Volume 218, number 1, 139–142 FEB 04812 June 1987

Evidence for interaction between smooth muscle tropomyosin and caldesmon

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Received 1 April 1987

The viscosity of chicken gizzard smooth muscle tropomyosin is enhanced 4.7-fold in the absence of salt and 1.43-fold in 0.1 M salt by the presence of stoichiometric amounts of gizzard caldesmon, indicating that the two proteins interact under these conditions. Since the thin filament regulation of smooth muscle contraction by caldesmon requires the presence of tropomyosin, these results suggest that the direct interaction between tropomyosin and caldesmon on the thin filament plays a role in this regulation.

Caldesmon; Tropomyosin; (Smooth muscle)

1. INTRODUCTION

Muscle consists of an interdigitating array of thick myosin and thin actin filaments which slide past each other during contraction. Rod-shaped tropomyosin molecules bind end-to-end along the actin filament. The sliding motion is due to the interaction between myosin and actin during the hydrolysis of ATP by the actomyosin complex. Striated muscle contraction is activated by the binding of Ca^{2+} to troponin, bound to both actin and tropomyosin, which then relieves tropomyosin's inhibition of the actomyosin ATPase activity [1–3]. In smooth muscle there appears to be a dual system of Ca^{2+} regulation. Ca^{2+} binding to calmodulin activates the phosphorylation of one of the light chains of myosin and switches on the thick filament (review [4]). It has been suggested that the thin filament is switched on by Ca^{2+} binding to calmodulin which then binds to actin-bound caldesmon, resulting in the release of caldesmon's inhibition of actomyosin ATPase activity [5–9].

The mechanism whereby caldesmon regulates the smooth muscle thin filament is not known. However, it has been shown that regulation by caldesmon requires the presence of actin-bound tropomyosin [7,10–13] and that tropomyosin increases the binding of caldesmon to actin [11,12,14]. Therefore, we considered the possibility that a direct interaction between tropomyosin and caldesmon may explain these observations. To see whether there is an interaction between gizzard smooth muscle tropomyosin and caldesmon, in the absence of actin, we measured the effect of caldesmon on tropomyosin's low-salt viscosity, since the viscosity of skeletal striated muscle tropomyosin is enhanced upon binding of troponin [1,15]. The high low-salt viscosity of tropomyosin, which is due to its end-to-end polymerization [16,17], is greater for gizzard tropomyosin compared to skeletal tropomyosin [18–20] and decreases dramatically with increasing salt for both proteins [18–21]. We have found that the viscosity of gizzard tropomyosin is increased by the presence of caldesmon, indicating that these proteins can interact directly.

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

2. MATERIALS AND METHODS

Caldesmon was prepared by the method of Bretscher [14] from chicken gizzard muscle obtained from freshly slaughtered chicken (Mayflower Poultry Co., Cambridge, MA). This caldesmon was then further purified by affinity chromatography on a calmodulin-Sepharose 4B column according to Sobue et al. [5]. Tropomyosin was prepared from a crude tropomyosin preparation which was prepared according to Ebashi et al. [22] from frozen gizzards (Pel-Freeze, Rogers, AK). Tropomyosin was precipitated from the crude tropomyosin at its isoelectric pH of 4.9. The precipitate was dissolved in 1 M NaCl, 5 mM Mops, 1 mM EDTA, pH 8.0, and further purified by collecting the fraction precipitating between 45 and 70% ammonium sulfate saturation. Tropomyosin and caldesmon were exhaustively dialyzed vs 2 mM Mops, 0.1 mM EDTA, pH 7.5, before viscosity measurements were performed. The concentration of tropomyosin was determined by the Lowry method using rabbit skeletal tropomyosin as a standard [23] and that of caldesmon was determined from the absorbance at 276 nm [14]. The reported molecular mass of a caldesmon polypeptide chain has ranged from 120 to 150 kDa [5,8, 14,24,25]. In this study the molecular mass of a caldesmon chain was taken to be 140 kDa and that of the tropomyosin molecule to be 66 kDa.

Viscosity was determined at 24°C in Ostwald-type viscometers (Cannon Instrument Co., State College, PA) which had buffer outflow times (t_{buffer}) of 67 s (model 100/B697) or 118 s (model 75/102). The former was used for measurements carried out in the absence of salt and the latter used for measurements performed in 0.1 M NaCl. Viscosity was measured for tropomyosin adjusted to 0.3 or 0.45 mg/ml with varying concentrations of caldesmon in 2 mM Mops, 0.1 mM EDTA, pH 7.5, in the absence or presence of 0.1 M NaCl. Specific viscosity = $(t_{sample}/t_{buffer}) - 1$.

