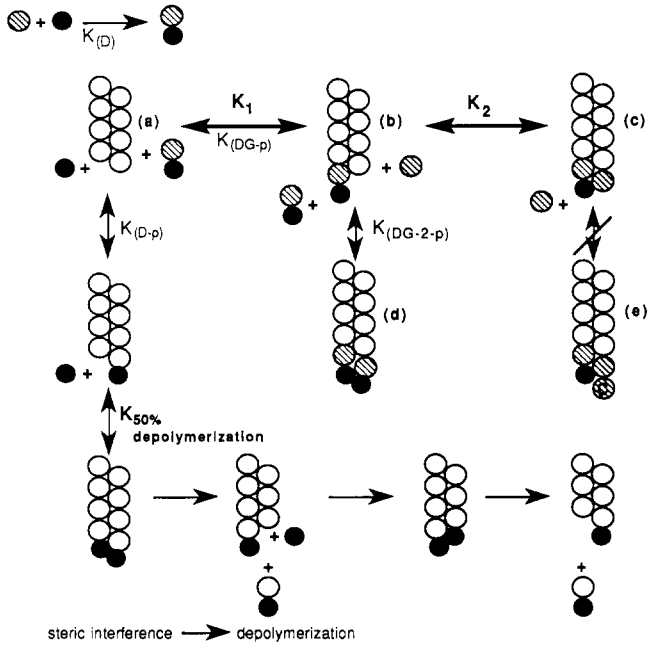

FIGURE 6: Binding of DNase, DNase–actin, and G-actin to the pointed end of actin filaments. The striped circle, monomeric actin, with the attached solid circle, DNase, together is the DNase–actin complex. It attaches to the recessed position on the pointed filament end (filament b) where the actin is bound to two surrounding actin molecules rather than to the protruding position (not shown here) where the actin could only form a dimeric bond which has a very low affinity (see text). Subsequent binding of an actin monomer (filament c) results in tighter binding of the DNase–actin by creating one more binding site on the actin filament. Any further G-actin binding is blocked by the DNase molecule, since attachment into position C (filament e) is prevented by the very low affinity of a dimer bond. K_1 ($K_{(DG-p)}$) = 0.3–0.7 μM; K_2 = 1–6.7 μM; $K_{(D)}$ = 1.0 nM; $K_{(D-p)}$ = 1.0 nM; $K_{(DG-2-p)}$ in the mM range. At high concentrations, DNase (solid circle) binds to both strands and increases the rate of depolymerization as indicated in the bottom row.

Table 1 [a]

experiment no.	K_1, μM	K_2, μM
9–17	0.68	2.3
3–4	0.26	6.7
2–18	0.50	1.0
2–23	0.27	3.9

[a] The values for K_1 and K_2 were obtained by minimizing the sum of squares of deviations between the observations and their values predicted by eq 2 in the text for both G-actin concentrations together, using a standard nonlinear optimization routine. The critical concentration at the pointed end (K_d of actin binding to the pointed end) in these experiments varied between 0.6 and 0.75 μM.

k_{off} from 0.1–0.2 to 0.0005 s^{-1} according to our observations (data not shown). Phalloidin, therefore, may be expected to strengthen DNase binding to the filament end since DNase, or for that matter any other blocking protein, is bound to the filament end only as strongly as the actin monomer to which it is attached [for a discussion, cf. Young et al. (1990) and Lin et al. (1991)]. As explained under Results, using the inhibition of the elongation rate as a marker for DNase binding, it is not possible to directly determine the binding constant of DNase for the pointed end. The observation that the inhibition was increased even though, as the result of increasing the G-actin concentration, the concentration of free DNase was decreased shows the insignificance of the contribution of free DNase to the inhibition of elongation. However, this binding constant can be calculated using the binding constant of DNase–actin for the pointed end and some other constants for free DNase, as shown below. The K_d value of DNase–actin between 0.3 and 0.7 μM is quite close to the value of 0.5–0.8 μM for the critical concentration at the pointed end which equals the K_d for the binding of free G-actin to this filament end. This would be expected if DNase binding does not significantly alter the thermodynamics of the transition from G- to F-actin. This is consistent with the crystallographic data which suggest that DNase does not significantly alter actin conformation since actin has the same structure in the DNase crystal (Kabsch et al., 1990) as in the crystal with the N-terminal fragment of gelsolin which binds to a cleft between domains I and III (McLaughlin et al., 1993).

Assuming microreversibility, one can calculate the K_d of DNase for the pointed end (prior to the binding of the last actin molecule to the second strand; Figure 6, filament b), $K_{(D-p)}$, from the K_d values of the other interactions between DNase, actin, and the filament end (Figure 6): DNase–actin monomer binding ($K_{(D)}$), DNase–actin binding to the pointed end ($K_{(DG-p)}$), and actin monomer binding to the pointed end (c_{oo-p}), $K_{(D-p)} = K_{(DG-p)}K_{(D)}/c_{oo-p}$. This value, 0.5 μM × 0.001 μM/0.5 μM = 1.0 nM, is close to that determined by Podolski and Steck (1988) for actin filaments in red blood cells. Subsequent binding of an actin molecule to the other actin strand could be expected to weaken DNase binding (Figure 6, filament c) because of interference between the two molecules. This is indicated by a comparison of the values for K_2 and c_{oo-p} in Table 1.

