Rate of Binding of Tropomyosin to Actin Filaments†

Albrecht Wegner* and Klaus Ruhnau
Institute of Physiological Chemistry, Ruhr-University Bochum, D-4630 Bochum, Federal Republic of Germany
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ABSTRACT: The decrease of the rate of actin polymerization by tropomyosin molecules which bind near the ends of actin filaments was analyzed in terms of the rate of binding of tropomyosin to actin filaments. Monomeric actin was polymerized onto actin filaments in the presence of various concentrations of tropomyosin. At high concentrations of monomeric actin (c_1) and low tropomyosin concentrations (c_t) ($c_1/c_t > 10$), actin polymerization was not retarded by tropomyosin because actin polymerization was faster than binding of tropomyosin to actin filaments. At low actin concentrations and high tropomyosin concentrations ($c_1/c_t < 5$), the rate of elongation of actin filaments was decreased because actin polymerization was slower than binding of tropomyosin at the ends of actin filaments. The results were quantitatively analyzed by a model in which it was assumed that actin-bound tropomyosin molecules which extend beyond the ends of actin filaments retard association of actin monomers with filament ends. Under the experimental conditions (100 mM KCl, 1 mM $MgCl_2$, pH 7.5, 25 °C), the rate constant for binding of tropomyosin to actin filaments turned out to be about 2.5×10^6 to 4×10^6 M^{-1} s^{-1}.

Many proteins have been isolated which regulate the assembly of actin filaments, and the mode of interaction of these proteins with actin filaments has been elucidated (Weeds, 1982). However, relatively little is known about the rates of the reactions occurring during the assembly of microfilaments with the exception of actin polymerization (Oosawa & Asakura, 1975; Korn, 1982; Frieden, 1985; Pollard & Cooper, 1986; Wegner et al., 1987). As the rates of reactions are important for the sequence of events occurring during the turnover of actin filaments, we determine the rate of assembly of one of the constituents of microfilaments, namely, tropomyosin, with actin filaments in this paper. We take advantage of the observation that tropomyosin can decrease the rate of actin polymerization (Keiser & Wegner, 1985; Lal & Korn, 1986). It has been proposed that tropomyosin, which binds near the ends of actin filaments, decreases the rate of association of actin monomers with the ends of filaments (Lal & Korn, 1986). If actin associates faster than tropomyosin, actin filaments polymerize at a high rate. If, however, the tropomyosin strands elongate faster at the ends of filaments than actin polymerizes, the rate of actin polymerization will be decreased by tropomyosin. We measure the rate of binding of tropomyosin by using this effect of tropomyosin on the rate of actin polymerization.

Materials and Methods

Preparation of Proteins. Rabbit skeletal muscle actin was prepared according to the method of Rees and Young (1967). The protein was applied to a Sephacryl S-200 column (2.5 × 90 cm) equilibrated with buffer A (0.5 mM ATP, 0.2 mM $CaCl_2$, 5 mM triethanolamine hydrochloride, pH 7.5, and 3 mM NaN_3). 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 (Faust et al., 1974; Detmers et al., 1981). The concentration of actin was determined photometrically at 290 nm using an absorption coefficient of 24 900 M^{-1} cm^{-1} (Wegner, 1976).

Rabbit skeletal muscle tropomyosin–troponin complex was extracted from the residue remaining after the preparation of actin (Spudich & Watt, 1971). Tropomyosin was separated from troponin and purified by chromatography on hydroxyapatite (Eisenberg & Kielley, 1974). All buffers contained 5 mM mercaptoethanol to produce tropomyosin reduced at cysteine-190 (Walsh & Wegner, 1980). The tropomyosin concentration was determined by measuring the absorbance at 276 nm using an extinction coefficient of 24 500 M^{-1} cm^{-1} (Wegner, 1979).

The gelsolin–actin complex was prepared from human platelet concentrate. Platelets were purified by differential centrifugation (Harris & Crawford, 1973); 20 nM prostaglandin E_1 was included in the suspension buffer to inhibit aggregation of the platelets (Selve & Wegner, 1986a).

After the first resuspension, the purification was continued according to the method of Lind et al. (1982). The platelet extract was applied to an agarose affinity column to which DNase I was covalently bound (1.2 × 12 cm; Affi Gel 10). The column was equilibrated with monomeric actin to produce immobilized actin (Lazarides & Lindberg, 1974). The concentration of the gelsolin–actin complex was determined by the method of Lowry et al. (1951). For calculation of the molar concentration, the molecular masses of gelsolin and actin were assumed to be 90 kDa and 42.3 kDa, respectively (Yin & Stossel, 1979; Elzinga et al., 1973).

Fluorescence. Actin polymerization was followed by the 2.2–2.5-fold greater fluorescence intensity of polymeric actin compared to that of monomeric actin (Detmers et al., 1981). Five percent of fluorescently labeled actin was copolymerized with unmodified actin. This low proportion of labeled actin does not significantly alter the polymerization rate or extent of assembly of unmodified actin (Wegner, 1982b). The excitation wavelength was 480 nm, and the fluorescence intensity was measured at 540 nm. The changes of the fluorescence intensities were evaluated in terms of concentrations of monomeric or polymeric actin. The fluorescence intensity of monomeric or polymeric actin was calibrated by measuring the fluorescence intensities of dilution series of monomeric or polymeric actin. Binding of tropomyosin to actin filaments

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* Address correspondence to this author.

