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Effect of Capping Protein on the Kinetics of Actin Polymerization†

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ABSTRACT: *Acanthamoeba* capping protein increased the rate of actin polymerization from monomers with and without calcium. In the absence of calcium, capping protein also increased the critical concentration for polymerization. Various models were evaluated for their ability to predict the effect of capping protein on kinetic curves for actin polymerization under conditions where the critical concentration was not changed. Several models, which might explain the increased rate of polymerization from monomers, were tested. Two models which predicted the experimental data poorly were (1) capping protein was similar to an actin filament, bypassing nucleation, and (2) capping protein fragmented filaments. Three models in which capping protein accelerated, but did not bypass, nucleation predicted the data well. In the best one, capping protein resembled a nondissociable actin dimer. Several lines of evidence have supported the idea that capping protein blocks the barbed end of actin filaments, preventing the addition and loss of monomers [Cooper, J. A., Blum, J. D., & Pollard, T. D. (1984) *J. Cell Biol. 99*, 217–225; Isenberg, G. A., Aebi, U., & Pollard, T. D. (1980) *Nature* (*London*) *288*, 455–459]. This mechanism was also supported here by the effect of capping protein on the kinetics of actin polymerization which was nucleated by preformed actin filaments. Low capping protein concentrations slowed nucleated polymerization, presumably because capping protein blocked elongation at barbed ends of filaments. High capping protein concentrations accelerated nucleated polymerization because of capping protein's ability to interact with monomers and accelerate nucleation.

Acanthamoeba capping protein is a protein which blocks the barbed end of actin filaments, binds to filaments, decreases the filament length distribution, and is not sensitive to calcium (Cooper et al., 1984; Isenberg et al., 1980). The protein also decreases the low-shear viscosity of actin filaments, an activity which initially defined the material (MacLean-Fletcher & Pollard, 1980). The protein is a heterodimer of two subunits, with a native molecular weight of 74 000, and is concentrated in the cortex of *Acanthamoeba*, where actin filaments are found (Cooper et al., 1984). These properties suggest that capping protein may function in cells to control filament length distribution, cytoplasmic viscosity, and filament location. A very similar protein has been discovered in vertebrate brain (Kilimann & Isenberg, 1982).

Actin polymerization and depolymerization may be an essential process in cell motility. In light microscopy of living *Acanthamoeba* and other motile cells, the hyaline ectoplasm, which is rich in actin filaments (Taylor et al., 1980; Pollard et al., 1970), changes its shape and moves about the cell on a rapid time scale. These changes could be due either to the movement of whole actin filaments from one place in the cell to another or to the disassembly of filaments in one place and assembly in another place. The time scale of these changes is probably too fast to allow for the diffusion of actin filaments, because by fluorescence photobleaching actin filaments in cells and at high concentrations in vitro are immobile (Wang et al., 1982; Tait & Frieden, 1982; Lanni et al., 1981). The filaments might be moved intact by bulk cytoplasmic flow. Actin monomers can diffuse rapidly, suggesting that disassembly and assembly may occur. In cells, the free monomer concentration is the critical concentration. However, at this concentration, on the basis of in vitro studies of actin, nucleation of polymerization is thermodynamically unfavorable and kinetically slow. A cell might therefore control filament formation by the use of a nucleating agent locally in the cytoplasm.

Several proteins, including villin (Craig & Powell, 1980), platelet gesolin (Kurth et al., 1983), and plasma ADF (Harris & Weeds, 1983), with activities similar to that of capping protein have been found to accelerate actin polymerization from monomers, and several possible explanations of this phenomenon have been proposed. In the experiments reported here, we analyzed the effect of capping protein on the kinetics of actin polymerization and used quantitative modeling studies to learn how capping protein affects polymerization. Capping proteins accelerated actin polymerization, and modeling studies showed that the data were consistent with capping protein acting as if it were a nondissociable actin dimer in a model where the nucleus was a trimer.

Certain preliminary experiments, which are not presented here, were presented at the 1981 Meeting of the American

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Society for Cell Biology (Cooper et al., 1981) and the 1982 Cold Spring Harbor Symposium (Pollard et al., 1982).

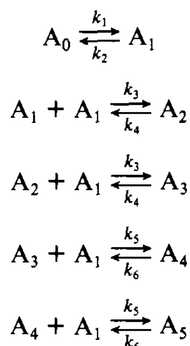
Materials and Methods

Proteins. Capping protein, rabbit skeletal muscle actin, *Acanthamoeba* actin, and the pyrene derivatives of the actins were prepared, and their concentrations were measured as previously described (Cooper et al., 1983a,b, 1984). The purity of the capping protein used in these experiments was high, like that depicted in Cooper et al. (1984). However, similar results were obtained when capping protein of only about 30% purity was employed (Cooper, 1983). Buffer A was 2 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 8.0, 0.2 mM ATP, 0.2 mM $CaCl_2$, and 0.5 mM dithiothreitol (DTT).

