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Interaction of plasma gelsolin with tropomyosin

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Horse plasma gelsolin labelled with benzophenone-4-isothiocyanate can be photochemically cross-linked to rabbit cardiac tropomyosin. The cross-linking proceeds with greater efficiency in calcium-containing buffers. Further evidence for interaction between these proteins is provided by retention of fluorescently labelled gelsolin on tropomyosin–agarose affinity columns and by the ability of tropomyosin to cause an increase in the fluorescence intensity of gelsolin labelled with fluorescein-5-isothiocyanate. Both of these effects require the presence of calcium ions.

Gelsolin; Tropomyosin

1. INTRODUCTION

Gelsolin is an actin filament severing, capping and nucleating protein that exists both as a cytoplasmic and as a secreted protein in vertebrates [1]. Horse plasma gelsolin resembles other gelsolins in its chemical and physical properties [2]. It is a globular protein with a molecular mass of 80.9 kDa, calculated from its amino acid composition, assuming it to consist of 739 amino acids as does pig plasma gelsolin [3].

The primary function of gelsolin in plasma apparently is to sever into short oligomers any F-actin filaments that might appear there as a result of cell death or injury [4,5]. If present, F-actin would increase the viscosity of the plasma and could severely restrict blood flow through microcirculatory vessels.

Tropomyosin (TM) is a rod-shaped protein composed of two parallel α-helical polypeptide chains wrapped around each other to form a coiled coil [6–8]. In skeletal and cardiac muscles, TM lies in a head-to-tail manner along the grooves of F-actin filaments and works with troponin to confer Ca^{2+} sensitivity to muscle contraction [9]. In non-muscle systems, the purpose of TM is less clearly defined, but it is likely to be relevant to stabilization of F-actin filaments [7].

2. MATERIALS AND METHODS

Horse plasma gelsolin was prepared as previously described [2]. Tropomyosin was prepared from both rabbit skeletal and cardiac muscles [10]. Concentrations of proteins in stock solutions were determined by absorbance measurements using a Perkin Elmer Lambda 4B spectrophotometer. Absorption coefficients of 1.4 $ml \cdot mg^{-1} \cdot cm^{-1}$ [2] at 280 nm for gelsolin and 0.345 $ml \cdot mg^{-1} \cdot cm^{-1}$ [11] at 277 nm for tropomyosin were employed.

Gelsolin was labelled with the sulfhydryl-selective fluorescent reagent, acrylodan (Molecular Probes), as described for modification of tropomyosin [12], to the extent of between one and two acrylodans per gelsolin. Three to five lysyl residues on gelsolin were modified with the amine-selective fluorescent reagent fluorescein-5-isothiocyanate (FITC, isomer I, Molecular Probes) essentially as described for modification of G-actin [13]. Approximately 2 mg of FITC in a minimal amount of 0.1 M NaOH was added to a gelsolin solution in 150 mM KCl, 50 mM HEPES, 1 mM EDTA, pH 8.5, and stirred for 24 h in a dark environment at 4°C. Unreacted FITC was removed by dialysis for 24 h against 150 mM KCl, 25 mM Tris-HCl, 1 mM EDTA, pH 8.0, followed by gel filtration through a 1.3 × 45 cm column of Bio-Gel P-2 (Bio-Rad Laboratories) equilibrated against the same buffer. Reaction of gelsolin with benzophenone-4-isothiocyanate (BITC, Molecular Probes) was carried out as detailed for FITC, except BITC initially was dissolved in a minimal amount of *N,N*-dimethyl-formamide. Microcentrifugation (15 min) to remove precipitated, unreacted BITC was followed by extensive dialysis against 25 mM Tris-HCl, 1 mM EDTA, pH 8.0.

Solutions of BITC-labelled gelsolin were mixed with rabbit cardiac TM in 25 mM Tris-HCl, pH 8.0, and either 1.0 mM $CaCl_2$ or 1.0 mM EDTA. Solutions were placed into a jacketed 1 cm path quartz cuvet (Hellma) and cooled to 4°C using a circulating water bath (Haake). Photolysis was conducted using a 200 W Hg arc-lamp (Osram) and a Corning #7-54 bandpass filter that cuts off light below 250 and above 400 nm. Aliquots of the mixture were removed at various times and examined by SDS-PAGE [14].

A tropomyosin–agarose affinity matrix was prepared using rabbit skeletal TM coupled to Affigel-15 (Bio-Rad Laboratories) according to the procedure recommended by the supplier. The matrix was poured into a 5 ml measuring pipette and equilibrated with 25 mM Tris-HCl, pH 8.0, and either 1 mM EDTA or 1 mM $CaCl_2$ at room temperature. Fluorescently labelled gelsolin was applied in the same buffer until the column was saturated, as detected by the elution of fluorescent gelsolin. The column was washed with the equilibration buffer until no fluorescence was detectable in the eluant. A 0–500 mM gradient of KCl in the equilibration buffer was applied to remove protein that had adhered to the affinity matrix. The fluorescence of the eluant was monitored with a Perkin Elmer LS-5B luminescence spectrometer equipped with a circulating water bath (Haake). The identity of the fluorescent protein eluted was confirmed to be gelsolin by SDS-PAGE.

