Renoir, J. M., Yang, C. R., Formstecher, P., Hustenberg, P., Wolfson, A., Redeuilh, G., Mester, J., Richard-Foy, H., & Baulieu, E. E. (1982) *Eur. J. Biochem. 127*, 71–79.
Schaffner, W., & Neissmann, C. (1973) *Anal. Biochem. 56*, 502–514.
Schneider, C., Newman, R. A., Sutherland, D. R., Asser, U., & Greaves, M. F. (1982) *J. Biol. Chem. 257*, 10766–10769.
Schrader, W. T., Birnbaumer, M., Hughes, M. P., Weigel, N., Grody, W. W., & O'Malley, B. W. (1980) *Recent Prog. Horm. Res. 37*, 583–633.
Sherman, M. R., Moran, M. C., Tuazon, F. B., & Stevers, Y. W. (1983) *J. Biol. Chem. 258*, 10366–10377.
Singh, H., & Kalnitsky, G. (1978) *J. Biol. Chem. 253*, 4319–4326.
Vu Hai, M. T., & Milgrom, E. (1978) *J. Endocrinol. 76*, 21–31.
Wrange, O., Carlstedt-Duke, J., & Gustafsson, J. A. (1979) *J. Biol. Chem. 254*, 9284–9290.
Wray, W., Boulikas, T., Wray, V. P., & Hancock, R. (1981) *Anal. Biochem. 118*, 197–203.

Equilibrium Constant for Binding of an Actin Filament Capping Protein to the Barbed End of Actin Filaments†

Michael Wanger and Albrecht Wegner*

Institute of Physiological Chemistry I, Ruhr-University Bochum, D-4630 Bochum, Federal Republic of Germany
Received February 1, 1984

ABSTRACT: Depolymerization of treadmilling actin filaments by a capping protein isolated from bovine brain was used for determination of the equilibrium constant for binding of the capping protein to the barbed ends of actin filaments. When the capping protein blocks monomer consumption at the lengthening barbed ends, monomers continue to be produced at the shortening pointed ends until a new steady state is reached in which monomer production at the pointed ends is balanced by monomer consumption at the uncapped barbed ends. In this way the ratio of capped to uncapped filaments could be determined as a function of the capping protein concentration. Under the experimental conditions (100 mM KCl and 2 mM $MgCl_2$, pH 7.5, 37 °C) the binding constant was found to be about 2×10^9 M^{-1}. Capping proteins effect the actin monomer concentration only at capping protein concentrations far above the reciprocal of their binding constant. Half-maximal increase of the monomer concentration requires capping of about 99% of the actin filaments. A low proportion of uncapped filaments has a great weight in determining the monomer concentration because association and dissociation reactions occur at the dynamic barbed ends with higher frequencies than at the pointed ends.

During the last years a number of proteins have been isolated that bind selectively either to the barbed or to the pointed ends of actin filaments. Most of the known proteins cap the barbed ends (Isenberg et al., 1980, 1983; Kilimann & Isenberg, 1982; Maruta & Isenberg, 1983). Proteins binding to the pointed ends of actin filaments have also been isolated (Maruyama et al., 1977; Southwick & Hartwig, 1982). These capping proteins have two effects. They inhibit association of monomers and dissociation of filament subunits at the ends where they bind. Furthermore, capping proteins shift the concentration of actin monomers coexisting with actin filaments (Brenner & Korn, 1979). When consumption of actin monomers at the lengthening barbed end of treadmilling actin filaments is blocked by capping proteins, filaments continue to produce monomers by release of subunits at the pointed ends. The actin monomer concentration increases. [For a recent review of treadmilling of actin, see Neuhaus et al. (1983).] Another group of actin filament binding molecules, the actin filament fragmenting proteins, has similar functions. They are able to insert themselves between the subunits in the actin filaments, causing them to disassemble. The actin filament fragmenting proteins cap the barbed ends of the broken filaments and inhibit them for reannealing (Yin & Stossel, 1979; Bretscher & Weber, 1980; Craig & Powell, 1980; Hasegawa et al., 1980; Hinssen, 1981).

In this study, we analyzed quantitatively the enhancement of the actin monomer concentration by a capping protein isolated from bovine brain. On the basis of known kinetic parameters of treadmilling of actin, it was possible to determine the equilibrium constant for binding of the capping protein to the barbed ends of actin filaments.

Materials and Methods

(*a*) *Preparation of the Proteins.* Actin was prepared according to the method of Rees & Young (1967). The protein was applied to a Sephacryl S-200 column (2.5 × 90 cm). Part of the protein was modified with *N*-ethylmaleimide at cysteine-374 and subsequently with 7-chloro-4-nitro-2,1,3-benzoxadiazole at lysine-373 to produce a fluorescently labeled actin (Detmers et al., 1981). The concentration of unmodified actin was determined photometrically at 290 nm by using an extinction coefficient of 24 900 M^{-1} cm^{-1} (Wegner, 1976). Fluorescently labeled actin was determined by the Lowry method (Lowry et al., 1951).