Polymerization Kinetics. Pyrene-labeled actin fluorescence has been documented as a valid assay for polymer weight concentration (Cooper et al., 1983a). Fluorescence was measured, and kinetic curves were digitized as described (Cooper et al., 1983b).

Theoretical Models and Fit to Experimental Data. Theoretical models were constructed, and kinetic curves were generated by numerical integration as described (Cooper et al., 1983b). Scheme I and eq 1–7 are reproduced below. A steady-state assumption is used in the calculations of the nucleation steps and of the steps where capping protein accelerates nucleation.

Scheme I



$$A_0 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} A_1$$
$$A_1 + A_1 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} A_2$$
$$A_2 + A_1 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} A_3$$
$$A_3 + A_1 \underset{k_6}{\overset{k_5}{\rightleftharpoons}} A_4$$
$$A_4 + A_1 \underset{k_6}{\overset{k_5}{\rightleftharpoons}} A_5$$

In this model, the nucleus size is a trimer. Unactivated monomer is A_0, active monomer is A_1, dimer is A_2, trimer is A_3, tetramer is A_4, and pentamer is A_5. The related equations for Scheme I are shown below.

$$\Delta A_0 = \Delta T(k_2A_1 - k_1A_0) \quad \text{activation} \quad (1)$$

$$A_2 = \frac{k_3A_1^2 + k_4A_3}{k_4 + k_3A_1} \quad \text{nucleation} \quad (2)$$

$$A_3 = \frac{k_3A_2A_1}{k_4 + k_5A_1 - k_6} \quad \text{nucleation} \quad (3)$$

$$\Delta N = \Delta T(k_3A_2A_1 - k_4A_3) \quad \text{nucleation} \quad (4)$$

$$\Delta N = \Delta TNF(P/N)^2 \quad \text{fragmentation} \quad (5)$$

$$\Delta P = \Delta TN(k_5A_1 - k_6) \quad \text{elongation} \quad (6)$$

$$A_1 = A_t - A_0 - 2A_2 - 3A_3 - P \quad (7)$$

The variables are as follows: A_0, unactivated monomer concentrations; A_1, active monomer concentration; A_2, dimer concentration; A_3, trimer concentration; N, polymer number concentration; P, polymer weight concentration (moles of actin protomers per liter); ΔT, time increment for the repetitive calculations; A_t, total actin concentration; F, fragmentation rate constant; k_1, forward activation rate constant; k_2, backward activation rate constant; k_3, forward nucleation rate constant; k_4, backward nucleation rate constant; k_5, forward elongation rate constant; k_6, backward elongation rate constant. Arbitrarily, k_4 was set equal to 500 s^{-1}. On the basis of previous data (Cooper et al., 1983a), k_5 was set equal to 10^7 M^{-1} s^{-1}. The critical concentration in the relevant buffer was 0.72 μM, so k_6 was set equal to 7.2 s^{-1}.

The general scheme was to measure the kinetics of polymerization for one actin concentration with different capping protein concentrations. The control experimental curve without capping protein was used to select the forward nucleation rate constant, k_3, which gave the best fit between the theoretical and experimental curves. This value was 1154 M^{-1} s^{-1}. k_3 was then kept fixed in all the subsequent models which attempted to predict the effect of capping protein. The rationale behind this decision was that k_3 only describes interactions between actin molecules without capping protein. A trimer nucleus fit better than a tetramer nucleus. The processes of activation and fragmentation each made insignificant improvements in the fit and were therefore omitted, which removed eq 1 and 5.

There were four different experimental curves with capping protein, representing the addition of four different volumes of capping protein solution to the reaction mixture. In models to predict the effect of capping protein, theoretical curves were generated for all four different capping protein volumes, and all four theoretical curves were compared to all four experimental curves to calculate the error of fit. The error of fit was the sum of the square of the difference between the theoretical and experimental curves at about 150 points along each curve. Each of the models took into account the different capping protein volumes and systematically varied one parameter, searching for the best fit between the four theoretical curves and the four experimental curves.

In the model where capping protein behaved like a filament, the filament number concentration, N, at time zero was not zero as in the usual case but instead was set equal to the capping protein volume multiplied by a value for the capping protein concentration in the stock. Capped filaments elongated only from their pointed ends, which meant that the elongation rate constants k_5 and k_6 were one-tenth the usual values. Although the capping protein stock concentration was known from A_{280}, this model had no rate constant to vary, so the capping protein stock concentration was the variable which was adjusted to achieve the best fit of theoretical to experimental curves.

