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Tropomyosin-troponin complex stabilizes the pointed ends of actin filaments against polymerization and depolymerization

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In striated muscle the pointed ends of polar actin filaments are directed toward the center of the sarcomer. Formed filaments keep a constant length of about 1 μm. As polymerization and depolymerization at free pointed ends are not sufficiently slow to account for the constant length of the filaments, we searched for proteins which occur in sarcomers and can stabilize the pointed ends of actin filaments. We observed that tropomyosin-troponin complex reduces the rate of association and dissociation of actin molecules at the pointed ends more than 30-fold. On the average, every 600 s one association or dissociation reaction has been found to occur at the pointed ends near the critical actin monomer concentration.

Actin; Tropomyosin; Troponin; Polymerization; Fluorescence

1. INTRODUCTION

In sarcomers of striated muscle actin filaments are arranged in a highly ordered manner. The pointed ends of the polar actin filaments are directed toward the center of the sarcomer while the barbed ends are attached to the Z-line. Formed filaments keep a uniform length of about 1 μm [1]. In vitro experiments show that at free pointed ends association of actin monomers and dissociation of filament subunits occur on the average every 5 s [2,3]. Association and dissociation at free pointed ends of actin filaments are not sufficiently slow to account for the stable constant length of actin filaments in sarcomers. In view of the constant length of the filaments, several attempts have been made to isolate capping proteins which bind to the pointed end to inhibit their alteration of the length by polymerization or depolymerization. It is being discussed whether β-actinin, a capping protein from skeletal muscle, binds to the pointed or to the barbed ends of actin filaments [4]. Another pointed end capping protein, namely acumentin, has been isolated from macrophages and human granulocytes [5]. Tropomyosin from skeletal muscle and non-muscle cells or tropomyosin-troponin complex has been reported to reduce the rate of assembly of the barbed ends only by a factor of 2 or 3 [6-9]. In this paper we investigate whether tropomyosin-troponin complex can stabilize the pointed ends to keep the length of actin filaments constant.

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2. MATERIALS AND METHODS

2.1. *Preparations of the proteins*

Rabbit skeletal muscle actin was prepared according to [10]. Part of protein was modified with *N*-ethylmaleimide at cysteine-374 and subsequently with 4-chloro-7-nitro-2-oxa-1,3-diazole at lysine-373 to produce a fluorescently labeled actin [11]. The concentration of actin was determined photometrically at 290 nm by using an absorption coefficient of 24900 $M^{-1} \cdot cm^{-1}$ [12]. Rabbit skeletal muscle tropomyosin was prepared as described previously [8]. The tropomyosin concentration was determined by measuring the absorbance at 276 nm using an extinction coefficient of 24500 $M^{-1} \cdot cm^{-1}$ [13]. The gelsolin-actin complex was isolated from chicken gizzard smooth muscle [14]. The muscle tissue was homogenized. The supernatant remaining after centrifugation was fractionated with ammonium sulfate (35-55%). The protein was applied to a DEAE-cellulose column. Further purification of the gelsolin-actin complex was achieved by using an agarose affinity column (1.5 × 12 cm Affi Gel 10, purchased from Bio Rad) to which DNase I was covalently bound [15]. The column was equilibrated with monomeric actin to produce immobilized actin [2]. The gelsolin-actin complex was eluted from the affinity column by using 5 mM EGTA buffer. The concentration of the gelsolin-actin complex was determined by its nucleation activity [2]. The isolation of troponin from bovine heart was based upon the preparation method described in [16], as modified in [17]. The concentration of troponin was determined according to [18].