Capping protein was isolated from bovine brain according to the method of Kilimann & Isenberg (1982) with the following alterations: After hydroxylapatite chromatography the capping protein was concentrated on a DEAE-cellulose column (1.5 × 10 cm). Following each chromatography step the capping activity of the fractions was tested by the depolymerizing effect of the capping protein. Aliquots of the capping protein fractions were added to samples to give final concentrations of 2 μM polymeric actin (5% fluorescently labeled

† Supported by the Deutsche Forschungsgemeinschaft (We 808/5-2, Sonderforschungsbereich 168).

0006-2960/85/0424-1035$01.50/0

actin) dissolved in 100 mM KCl, 2 mM $MgCl_2$, 0.5 mM ATP, 5 mM triethanolamine hydrochloride (pH 7.5), 1 mM dithiothreitol, and 200 mg/L NaN_3. The purity of the capping protein was tested by sodium dodecyl sulfate (SDS)[1] gel electrophoresis (Laemmli, 1970). The gels revealed two protein bands similar to the pattern described by Kilimann & Isenberg (1982). The weight concentration of the capping protein was determined by the Lowry method (Lowry et al., 1951). For calculation of molar concentrations the molecular weight of the capping protein was assumed to be 65000 (Kilimann & Isenberg, 1982).

(*b*) *Fluorescence.* Actin polymerization and depolymerization were followed by the 2.2–2.5-fold greater fluorescence intensity of polymeric actin compared to monomeric actin (Detmers et al., 1981). Five percent of fluorescently labeled actin was copolymerized with unmodified actin. This low proportion of labeled actin does not significantly alter the polymerization rate or extent of assembly of the unmodified actin (Wegner, 1982). The fluorescence intensity was measured by using a Jobin-Yvon 3D fluorometer equipped with a mercury–xenon lamp. The excitation wavelength was 436 nm, and the fluorescence intensity was measured at 530 nm. The fluorescence intensity of monomeric actin was calibrated by measuring the fluorescence intensity of a dilution series of monomeric actin dissolved in a depolymerizing buffer containing 0.5 mM ATP, 10 μM $MgCl_2$, 5 mM triethanolamine hydrochloride (pH 7.5), 1 mM dithiothreitol, and 200 mg/L NaN_3. The fluorescence intensity of polymeric actin was calibrated correspondingly by using a dilution series of polymeric actin dissolved in the final sample buffer described in section d.

(*c*) *Centrifugation.* Actin monomers and filaments were separated by centrifugation for 40 min at 135000*g*. The rotor was preincubated at 37 °C. The upper two-thirds of the supernatant were used for determination of the activity of capping protein that was not bound to actin filaments.

(*d*) *Experimental Design.* Samples were prepared by combining an ATP buffer, a magnesium buffer, a potassium buffer, an actin, and a capping protein solution. The buffers had the following composition: ATP buffer contained 0.5 mM ATP, 0.2 mM $CaCl_2$, 5 mM triethanolamine hydrochloride (pH 7.5), 1 mM dithiothreitol, and 200 mg/L NaN_3; magnesium buffer contained 100 mM $MgCl_2$ and 200 mg/L NaN_3; potassium buffer contained 300 mM KCl, 5 mM triethanolamine hydrochloride (pH 7.5), and 200 mg/L NaN_3. Actin was dialyzed against ATP buffer. Capping protein was dialyzed against a buffer containing 100 mM NaCl, 1 mM EGTA, 200 mM imidazole hydrochloride (pH 7.2), and 1 mM dithiothreitol. The buffers and protein solutions were mixed in such a ratio that the final composition of the samples was 3 mM $MgCl_2$, 0.13 mM $CaCl_2$, 100 mM KCl, 5 mM NaCl, 0.05 mM EGTA, 0.32 mM ATP, 5 mM triethanolamine, 1 mM imidazole hydrochloride (pH 7.5), 1 mM dithiothreitol, and 190 mg/L NaN_3. Always the buffers were combined first and then the protein solutions were added. All experiments were performed at 37° C.

Results

(*a*) *Effect of the Capping Protein on Actin Polymerization and Depolymerization.* Monomeric or polymeric actin was added to solutions containing various concentrations of capping protein. The total actin concentration was 2 μM. The final actin monomer concentration was found to increase with the concentration of capping protein (Figure 1). The final monomer concentrations reached by depolymerization or polymerization usually differed by about 0.1 μM. A small difference between the final monomer concentrations has been observed in a previous study, too (Wegner & Isenberg, 1983). The formation of monomers from filament subunits was induced by substoichiometric amounts of capping protein; 25 nM capping protein caused 0.4 μM actin to be depolymerized (Figure 1). Similar observations have been reported for cytochalasin (Brenner & Korn, 1979).