A fragmentation model was also constructed to conform to current notions about how capping proteins might fragment filaments. In this model, one capping protein molecule could fragment one filament, resulting in two shorter filaments, one of which could elongate at both ends (the usual elongation rate constants) and one of which could elongate only at the pointed end (one-tenth the usual rate constants). Then that capping protein molecule was subsequently not allowed to fragment any more filaments. In this model, the usual fragmentation rate equation was substituted with eq 8, where k_{cp} was the

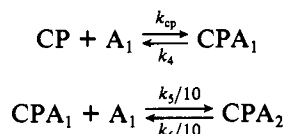
$$\Delta N = \Delta Tk_{cp}[CP]N(P/N)^{0.5} \quad (8)$$

forward rate constant, [CP] was the capping protein concentration, and P/N was the average filament length. The rate should be proportional to the length of filaments since a long filament is more likely to collide with a capping protein than a short filament. Equation 8 shows the rate as proportional to the length to a power of 0.5. The power should not be as high as 1 because all the protomers in a filament are not independent, which that would imply. Several values for the

power, from 0.2 to 1.5, were tested in the modeling procedure, and the fit was about the same for all of them. The best value was 0.5, and those results are the ones presented later. Annealing was not included. In the fit routine using this model, the rate constant was varied to achieve the best fit. The total capping protein concentration was the product of the capping stock concentration and the volume of stock solution, which was specified for each experimental curve. The capping protein stock concentration was set equal to the measured value and was not varied.

The model where capping protein was similar to a nondissociable actin dimer was a bit more complex (Scheme II). Capping protein was similar to a nondissociable actin dimer, in this model where the nucleus was a trimer.

Scheme II



The addition of the first actin monomer was governed by a forward rate constant, k_{cp}, which was varied and a backward rate constant, k_4, set equal to its usual value of 500. The addition of the second actin monomer to the capping protein–actin complex, CPA_1, was governed by the elongation rate constants for the pointed end. Capping protein was similar to a "nondissociable" actin dimer because capping protein was not allowed to dissociate or become inactive, unlike a normal actin dimer which is unstable and readily dissociates into two actin monomers. In the computer program for stepwise numerical integration, eq 9–11 were then added to the ones above.

$$[CPA_1] = \frac{k_{cp}[CP]A_1}{k_4 + (k_5/10)A_1 - k_6/10} \quad (9)$$

$$\Delta N = \Delta T(k_{cp}[CP]A_1 - k_4[CPA_1]) \quad (10)$$

$$\Delta[CP] = -\Delta T(k_{cp}[CP]A_1 - k_4[CPA_1]) \quad (11)$$

These equations require the same assumptions as those required for the simple actin polymerization model which were modified from Wegner & Engel (1975): (1) The intermediates CP and CPA_1 are in rapid preequilibrium, and (2) the concentration of CPA_1 is equal to the concentration of CPA_2. The best fit was achieved by systematically varying k_{cp}. Again, the total capping protein concentration was the product of the capping protein stock concentration and the volume of the stock solution added to each reaction mixture. The capping protein stock concentration was set equal to the measured value and was not varied.

Two other models in which capping protein accelerated nucleation without bypassing it were tested. In the "dimer capture" model, capping protein bound to actin dimers. This complex then polymerized, with the elongation rate constants for the pointed end. Equations 12–14 were used in place of eq 9–11.

$$[CPA_2] = \frac{k_{cp}[CP]A_2}{k_4 + (k_5/10)A_1 - k_6/10} \quad (12)$$

$$\Delta N = \Delta T(k_{cp}[CP]A_2 - k_4[CPA_2]) \quad (13)$$

$$\Delta[CP] = -\Delta T(k_{cp}[CP]A_2 - k_4[CPA_2]) \quad (14)$$

In the "monomer" model, capping protein was similar to an actin monomer, again in the scheme where the nucleus was a trimer. This complex added another actin monomer with the usual nucleation rate constants, k_3 and k_4, and then elongated with pointed end rate constants, $k_5/10$ and $k_6/10$. Equations 15–18 were used in place of eq 9–11.

$$[CPA_1] = \frac{k_{cp}[CP]A_1 + k_4[CPA_2]}{k_4 + k_3A_1} \quad (15)$$

$$[CPA_2] = \frac{k_3[CPA_1]A_1}{k_4 + (k_5/10)A_1 - k_6/10} \quad (16)$$

$$\Delta N = \Delta T(k_3[CPA_1]A_1 - k_4[CPA_1]) \quad (17)$$

$$\Delta[CP] = -\Delta T(k_3[CPA_1]A_1 - k_4[CPA_1]) \quad (18)$$

These models also require an assumption of rapid preequilibrium or steady state among the capping protein intermediates in nucleation. Equations 12, 15, and 16 use this assumption.

