Interaction of actin with dansyl-tropomyosin

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5-[Dimethylamino]naphthalene-1-sulfonyl chloride (dansyl chloride) reacts with rabbit skeletal muscle tropomyosin (TM) to yield a highly fluorescent product, DNS-TM. The extent of modification of TM can be regulated over a wide range, 0.3–15.5 dansyl groups per TM, depending upon the temperature and duration of the reaction. However, under all conditions employed, about 15 different fluorescent tryptic peptides of TM were produced. DNS-TM undergoes end-to-end polymerization at low ionic strengths, but to a somewhat lesser extent than unlabelled TM does. DNS-TM also binds muscle F-actin. This interaction may be followed fluorimetrically by observing a blue-shift in emission maximum, an increase in fluorescence intensity or an increase in fluorescence polarization of the DNS-TM complex with F-actin.

Actin *Tropomyosin* *Fluorescence polarization* *Polymerization*

1. INTRODUCTION

Striated muscle TM is a highly helical protein composed of two polypeptide chains, each of 33 kDa, coiled around each other to form a rod some 41 nm long (review [1]). TM binds along the length of F-actin filaments, and in striated muscle, functions with troponin to confer calcium sensitivity to the interaction of myosin with actin (review [2]). TM is also found bound to actin filaments in non-muscle cells (review [3]). Its role in such systems is not well understood.

Each α- and β-chain of TM has 39 lysine residues distributed along its sequence [4]. Many of these lysines are reactive toward acetic anhydride [5,6]. However, other than the high reactivity of Lys 5, 6 and 7 at the amino-terminal, no strong correlation of position in the sequence with reactivity was found. We decided to label TM with a fluorescent reagent, dansyl chloride, which reacts selectively with lysine side chains. We expected random labelling of lysines along the length of TM and thought that such a modified TM should exhibit significant changes in its fluorescence properties when bound to actin.

Abbreviations: TM, tropomyosin; DNS, dansyl, dimethylaminonaphthalenesulfonyl; DTT, DL-dithiothreitol

2. MATERIALS AND METHODS

TM was prepared from frozen rabbit skeletal muscle (Pel-Freez Biologicals) according to Smillie [7]. We used a fraction that eluted from a hydroxyapatite column as a mixture of TM α- and β-chains. Actin was extracted from rabbit skeletal muscle powder as described by Spudich and Watt [8]. Both proteins were greater than 95% pure as judged by polyacrylamide gel electrophoresis in the presence of SDS [9].

Protein concentrations in solutions were determined spectrophotometrically using a molar absorption coefficient at 277 nm of 21780 $M^{-1} \cdot cm^{-1}$ [10] for TM and a molar absorption coefficient at 290 nm of 26230 $M^{-1} \cdot cm^{-1}$ [11] for G-actin. Concentrations of labelled proteins in solution were determined according to Bradford [12]. For each such determination, a standard curve was prepared using unlabelled skeletal TM. The concentration of the label in solutions containing labelled protein was estimated spectrophoto-

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metrically using an absorption coefficient at 340 nm of 43000 $M^{-1} \cdot cm^{-1}$ [13].

Labelling of TM with dansyl chloride (Sigma) was performed by first dissolving the reagent in acetone and adding it dropwise to a stirred solution of TM (1.5–5.0 mg/ml) that had been dialysed against 0.5 M KCl, 40 mM Na borate, 1 mM DTT, pH 8.0. Reaction conditions varied with respect to temperature (0–38°C), time (1–3 h) and molar excess of label to TM (10–100-fold).

Tryptic digestion of the labelled TM and separation of the peptides by thin-layer electrophoresis followed by thin-layer chromatography were carried out as described [14]. Fluorescent spots were viewed under a hand-held ultraviolet lamp.

Viscosity measurements were conducted in a thermostatted water bath held at 27°C in a Cannon-Manning semi-microviscosimeter with a charge of 1.0 ml of solution at protein concentrations near 2.0 mg/ml. The flow time for water in this viscosimeter was about 2 min.

Fluorescence intensity and polarization measurements were recorded using a fluorimeter constructed in this laboratory [15,16]. Polarization in emission, $p = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$, was determined as described by Ehrenberg [17] who used a similar instrument. Excitation of the DNS-TM at 365 nm was achieved using a 200 W Hg arc lamp (Osram), a Spex Micromate monochromator and a Schott UG11 black glass filter. Emission was detected through a Spex Doublemate double monochromator, equipped with a wavelength drive mechanism, after passage through a 1.0 cm path of 2 M $NaNO_3$, which absorbs light of wavelengths shorter than 400 nm. The emission spectra reported here have not been corrected for instrumental response.

3. RESULTS AND DISCUSSION

The extent of incorporation of the DNS label into TM varied with reaction conditions, particularly temperature. When the reaction was performed on ice at a molar excess of label to TM of 20, 0.3 labels were incorporated per TM. At 38°C, 15.5 labels per TM were introduced. Tryptic digestion of DNS-TM, whether labelled extensively or at substoichiometric ratios, produced a large number (about 15 that showed up more or less clearly depending upon the reaction conditions) of fluorescently labelled peptides. It is apparent that our labelling conditions do not result in selective labelling of a particular lysine or small group of lysine residues, but rather produce fractional labelling at a large number of sites.

Modification of lysine residues on TM with acetic anhydride inhibits its ability to undergo end-to-end polymerization at low ionic strengths [5]. In contrast, while DNS-TM does demonstrate a somewhat impaired ability to polymerize, it produces solutions of high relative viscosity at low ionic strength when compared to unlabelled TM (fig.1). Either the conditions of labelling or the effects of labelling are less drastic than those with acetic anhydride.

