Equilibrium self-association of tropomyosin

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ABSTRACT

It has recently been reported that tropomyosin exists exclusively as a dimer in physiological salt conditions. It is shown in the present work using analytical ultracentrifugation that, on the contrary, tropomyosin is in equilibrium between monomer, dimer and tetramer with a weak tendency to dimerize and tetramerize. Such a finding has consequences for the assembly of the tropomyosin–actin complex.

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1. Introduction

The tropomyosin molecule is a 66 kDa (muscle tropomyosin) coiled-coil of two parallel polypeptide chains that bind end-to-end, i.e. head-to-tail, along actin filaments [1–4]. Tropomyosin controls the stability of actin filaments in non-muscle cells [3,5] and its end-to-end interaction adds cooperativity to its binding to actin [6] and to the switching on/off of actin filaments in muscle cells [7]. The nature of the oligomeric size of free tropomyosin, i.e. unbound to actin, might determine the nature and kinetics of its binding to actin and thus determine its function in space and time. A recent report has concluded that free tropomyosin from skeletal muscle and non-muscle sources is a monodisperse dimer of tropomyosin molecules, i.e. a tetramer of polypeptide chains, without the equilibrium presence of monomers or higher order oligomers [8]. We re-investigated this question with analytical ultracentrifugation for two main reasons. First of all, we would have expected tropomyosin dimers to be in equilibrium with the monomer and/or larger species based on our recent electron microscopy study [9] and on the general understanding that the increased viscosity of skeletal muscle tropomyosin solutions with decreasing ionic strength [10] is due to end-to-end polymer formation [10–14]. Second, the techniques of gel filtration and sucrose gradient ultracentrifugation used in the previous report [8] can result in misleading conclusions. The technique of analytical ultracentrifugation does not share these shortcomings.

2. Materials and methods

Tropomyosin from rabbit skeletal striated muscle was prepared as reported [15] and its concentration determined from its absorption at 277 nm using an extinction coefficient $A^{1\%}_{277\,nm} = 2.4\ cm^{-1}$ [16]. All experiments were conducted at 20 °C on tropomyosin dialyzed extensively vs. Buffer A (150 mM KCl, 10 mM Mops, 2 mM DTT, 0.1 mM EDTA, 0.01% NaN_3, pH 7.5). Analytical ultracentrifugation [17] and its analysis using SENDAL software [18], available from http://sedanal/bbri.org/, have been described previously. The order at which speeds of the different sedimentation equilibrium runs were performed was important: If the higher speed was run first, we were unable to attain equilibrium at the lower speed because the protein at the bottom of the cell equilibrated only extremely slowly upon lowering the speed. However, if the lower speeds were run first we were able to attain equilibrium at each speed. The approach to equilibrium was monitored using the sensitive program WinMatch (available on-line from the University of Connecticut: http://www.biotech.uconn.edu/auf/ or at RASMB software archives: http://rasmb.org/). Equilibrium was considered established when there were no further changes over a 12 h period by least squares criteria.

3. Results and discussion

Sedimentation velocity of skeletal muscle tropomyosin was run in ionic conditions close to physiological, as used in the previous report [8]. Sedimentation velocity exhibited a single broad peak whose weight average sedimentation coefficient shifted with dilution from $s_{w(20,w)} = 3.03$ S to a value of 2.80 S over a concentration

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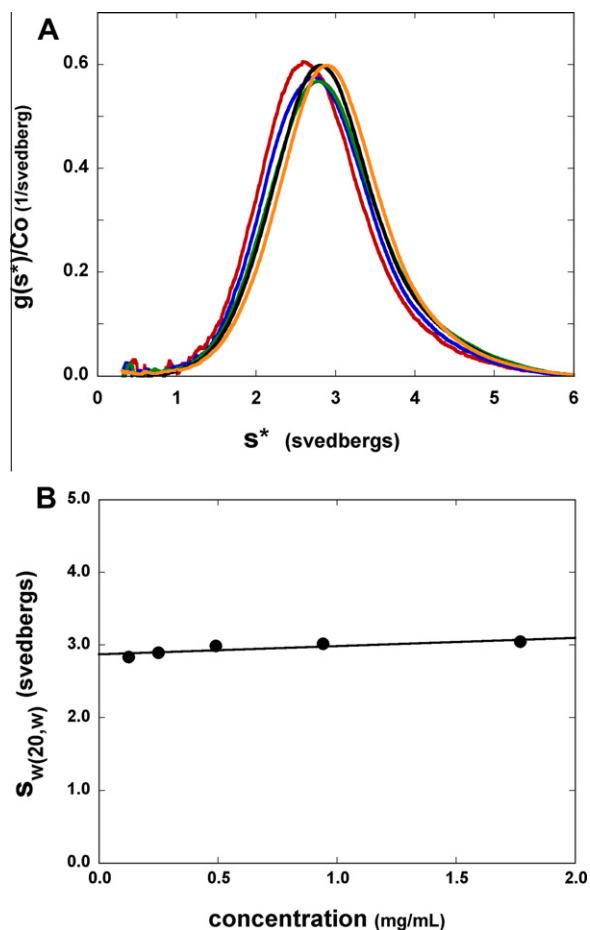*E-mail address:* Graceffa@bbri.org (P. Graceffa).