Table I: Error of Fit for Various Models[a]

model	error of fit
control (no capping protein), 4 × 0.007 =	0.03
nondissociable dimer	0.28
fragmentation	11.2
filament	9.1
dimer capture	0.40
monomer	0.44

[a] The values listed are a measure of the difference between the experimental and theoretical curves, calculated by summing the square of the difference between the experimental and theoretical curves at about 150 points along the curve, for the four curves. The value for the control is multiplied by 4 for comparison with the other values since only one curve was fit in this routine. A difference of 1% between experimental and theoretical values would result in a value of 0.1 for the error of fit.

Results

Effect of Capping Protein on the Kinetics of Actin Polymerization. Capping protein accelerated the polymerization from monomers of both rabbit skeletal muscle and *Acanthamoeba* actin, in both the presence and absence of calcium (Figure 1). In the absence of calcium, capping protein decreased the final extent of polymerization, and small amounts of capping protein slowed the rate of actin polymerization (Figure 1A,C). The lag time was decreased, even when the overall rate was decreased. The final extent of polymerization decreased because the critical concentration increased. The effect of capping protein on the critical concentration was measured previously (Cooper et al., 1984), and the quantity of the decrease in the final extent of polymerization measured here was the same as the previously measured increase in the critical concentration for these buffer conditions.

Prediction of Experimental Data with Theoretical Models. The curves of Figure 1B were chosen for the experimental data because the equilibrium value for the fluorescence was unaffected by capping protein. The forward nucleation rate constant was chosen by fitting a simple theoretical model with a trimer nucleus to the experimental control curve in the absence of capping protein. The theoretical and experimental curves fit well (Figure 2). The value of k_3 was 1154 $M^{-1}\ s^{-1}$. This forward nucleation rate constant was used in subsequent models to attempt to predict the effect of capping protein. Figure 3 shows the experimental curves along with the best theoretical curves generated by three models tested, and Table I lists a quantitative measure of the fit. The models include (1) capping protein was similar to an actin filament, (2) capping protein fragmented filaments, and (3) capping protein was similar to a nondissociable actin dimer. The fits for the first two models were poor (Figure 3E–H). The third model