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has no effect on the fluorescence intensity of actin (Wegner, 1982a).

Light Scattering. Binding of tropomyosin to actin filaments was measured by light scattering according to Wegner (1979). The samples were prepared as described under Experimental Design. The actin–tropomyosin mixtures were incubated for about 12 h at room temperature to form the actin–tropomyosin complex. The scattering intensity was measured at a 90° observation angle using a Jobin Yvon fluorometer. All measurements were made at 546 nm.

When tropomyosin molecules bound along rodlike actin filaments induce a small inhomogeneity of the mass to length ratio, the scattering intensity (R_T) has been shown to be given by (Wegner, 1979)

$$R_T/R_A = (C_A + C_T)^2/C_A^2 \quad (1)$$

where R_A is the scattering intensity of actin filaments, C_A is the weight concentration of filaments, and C_T is the weight concentration of bound tropomyosin. The average number (n) of tropomyosin molecules bound per seven actin filament subunits is given by (Wegner, 1979)

$$n = \frac{1}{7}\frac{C_T/M_T}{C_A/M_A} = \frac{1}{7}(\sqrt{R_T/R_A} - 1)\frac{M_A}{M_T} \quad (2)$$

M_A is the molecular mass of actin, and M_T is the molecular mass of tropomyosin. M_A and M_T were assumed to be 42.3 kDa and 65.4 kDa, respectively (Elzinga et al., 1973; Hodges et al., 1972).

Experimental Design. Fluorescence samples were prepared by mixing a 20 mM $MgCl_2$ solution, a 1 M KCl solution, buffer A, a 4 μM tropomyosin solution, a 20 μM monomeric actin solution, and a 20 μM polymeric actin solution. Tropomyosin was dialyzed against buffer B (300 mM KCl, 5 mM triethanolamine hydrochloride, pH 7.5, 3 mM NaN_3, and 5 mM mercaptoethanol). Monomeric actin was contained in buffer A. Polymeric actin was obtained by the addition of 100 mM KCl and 1 mM $MgCl_2$ to monomeric actin. The solutions were mixed in such a ratio that the final composition was 100 mM KCl, 1 mM $MgCl_2$, 0.18 mM $CaCl_2$, 0.45 mM ATP, 4.5 mM triethanolamine hydrochloride, pH 7.5, 2.7 mM NaN_3, and the desired concentrations of tropomyosin and of monomeric and polymeric actin. Always first the salt solutions and buffer A were combined, and then the protein solutions were added. The time course of polymerization turned out to be practically independent of the sequence of addition of proteins.

Light-scattering samples were prepared by mixing 1 mL of salt solution, 1 mL of tropomyosin solution, and 1 mL of monomeric actin solution. The salt solution contained 3 mM $MgCl_2$ in buffer A. Tropomyosin was dialyzed against buffer B. Monomeric actin was contained in buffer A. Before being mixed in light-scattering cells, all solutions were centrifuged for 1 h at 100000g to remove dust. All experiments were performed at 25 °C.

Results

Effect of Tropomyosin on the Elongation of Actin Filaments. The effect of tropomyosin on the elongation of actin filaments was investigated by nucleated polymerization. Various concentrations of monomeric actin (0.5–6 μM) were polymerized onto actin filaments (1 μM polymeric actin) in the presence of various concentrations of tropomyosin. The time course of incorporation of monomers into filaments was measured by fluorescence. The effect on actin polymerization of tropomyosin is depicted in Figure 1. Tropomyosin retards