2.2. *Experimental design*

Actin polymerization and depolymerization were followed by the 2.2-2.5-fold greater fluorescence intensity of fluorescently labeled polymeric actin compared to that of monomeric actin [11]. Fluorescence was measured and evaluated as described previously [8]. Gelsolin-capped filaments were prepared by combining a 1 M KCl solution, a 20 mM $MgCl_2$ solution, buffer A (5 mM triethanolamine-HCl pH 7.5, 100 mM KCl, 0.5 mM ATP, 0.1 mM $CaCl_2$, 2 mM $MgCl_2$, 200 mg/l NaN_3), monomeric actin in buffer B (40 μM actin, 5 mM triethanolamine-HCl pH 7.5, 0.5 mM ATP, 0.2 mM $CaCl_2$, 200 mg/l NaN_3) and gelsolin-actin complex in buffer C (1 μM complex, 20 mM imidazole-NaOH, pH 7.8, 0.5 mM ATP, 5 mM EGTA, 150 mM NaCl). The solutions were mixed in such a ratio that the final composition was: 100 mM KCl, 2 mM $MgCl_2$, 4 μM monomeric actin and

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50 nM gelsolin-actin complex. Tropomyosin-troponin complex was formed by mixing a 1 M KCl solution, a tropomyosin solution (50 μM, 5 mM triethanolamine-HCl pH 7.5), a troponin solution (25 μM, 20 mM Tris-HCl, pH 7.5, 500 mM KCl, 15 mM mercaptoethanol) and finally 5 mM triethanolamine-HCl pH 7.5. The solutions were mixed in such a ratio that tropomyosin and troponin occurred in equimolar concentrations and the KCl concentration was 100 mM. Polymerization or depolymerization of actin was measured following the combination of buffer A, a 4 mM $MgCl_2$ solution, a 200 mM KCl solution, a 5 μM monomeric actin solution and the gelsolin-capped polymeric actin solution. The samples contained finally 2 mM $MgCl_2$, 100 mM KCl, different concentrations of monomeric actin and gelsolin-capped actin filaments. When polymerization and depolymerization were measured in the presence of tropomyosin-troponin complex, 1 μM of the complex was added to the preparation of the gelsolin-capped actin filaments and to the samples. All experiments were performed at 25°C.

3. RESULTS

3.1. *Effect of the tropomyosin-troponin complex on actin polymerization and depolymerization at the pointed ends*

Actin filaments which could polymerize or depolymerize solely at the pointed ends, were produced by polymerization of actin monomers onto 1:1 gelsolin-actin complex. These barbed end-capped filaments were mixed with different concentrations of monomeric actin. Polymerization or depolymerization at the free pointed ends was followed by the change of the fluorescence. The time course of actin assembly and disassembly in the presence and absence of tropomyosin-troponin complex is depicted in figs 1 and 2, respectively. Actin polymerization and depolymerization appear to be retarded by tropomyosin-troponin complex. Especially at low actin monomer concentrations (0–0.4 μM) the polymerization and depolymerization rates are strongly reduced. At higher actin monomer concentrations polymerization is only moderately retarded. This effect is independent of the Ca^{2+} concentration.

3.2. *Rate of binding and dissociation of actin at the pointed ends*

In fig.2 the entire time course of actin polymerization in the absence of tropomyosin-troponin complex is depicted. The rate of change of the polymer concentration (c_f) or monomer concentration (c_1) is given by:

$$-dc_f/dt = dc_1/dt = -k_p^+ \times c_1 \times c_g + k_p^- \times c_g \quad (1)$$

or

$$c_1 - \bar{c}_{1p} = (c_{1i} - \bar{c}_{1p}) \times \exp[-(k_p^-/\bar{c}_{1p}) \times c_g \times t] \quad (2)$$

where k_p^+ and k_p^- are the rate constants of association and dissociation of actin molecules at the pointed ends, respectively; c_g is the gelsolin-actin complex concentration which is assumed to be equal to the concentration of the pointed ends of filaments; $\bar{c}_{1p}$ is the critical monomer concentration of the pointed ends

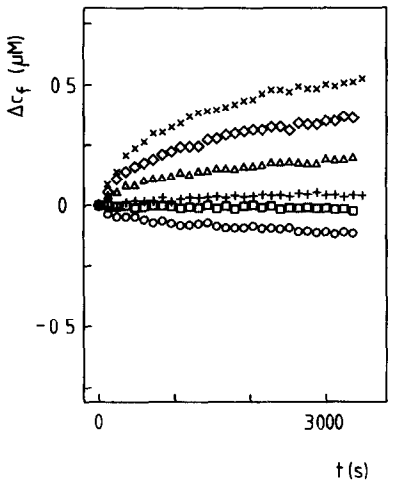

Fig.1. Polymerization and depolymerization of the pointed ends of actin filaments in presence of 1 μM tropomyosin-troponin complex. Δc_f, change of the concentration of polymerized actin. Gelsolin-capped filaments (12.5 nM gelsolin plus 1 μM actin) were mixed with the following concentrations of monomeric actin: ○, 0 μM; □, 0.2 μM; +, 0.4 μM; △, 0.6 μM; ◇, 0.8 μM; ×, 1 μM.