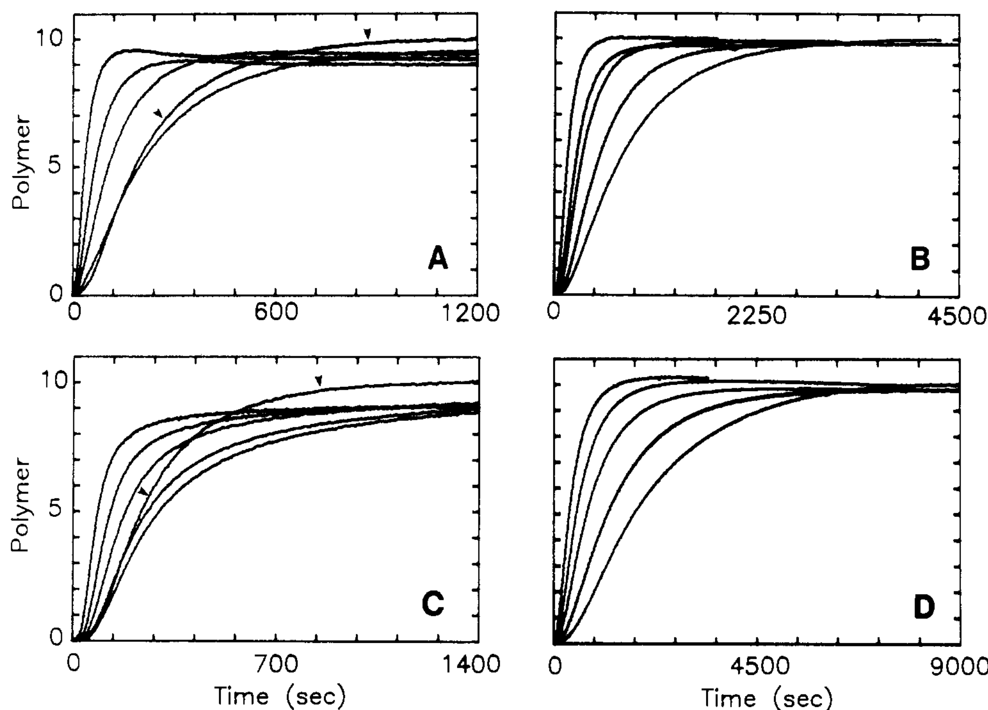


FIGURE 1: Effect of capping protein on the kinetics of actin polymerization from monomers. Increasing amounts of capping protein were added to *Acanthamoeba* (panels A and B) and muscle (panels C and D) actin in the presence (panels B and D) and absence (panels A and C) of calcium at the onset of polymerization. Polymer weight concentration in arbitrary units, on the basis of pyrene-labeled actin fluorescence, is plotted vs. time in seconds. Note that the different panels have different time scales. (A) 7 μM *Acanthamoeba* actin (5% pyrene labeled), 0.1 M KCl, 2 mM $MgCl_2$, 1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 10 mM imidazole hydrochloride, pH 7.0, and 41% (v/v) buffer A. Arrowheads mark the curve without capping protein. Capping protein concentrations (nM) of the curves from slow to fast: 50, 133, 330, and 1040. The critical concentration was 0.1 μM. (B) 10 μM *Acanthamoeba* actin (5% pyrene labeled), 0.1 M KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM imidazole hydrochloride, pH 7.0, and 60% (v/v) buffer A. Capping protein concentrations (nM) of the curves from slow to fast: 0, 50, 167, 330, and 1040. The critical concentration was 0.72 μM. (C) Identical with panel A, except muscle in place of *Acanthamoeba* actin. Arrowheads mark the curve without capping protein. Capping protein concentrations (nM) of the curves from slow to fast: 0, 23, 50, 133, 330, and 1040. (D) 10 μM muscle actin (5% pyrene labeled), 0.1 M KCl, 10 mM imidazole hydrochloride, pH 7.0, and 50% (v/v) buffer A. Capping protein concentrations (nM) of the curves from slow to fast: 0, 133, 330, 667, and 1350.

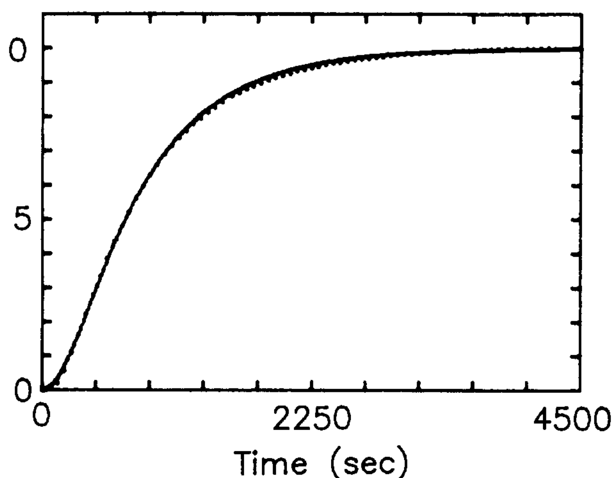


FIGURE 2: Experimental (dotted line) and theoretical (solid line) curves for actin polymerization from monomers in the absence of capping protein. The experimental curve is the curve of Figure 1B without capping protein. The theoretical curve was generated by a polymerization model described in the text. The forward nucleation rate constant, k_3, was varied to achieve the best fit.

fit the data very well (Figure 3A–D). In this model, the rate constant, k_{cp}, was varied to achieve the best fit. The value chosen was 464 M^{-1} s^{-1}. For the filament model, the concentration of the capping protein stock solution was varied to achieve the best fit. The value chosen was 24 nM, which was less than 1% of the actual value of 3.3 μM. When 3.3 μM was the value, the fit was much worse, with an error of fit of 180 as compared to 9.1 (Table I). We chose to vary the concentration in order to let the model have one parameter to vary, as the other models did. The theoretical curves for the filament model are poor because they lack the lag phase present in the experimental data. Filaments bypass nucleation entirely and immediately begin the rapid process of elongation. In the fragmentation model, the rate constant, *F*, was varied to achieve the best fit. The value chosen was 3340 M^{-1} s^{-1}. The theoretical curves for the fragmentation model are poor, by contrast, because their shape is more sigmoidal than that of the experimental curves. Early in the time course the curves are too slow, and later they are too fast.

Two other models in which capping protein accelerated, but did not bypass, nucleation also fit the data well but not as well as the nondissociable dimer model (Table I). In one of these models, called "dimer capture", capping protein bound to actin dimers and allowed further polymerization. In another model, called "monomer", capping protein resembled an actin monomer and interacted with actin monomers to accelerate nucleation. The theoretical curves for these models are not shown but are similar to those of Figure 3A–D.