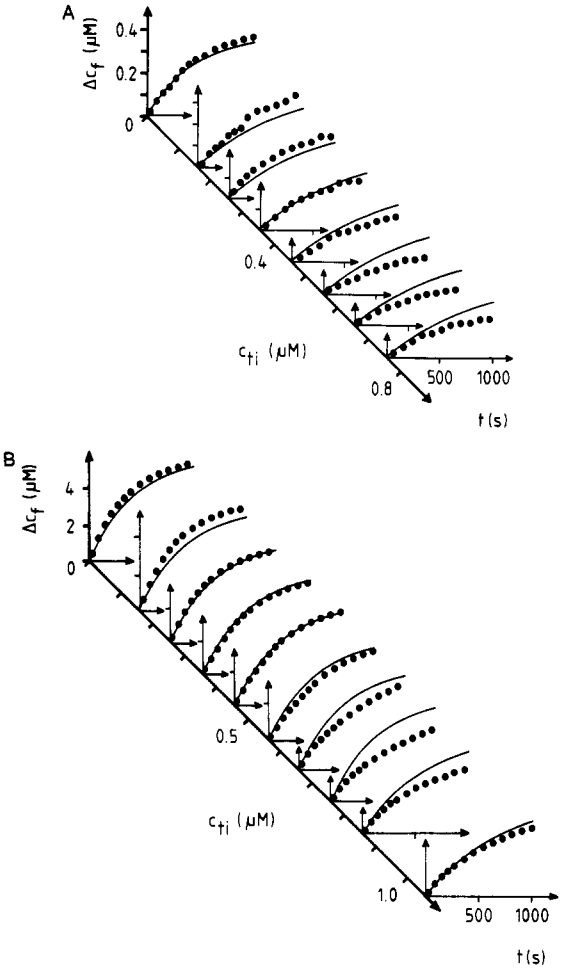

FIGURE 1: Retardation of nucleated actin polymerization by tropomyosin. The polymerization of 0.5–6 μM monomeric actin was nucleated by 1 μM polymeric actin in the presence of 0–1.2 μM tropomyosin. Δc_f, increase of the concentration of polymeric actin; c_{ti}, initial free tropomyosin concentration. Initial concentrations of monomeric actin: (A) 0.5 μM; (B) 6 μM. The continuous lines were calculated for the assumption that one tropomyosin molecule extending beyond the end of actin filaments retards the elongation of actin filaments. The rate constants are summarized in Table I.

the rate of elongation of actin filaments. Similar results have been reported by Keiser and Wegner (1985) and by Lal and Korn (1986). The factor by which the rate of elongation is decreased depends on the concentrations both of monomeric actin and of tropomyosin. If the initial concentration of monomeric actin was 0.5 μM, 0.1 μM free tropomyosin was required for half-maximal decrease of the elongation rate, while at 6 μM monomeric actin 0.6 μM free tropomyosin was required for half-maximal decrease of the elongation rate (Figure 1). Lal and Korn (1986) have proposed that the rate of binding and dissociation of actin molecules at the ends of filaments is decreased by tropomyosin which binds at the ends of filaments and extends beyond the ends. If tropomyosin binds faster at the ends than actin, the rate of elongation is decreased. If tropomyosin associates slower at the ends of filaments than actin, tropomyosin has no effect on the rate of elongation. At half-maximal decrease of the elongation rate, binding of tropomyosin and actin polymerization may occur at similar rates. As one tropomyosin molecule binds per seven actin filament subunits, the rate constants of binding of tropomyosin (k_t^+) and of actin (k_a^+) can be roughly correlated:

$$7k_t^+ tm_{1/2} \approx k_a^+ a_{1/2} \quad (3)$$

where $tm_{1/2}$ and $a_{1/2}$ are the concentrations of free tropomyosin

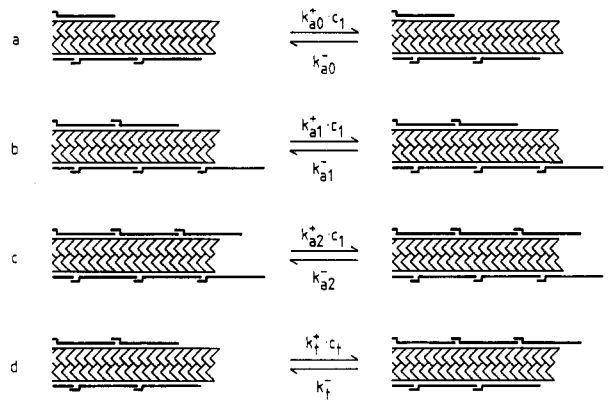

FIGURE 2: Reaction scheme of actin polymerization and binding of tropomyosin to actin filaments. Actin filament subunits are represented by chevrons. Tropomyosin molecules are represented by lines along the actin filaments. (a) Binding of an actin monomer to a free filament end. (b) Binding of an actin monomer to a filament end with one extending tropomyosin strand. [The upper tropomyosin strand of the left filament does not cover 9 terminal subunits ($i = 9$); the lower tropomyosin strand of the left filament extends by 10 subunit binding sites beyond the end ($j = -10$).] (c) Binding of an actin monomer to a filament end with two extending tropomyosin strands. (d) Binding of tropomyosin to an actin filament.

and of monomeric actin at half-maximal decrease of the elongation rate, respectively. One can calculate that the rate constants of binding of tropomyosin and of actin are similar [$tm_{1/2} = 0.1$ μM and $a_{1/2} = 0.5$ μM or $tm_{1/2} = 0.6$ μM and $a_{1/2} = 6$ μM (Figure 1)]. As the rate constant for binding of actin to filament ends (k_a^+) has been reported to range from 10^6 to 10^7 M^{-1} s^{-1} (Pollard & Mooseker, 1981; Bonder et al., 1983; Lal et al., 1984; Selve & Wegner, 1986b), tropomyosin binds presumably also at a rate of 10^6–10^7 M^{-1} s^{-1}. A more rigorous treatment which is based on kinetic rate equations will be given in the next sections.

Kinetic Model of Binding of Tropomyosin to Polymerizing Actin Filaments. A reaction scheme of binding of tropomyosin to polymerizing actin filaments is depicted in Figure 2. The rate of binding of actin to a filament end is assumed to depend on the number of tropomyosin molecules binding at the ends. In Figure 2 parts a, b, and c representations of actin filaments are displayed which have no, one, or two bound tropomyosin molecules at the ends, respectively. Tropomyosin molecules are assumed to bind end-to-end when they associate with actin filaments (Figure 2). This assumption is reasonable because tropomyosin is known to bind highly cooperatively to actin filaments. According to an analysis of the equilibrium of assembly of tropomyosin with actin filaments, tropomyosin associates several hundredfold more likely end-to-end than when isolated along actin filaments (Wegner, 1979; Yang et al., 1979). Under the experimental conditions (1 mM $MgCl_2$, 100 mM KCl, tropomyosin concentration <1.2 μM), unbound tropomyosin does not aggregate to form dimers or oligomers (Wegner, 1979).

