THE INTERACTION OF α,α- AND α,β-TROPOMYOSIN WITH ACTIN FILAMENTS

Albrecht WEGNER

School of Medicine, Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19174, USA

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1. Introduction

Tropomyosin from adult skeletal muscle consists of 2 identical polypeptide chains (α,α-tropomyosin) or 2 different chains (α,β-tropomyosin) [1–3]. The amino acid sequence of both polypeptide chains differ by 39 residues [4]. Of these amino acid residues, 23 are situated on the surface of the molecule. Two amino acid differences occur in the last 9 residues near the C-terminus where tropomyosin molecules form a polar end-to-end contact with the N-terminus of an adjacent tropomyosin molecule when bound along the actin filament [5–7].

Here, we investigate whether these amino acid differences cause alterations in the interaction of tropomyosin with actin filaments or in the end-to-end contact of tropomyosin molecules bound along actin filaments. Binding isotherms of α,α- and α,β-tropomyosin to actin filaments were measured by light scattering [8,9]. The binding curves were quantitatively analyzed thereby separating the affinity of tropomyosin for actin filaments and the affinity for the end-to-end contact of tropomyosin molecules. In terms of free energy, the differences in the interaction with actin filaments or in the end-to-end contact of the 2 multiple forms of tropomyosin were found to be <1 kJ.

2. Material and methods

2.1. *Preparation of the proteins*

Actin was prepared by the method in [10] and was applied to a Bio-Gel P-150 column (2.5 × 90 cm) [11] equilibrated with a buffer containing 500 μM ATP, 200 μM $CaCl_2$, 200 mg NaN_3/l, 0.5 mM ascorbic acid and 5 mM triethanolamine–HCl (pH 7.5) [12]. The [actin] was determined photometrically at 290 nm using $\epsilon = 24\,900\ M^{-1}\ cm^{-1}$ [13].

The tropomyosin–troponin complex was extracted and purified by ammonium sulphate fractionation as in [10]. Tropomyosin was separated from troponin by hydroxylapatite chromatography [14]. By this procedure, tropomyosin was partially separated into α,α- and α,β-tropomyosin [14]. The first third of the fractions containing tropomyosin were combined and also the last third were combined. The first fractions contained <5% β-tropomyosin chains or 10% α,β-tropomyosin as tested by electrophoresis [1,15]. The last fractions contained α- and β-chains at ~0.6:0.4 so that α,α- and α,β-tropomyosin were present at 4:1. In order to reduce tropomyosin at Cys_{190} [16–18] the protein was incubated with 20 mM dithiothreitol for 1 h at 40°C [18]. The [tropomyosin] was determined at 276 nm using $\epsilon = 24\,500\ M^{-1}\ cm^{-1}$ [8].

2.2. *Light scattering*

The actin–tropomyosin complex was formed by mixing equal volumes of an actin solution, a tropomyosin solution and a buffer containing salt. Actin was dialyzed against 750 μM ATP, 30 μM $CaCl_2$ or $MgCl_2$, 0.5 mM dithiothreitol, 200 mg NaN_3/l and 5 mM triethanolamine–HCl (pH 7.5). Tropomyosin was dialyzed against 240 mM KCl, 0.5 mM dithiothreitol, 200 mg NaN_3/l and 5 mM triethanolamine (pH 7.5). The salt buffer contained 750 μM ATP, 2.97 mM $MgCl_2$, 0.5 mM dithiothreitol, 200 mg NaN_3/l and 5 mM triethanolamine (pH 7.5). Hence, the actin–tropomyosin complex was dissolved in a buffer containing 500 μM ATP, 1 mM $MgCl_2$, 80 mM KCl, 0.5 mM dithiothreitol, 200 mg NaN_3/l and 5 mM triethanolamine (pH 7.5). Before mixing in light scattering cells, all solutions were centrifuged at 100 000 × *g* for 1 h to remove dust.