These models each had one parameter to vary to find the best fit between the experimental and theoretical curves. The capping protein stock concentration was varied in the filament model but was held constant at its measured value in the other models. In separate computer-assisted fits, this concentration was also varied along with the rate constant in these models. In these cases, there were two independent variables which had to allow for a better fit compared to the cases of one variable. Even so, the fits were only improved minimally, and the value chosen for the capping protein stock concentration

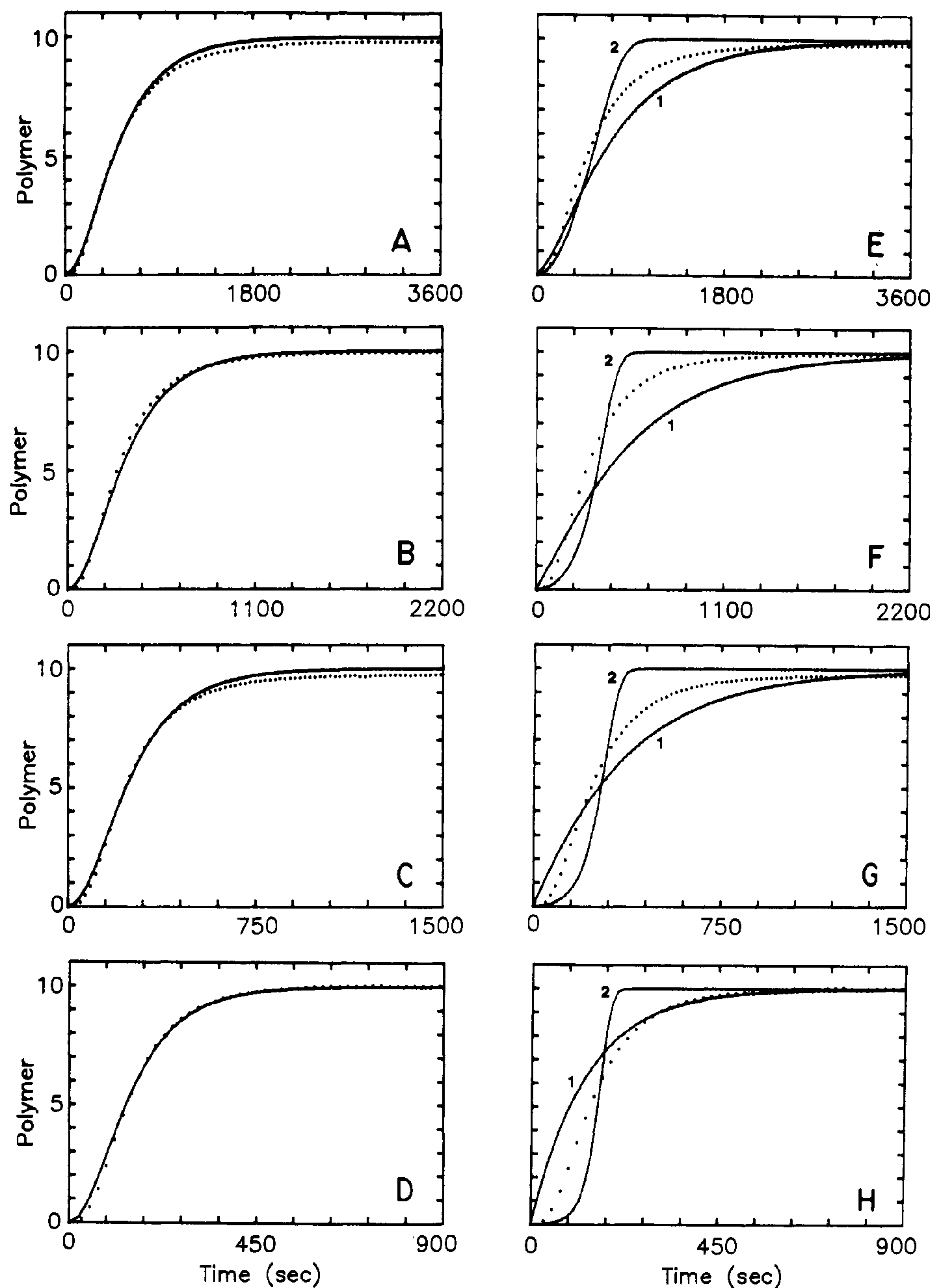


FIGURE 3: Experimental (dotted lines) and theoretical (solid lines) curves for actin polymerization from monomers in the presence of capping protein. The theoretical curves were generated by a model in which capping protein was similar to a nondissociable actin dimer (A–D) or a filament (E–H, curves 1) or where capping protein fragmented filaments (E–H, curves 2). The experimental curves are those shown in Figure 1B. Capping protein concentrations (nM): panels A and E, 50; panels B and F, 167; panels C and G, 330; panels D and H, 1040. Note that the different panels have different time scales.

was close to the measured value.

Effect of Capping Protein on the Kinetics of Actin Polymerization Nucleated by Actin Filaments. The rate of polymerization of actin monomers can be increased by the addition of actin filaments. Actin monomers were polymerized with different concentrations of capping protein, and these mixtures were employed to accelerate actin polymerization (Figure 4). At low capping protein concentrations, the initial rate of polymerization was slow relative to that for no capping protein, reaching a minimum value at about 200 nM capping protein. As the capping protein concentration increased above this value, the rate increased. This experiment was performed with *Acanthamoeba* actin. Similar results were obtained with muscle actin, using capillary viscometry instead of pyrene-labeled actin fluorescence to measure polymerization (Pollard et al., 1982).