In Figure 2, a scheme of the reactions occurring at a barbed end is displayed. As assembly of actin at the barbed end is 10–40-fold faster than at the pointed end, the pointed ends contribute little to the overall elongation of actin filaments. Therefore, the reactions at the pointed ends can be neglected. Actin polymerization can be treated as unidirectional polymerization (Woodrum et al., 1975; Kondo & Ishiwata, 1976; Selve & Wegner, 1986b; Pollard, 1986).

The rate of monomer consumption (c_1) or of the increase of the concentration of polymeric actin (Δc_f) is given by

$$\frac{dc_1}{dt} = -\frac{d\Delta c_f}{dt} =$$
$$-[k_{a0}^+c_1\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}p_{ij} + k_{a1}^+c_1(\sum_{i=0}^{-13}\sum_{j=1}^{\infty}p_{ij} + \sum_{i=1}^{\infty}\sum_{j=0}^{-13}p_{ij}) + k_{a2}^+c_1\sum_{i=0}^{-13}\sum_{j=0}^{-13}p_{ij}$$
$$- k_{a0}^-\sum_{i=2}^{\infty}\sum_{j=2}^{\infty}p_{ij} - k_{a1}^-(\sum_{i=1}^{-13}\sum_{j=2}^{\infty}p_{ij} + \sum_{i=2}^{\infty}\sum_{j=1}^{-13}p_{ij}) - k_{a2}^-\sum_{i=1}^{-13}\sum_{j=1}^{-13}]c_p \quad (4)$$

c_p is the concentration of filament ends. The rate constants are defined in Figure 2. The rate of tropomyosin consumption (c_t) is given by

$$\frac{dc_t}{dt} = -[2k_t^+c_t\sum_{i=1}^{\infty}\sum_{j=1}^{\infty}p_{ij} - k_t^+c_t(\sum_{i=0}^{-13}\sum_{j=1}^{\infty}p_{ij} + \sum_{i=1}^{\infty}\sum_{j=0}^{-13}p_{ij}) -$$
$$2k_t^-\sum_{i=-13}^{\infty}\sum_{j=-13}^{\infty}p_{ij}]c_p \quad (5)$$

p_{ij} ($i \geq 0, j \geq 0$) is the probability that one strand of tropomyosin bound along actin filaments does not cover i terminal subunits and the other strand does not cover j terminal subunits. For negative values of i and j, p_{ij} gives the probability that tropomyosin extends by i or j actin filament subunit binding sites beyond the filament ends (for illustration, see Figure 2). It should be mentioned that because of the equivalence of the binding sites of tropomyosin to actin, the two strands are always staggered by an odd number of subunits (Hodges et al., 1972; McLachlan & Stewart, 1975). Only combinations of an odd i with an even j or an even i with an odd j can occur. The probabilities p_{ij} of combinations of even i and j or of odd i and j are zero.

For solving eq 4 and 5, it is necessary to calculate the probabilities p_{ij}. Kinetic rate equations for p_{ij} can be formulated according to the reaction scheme depicted in Figure 2:

$$\frac{dp_{ij}}{dt} = -k_{a0}^+c_1p_{ij} - k_{a0}^-p_{ij} - 2k_t^+c_tp_{ij} - 2k_t^-p_{ij} + k_{a0}^+c_1p_{i-1,j-1} +$$
$$k_{a0}^-p_{i+1,j+1} + k_t^+c_t(p_{i+14,j} + p_{i,j+14}) + k_t^-(p_{i-14,j} + p_{i,j-14}) \quad (6)$$

where $i > 1, j > 1$. Rate equations for $i \leq 1$ or $j \leq 1$ can be formulated accordingly. Steady-state probabilities p_{ij} are achieved if actin filaments polymerize at a constant rate in the presence of constant concentrations of monomeric actin and free tropomyosin. These steady-state probabilities can be calculated by applying the steady-state approximation to eq 6:

$$dp_{ij}/dt = 0 \quad (7)$$

The steady-state approximation can also be applied if the concentrations of monomeric actin and free tropomyosin change sufficiently slowly. In Figure 3, an example for the rate at which steady-state polymerization is reached is depicted. The plot has been calculated for rate constants that are realistic for actin polymerization and for the assembly of tropomyosin with actin filaments. Figure 3 demonstrates that within a few seconds following a change of the concentrations of monomeric actin and free tropomyosin, a new steady state is established. As in the experiments, the time necessary for polymerization of half of the monomers was in the range of 300 s (Figure 1); it is justified to apply the steady-state approximation to calculations of the time course of actin polymerization.

