CONFORMATIONAL CHANGES IN TROPONIN INDUCED BY Ca^{++}[1]

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Received November 17, 1969

SUMMARY

Troponin undergoes reversible changes in fluorescense emission intensity and emission maximum when Ca^{++} concentration is varied between 10^{-5} and 10^{-4}M at pH 7.5 and 25°. These changes suggest infolding of tryptophan fluorophores of the protein into hydrophobic regions as Ca^{++} concentration is increased. A Ca^{++}-induced change in light scattering also occurs, indicating an aggregation of the protein, but the dependency on Ca^{++} concentration and the kinetics of the aggregation process suggest that it follows in time the Ca^{++}-induced conformational changes in troponin.

The contractile state of muscle appears to be governed by changes in Ca^{++} concentration in the myofibrillar space (1,2,3). This control is mediated through troponin, a protein component of the thin myofilaments, which has a unique affinity for Ca^{++}(4).

Thin filaments are composed of at least three proteins: F-actin, tropomyosin and troponin (4). Using spin-labelling techniques, Tonomura et. al. (5) have recently found evidence to support the suggestion that the force-generating interaction between F-actin and myosin of thick myofilaments, the fundamental contractile process of muscle, is influenced by Ca^{++}-induced conformational changes in the troponin-tropomyosin system. Wakabayashi and Ebashi (6) found that aggregation of troponin was influenced by variation in Ca^{++} concentration.

We studied, using ultraviolet fluorescence emission (7,8), the effect of Ca^{++} on the conformation of troponin in the absence of tropomyosin and found evidence of a reversible conformational change in troponin depending on Ca^{++}

1. Supported in part by USPHS grants HE-01584 and GM13793 and by grants from the Graduate School of the University of Minnesota.

concentration. An aggregation of troponin followed the Ca^{++}-induced changes in conformation.

METHODS

Troponin and tropomyosin were prepared from ethanol-treated rabbit skeletal muscle by methods described by Yasui et. al. (9) using isoelectric and ammonium sulfate precipitation. The procedures were slightly modified, omiting dithiothreitol; thus, the extracted proteins probably correspond to S-S troponin and S-S tropomyosin (9). In the final step of the procedure, the protein solutions were dialyzed against 2mM Tris-HCl buffer (pH 7.5). The resulting preparations were soluble in the dialyzing medium, but tropomyosin in high concentration was highly viscous; accordingly, the protein solution was diluted with either 2mM Tris-HCl buffer or 1M KCl solution before use to obtain a final protein concentration of 0.2 to 0.5 mg/ml. All protein preparations appeared to be homogenous on acrylamide gel electrophoresis. A 1:1 complex of the two proteins demonstrated an inhibitory effect on superprecipitation of synthetic actomyosin in the presence of 0.1mM EGTA (9). We observed that 0.1 mg/ml of the protein complex caused about 60 per cent inhibition of superprecipitation.

Ultraviolet spectra were determined in a Cary 15 spectrophotometer. Fluorescence emission spectra and the amount of light scattering were measured in an Aminco Bowman spectrophotofluorometer. Excitation wave lengths were set at 275 mμ and 290 mμ for tyrosine and tryptophan fluorescence respectively. All experiments were carried out in 2mM Tris-HCl buffer (pH 7.5) at 25°. Final concentrations of tropomyosin and troponin used were about 0.2 - 0.5 mg/ml and 0.1 - 0.2 mg/ml respectively.

RESULTS AND DISCUSSION

Ultraviolet absorption spectra of troponin and tropomyosin are seen in Fig. 1. Tropomyosin has an absorbance maximum at 275 mμ and the spectrum is similar to that of tyrosine. Troponin, however, showed a 260 mμ absorbance maximum, which can be attributed to nucleotides in troponin A (10) or