Discussion

Capping protein increased the rate of polymerization from monomers of *Acanthamoeba* and muscle actin, in the presence and absence of calcium. Two general ideas for how this might happen were considered. One general idea was that capping protein "nucleates" actin polymerization. A simple notion of nucleates is that capping protein is similar to an actin filament. Capping protein binds to and blocks the barbed end of actin filaments; therefore, capping protein might resemble a pointed end of an actin filament. Such a model was devised, and theoretical kinetic curves for actin polymerization were gen-

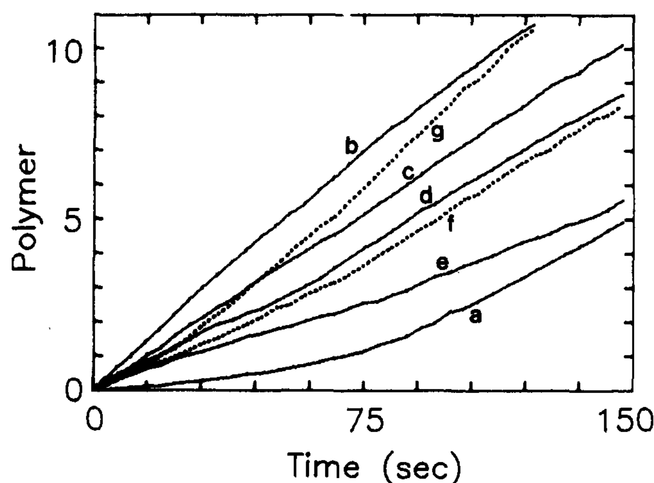


FIGURE 4: Effect of capping protein on the kinetics of actin polymerization nucleated by actin filaments. Low concentrations of capping protein slowed polymerization, and high concentrations accelerated polymerization. Polymer weight concentration, in arbitrary units derived from pyrene-labeled actin fluorescence, is plotted vs. time in seconds. Conditions common to all curves: 5 μM monomeric *Acanthamoeba* actin (5% pyrene labeled), 0.1 M KCl, 2 mM $MgCl_2$, 1 mM EGTA, 10 mM imidazole hydrochloride, pH 7.0, and 30% (v/v) buffer A. At time zero, polymerization was initiated by mixing actin monomers, salt, and a small amount of native actin filaments which had been polymerized for 4 h with various concentrations of capping protein. The solutions of filaments and variable capping protein contained the same concentrations listed above, and the volume added was 10% of the total volume. Curve a is a control without actin filaments or capping protein. Curve b is a control with actin filaments but without capping protein. Curves c–g represent increasing concentrations of capping protein in the solution with filaments and capping protein. Those concentrations (nM) were (c) 19, (d) 48, (e) 190, (f) 950, and (g) 1900. The final concentrations were one-tenth of these. Curves f and g are dotted for clarity.

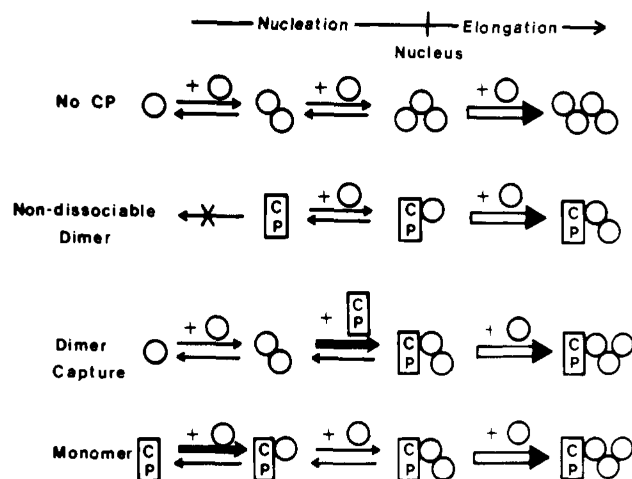


FIGURE 5: Diagram of nucleation and the models where capping protein accelerates nucleation. All the models predict the experimental data reasonably well. The models are described in detail under Materials and Methods and under Results. The open circles represent actin monomers, and the rectangles with CP represent capping protein molecules. Capping protein binds to actin filaments at their barbed ends, but no other structural details of actin filaments and their complex with capping protein are implied.

erated on the basis of this model. The fit between these curves and the actual experimental curves was poor. The experimental curves were sigmoidal, with a lag phase early in the time course. The theoretical curve for this model did not contain this lag phase because filaments present at time zero immediately polymerized at the rapid rate characteristic of elongation.