The Number and Position of DNase–G-Actin Complexes at the Pointed End. Holmes and Kabsch (personal communication) determined by modeling that only one DNase molecule fits comfortably at the pointed filament end. This is consistent with our binding kinetics which suggest that two DNase–G-actin complexes (Figure 6, filament d) cannot be bound simultaneously to the filament end with equal affinity (Figure 4, straight line instead of downwards sloping curve). Podolski and Steck (1988) also concluded that only one DNase molecule is bound to each pointed filament end on the basis of a comparison of the number of estimated actin filaments in a red blood cell with the measured number of bound DNase molecules. A potential problem with this estimate is that their preparations most probably contained another pointed-end binding protein, tropomodulin (Weber et al., 1993); we do not know whether it could have competed with DNase for binding to the filament end. This one DNase molecule binds with high affinity to either one of the two strands as shown by the results described by Figure 5 and Table 1, with preference for the outermost position.

Increase in the Pointed-End Depolymerization Rate Constants at High Concentrations of DNase

DNase does not affect the rate of depolymerization of uncapped actin significantly (Mannherz et al., 1980), since depolymerization takes place primarily at the barbed end with rate constants about 10 times higher than the rate constants at the pointed end (Bonder & Mooseker, 1983; Pollard & Cooper, 1986; Walsh et al., 1984). Once the barbed end is capped, the effects of DNase can be observed.

The factor by which DNase increased the depolymerization rate was independent of the initial concentration of filament ends. This indicates that DNase increased the depolymerization rate constant and rules out a mechanism that acts by increasing the number of filament ends, such as filament cutting, for the reasons given in Results. This is consistent with the finding that DNase does not significantly increase the depolymerization rate of uncapped actin filaments since

cutting would have also increased the concentration of barbed filament ends.

The most plausible explanation for the DNase effect on depolymerization is steric interference between the two DNase molecules which destabilizes the binding of the attached actin monomers to the filament. The increase in K_d for the second DNase molecule by a factor of 10^3–10^4 indicates a loss in binding energy of about 4000–5000 cal/mol. DNase so far is the only known actin-binding protein that increases the rate of depolymerization by increasing its rate constant.

In conclusion, DNase in the nanomolar range (K_d = 1.0 nM) binds to domains II and IV of the actin molecule both in actin monomers and at the pointed ends of the actin filament at one of the two strands. This binding has no significant effect on the affinity of the attached actin for the pointed filament end. In the 1–100 μM range ($K_{50\%}$ = 8 μM), at subcritical G-actin concentrations, DNase increases the depolymerization rate constant, plausibly as the result of steric interference caused by the binding of a second DNase molecule to the other strand of the actin filament. At very high concentrations, K_d = 100 μM, DNase binds alongside the actin filament. This binding does not cause filament cutting, and the site for this weak interaction has not been identified.

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APPENDIX

Derivation of Eqs 1 and 2 in Results

Using the notation: F—uncapped filaments, F-D—singly-capped filaments, F-D_2—doubly-capped filaments, F-DG—capped filaments with DNase–actin occluded by an additional G-actin, and F_T—total filaments in all states, and noting that the binding of two DNase–actins is obligatorily sequential, *not* random, we get the equations

$$[\text{F-D}] = [\text{F}][\text{DNase–actin}]/K_1$$

$$[\text{F-D}_2] = [\text{F-D}][\text{DNase–actin}]/K_1 = [\text{F}][\text{DNase–actin}]^2/K_1^2$$

$$[\text{F-DG}] = [\text{F-D}][\text{G-actin}]/K_2 = [\text{F}][\text{DNase–actin}][\text{G-actin}]/(K_1K_2)$$

$$[\text{F}_T] = [\text{F}] + [\text{F-D}] + [\text{F-D}_2] + [\text{F-DG}]$$

These yield

$$\frac{\text{F}}{\text{F}_T} = \frac{1}{1 + \frac{[\text{DNase–actin}]}{K_1}\left(1 + \frac{[\text{DNase–actin}]}{K_1} + \frac{[\text{G-actin}]}{K_2}\right)} = \frac{\text{rate}}{\text{control rate}}$$

If the binding of the second DNase–actin, in contrast to the above, is not characterized by K_1 but is with substantially lower affinity than the first, then at moderate [DNase–actin] we can neglect [F-D_2] and this reduces to

$$\frac{\text{rate}}{\text{control rate}} = \frac{1}{1 + \frac{[\text{DNase–actin}]}{K_1}\left(1 + \frac{[\text{G-actin}]}{K_2}\right)} \quad (2)$$

Alternatively, if the second DNase–actin binds with an affinity similar to the first, characterized as in the equation for [F]/[F_T] above by K_1, we can rearrange that equation to

$$1 - \frac{\text{rate}}{\text{control rate}} = \frac{\frac{[\text{DNase–actin}]}{K_1}\left(1 + \frac{[\text{DNase–actin}]}{K_1} + \frac{[\text{G-actin}]}{K_2}\right)}{1 + \frac{[\text{DNase–actin}]}{K_1}\left(1 + \frac{[\text{DNase–actin}]}{K_1} + \frac{[\text{G-actin}]}{K_2}\right)}$$

and

$$\frac{1}{1 - \frac{\text{rate}}{\text{control rate}}} = 1 + \frac{K_1}{[\text{DNase–actin}]\left(1 + \frac{[\text{DNase–actin}]}{K_1} + \frac{[\text{G-actin}]}{K_2}\right)} \quad (1)$$

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