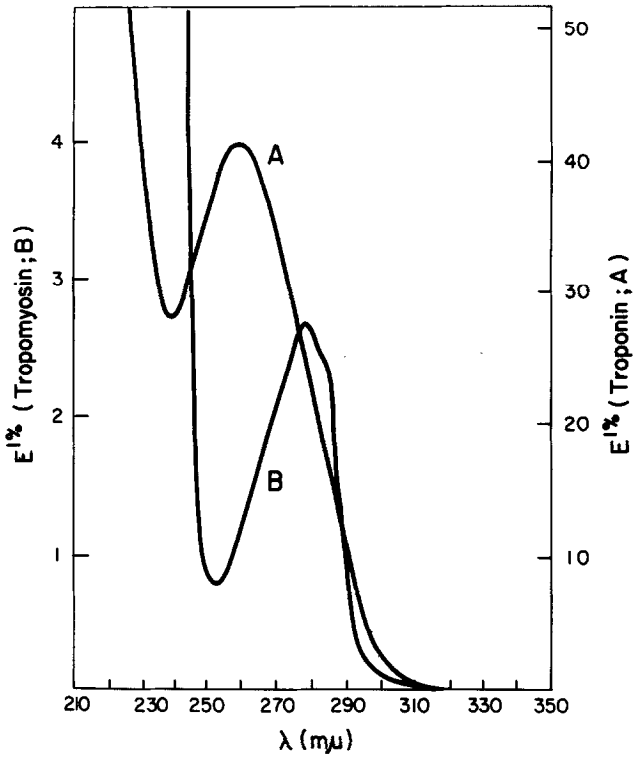

Figure 1. UV spectra of troponin (A) and tropomyosin (B). Troponin was diluted in 2mM Tris-HCl buffer (pH 7.5) and tropomyosin in the same buffer containing 1 M KCl.

ribonucleic acid which has been found in myofibrils bound to myofibrillar proteins (11,12).

The fluorescence emission spectrum of tropomyosin showed a typical tyrosine fluorescence with the emission maximum at 305 mμ as demonstrated by Cowgill (8). On the other hand, troponin demonstrated an emission peak at 352 mμ which is close to that of tryptophan in aqueous solution (355 mμ). Since fluorescence yield of nucleotides or nucleic acids is very low at neutral pH (13), the fluorescence emission may be accredited to tryptophan residues of troponin. It was very difficult to estimate relative quantum yields of troponin without obtaining correct estimation of protein absorption at 290 mμ. Relative fluorescence yields of troponin varied from 20 to 40% of that of tryptophan. The variation in yield depended on the content of

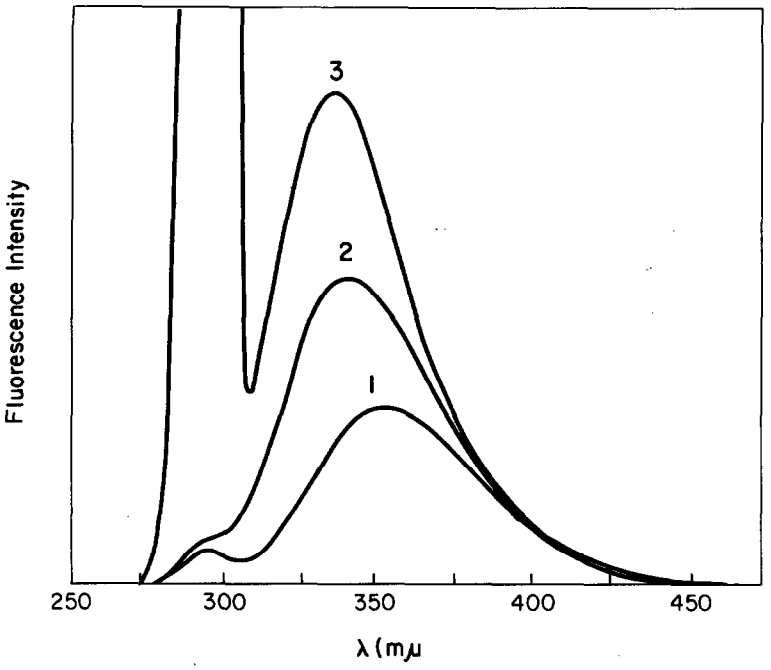

Figure 2. Effect of Ca^{++} on the fluorescence of troponin. Fluorescence was determined at pH 7.5 and 25°. Troponin concentration used was 0.1 mg/ml and Ca^{++} concentrations were as follows: (1) no Ca^{++} added, (2) 9.2×10^{-5}M, and (3) 2.3×10^{-4}M.

the 260 mμ absorbing component.