Fig. 1. Sedimentation velocity of skeletal muscle tropomyosin. (A) Concentration-normalized sedimentation distribution patterns, g(s*)/C$_o$, vertical axis, versus apparent sedimentation coefficient, s*, horizontal axis. Tropomyosin concentrations: 0.13 (red), 0.26 (blue), 0.5 (green), 0.94 (black), 1.8 (orange) mg/ml. (B) Weight average sedimentation coefficient, $s_{w(20,w)}$, versus concentration of runs in (A).

range of 1.8 to 0.13 mg/mL (Fig. 1). A shift in sedimentation coefficient indicates that the tropomyosin is in equilibrium between at least two species. Since tropomyosin in solution is thought to associate in an end-to-end fashion, the sedimentation coefficient could remain relatively constant with dilution even though there might be a major shift in the equilibrium species present. Therefore it was necessary to perform sedimentation equilibrium in order to determine the stoichiometry and equilibrium states of tropomyosin.

Sedimentation equilibrium data were fit globally with a number of tropomyosin monomer–dimer–trimer–tetramer–pentamer–hexamer models and a model for dimer alone. The root mean square deviation for all models varied between ±0.0134 fringes for the best bit (Fig. 2) to ±0.057 fringes for dimer alone, the worst fit (Fig. 3). The data were best fit (Fig. 2) with a monomer–dimer–tetramer equilibrium model with a monomer–dimer dissociation constant $K_d = 42.9 \pm 1.6\ \mu M$ and a dimer–tetramer dissociation constant $K_d = 18 \pm 12\ \mu M$. Global fitting also revealed the presence of about 2% (mole/mole) of a species with molar mass corresponding to tropomyosin tetramer not participating in the reversible equilibria and referred to as incompetent tetramer. At a concentration of 1 mg/ml tropomyosin, this would translate to an equilibrium mixture consisting of 9.7 μM monomer, 2.2 μM dimer, 0.27 μM tetramer and 0.092 μM incompetent tetramer. This indicates that the tropomyosin is predominantly in the monomer state with a weak tendency to dimerize and tetramerize. Since most of the tropomyosin (about 6 mg/ml [19,20]) in a muscle cell is bound to actin, the above numbers are most likely an upper limit to the proportion of free tropomyosin dimer and tetramer in a muscle cell. A previous work has also demonstrated a heterogeneous population of cardiac muscle tropomyosin species over a wide range of ionic strengths [21]. Whenever tropomyosin trimer was included in modeling its equilibrium concentration was vanishingly small, indicating that it must be thermodynamically unstable compared to the dimer and tetramer. It is not clear what the incompetent tetramer species is, possibly a denatured tropomyosin aggregate.