The probabilities p_{ij} were calculated in the following way: If tropomyosin binds faster at the ends of actin filaments than actin monomers, the actin subunits which are near the center of actin filaments were assumed to be completely covered with

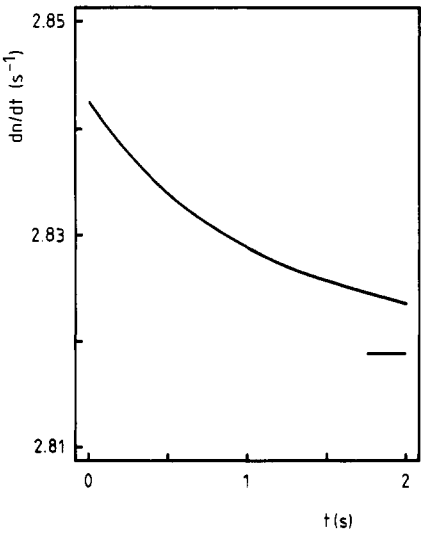

FIGURE 3: Rate at which following a change of the concentrations of actin and tropomyosin a new steady state of filament elongation is reached. dn/dt, number of filament subunits (n) by which a filament elongates per time. The concentrations of monomeric actin and of tropomyosin were assumed to decrease at time $t = 0$ from 2.8 and 0.5 μM to 2.1 and 0.4 μM, respectively. The bar at the right gives the steady-state elongation rate at 2.1 μM monomeric actin and 0.4 μM tropomyosin.

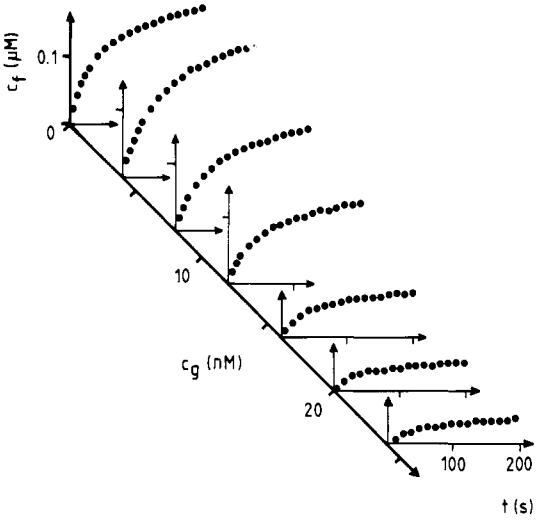

FIGURE 4: Titration of the barbed ends of actin filaments by the gelsolin–actin complex. 0.3 μM fluorescently labeled monomeric actin was added to 18 μM unlabeled polymeric actin in the presence of various concentrations of gelsolin–actin complex (c_g). c_f, concentration of fluorescently labeled polymeric actin.

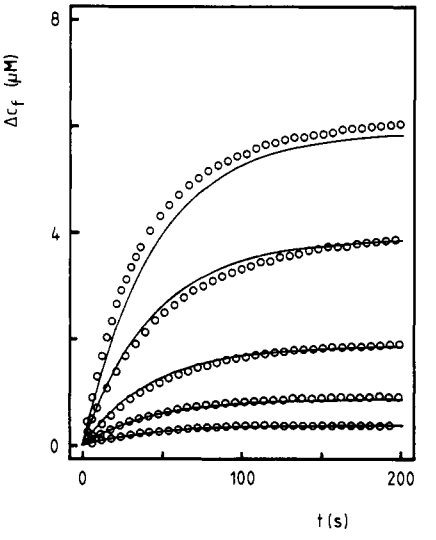

FIGURE 5: Polymerization of actin nucleated by 1 μM polymeric actin. Δc_f, increase of the concentration of polymeric actin. (O) Experimental data; initial monomer concentrations from bottom to top: 0.5, 1.0, 2, 4, and 6 μM. The continuous lines were calculated for a concentration of filament ends $c_p = 0.9$ nM and for the rate constants given in Table I.

tropomyosin. In that case, 30 terminal subunits ($i \leq 30, j \leq 30$) were considered by our calculations. The probabilities p_{ij} were computed by numerical integration of eq 6 until constant steady-state values were reached (eq 7). At the beginning of the integration ($t = 0$), the probabilities p_{ij} had the same values, and the sum of the probabilities p_{ij} was equal to 1. If actin polymerization was faster than binding of tropomyosin, the rate of polymerization was equal to the rate of actin assembly.

Concentration of Filament Ends. The kinetic rate equations contain measurable concentrations and concentrations which are unknown. The concentration of monomeric or polymeric actin can be determined by fluorescence. The initial concentration of tropomyosin is known. The concentration of filament ends c_p is not known but can be measured by titration of the barbed ends of filaments using the gelsolin–actin complex (Selve & Wegner, 1986b); 0.3 μM fluorescently labeled monomeric actin was polymerized onto 18 μM unlabeled polymeric actin in the presence of various concentrations of gelsolin–actin complex. This combination of labeled and unlabeled actin was applied for reasons of the signal/noise ratio. The concentration of monomeric actin (0.3 μM) was kept below the critical concentration of the pointed end (0.6 μM) because above the critical concentration of the pointed end the gelsolin–actin complex would increase the number of filaments by nucleation of new filaments (Tellam & Frieden, 1982; Wegner & Isenberg, 1983). The results are depicted in Figure 4. Above 16 nM gelsolin–actin complex, actin polymerization was inhibited almost completely, indicating that 16 nM gelsolin–actin complex caps the barbed ends of 18 μM polymeric actin. The filaments contain on the average $\bar{n} =$ 1125 (18 μM/16 nM) subunits. This value is in reasonable agreement with the length of actin filaments determined by electron microscopy (Kawamura & Maruyama, 1970). In the experiments summarized in Figure 4, actin polymerization was nucleated by 1 μM polymeric actin. Thus, the concentration of filament ends c_p can be calculated to be about 0.9 nM (1 μM/1125).