The ratio of the scattering intensities of long rod-like actin filaments (R_a) and of actin filaments to which ligands such as tropomyosin are bound (R_t)

has been shown to be given by [8,9]:

$$\frac{R_t(\vartheta)}{R_a(\vartheta)} = \frac{(C_a + C_t)^2}{C_a^2} \quad (1)$$

C_a is the weight concentration of polymeric actin, C_t is the weight concentration of tropomyosin bound by actin filaments and ϑ is the observation angle. The ratio R_t/R_a was measured under $\theta = 90°$ at 546 nm using a Perkin Elmer MPF3 fluorimeter. For the calculation of the molar concentrations, actin and tropomyosin M_r values were assumed to be 42 300 [19] and 65 400 [20], respectively.

2.3. *SDS gel electrophoresis*

SDS–polyacrylamide electrophoresis was performed as in [15]. The gels were stained with fast-green. For a semiquantitative determination of the ratio of α- and β-tropomyosin chains, the gels were scanned at 625 nm.

3. Results and discussion

3.1. *Binding curves of α,α- and α,β-tropomyosin to actin*

Binding curves of α,α- and α,β-tropomyosin to actin filaments were measured by light scattering. Actin was kept constant at 5 μM whereas tropomyosin was varied from 0.2–3 μM. The scattering intensity of pure actin (R_a) was temperature-independent. At the [salt] used, the [monomeric actin] coexisting with polymeric actin was ~0.1 μM [8,9] so that almost all actin was in the polymeric form. At 43°C, the light scattering intensities of the actin–tropomyosin mixtures were similar to the scattering intensity of pure actin indicating that actin filaments were bare of tropomyosin. When tropomyosin was present in a stoichiometric excess over actin the scattering intensity increased on cooling to 25°C by ~50% due to covering of actin filaments with tropomyosin. The binding curves were measured at intermediate temperatures where the filaments were partially covered with tropomyosin (fig.1,2). The scattering intensities of the solutions were reversible with temperature changes indicating that the actin–tropomyosin association had reached an equilibrium. The av. no. bound tropomyosin molecules/actin subunit (binding density υ) was calculated from the light scattering intensity as in section 2.2. The results are depicted in figs. 1,2.

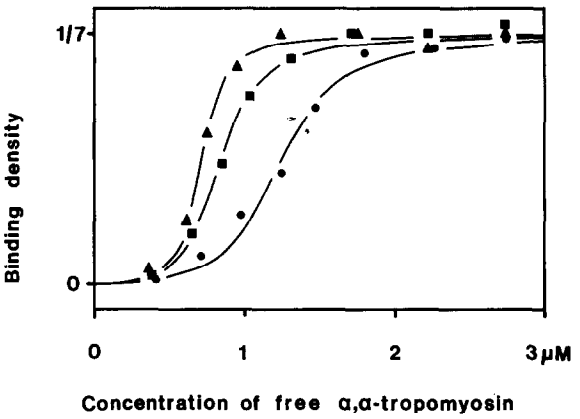

Fig.1. Binding curves of uncrosslinked α,α-tropomyosin to actin filaments: total actin 5.3 μM; (▲) 38.2°C; (■) 39.4°C; (●) 40.7°C. The continuous lines were calculated using the fitted equilibrium constants given in table 1.

An attempt was made to measure also binding curves of α,α- and α,β-tropomyosin crosslinked at Cys_{190}. However the binding curves of crosslinked α,β-tropomyosin were found to be irreproducible.

3.2. *Analysis of the binding curves*

Tropomyosin can bind to 3 types of binding sites on actin filaments (fig.3):

1. Isolated binding sites: The termini of tropomyosin form no end-to-end contact with adjacent ligands.
2. Singly contiguous binding sites: One terminus forms an end-to-end contact with an adjacent ligand.