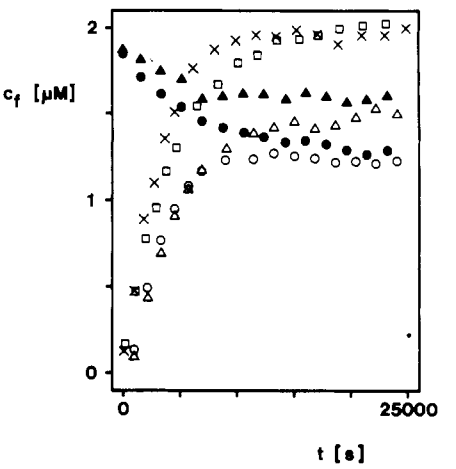

FIGURE 1: Effect of various concentrations of capping protein on actin polymerization and depolymerization. Zero (×), 5 (□), 25 (△), or 50 nM (○) capping protein was added to 2 μM monomeric actin; 25 (▲) or 50 nM (●) capping protein was mixed with 2 μM polymeric actin. Capping protein at 5 nM did not have any significant effect on the concentration of polymeric actin (not displayed). c_f, concentration of actin filament subunits; t, time.

Addition of capping protein to actin monomers did not increase the overall rate of actin polymerization, suggesting that the brain capping protein has no major effect on the nucleation of actin polymers (Figure 1). The half-time of depolymerization was several hours whereas a fragmenting protein such as severin induces depolymerization within seconds or minutes (Yamamoto et al., 1982). Fragmentation increases the number of pointed ends so that more monomers are produced per time. The slow depolymerization observed in our experiment supports previous findings that the brain capping protein caps the barbed filament ends but does not fragment filaments (Kilimann & Isenberg, 1982; Füchtbauer et al., 1983).

In a second experiment, various amounts of polymeric actin were added to solutions containing a constant capping protein concentration. The total actin concentrations ranged from 2 to 10 μM. In Figure 2, the time course of depolymerization is depicted. The final concentration of depolymerized monomeric actin was about 0.4 μM, essentially independent of the total actin concentration (Figure 2). At high total actin concentrations, the fluorescence intensity is changed by depolymerization only by a few percent. Thus, the concentration of depolymerized actin can be more reliably measured at low total actin concentrations than at high total actin concentrations.

In a third experiment, the question investigated was whether or not most of the capping protein is bound to the ends of polymeric actin. Monomeric or polymeric actin (2 μM) was incubated with different concentrations of capping protein. The final monomer concentrations were measured by fluorescence intensity. Figure 3 shows that the concentration of actin monomers coexisting with filaments depends on the

[1] Abbreviations: SDS, sodium dodecyl sulfate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid.

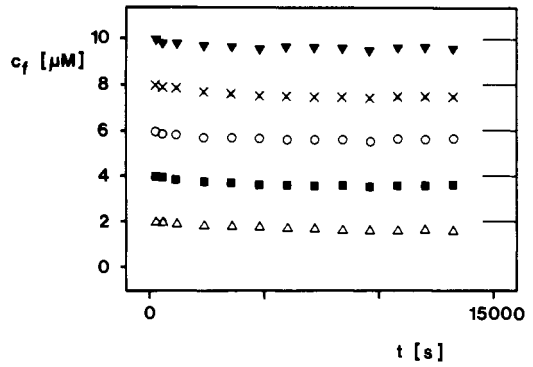

FIGURE 2: Effect of 25 nM capping protein on various concentrations of polymeric actin. Total actin concentrations: (△) 2, (■) 4, (○) 6, (×) 8, and (▼) 10 μM. c_f, concentration of actin filament subunits; t, time. For comparison, the initial actin filament subunit concentrations are displayed as lines at the right side of the plot.

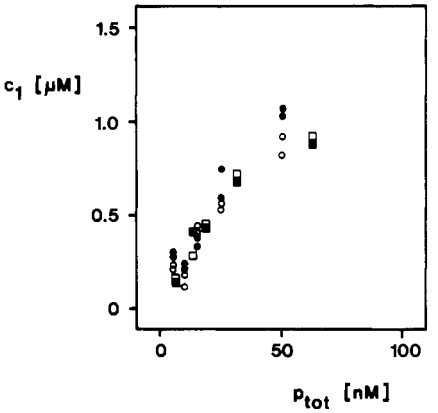

FIGURE 3: Determination of free capping protein. The monomer concentration c_1 coexisting with filaments was measured after incubating different concentrations of capping protein p_{tot} with 2 μM monomeric or polymeric actin. (□) Initially monomers were present; (■) initially polymers were present. After centrifugation, the supernatants were adjusted to 2 μM actin by adding monomeric or polymeric actin. The steady-state monomer concentrations were drawn in at the capping protein concentrations before centrifugation, with the capping protein concentrations corrected for the dilution due to addition of actin. (○) Monomeric actin was added to the supernatants; (●) polymeric actin was added to the supernatants.