3. RESULTS AND DISCUSSION

Fig.1 shows that the viscosity of tropomyosin in the absence of salt is enhanced by the presence of caldesmon. The enhancement increases with increasing concentration of caldesmon, leveling off

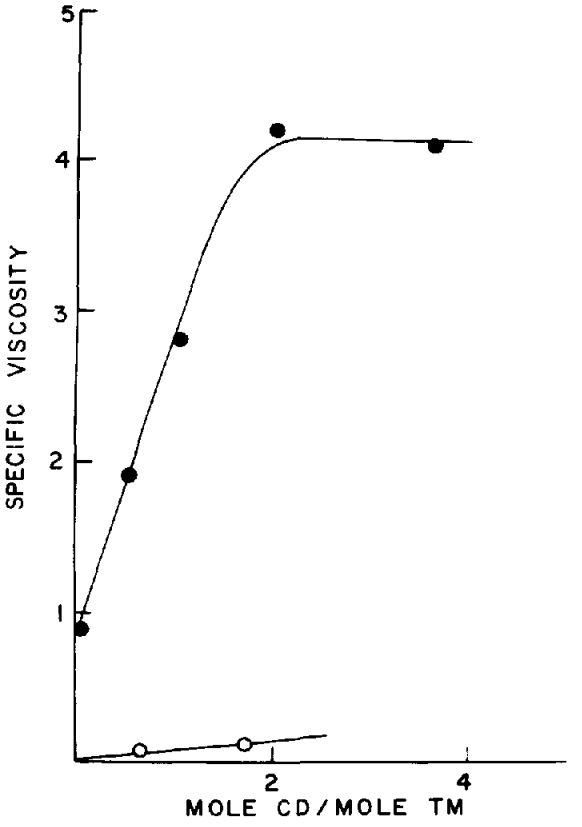

Fig.1. Specific viscosity at 24°C in 2 mM Mops, 0.1 mM EDTA, pH 7.5, of (●) tropomyosin (TM) at 0.3 mg/ml vs increasing concentration of caldesmon (CD) and (○) caldesmon alone at the same concentration as in the mixture. The viscosity is plotted vs the molar ratio of CD polypeptide chain to tropomyosin molecule.

at 4.7-times the viscosity of tropomyosin alone at approx. 2.0 caldesmon polypeptide chains per tropomyosin molecule. The viscosity of caldemon alone, under these conditions, is very low (fig.1) and thus cannot account for the increase in viscosity. These results indicate that tropomyosin and caldesmon bind under these low-ionic conditions, resulting in an increased viscosity, and furthermore suggest a binding stoichiometry of two caldesmon polypeptide chains per tropomyosin molecule. At present it is controversial whether the caldesmon molecule is a monomer or dimer [5,14,25,26].

The ratio of caldesmon polypeptide chains to tropomyosin when both proteins are bound to actin is, however, less than 2.0. Studies of caldesmon

Table 1

Enhancement of tropomyosin viscosity by caldesmon in 0.1 M NaCl

	Specific viscosity
TM alone	0.17
CD alone	0.13
TM + CD	0.43
Percent enhancement	43

Specific viscosity (N) of tropomyosin (TM), at 0.45 mg/ml, and caldesmon polypeptide chain (CD), at twice the molarity of TM, alone or together in 0.1 M NaCl, 2 mM Mops, 0.1 mM EDTA, pH 7.5, at 24°C. Percent enhancement = $100 \times \{[(N_{TM+CD})/(N_{TM\,alone} + N_{CD\,alone})] - 1\}$

binding to actin and actin-tropomyosin indicate a maximum caldesmon polypeptide chain to tropomyosin molar ratio of about 1.0 [14]. This difference between the stoichiometry of caldesmon binding directly to tropomyosin and their ratio when both are bound to actin may indicate that caldesmon and tropomyosin on the actin filament are constrained to interact in a stoichiometry different from that found in the absence of actin. Another possibility is that the interaction stoichiometry of caldesmon-tropomyosin may be different under physiological ionic conditions.

Caldesmon also enhances the viscosity of tropomyosin at a more physiological ionic strength. Measurements were performed at 0.1 M NaCl where the viscosity of tropomyosin is less than one-tenth that in the absence of salt. Table 1 shows that in 0.1 M NaCl the specific viscosity of a solution of tropomyosin and caldesmon, in a ratio of 2.0 caldesmon chains per tropomyosin molecule, is enhanced 43% over the sum of the specific viscosities of solutions of tropomyosin and caldesmon alone. The specific viscosity of a solution of non-interacting molecules would be expected to equal the sum of the specific viscosities of solutions of the individual molecules alone. These results indicate that tropomyosin and caldesmon interact under ionic conditions close to physiological.

Since actin-bound tropomyosin is necessary for regulation by caldesmon [7,10–13], the present findings suggest that a direct interaction between tropomyosin and caldesmon, both bound to actin, plays a role in the Ca^{2+}-sensitive, thin filament regulation of smooth muscle contraction. This would be somewhat analogous to striated muscle, where the presence of tropomyosin is necessary for the Ca^{2+}-sensitive, thin filament regulation by troponin which interacts directly with tropomyosin on the filament [1,3].

During the preparation of this manuscript a paper appeared which reported the interaction of vascular smooth muscle tropomyosin with caldesmon covalently linked to Sepharose [27].

ACKNOWLEDGEMENTS

I am very grateful to Miss Qian Zhan and Dr Chih-Lueh Albert Wang for their major contribution to the preparation of caldesmon. I would also like to thank Dr Walter Stafford for helpful discussions, and Drs John Gergely, Terence Tao and Chih-Lueh Albert Wang for reading the manuscript.

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