Rate Constants of Binding and Dissociation of Actin in the Absence of Tropomyosin. In Figure 5, the time course of polymerization of various concentrations of actin nucleated by 1 μM polymeric actin is depicted. In the absence of tropomyosin, the rate of change of the concentration of monomeric (c_1) or polymeric actin (Δc_f) is given by (Figure 2)

$$\frac{dc_1}{dt} = -\frac{d\Delta c_f}{dt} = -k_{a0}^{+}c_1c_p + k_{a0}^{-}c_p = -k_{a0}^{+}(c_1 - \bar{c}_1)c_p$$

or

$$\Delta c_f = c_{1i} - c_1 = (c_{1i} - \bar{c}_1)[1 - \exp(-k_{a0}^{+}c_p t)] \qquad (8)$$

$\bar{c}_1$ is the critical monomer concentration of the barbed end ($\bar{c}_1 = k_{a0}^{-}/k_{a0}^{+}$). c_{1i} is the initial monomer concentration. Curves calculated according to eq 8 are also depicted in Figure 5. All experimental curves can be fitted by exponentials which have been calculated for the same value of $k_{a0}^{+}c_p = 2.25 \times 10^{-3}$ s^{-1}. As the concentration of filament ends c_p is known to be 0.9 nM, the rate constant for binding of actin to filament ends

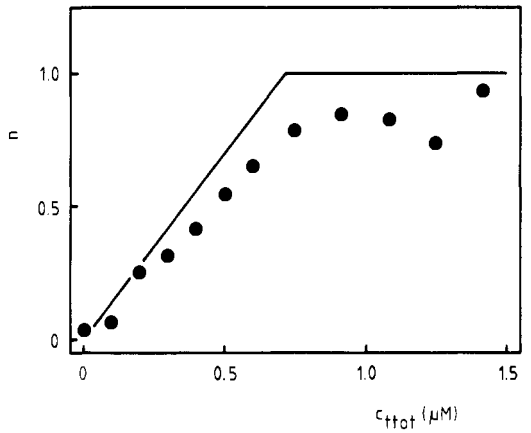

FIGURE 6: Binding curve of tropomyosin to actin filaments. Concentration of polymeric actin, 5 μM. c_{ttot}, total concentration of tropomyosin. n, average number of tropomyosin molecules bound per seven actin filament subunits. (●) Experimental data. (—) Curve calculated for irreversible binding of tropomyosin to actin filaments.

k_{a0}^+ is 2.5 × 10^6 M^{-1} s^{-1}. This value is in good agreement with rate constants reported by others (Pollard & Mooseker, 1981; Lal et al., 1984; Selve & Wegner, 1986b; Pollard, 1986).

The critical monomer concentration $\bar{c}_1$ was measured as described previously (Wegner, 1982b). This monomer concentration was determined to be 0.12 μM. Consequently, the rate constant of dissociation of a subunit from a filament end k_{a0}^- can be calculated to be 0.3 s^{-1} ($k_{a0}^- = {}^+_{a0}\bar{c}_1$). In agreement with the results reported by Lal and Korn (1986) also in the presence of tropomyosin, the critical concentration of actin was found to be 0.12 μM ($k_{a0}^-/k_{a0}^+ = k_{a1}^-/k_{a1}^+ = k_{a2}^-/k_{a2}^+ = \bar{c}_1$). Furthermore, the results of Figure 5 demonstrate that within the range of concentrations applied in the experiments nucleation of actin filaments from monomers is negligibly slow. The concentration of filament ends c_p is unchanged in all samples.

Binding of Tropomyosin to Actin Filaments. Binding of tropomyosin to actin filaments was followed by the increase of the light-scattering intensity. A binding curve, which was measured at 25 °C, is displayed in Figure 6. Various concentrations of tropomyosin were incubated with 5 μM actin. Above 0.75 μM total tropomyosin concentration, maximal binding of 0.85 tropomyosin molecule per 7 actin filament subunits was observed. Below 0.75 μM total tropomyosin, the concentration of bound tropomyosin increases linearly with the total tropomyosin concentration. At higher temperature, the binding curves of tropomyosin to actin filaments have been reported to reveal a sigmoidal shape (Wegner, 1979; Yang et al., 1979). The sigmoidal shape has been interpreted in terms of binding cooperativity. Evidently, at the low temperature of the experiments presented in this paper, a sigmoidal shape cannot be observed because all tropomyosin binds irreversibly to actin filaments. A calculated curve of irreversible binding is depicted in Figure 6. Within the limits of experimental error, the experimental data can be fitted by the calculated curve. Because of the irreversibility of binding of tropomyosin to actin filaments, the rate constant of dissociation of tropomyosin from actin filaments was set equal to zero ($k_t^- = 0$).