concentration of capping protein. Thus, the capping protein concentration can be determined by its depolymerizing activity. Actin filaments were separated by centrifugation. The concentration of unbound capping protein that was not cosedimented with actin filaments was measured by the depolymerizing activity of the supernatants. Monomeric or polymeric actin was added to the supernatants of centrifuged solutions to give a final total actin concentration of 2 μM. The final steady-state monomer concentrations reached in the supernatants are displayed in Figure 3. The monomer concentrations were drawn in at the capping protein concentrations before centrifugation, with the capping protein concentrations corrected for dilution due to addition of actin after centrifugation. The capping protein in the supernatants has almost the same activity as in actin solutions before centrifugation, indicating that most of the capping protein remains in the supernatant and is not bound to the ends of actin filaments.

(*b*) *Equilibrium of the Binding of a Capping Protein to the Barbed End of Actin Filaments.* The substoichiometric depolymerization of actin by a capping protein can be explained by the treadmilling polymerization of actin (Wegner, 1976; Brenner & Korn, 1979, Hill & Kirschner, 1982). A reaction scheme of treadmilling and of the association of a capping protein with the barbed ends of actin filaments is displayed

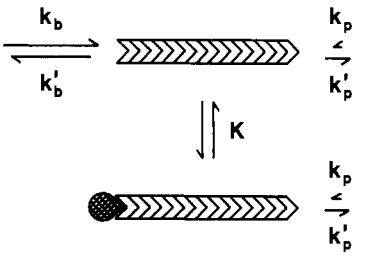

FIGURE 4: Reaction scheme of binding of a capping protein to the barbed end of a treadmilling actin filament. The polar arrangement of the subunits in the filament is represented by the chevron symbol. The rate constants of association and dissociation at the barbed (k_b, k_b') and the pointed end (k_p, k_p') and the equilibrium constant for binding of a capping protein to the barbed end K are drawn in.

in Figure 4. The rate constants of actin assembly and disassembly and the equilibrium constant for binding of a capping protein are defined in this figure. Actin filaments elongate preferentially at the barbed ends and shorten preferentially at the pointed ends. Capping the barbed ends inhibits consumption of monomers by elongation of the barbed end. However, monomers continue to be produced by shortening of the pointed ends until the monomer concentration has increased to a value where production of monomers at the pointed ends is balanced by consumption of monomers at the uncapped barbed ends.

The equilibrium constant for binding of a capping protein to the barbed ends of actin filaments is given by

$$K = \frac{\mathrm{pf}}{pf} \tag{1}$$

where p is the concentration of free capping protein, f is the concentration of uncapped barbed filament ends, and pf is the concentration of capped barbed filaments ends. The results described in section a and summarized in Figure 3 show that most of the capping protein molecules are free. Thus, eq 1 can be rewritten as

$$Kp_{tot} \approx \frac{\mathrm{pf}}{f} \tag{2}$$

where p_{tot} is the total capping protein concentration. A constant monomer concentration (c_1) coexists with capped (pf) and uncapped filaments (f) if consumption of monomers at the uncapped barbed ends is balanced by production of monomers at the pointed ends (f + pf):

$$-(k_b c_1 - k_b')f = (k_p c_1 - k_p')(f + \mathrm{pf}) \tag{3}$$

This equation of a binding curve can be rearranged by replacing the rate constants by three parameters that are experimentally available.

(i) the net critical monomer concentration $\bar{c}_1$ is the monomer concentration at which association of monomers with filaments and dissociation of subunits from filaments occur with the same frequency in the absence of capping protein (Oosawa & Kasai, 1962): $(k_b\bar{c}_1 + k_p\bar{c}_1)f = (k_b' + k_p')f$ or

$$\bar{c}_1 = \frac{k_b' + k_p'}{k_b + k_p} \tag{4}$$

(ii) The critical concentration of the pointed end c_p is the monomer concentration at which association of monomers with the pointed ends occurs with the same frequency as dissociation of subunits from the pointed ends: $k_p c_p f = k_p' f$ or