On excitation at 365 nm, DNS-TM emission was maximal near 515 nm (fig.2). The emission maximum did not vary with extent of labelling.

To follow up on the effects of salt on the viscosity of DNS-TM solutions, we studied polarization in the emission of samples of DNS-TM at various ionic strengths. The wavelength of maximum emission did not alter significantly for samples of ionic strengths from 10 to 300 mM. However, emission intensities declined by nearly a quarter and

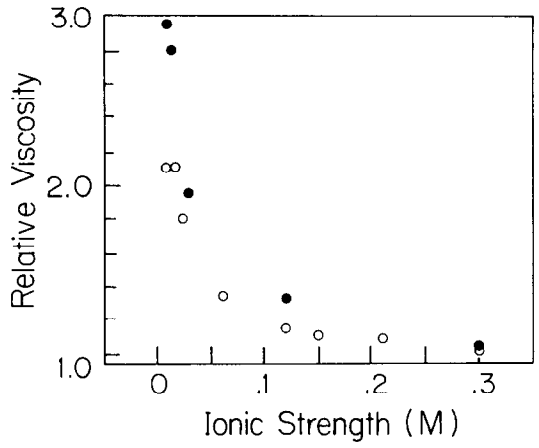

Fig.1. Viscosities of DNS-TM and unlabelled TM solutions. DNS-TM (9.8 labels/TM, ○) and unlabelled TM (●) were dialysed against 2 mM Tris-HCl, 1 mM DTT, pH 8.0. Samples were prepared from these stock solutions by addition of both dialysate and a solution of 3 M KCl such that the final protein concentration in each sample was 1.9 mg/ml and the ionic strength of the sample was at a desired value between 2 and 300 mM. Viscosities were measured relative to that of the dialysate at 27°C.

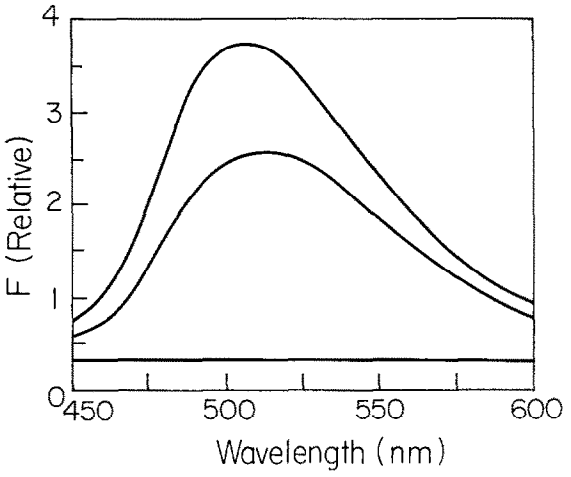

Fig.2. Fluorescence emission spectra. The upper spectrum is that of a sample formed by mixing 1.00 ml of DNS-TM (9.8 labels/TM, 1.64 mg/ml) in its dialysate (0.3 M KCl, 25 mM Tris-HCl, 1 mM DTT, pH 8.0) with 0.20 ml of G-actin (5.25 mg/ml) in its dialysate (2 mM Tris-HCl, 1 mM DTT, 0.2 mM ATP, 0.2 mM $CaCl_2$, pH 7.6). The lower spectrum is that for a sample prepared by mixing 1.00 ml DNS-TM in its dialysate with 0.20 ml of the actin dialysate (no actin present). Each sample was allowed to sit at room temperature for 1 h prior to recording its spectrum in order to allow the actin to polymerize. Excitation was at 365 nm. Excitation and emission bandpasses were each 10 nm.

fluorescence polarization declined from 0.24 to 0.21 over this range of ionic strengths. This is consistent with the presence of extended TM filaments in low ionic strength solutions, as suggested by the viscosity measurements.

Addition of actin to solutions of DNS-TM in 0.5 M KCl, 10 mM Tris-HCl, 1 mM DTT, pH 8.0, caused both an increase in fluorescence intensities and a blue-shift in the emission maximum of the samples (fig.2). In addition, the polarization values for the samples increased with added actin, from $p = 0.21$ in the absence of actin to $p = 0.33$ in its presence. These effects reached plateau levels at actin to TM mole ratios near 7. Clearly, actin interacts with DNS-TM. The filamentous F-actin in these solutions is decorated along its length with DNS-TM molecules. The reduced exposure of some of the DNS labels to solvent on interaction with actin accounts for the blue-shifted and intensified emission. The increased mass and asymmetry of the entity (F-actin plus bound TM) to which the label is bound account for the increased polarization of its fluorescence. It is of interest to note that the observed polarization value for DNS-TM bound to F-actin is the same as that observed by Cheung et al. [18] for F-actin labelled directly with dansylcystine.

4. CONCLUSIONS

Dansyl chloride reacts with TM at several sites along its length. The extent of labelling can be regulated by altering the temperature or duration of the reaction, but the specificity of the reaction is not improved by lowering the extent of reaction. DNS-TM retains to a great extent its ability to polymerize in an end-to-end fashion at low ionic strengths. Addition of salt disrupts the interaction, as observed in viscosimetric and fluorescence polarization studies. DNS-TM also retains its ability to bind to actin filaments. This interaction can be followed by observing changes in the wavelength of maximal emission. The intense fluorescence and the ability of the DNS labels to report on these properties of muscle TM will make DNS-TM a useful tool in the study of TM interactions with actin as they pertain to the regulation of muscle contraction or to the regulation of actin filament assembly and disassembly in non-muscle systems.

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