Rate Constant of Binding of Tropomyosin to Actin Filaments. In Figure 1, the time course of actin polymerization in the presence of various tropomyosin concentrations is displayed. At high tropomyosin concentrations, a maximal decrease of the polymerization rate is reached. Consistently at all monomer concentrations tested (0.5–6 μM), the rate of polymerization is decreased maximally by a factor of about 1.9. Within the limits of the proposed model, this finding can be interpreted in the following way. Actin binds to the ends of actin filaments at a rate of 1.33 × 10^6 M^{-1} s^{-1} ($k_{a0}^+/1.9$) if tropomyosin extends beyond the ends of actin filaments.

Table I: Rate Constants for Binding of Tropomyosin to Actin Filaments and for Actin Polymerization in the Presence of Tropomyosin[a]

constants	determined values	model
k_t^+ (M^{-1} s^{-1})	2.5 × 10^6	one tropomyosin molecule[b]
k_{a0}^+ (M^{-1} s^{-1})	2.5 × 10^6	
k_{a0}^- (s^{-1})	0.3	
$k_{a1}^+ = k_{a2}^+$ (M^{-1} s^{-1})	1.33 × 10^6	
$k_{a1}^- = k_{a2}^-$ (s^{-1})	0.16	
k_t^+ (M^{-1} s^{-1})	4 × 10^6	two tropomyosin molecules[c]
$k_{a0}^+ = k_{a1}^+$ (M^{-1} s^{-1})	2.5 × 10^6	
$k_{a0}^- = k_{a1}^-$ (s^{-1})	0.3	
k_{a2}^+ (M^{-1} s^{-1})	1.33 × 10^6	
k_{a2}^- (s^{-1})	0.16	

[a] The rate constants are defined in Figure 2. [b] One tropomyosin molecule extending beyond the ends of actin filaments is assumed to be sufficient to retard actin polymerization. [c] Two tropomyosin molecules extending beyond the ends of actin filaments are assumed to be necessary to retard actin polymerization.

It is not known whether only one tropomyosin strand extending beyond the ends is sufficient for a decrease of the rate of actin polymerization or whether two extending tropomyosin strands are necessary for this effect. We, therefore, calculated rate constants of binding of tropomyosin to actin k_t^+ for both the model of one and of two extending tropomyosin strands. Good agreement of the calculated curves with the experimental data could be achieved if one extending tropomyosin strand was assumed to bring about a decrease of the rate of actin polymerization. Curves calculated for k_t^+ = 2.5 × 10^6 M^{-1} s^{-1} are depicted in Figure 1. The agreement of calculated curves with the experimental data was not as good if two extending tropomyosin strands were assumed to decrease the rate of actin polymerization. (calculated curves not shown). For both models, the rate constants of binding of tropomyosin to actin filaments are similar (Table I), suggesting that tropomyosin binds to actin filaments at a rate of 2.5 × 10^6 to 4 × 10^6 M^{-1} s^{-1}.

Only the first part of the polymerization curves could be satisfactorily fitted to the experimental data (Figure 1). The fit is poor near the critical monomer concentration. It was not possible to find a better fit of the proposed model to the experimental data near the critical monomer concentrations. Reasons for this deviation will be discussed under Discussion.

Discussion

In this paper, tropomyosin has been found to bind to actin filaments at a rate of 2.5 × 10^6 to 4 × 10^6 M^{-1} s^{-1}. Thus, the rate of assembly of tropomyosin with actin is not very much slower than diffusion controlled. This fast rate underlines the dynamics of the turnover of microfilaments. The calculated curves deviate systematically from the experimental data near the critical monomer concentration. Probably, the model of assembly of tropomyosin with actin filaments proposed in this paper has to be extended by additional reaction steps (e.g., conformational changes) to achieve a better agreement of calculated curves with experimental data. In this context, it is interesting to note that also actin polymerization in the absence of tropomyosin cannot be described as an association–dissociation reaction. A conformational change or hydrolysis of ATP has been found to affect the rate of actin polymerization near the critical monomer concentration (Keiser et al., 1986; Carlier et al., 1984).

Tropomyosin also has other effects on actin polymerization. Tropomyosin has been reported to inhibit spontaneous fragmentation of actin filaments (Wegner, 1982a). Inhibition of spontaneous fragmentation decreases the overall rate of actin polymerization because the concentration of filament ends which consume actin monomers does not increase. However, the good agreement of the calculated curves with the nucleated polymerization curves in the absence of tropomyosin (Figure 5) suggests that even in the absence of tropomyosin the concentration of filament ends c_p remains constant. There is no evidence that filaments fragment spontaneously under the experimental conditions. Spontaneous fragmentation can be observed if filaments are longer and the temperature is higher (Wegner & Savko, 1982; Cooper et al., 1983; Hill, 1983).

The assembly of tropomyosin with actin filaments has been investigated on human lung cells in tissue culture (Lazarides, 1976). Tropomyosin has been found to exhibit a lag of some minutes in its association with newly forming filament bundles although free tropomyosin has been present. This delay of assembly of tropomyosin with actin filaments cannot be explained by the fast rates of assembly determined in this paper. It is conceivable that binding of tropomyosin to actin filaments is regulated by other proteins which may bring about a delay of assembly. It is also possible that in human lung cells different isoforms of tropomyosin occur which bind to actin at different rates. Differences of the affinity of tropomyosin isoforms to actin filaments have been reported (Cohen & Cohen, 1972; Tanaka & Hatano, 1972; Fine et al., 1973; Keiser & Wegner, 1985; Broschat & Burgess, 1986; Hitchcock-DeGregori & Heald, 1987).