$$c_p = \frac{k_p'}{k_p} \tag{5}$$

(iii) The treadmilling efficiency parameter s gives the number

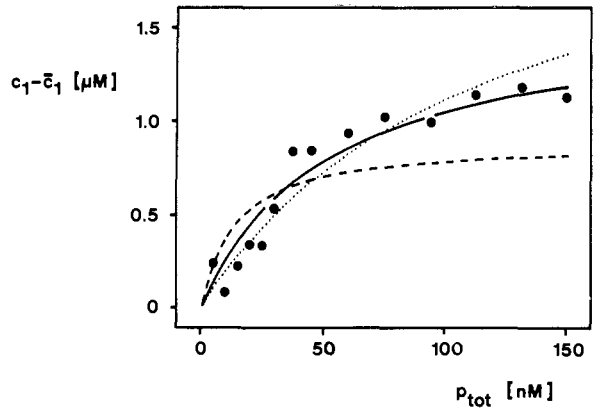

FIGURE 5: Difference between the final monomer concentration c_1 and the net critical concentration $\bar{c}_1$ vs. the total concentration of capping protein p_{tot}. (●) Experimental results. (—) Curve calculated for the adjusted parameters: $\bar{c}_1 = 0.12$ μM, $c_p = 1.7$ μM, and $sK = 0.25 \times 10^9$ M^{-1}. Two other curves are displayed in order to show the sensitivity of the fit to the choice of K and c_p: (- - -) $c_p = 1$ μM and $sK = 0.54 \times 10^9$ M^{-1}; (···) $c_p = 2.5$ μM and $sK = 0.17 \times 10^9$ M^{-1}.

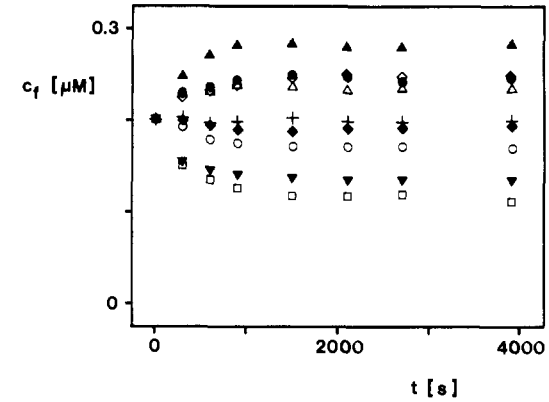

FIGURE 6: Determination of the net critical monomer concentration of actin $\bar{c}_1$. Various concentrations of actin monomers were added to 0.2 μM polymeric actin, and the time course of the filament subunit concentration c_f was followed. Concentrations of added monomers: (□) 0.04, (▼) 0.06, (○) 0.08, (♦) 0.10, (+) 0.12, (△) 0.14, (●) 0.16, (◇) 0.18, and (▲) 0.20 μM. Association and dissociation reactions were balanced at a monomer concentration of 0.12 μM ($\bar{c}_1 = 0.12$ μM).

of subunits by which, on the average, actin filaments lengthen at the barbed end during the time that, on the average, one association reaction lasts at the two ends (Wegner, 1976).

$$s = \frac{k_b\bar{c}_1 - k_b'}{(k_b + k_p)\bar{c}_1} \quad (6)$$

The meaning of the treadmilling efficiency parameter s is illustrated in Figure 4. The length of the arrows represents the rates of association or dissociation in arbitrary units. As at the lengthening end the association steps are partially reversed by dissociation reactions, filaments lengthen at the barbed ends by less than one subunit per association. The efficiency parameter s of the filament sketched in Figure 4 is 0.15.

Combining eq 2–6 yields a useful binding equation:

$$c_1 - \bar{c}_1 = \frac{(c_p - \bar{c}_1)\bar{c}_1 sKp_{tot}}{c_p - \bar{c}_1 + \bar{c}_1 sKp_{tot}} \quad (7)$$

As in the binding equation the total actin concentration does not appear, the final monomer concentration c_1 is expected to be independent of the total actin concentration. The results of Figure 2 demonstrate that in this respect the model proposed is in agreement with the system of actin and capping protein.

(*c*) *Binding Curve.* Various concentrations of capping protein were added to 2 μM monomeric or polymeric actin. After 6–8 h, samples containing the same concentrations of capping protein had reached a similar fluorescence intensity (Figure 1). The concentration of actin monomers coexisting with capped and uncapped filaments (c_1) was calculated from the difference between the initial fluorescence intensity of monomeric or polymeric actin and the final constant fluorescence intensity after addition of capping protein. In Figure 5 the results are displayed as a plot of the difference between the final monomer concentration c_1 and the net critical concentration $\bar{c}_1$ vs. the total capping protein concentration p_{tot}. The net critical concentration $\bar{c}_1$ is 0.12 μM (see next section).