The data were also fit to a model of dimer alone, an example of which is shown in Fig. 3. The systematic varying residuals and obvious poor fit, conclusively rules out the sole presence of dimer.
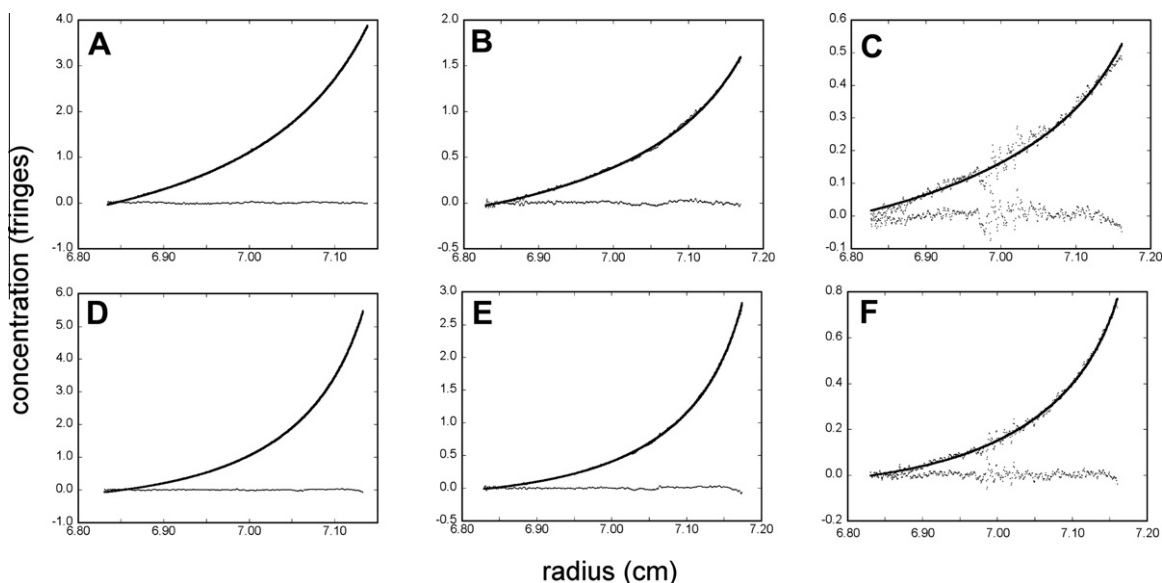

Fig. 2. Sedimentation equilibrium of skeletal muscle tropomyosin. Runs at three loading concentrations: C$_o$ (A, D), 1 mg/ml; C$_o$/3 (B, E); C$_o$/9 (C, F) and two speeds: 8000 rpm (A, B, C); 11 000 rpm (D, E, F). Vertical axis: concentration in fringes; and horizontal axis: cell radius in cm. Solid lines through the data points represent a fit to the points using a model of tropomyosin in equilibrium between monomer, dimer, and tetramer. Residuals are shown below each plot. In some cases the data points cannot be distinguished from the fitted solid line.
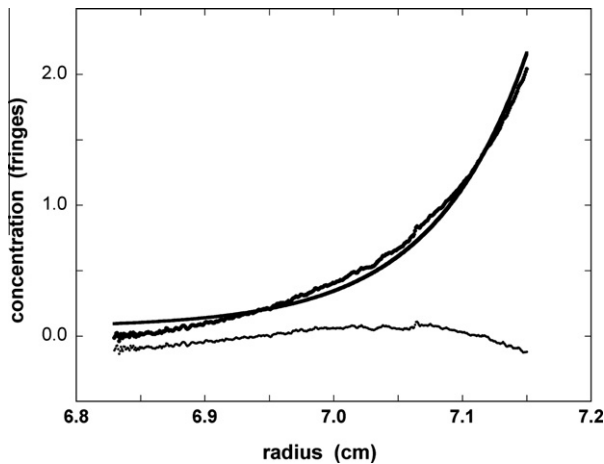
Fig. 3. Sedimentation equilibrium of skeletal muscle tropomyosin fit to a monodisperse dimer model. Run at 11 000 rpm at 0.333 mg/ml tropomyosin. Vertical axis: concentration in fringes; horizonal axis: cell radius in cm. Solid line through data points represent a fit to the points using a model of tropomyosin as a monodisperse dimer. Residuals are shown below the plot.

These results are in contrast to the previously reported work [8] that concluded that the tropomyosin was exclusively a dimer molecule (a tetramer of chains) under these conditions. The exclusive formation of a dimer would imply that tropomyosin binds strongly to itself head-to-head or tail-to-tail. Since tropomyosin binds head-to-tail on actin filaments, a head-to-head or tail-to-tail dimer would have to undergo significant rearrangement upon binding to actin. However the present results of tropomyosin in solution together with electron microscopic [9] or X-ray crystallographic [22] studies of fixed tropomyosin indicate that tropomyosin binds to itself head-to-tail which would present a much lower barrier to its binding to actin and consequently a more rapid binding. A rapid binding might be critical to the response time of tropomyosin in its stabilization of actin filaments during its role in the motility of non-muscle cells [3,5].

We suspect that the methods used in the previous work [8] resulted in misleading conclusions. Their use of the method of Siegel & Monty [23], i.e. a combination of the results from the two separate techniques of sucrose density sedimentation and gel filtration chromatography, is nearly impossible to apply to a reversibly interacting system since the concentrations are not well defined because both are zone transport methods [24]. Moreover, the typical concentrations of sucrose used in density gradient sedimentation have been well documented to induce protein dimerization and aggregation [25–27].

Acknowledgments

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