In this paper, the elongation of tropomyosin strands toward the barbed ends of actin filaments has been treated. Other interactions of tropomyosin with actin filaments are still to be investigated. Tropomyosin binds to actin filaments highly cooperatively (Wegner, 1979; Yang et al., 1979). Thus, the elongation of existing tropomyosin strands along actin filaments is more favored than formation of a new strand (nucleation). The rate of nucleation of tropomyosin strands is still unknown. Another problem to be investigated is the elongation of tropomyosin strands toward the pointed ends of actin filaments. The rate of this reaction has not yet been measured. It also has to be explored whether or not tropomyosin bound near the pointed ends of actin filaments decreases the rate of polymerization and depolymerization of the pointed ends. This question is important because it is still unclear which molecules protect the pointed ends in sarcomeres against polymerization and depolymerization (Oosawa et al., 1987).

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Comparison of the Structure and Dynamics of Chicken Gizzard and Rabbit Cardiac Tropomyosins: ^{1}H NMR Spectroscopy and Measurement of Amide Hydrogen Exchange Rates†

Clive Sanders, Brian D. Sykes,* and Lawrence B. Smillie
MRC Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

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ABSTRACT: The side chain and backbone mobilities of chicken gizzard tropomyosin (TM) and its nonpolymerizable derivative have been investigated by ^{1}H NMR spectroscopy and amide hydrogen exchange kinetics and compared to those of rabbit cardiac TM and its nonpolymerizable derivative. Analysis of the 300-MHz ^{1}H NMR spectra of native chicken gizzard and rabbit cardiac TMs and their nonpolymerizable derivatives showed that the line widths of the aromatic and histidine residues were within a factor of 2 for all four proteins, demonstrating that the side chain mobility of these residues is similar in the different TMs. Direct proton exchange-out kinetics were determined in D_2O in the pD range 1.5-3.0 at 25 °C by ^{1}H NMR spectroscopy. Multiple exponential fitting of the exchange data indicated the presence in gizzard TM of at least three kinetically distinct classes of amide hydrogens at pD 1.7 with average population sizes of 147, 74, and 61, whose rates were retarded by a factor of 10, 10^3, and 10^5, respectively, relative to the random-coil peptide poly(DL-alanine). Measurement of the direct exchange kinetics of both rabbit cardiac and nonpolymerizable gizzard TMs showed that their rate constants and population sizes were within experimental error of those for the gizzard protein, except that the fast exchanging class for cardiac TM was increased in size while that of the nonpolymerizable gizzard TM was reduced, relative to that for gizzard TM. Comparison of the exchange-out kinetics for the cardiac and gizzard proteins at pH 2.0 and 55 °C, where only the two slowly exchanging amide hydrogen sets are measured, again demonstrated the similarity of their kinetic parameters. Amide hydrogen exchange in the pH range 6-11 was studied by NMR measurement of the transfer of saturation from water to the amide hydrogens. The loss in amide hydrogen intensity of the nonpolymerizable forms of the gizzard and cardiac TMs paralleled each other and was modeled using the kinetic parameters derived at acidic pH for the gizzard protein. The retardation factors for the fast and intermediate classes of amide hydrogens were decreased at the higher pHs so that they were only 2-fold and 10-fold, respectively, slower than the rate for the random-coil polypeptide. Differential scanning calorimetry of gizzard TM and its nonpolymerizable derivative indicated the presence of domains of differing thermal stability in both proteins at acidic and alkaline pH. The thermal unfolding properties of the two gizzard proteins were very similar to each other, both being considerably more stable at pH 2.0 than at pH 7.0, an observation which has been previously made with rabbit skeletal TM. Circular dichroism measurements on the variation of the α-helical content with temperature of gizzard TM and its nonpolymerizable derivative at pH 2.0 and 7.0 also showed that the two proteins' structures were of comparable stability. We conclude from these results that the relative mobility and stability of the gizzard and cardiac proteins are very similar and that whereas 20% of each molecule is in a relatively immobile and stable conformation, over half is in a more mobile, less stable conformation.

Tropomyosin (TM)[1] is of central importance in the regulation of contraction in muscle and cellular motile systems [see Smillie (1979); Talbot and Hodges (1982); Côté (1983); Leavis and Gergely (1984); and Payne and Rudnick (1985)]. The function of TM is best understood in striated muscle, where it serves to propagate conformational changes which start in the troponin complex (in response to changes in [Ca^{2+}]) to actin and thereby regulate the actomyosin ATPase. The structural features of TM responsible for its interaction with actin and the members of the troponin complex have been investigated as have those responsible for the stabilization of its coiled-coil structure and heat to tail overlap [for a review, see Leavis and Gergely (1984)]. In comparison, the role of TM in smooth muscle is more obscure. Here, contraction is

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[1] Abbreviations: TM, tropomyosin; NPTM, nonpolymerizable tropomyosin; DSS, disodium 2,2-dimethyl-2-silapentane-5-sulfonate; PDLA, poly(DL-alanine); NMR, nuclear magnetic resonance.

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