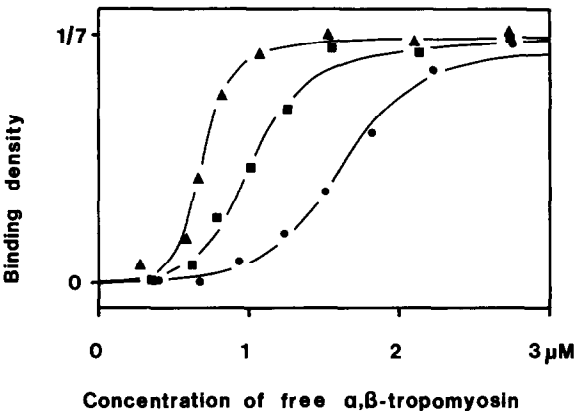

Fig.2. Binding curves of uncrosslinked α,β-tropomyosin to actin filaments: total actin 5.1 μM; (▲) 38.2°C; (■) 39.4°C; (●) 40.7°C. The continuous lines were calculated using the fitted equilibrium constants given in table 1.

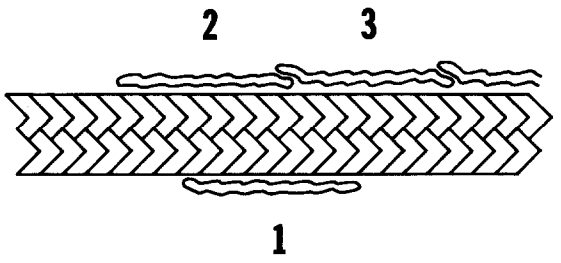

Fig.3. Types of binding sites of tropomyosin (bars) along the actin filament (chevrons): (1) isolated binding site; (2) singly contiguous binding site; (3) doubly contiguous binding site.

Table 1
Equilibrium constants of binding of uncrosslinked α,α- and α,β-tropomyosin to actin filaments

Temp. (°C)	α,α-tropomyosin		α,β-tropomyosin	
	K (M^{-1})	ω	K (M^{-1})	ω
38.2	3.1×10^3	450	4.1×10^3	350
39.4	4.6×10^3	250	4.7×10^3	200
40.7	3.1×10^3	250	2.4×10^3	250

K, binding constant for isolated binding sites; ω, equilibrium constant for moving a tropomyosin molecule from an isolated binding site to a singly contiguous binding site

3. Doubly contiguous binding sites: Both termini have an end-to-end contact with adjacent ligands.

This equilibrium may be described by 2 independent equilibrium constants:

1. Equilibrium constant for the association of a tropomyosin molecule with an isolated binding site (K).
2. Equilibrium constant for moving a tropomyosin molecule from an isolated binding site to a singly contiguous binding site (ω).

Binding of tropomyosin to singly and doubly contiguous binding sites can be thought to be composed of association with isolated binding sites and moving from isolated binding sites to singly contiguous binding sites. The equilibrium constant K represents the affinity of tropomyosin for actin filaments and the equilibrium constant ω represents the affinity of the end-to-end contact of tropomyosin molecules.

This complex equilibrium has been considered [21] in a model of co-operative binding of large ligands as a one-dimensional homogeneous lattice. The free [tropomyosin] t has been related to the binding density v and the equilibrium constants K and ω thus:

$$t = \frac{1}{K}\frac{v}{1-7v}\left(\frac{2(\omega-1)(1-7v)}{(2\omega+1)(1-7v)+(v-R)}\right)^6\left(\frac{2(1-7v)}{(1-8v)+R}\right)^2 \quad (2)$$

where

$$R = \sqrt{((1-8v)^2 + 4\omega v(1-7v))}$$

To fit the binding constants that set of parameters K and ω was sought which leads to the smallest standard deviation from the measured binding density. The values of K and ω are summarized in table 1.

The differences in the affinity of α,α- and α,β-tropomyosin for actin filaments (represented by K) as well as of the end-to-end contact of the two multiple forms of tropomyosin (represented by ω) are small. In terms of free energy, the differences are <1 kJ. Tropomyosin has been shown to be located in the groove of the double helical actin filament at a position similar to that occupied by the tropomyosin–troponin complex on actin in the presence of calcium [22–25]. It remains to be established whether the actin–tropomyosin interaction is also similar for both multiple forms of tropomyosin when the tropomyosin–troponin complex is located at the periphery of the actin filament, i.e., in the absence of calcium.

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