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Abbreviations: TM, tropomyosin; FITC, fluorescein-5-isothiocyanate; BITC, benzophenone-4-isothiocyanate.

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3. RESULTS AND DISCUSSION

BITC-labelled gelsolin can be photochemically cross-linked to cardiac tropomyosin (Fig. 1). The appearance of low mobility, high molecular mass bands on SDS-polyacrylamide gels confirms that a complex can form between gelsolin and tropomyosin in the calcium-containing buffer employed. When the buffer contained EDTA in place of calcium, only trace amounts of the cross-linked species could be detected (data not shown).

Gelsolin is labelled at multiple sites with BITC, so it is likely that cross-links, if formed, would be made to both chains of an adjacent TM molecule. The migration of such an entity on SDS-polyacrylamide gels could, therefore, correspond to a protein of M_r equal to the sum of the apparent M_r values for gelsolin (90 kDa) and two α-TM chains (33 kDa each). The lower band of the high M_r doublet observed in Fig. 1 for samples that contained both gelsolin and TM corresponds to a protein of M_r 150 kDa. This M_r value has considerable uncertainty associated with it, partly because the ratio of bandwidth-to-distance migrated is great, and partly because of possibly anomalous migration of a multiply cross-linked complex that is expected to involve at least three polypeptide chains. A second source of uncertainty in Fig. 1 is the different banding pattern in the 80–90 kDa region of samples that contained both gelsolin and TM when compared to the lane that contained irradiated BITC-gelsolin alone.

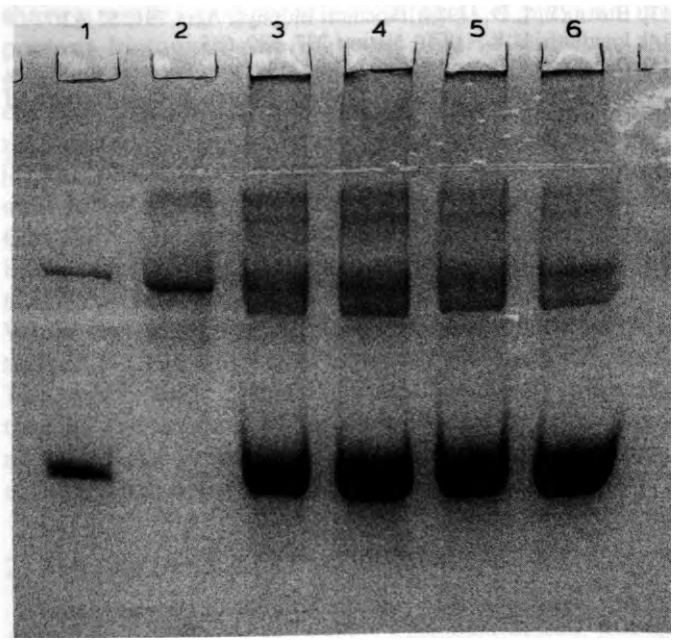

Fig. 1. Photochemical cross-linking of cardiac tropomyosin with BITC-labelled gelsolin. A 10:1 mole ratio of C-TM to BITC-gelsolin was prepared in 25 mM Tris-HCl, 1 mM EDTA, pH 8.0, made up to 2 mM in $CaCl_2$ and irradiated as described in the text. Aliquots were removed at 15, 30, 45 and 60 min and 12 μl samples, each containing 4.9 μg of gelsolin, were subjected to SDS-PAGE (lanes 3–6, respectively). Lane 1: a sample of BITC-gelsolin and C-TM that had not been irradiated; it provides M_r markers of 90 and 33 kDa. Lane 2: 3.6 μg of BITC-gelsolin that had been irradiated for 60 min under the same conditions as samples that contained both BITC-gelsolin and TM.

To provide further evidence for complex formation and to investigate the calcium-sensitivity of the interaction, we next investigated the ability of gelsolin to be retained by a tropomyosin–agarose affinity matrix. Enhanced sensitivity in detection of gelsolin in low concentration solutions was achieved using fluorescently labelled protein. Both FITC- and acrylodan-labelled gelsolin were retained by the affinity column only in the presence of free Ca^{2+}. Elution could be achieved with a salt gradient (Fig. 2). Confirmation that the eluted protein was gelsolin was provided by SDS-PAGE, which resulted in a single fluorescent band at a position matching that of unlabelled gelsolin when viewed after staining with Coomassie blue. The retention of gelsolin at ionic strengths up to approximately physiological levels suggests that the interaction may have a biological function.