A more complex notion of "nucleates" is that capping protein is similar to one of the intermediate species between monomer and filament. Since the nucleus size was a trimer, we considered that capping protein might be similar to a nondissociable actin dimer. In this model, capping protein increased the nucleation rate but did not entirely bypass the nucleation step. The fit between the theoretical curves generated by this model and the experimental curves was good. The fit routine chose a forward rate constant, k_{cp}, of 464 $M^{-1}\ s^{-1}$, and therefore the equilibrium constant for the capping protein–actin monomer interaction was $464/500 = 0.9\ M^{-1}$. By contrast, that constant was $1154/500 = 2.3\ M^{-1}$ for the actin monomer–actin monomer and actin dimer–actin monomer interactions and $10^7/7.2 = 1.4 \times 10^6\ M^{-1}$ for the actin filament–actin monomer interaction. By this assessment, capping protein was not as effective as an actin dimer in interacting with monomers to increase nucleation. Several alternative ways exist to write a model in which capping protein accelerates without bypassing it entirely. Two other models were tested, and both predicted the data well but not quite as well as the nondissociable dimer model. In one model, capping protein bound to actin dimers, and in the other model, capping protein was similar to an actin monomer. Both models selected high forward rate constants so that the equilibrium constants analogous to those above were 45 900 M^{-1} for the former and 52 100 M^{-1} for the latter. These models are illustrated in a diagram (Figure 5).

Another possible mechanism for increasing the bulk rate of actin polymerization is to increase the fragmentation rate of filaments. This possibility is supported by the observation that capping protein decreases the length distribution of actin filaments. The theoretical kinetic curves generated by such a model fit the experimental curves poorly. Notably, the lag phase was prolonged, not shortened, and the shape of the curves was too sigmoidal. This poor shape was a result of the fact that capping protein acted on polymers. The curve was too slow early in the time course because little polymer had formed, and the curve was too fast later because much polymer had formed and was available to be broken.

Our interpretation of the results from the modeling studies is that the nondissociable actin dimer model is most consistent with the experimental data; the other nucleation models are also consistent, but the actin filament model and the fragmentation model are not consistent. The results demonstrate two important points about the interpretation of studies of this type. Some models predict the data well, and others do not. All the models that predict the data well are possibly correct, but the "best" model (which gives the lowest error of fit) is certainly not the one and only correct one. The models which predict the data poorly are probably not correct; however, if they were made more complicated and were allowed more parameters to vary, then they might predict the data well. These studies assist in a systematic evaluation of these mechanisms by pointing out the plausibility of certain models and suggesting further experiments which may help to discern more accurately the correct mechanism. For example, one might attempt to detect an interaction between capping protein and actin monomers and measure the rate constants governing that interaction. The ultimate goal is knowledge of what species exist, what their concentrations are, and what their rate constants are. This approach, the modeling of the full length of time courses, can extract certain fundamental pieces of information from these complex sets of data but is not yet capable of providing the details.

One unexplained aspect of these data is that at high capping protein concentrations, in the absence of calcium, the fluorescence transiently exceeded its final level (Figure 1A). This phenomenon has also been observed in our laboratory

under certain conditions for actin polymerization alone, measured by fluorescence or light scattering (D. L. Rimm, J. A. Cooper, and T. D. Pollard, unpublished results). Clearly, the critical concentration, and therefore the elongation rate constants, must be changing with time. A more complex model which describes the interaction of capping protein with actin filaments in more detail will probably be required to predict this phenomenon.

Given the previous evidence that capping protein blocks the barbed end for polymerization (Cooper et al., 1984; Isenberg et al., 1980), one might expect capping protein to slow the elongation phase of polymerization. In experiments starting with monomers, low concentrations of capping protein decreased the lag time, implying acceleration of nucleation, but slowed polymerization during the later, elongation, phase of the time course (Figure 1A,C). Also, in experiments where actin filaments induced the polymerization of monomers, reflecting elongation, low concentrations of capping protein slowed polymerization (Figure 4). The rate of polymerization reached a minimum at about 200 nM capping protein and increased with higher concentrations of capping protein. Our interpretation of these results is that at low capping protein concentrations, barbed ends of filaments were blocked and the polymerization rate decreased. At higher capping protein concentrations, the rate increased because there were so many more short filaments (even though their barbed end was capped) or because there was free capping protein which interacted with monomers to increase nucleation. Our previous data concerning the effect of capping protein on filament length (Cooper et al., 1984) support the notion that free capping protein could exist at these concentrations.

In summary, the kinetic data are consistent with the previous idea that capping protein blocks the barbed end of actin filaments. The ability of capping protein to accelerate polymerization was best predicted by considering capping protein to act like a nondissociable actin dimer. Capping protein therefore accelerates nucleation but does not entirely bypass the nucleation process.

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