As shown in Fig. 2, the fluorescence intensity of troponin increased on addition of Ca^{++} and the emission maximum shifted toward the blue. These observations suggest that Ca^{++} induces a conformational change in troponin affecting the electronic properties of the tryptophan fluorophores.

Relative changes in fluorescence of troponin and shifts in emission maximum as a function of Ca^{++} concentration are seen in Fig. 3-a. Changes in emission intensity are noted at a concentration of Ca^{++} of 4×10^{-5}M and the fluorescence change reached maximum at a concentration of 2×10^{-4}M. With increase in fluorescence emission, there was progressive shift of the emission maximum from 352 mμ to 337 mμ. Fig. 3-a also shows a sharp transition curve for the Ca^{++}-induced fluorescence changes suggesting that the conformational change in troponin is highly cooperative in type. From a Hill plot (14)

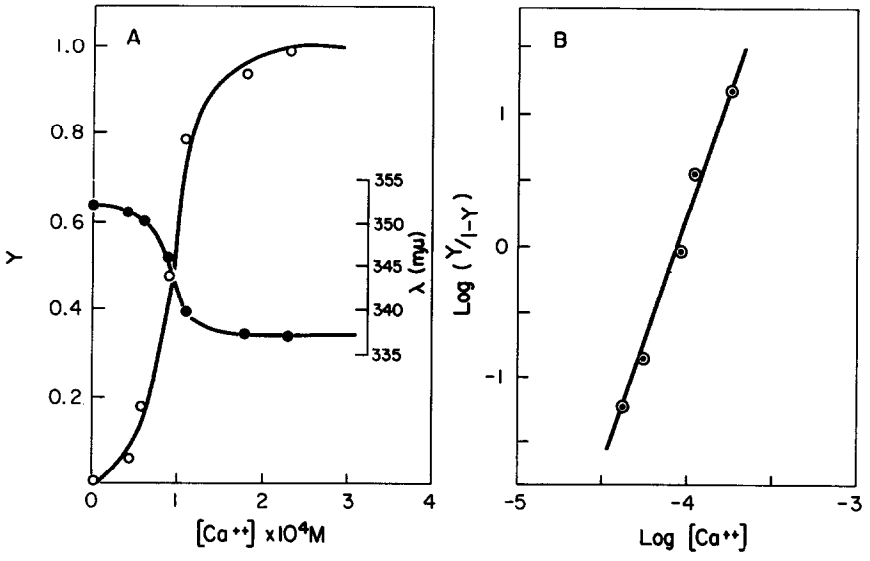

Figure 3. (a) Cooperative transition of the Ca^{++}-induced fluorescence changes. Relative fluorescence emission (o-o) and emission maxima (●-●) were plotted as a function of Ca^{++} concentration. Experimental conditions were the same as in Fig. 2. Y indicates relative fluorescence emission.

(Fig. 3-b), we have estimated that n=4 and the apparent dissociation equilibrium constant for the troponin-Ca^{++} complex is thus about 9 x 10^{-5}M.

Kinetic patterns of the changes in fluorescence and light scattering are seen in Fig. 4. This experiment demonstrates that change in light scattering follows the change in fluorescence emission. The half-time of change in fluorescence intensity is 20 seconds whereas that of the change in light scattering is about 4-5 minutes in the presence of 2.3 x 10^{-4}M Ca^{++} at pH 7.5 and 25°. There was no increase in light scattering at Ca^{++} concentrations lower than 2 x 10^{-4}M Ca^{++}. The Ca^{++}-induced changes in fluorescence emission and in light scattering of troponin were reversed by addition of an excess amount of EGTA (5 x 10^{-4}M) or by adjusting KCl concentration to 0.05M.