($\bar{c}_{1p} = k_p^-/k_p^+$); c_{1i} is the initial monomer concentration. Curves calculated according to eqn 2 are displayed in fig.2. The dissociation constant k_p^- was found to be $1/18\ s^{-1}$. At steady-state one association and dissociation reaction takes place on the average every 18 s at a single pointed end. It turned out that the time course of polymerization or depolymerization in the presence of tropomyosin-troponin complex (fig.1) cannot be approximated by exponentials calculated according to eqn

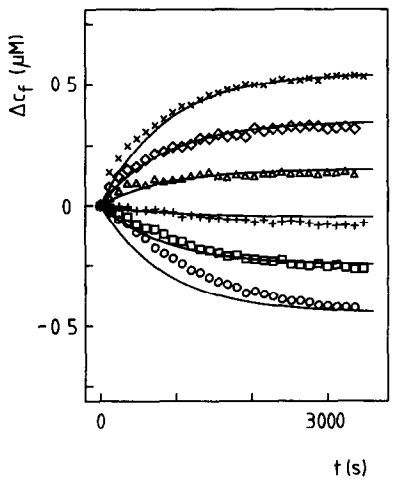

Fig.2. Polymerization and depolymerization of the pointed ends of actin filaments in absence of tropomyosin-troponin complex. Δc_f, change of the concentration of polymerized actin. Gelsolin-capped filaments (12.5 nM gelsolin plus 1 μM actin) were mixed with the following concentrations of monomeric actin: ○, 0 μM; □, 0.2 μM; +, 0.4 μM; △, 0.6 μM; ◇, 0.8 μM; ×, 1 μM. The continuous lines were calculated for $k_p^- = 1/18\ s^{-1}$ (dissociation rate constant of the pointed end) and $\bar{c}_{1p} = 0.6\ \mu$M (critical concentration of the pointed end).

2. However, based on fig.1 one can estimate the number per time of actin molecules which dissociate from the pointed ends of tropomyosin-troponin covered filaments. If no monomers are present about 120 nM subunits dissociate from 12.5 nM pointed ends within one hour. Thus, at steady-state, i.e. when association is balanced by dissociation, every 600 s one association and one dissociation reaction occurs at a single pointed end. Thus, the frequency of actin monomer binding and dissociation is decreased by tropomyosin-troponin complex by more than 30-fold.

4. DISCUSSION

An investigation on the effect of tropomyosin-troponin complex on actin polymerization has been reported previously [7]. In that paper high concentrations of actin (6 μM) were polymerized predominantly onto the barbed ends of actin filaments. Only a 2–3-fold retardation of actin polymerization has been observed. Also we found only a moderate retardation of actin polymerization at the pointed ends, if the actin monomer concentration was high (fig.1). The stronger retardation at low actin monomer concentrations compared to the effect at high actin monomer concentrations has also been reported for actin polymerization in the presence of tropomyosin [8]. According to the interpretation given for the actin-tropomyosin system at high actin monomer concentrations, actin monomers bind at the ends of filaments faster than tropomyosin-troponin complex so that the rate of polymerization is only slightly decreased. At low actin monomer concentrations, tropomyosin-troponin complex can inhibit actin polymerization because tropomyosin-troponin complex binds faster at filament ends than actin. In striated muscle, actin is preodominantly in the form of filaments and only a minor amount occurs in the monomeric state. Thus, in muscle association and dissociation reactions of actin at the pointed ends are expected to be strongly retarded by tropomyosin-troponin complex which is bound to the actin filaments in sarcomers. Tropomyosin-troponin complex may be one of the molecules which stabilize the thin filaments in muscle against polymerization and depolymerization and keep the length of formed filaments constant.

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