Further support for a Ca^{2+}-sensitive interaction between gelsolin and tropomyosin at physiological ionic strength is provided by direct titration of FITC-labelled gelsolin with tropomyosin. Treatment of this data as described by Bagshaw and Harris [15] (Fig. 3) allows an explanation in which two independent sites on gelsolin bind TM. From the mole ratio of total tropomyosin-to-total gelsolin at the half-completion point of the titration, multiplied by the gelsolin concentration at that point, the maximum possible dissociation constant for each site is estimated bo te 0.6 μM.

The idea to look for an interaction between gelsolin and tropomyosin arose from earlier studies in this laboratory to characterize the interaction between tro-

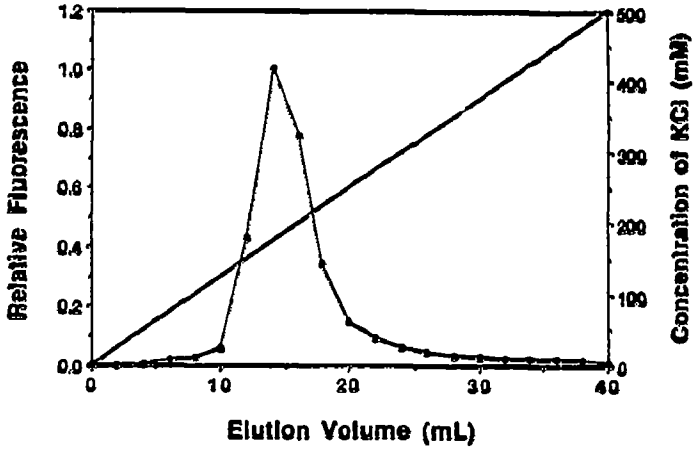

Fig. 2. Elution of FITC-labelled gelsolin from a skeletal tropomyosin-agarose affinity gel. FITC-gelsolin was loaded onto a TM–agarose column equilibrated with 25 mM Tris-HCl, 1 mM $CaCl_2$, pH 8.0. The column was washed with the equilibration buffer until no fluorescence was detectable in the eluant. A KCl gradient (0–500 mM) was applied in the equilibration buffer and 2 ml fractions were collected. The fluorescence intensity at 520 nm of each fraction was measured on excitation at 490 nm at 25°C.

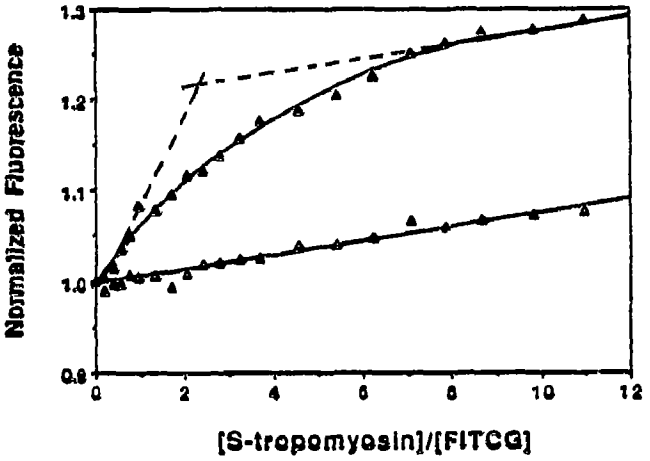

Fig. 3. Titration of FITC-labelled gelsolin with skeletal tropomyosin. The open triangles are data collected on addition of a stock solution of S-TM (24 μM) in 150 mM KCl, 25 mM Tris-HC, 1 mM EDTA, pH 8.0, to a sample containing 0.25 μM FITC-gelsolin in the same buffer. The data were corrected for dilution effects by dividing the fluorescence intensity recorded for each sample by the fluorescence intensity of an identical sample to which buffer alone was added in place of the S-TM solution. The closed triangles are data collected as described above, but for samples in a buffer containing 1 mM $CaCl_2$ in place of EDTA. Extrapolation of the two dashed lined to their intersection [15] allows an estimation of two binding sites on gelsolin for TM. Titrations were performed at 25°C, with 490 nm excitation and 520 nm emission settings on the fluorometer.

pomyosin and another actin-binding protein, DNase I [16]. Is it possible that interaction of tropomyosin with other proteins that affect the stability of F-actin is a general phenomenon? The results of this study with plasma gelsolin contribute evidence for a positive answer to this question. Perhaps in performance of their own actin-related functions, such proteins are assisted by an ability to bind to tropomyosin. The Ca^{2+}-dependent severing activity of gelsolin on actin can be diminished by tropomyosin [17]. Perhaps direct interaction between gelsolin and tropomyosin is a part of this process.

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