For the determination of the equilibrium constant K the values of the net critical concentration $\bar{c}_1$, the efficiency parameter s, and the critical concentration of the pointed end c_p have to be known (eq 7). The net critical concentration $\bar{c}_1$ was measured according to an assay described previously (Wegner, 1982). Samples containing various concentrations of monomeric actin (0.04–0.20 μM) were mixed with 0.2 μM polymeric actin. If the concentration of added monomers is below the net critical concentration $\bar{c}_1$, filaments lose subunits until the net critical concentration is reached (Figure 6). If the monomer concentration is above the net critical concentration $\bar{c}_1$, filaments bind monomers at their ends. Figure 6 shows that at a monomer concentration of 0.12 μM filaments neither produce nor consume monomers, indicating that the net critical monomer concentration is in the range of 0.12 μM. Similar values have been reported by others (Pollard & Mooseker, 1981; Wegner, 1982; Bonder et al., 1983).

The treadmilling efficiency parameter s has been previously measured by two different methods. Under experimental conditions almost identical with the conditions of this study the s value has been found to be 0.25 (Wegner, 1982). This value has been determined by a fluorescence assay. In another type of experiment, the rate constants of assembly (k_b, k_p) and disassembly (k_b', k_p') of the two ends have been measured by direct electron microscopic observation (Pollard & Mooseker, 1981; Bonder et al., 1983). s values of 0.2 and 0.1 have been reported. We base the determination of the equilibrium constant K on a treadmilling parameter s of 0.1–0.25. Brenner & Korn (1983) have suggested that the s value may be very much smaller than 0.1. This possibility will be commented on under Discussion.

The equilibrium constant K and the critical monomer concentration of the pointed end c_p were fitted by seeking that value of K and c_p for which the standard deviation of the calculated from the measured action monomer concentrations c_1 reaches a minimum. The equilibrium constant K turned out to be 1×10^9 (for $s = 0.25$) to 2.5×10^9 M^{-1}) (for $s = 0.1$). The critical monomer concentration of the pointed end c_p was calculated to be 1.7 μM. The calculated binding curve that is in best agreement with the experimental results is displayed in Figure 5. In this figure, curves calculated for two other sets of parameters are also displayed in order to show the sensitivity of the fit to the choice of K and c_p. The equilibrium constant K was always in the range of 10^9 M^{-1}.

Discussion

The equilibrium constant for binding of the actin filament capping protein from bovine brain to the barbed end of actin filaments has been found to be about 2×10^9 M^{-1}. At a free capping protein concentration of $K^{-1} = 0.5$ nM, half of the

filaments are capped. Figure 5 shows that these low concentrations of capping protein have nearly no effect on the actin monomer concentration. Capping of most of the actin filaments is required for a substantial increase of the monomer concentration. Half-maximal increase of the monomer concentration is achieved at a capping protein concentration of 50 nM (Figure 5). At this capping protein concentration 99% of the actin filaments are capped. The high degree of capping required for regulation of the monomer concentration has to do with the dynamics of the barbed filament ends. The association rate constant for binding of an actin monomer to the barbed end is about 10-fold higher than for binding to the pointed end (Woodrum et al., 1975; Kondo & Ishiwata, 1976; Pollard & Mooseker, 1981; Tilney et al., 1983; Bonder et al., 1983). Also the dissociation of a filament subunit from the barbed end is faster than from the pointed end (Pollard & Mooseker, 1981; Wegner, 1982; Bonder et al., 1983). The ratio of the length of the arrows representing the association and dissociation reacitons in Figure 4 reflects a realistic picture of the frequency of assembly and disassembly reactions at the two ends. The barbed ends have a great weight in determining the monomer concentration because most of the association and dissociation reactions occur at these ends. The pointed ends can effect the monomer concentraiton only significantly if most of the barbed filament ends are capped.

In this analysis, the filament number concentration was assumed to be much smaller than the capping protein number concentration so that most capping protein molecules are unbound. The brain capping protein has been shown to have the same effect on actin filaments as the fungal alkaloid cytochalasin (Füchtbauer et al., 1983). Both agents inhibit monomer addition to the barbed ends of actin filaments but have little effect on the actin filament number. In our assay, the rate of actin polymerization was not increased by the addition of capping protein to actin monomers, suggesting that the brain capping protein has no major effect on the nucleation of actin polymers (Figure 1). On the contrary, Kilimann & Isenberg (1982) observed an increase of the rate of actin assembly induced by the addition of brain capping protein. Differences in the experimental conditions may account for these discrepancies.

As a further result of this study, the critical concentration of the pointed end was determined. The found value of 1.7 μM is in good agreement with the previously reported value of 1.5 μM measured under almost identical conditions (Wegner & Isenberg, 1983). Values determined by others range from 0.5 to 1 μM (Pollard & Mooseker, 1981; Tilney et al., 1982). Recently, in a careful study Bonder et al. (1983) measured a critical concentration of the pointed end of 0.5 μM. Differences in the salt concentration and temperature may account for the variation (Wegner & Neuhaus, 1981; Pollard & Mooseker, 1981; Wang & Taylor, 1981; Tellam & Frieden, 1982; Asai et al., 1983). In this study, the treadmilling efficiency parameter s was assumed to be 0.1–0.25. These values have been measured by exchange of actin monomers and filament subunits at non-steady-state monomer concentrations or by determining the rate of growth of the two ends of actin filaments by electron microscopy (Wegner, 1982; Pollard & Mooseker, 1981; Bonder et al., 1983). On the basis of measurements of the exchange of actin monomers and filament subunits at steady-state monomer concentrations Brenner & Korn (1983) have suggested that the s value may be considerably smaller than 0.1. If this small s value was correct, the equilibrium constant K would be considerably higher than 10^9 M^{-1} (see eq 7).