The kinetic results suggest that the protein will aggregate in the presence of a relatively high concentration of Ca^{++} after completion of Ca^{++}-induced conformational changes. Ca^{++}-dependent aggregation of troponin has been demonstrated by Arai and Watanabe (15). Wakabyashi and Ebashi (6) on

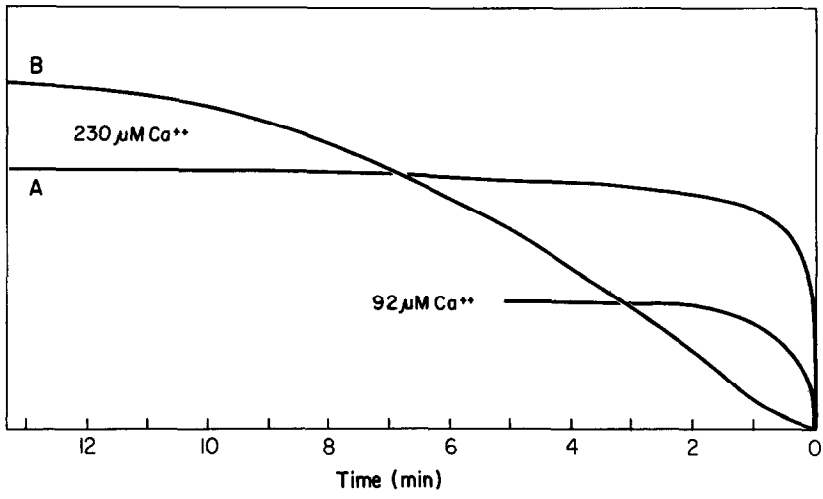

Figure 4. Time-dependent changes in fluorescence and light scattering of troponin induced by Ca^{++}. (This fugure shows actual kinetic traces on a strip-chart recorder which provides the time sequences from right to left. Ordinate is in an arbitrary relative unit.) 0.18 mg/ml of troponin was used. Experiments were carried out at pH 7.5 and 25°. (A): fluorescence change at 245 mu and (B): light scattering change at 290 mu. There was no observable light scattering changes at concentration of Ca^{++} of 9.2×10^{-5}M.

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the other hand, reported that Ca^{++}-free troponin tends to form an aggregate, whereas, the aggregation is suppressed in the presence of a trace of Ca^{++} due presumably to a conformational modification of the protein.

The characteristic fluorescence changes in troponin described above are similar to those of the tryptophan fluorophores exposed to differing dielectric environments (16), suggesting that the tryptophan fluorophores are increasingly folded within the protein, presumably in hydrophobic regions, when binding of Ca^{++} occurs. Thus, at very low concentrations of Ca^{++}, concentrations at which the protein-Ca^{++} complex is dissociated, the tryptophan residues lie in a region of the protein exposed to solvent. As Ca^{++} concentration is increased and, thus, concentration of troponin-Ca^{++} complex increases, troponin assumes an increasingly compact conformation, reflected by changes in fluorescence properties of the tryptophan residues.

We have failed to demonstrate changes in tyrosine fluorescence of

tropomyosin and in tryptophan fluorescence of F-actin on addition of Ca^{++} under the same conditions applied to troponin. It was observed, however, that increasing concentration of Ca^{++} (in the range of 10^{-3} - 10^{-2}M) can cause changes in light scattering due presumably to increased aggregation of these proteins. The aggregation of F-actin and tropomyosin by Ca^{++} was reversible on addition of 0.1 M KCl.

The conformational change in troponin induced by Ca^{++} which our studies suggest is probably related in a highly specific way to the characteristic Ca^{++}-dependent effect of troponin on actomyosin adenosine triphosphatase and, thus, on the force-generating interaction between F-actin and myosin in the contractile system of muscle. It is possible that the effect of this conformational change is transmitted via tropomyosin to the sites of F-actin-myosin interaction in the myofilaments as suggested by Tonomura et. al. (5).

The effect of the presence of the nucleotide or nucleic acid moiety on the Ca^{++}-induced changes in the conformation of troponin is not clear at this time. We are studying the identity of this component and its significance to the Ca^{++}-dependent properties of troponin.

We wish to express our thanks to Miss Karen Schroeder for her assistance in preparation of troponin and tropomyosin.

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