Only limited information about the affinity for binding of other capping proteins to the ends of actin filaments is available. Walsh et al. (1984) estimated the binding constant of villin in the presence of calcium to be 10^{11} M^{-1}. The brain capping protein appears to be less active as compared to villin.

In different types of cells, great variation of the amount of unpolymerized actin has been observed. Whereas in muscle no monomers are detectable, large amounts of unpolymerized actin have been isolated from brain (Bray & Thomas, 1976). Carlsson et al. (1977) have elucidated this observation by purifying a protein (profilin) that forms a complex with actin and inhibits assembly of monomers. The concentration of unpolymerized actin is above 10 μM and clearly higher than the critical concentration of the pointed ends of actin filaments. Capping of the barbed ends by capping proteins cannot explain the occurrence of large amounts of unpolymerized actin in cells. However, the concentration of actin unpolymerizable due to complexation by profilin can be amplified by increasing the free actin monomer concentration. Changes of the monomer concentration by capping and the amplification of this effect by profilin may be a mechanism that is important for shifting the actin monomer–polymer distribution in cells (Tobacman & Korn, 1982). A thorough discussion of the determined binding constant K with regard to the regulation of actin polymerization in cells requires more detailed knowledge on the concentration of actin, capping protein, profilin-like molecules, and perhaps other components involved in this process.

ACKNOWLEDGMENTS

We thank E. Werres for excellent technical assistance.

REFERENCES

Asai, H., Nagai, Y., & Tsuchiya, T. (1983) in *Actin: Structure and Function in Muscle and Non-Muscle Cells* (Dos Remedios, C. G., & Barden, J. A., Eds.) pp 161–168, Academic Press, Sydney.

Bonder, E. M., Fishkind, D. J., & Mooseker, M. S. (1983) *Cell (Cambridge, Mass.) 34*, 491–501.

Bray, D., & Thomas, C. (1976) *J. Mol. Biol. 105*, 527–544.

Brenner, S. L., & Korn, E. D. (1979) *J. Biol. Chem. 254*, 9982–9985.

Brenner, S. L., & Korn, E. D. (1983) *J. Biol. Chem. 258*, 5013–5020.

Bretscher, A., & Weber, K. (1980) *Cell (Cambridge Mass.) 20*, 839–847.

Carlsson, L., Nyström, L.-E., Sundkvist, I., Markey, F., & Lindberg, U. (1977) *J. Mol. Biol. 115*, 465–483.

Craig, S. W., & Powell, L. D. (1980) *Cell (Cambridge, Mass.) 22*, 739–746.

Detmers, P., Weber, A., Elzinga, M., & Stephens, R. E. (1981) *J. Biol. Chem. 256*, 99–105.

Füchtbauer, A., Jockusch, B. M., Maruta, H., Kilimann, M. W., & Isenberg, G. (1983) *Nature (London) 304*, 361–364.

Hasegawa, T., Takahashi, S., Hayashi, H., & Hatano, S. (1980) *Biochemistry 19*, 2677–2683.

Hill, T. L., & Kirschner, M. W. (1982) *Int. Rev. Cytol. 78*, 1–125.

Hinnsen, H. (1981) *Eur. J. Cell Biol. 23*, 225–240.

Isenberg, G., Aebi, U., & Pollard, T. D. (1980) *Nature (London) 288*, 455–459.

Isenberg, G., Ohnheiser, R., & Maruta, H. (1983) *FEBS Lett. 163*, 225–229.

Kilimann, M. W., & Isenberg, G. (1982) *EMBO J. 1*, 889–894.

Kondo, H., & Ishiwata, S. (1976) *J. Biochem. (Tokyo) 79*, 159–171.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Maruta, H., & Isenberg, G. (1983) *J. Biol. Chem. 258*, 10151–10158.
Maruyama, K., Kimura, S., Ishii, T., Kuroda, M., Ohashi, K., & Muramatsu, S. (1977) *J. Biochem. (Tokyo) 81*, 215–232.
Neuhaus, J.-M., Wanger, M., Keiser, T., & Wegner, A. (1983) *J. Muscle Res. Cell Motil. 4*, 507–527.
Oosawa, F., & Kasai, M. (1962) *J. Mol. Biol. 4*, 10–21.
Pollard, T. D., & Mooseker, M. S. (1981) *J. Cell. Biol. 88*, 654–659.
Rees, M. K., & Young, M. (1967) *J. Biol. Chem. 242*, 4449–4458.
Southwick, F. S., & Hartwig, J. H. (1982) *Nature (London) 297*, 303–307.
Tellam, R., & Frieden, C. (1982) *Biochemistry 21*, 3207–3214.
Tilney, L. G., Bonder, E. M., Coluccio, L. M., & Mooseker, M. S. (1983) *J. Cell. Biol. 97*, 112–124.
Tobacman, L. S., & Korn, E. D. (1982) *J. Biol. Chem. 257*, 4166–4170.
Walsh, T. P., Weber, A., Higgins, J., Bonder, E. M., & Mooseker, M. S. (1984) *Biochemistry 23*, 2613–2621.
Wang, Y., & Taylor, D. L. (1981) *Cell (Cambridge, Mass.) 27*, 429–436.
Wegner, A. (1976) *J. Mol. Biol. 108*, 139–150.
Wegner, A. (1982) *J. Mol. Biol. 161*, 607–615.
Wegner, A., & Neuhaus, J.-M. (1981) *J. Mol. Biol. 153*, 681–693.
Wegner, A., & Isenberg, G. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 4922–4925.
Woodrum, D. T., Rich, S. A., & Pollard, T. D. (1975) *J. Cell Biol. 67*, 231–237.
Yamamoto, K., Pardee, J. E., Reidler, J., Stryer, L., & Spudich, J. A. (1982) *J. Cell Biol. 95*, 711–719.
Yin, H. L., & Stossel, T. P. (1979) *Nature (London) 281*, 583–586.

Efficiency of in Vitro Transcription of *Dictyostelium discoideum* Actin Gene Is Affected by the Nucleotide Sequence of the Transcription Initiation Region†

Shigeharu Takiya,‡ Kazuhiro Takahashi, and Masaki Iwabuchi*

Department of Biology, Faculty of Science, Hokkaido University, Sapporo, 060 Japan

Yoshiaki Suzuki

Department of Developmental Biology, National Institute for Basic Biology, Myodaiji, Okazaki, 444 Japan

Received July 2, 1984

ABSTRACT: The actin gene of *Dictyostelium discoideum* is transcribed faithfully but with very low efficiency in a cell-free system containing HeLa cell extract [Takiya, S., Tabata, T., Iwabuchi, M., Hirose, S., & Suzuki, Y. (1984) *J. Biochem. (Tokyo) 95*, 1367–1377]. Using the same in vitro system, we determined that the promoter activity of the actin 5 gene is 100–200 times weaker than that of the silkworm fibroin gene. To clarify the cause of the low transcription efficiency, various chimeric genes were constructed from the actin and fibroin genes, and their transcription efficiencies were examined in vitro. Both the TATA box and the transcription initiation site of the two natural genes functioned in the transcription of the chimeric genes, the efficiency of which was especially affected by the transcription initiation region. In chimeric genes having the upstream sequence of the actin gene and a downstream sequence including the transcription initiation site of the fibroin gene, the transcription efficiency was higher than one-third of that of the natural fibroin gene. In chimeric genes having the actin transcription initiation region and an upstream sequence of the fibroin gene, the transcription efficiency was as low as that of the natural actin gene. We concluded that the transcription initiation site is a part of the promoter and an essential region for directing faithful and efficient initiation of gene transcription.

Attempts to reveal the promoter region of eukaryotic class II genes have yielded information about nucleotide sequences essential for transcription. One conserved sequence, the TATA box, in the 5′-flanking region of the class II genes is indispensable for accurate and efficient gene transcription (Corden et al., 1980; Grosschedl et al., 1980; Wasylyk et al., 1980; Grosveld et al., 1981; Tsujimoto et al., 1981; Hirose et al., 1982; Tokunaga et al., 1984). Other functional elements promoting or modulating transcription have been found in the region surrounding the TATA box or far upstream from it (Melon et al., 1981; Tsuda & Suzuki, 1981, 1983; Grosschedl & Birnstiel, 1982). However, inconsistent results have been reported on the role of the transcription initiation site (Corden et al., 1980; Tsujimoto et al., 1981; Grosveld et al., 1981; Dierks et al., 1983). Of these functional elements, the TATA

† Supported in part by Grants-in-Aid for Special Project Research from the Ministry of Education, Science and Culture, Japan (to M.I.), by the Naito Foundation Research Grant for 1983 (to M.I.), and by a grant for the Joint Studies Program of the National Institute for Basic Biology (to M.I. and Y.S.).

‡ Present address: Department of Developmental Biology, National Institute for Basic Biology, Myodaiji, Okazaki, 444 Japan.

0006-2960/85/0424-1040$01.50/0