Troponin-C-mediated Calcium-sensitive Changes in the Conformation of Troponin I Detected by Pyrene Excimer Fluorescence*

Gale M. Strasburg‡, Paul C. Leavis, and John Gergely

From the Department of Muscle Research, Boston Biomedical Research Institute, Boston, Massachusetts 02114, Departments of Neurology and Biological Chemistry, Harvard Medical School, and Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts 02114

(Received for publication, June 4, 1984)

Troponin I (TnI) from rabbit white skeletal muscle was labeled its cysteines 48 and 64 with the fluorescent reagent N-(1-pyrene)maleimide. The fluorescence spectra of pyrene-labeled TnI (pyr-TnI) exhibit peaks characteristic of pyrene in its monomeric form and an additional peak resulting from formation of excited dimers (excimers), indicating that the labeled cysteines are close together. Formation of a pyr-TnI-TnC complex in the absence of Ca²⁺ has little effect on the spectrum, but when Ca²⁺ is bound to the low-affinity sites of TnC there is a substantial decrease in excimer and a corresponding increase in monomer fluorescence. The involvement of the low-affinity sites in the Ca²⁺-induced effect is consistent with the fact that Mg²⁺ has no effect on pyrene fluorescence. On rapid mixing of the pyr-TnI-TnC complex with Ca²⁺ in a stopped-flow apparatus, most of the excimer decrease is complete within the instrumental dead time, indicating a rate constant \( k > 350 \text{ s}^{-1} \), which is comparable to that of the conformational change in TnC resulting from Ca²⁺ binding to the low-affinity sites. Rapid mixing of the Mg₂⁻TnC-pyr-TnI complex with Ca²⁺ yields similar results, suggesting that the type of metal ion present at the high-affinity sites has little, if any, effect on the probe. It has been suggested previously that Cys 48 and 64 are located in a TnT-binding region of TnI (Chong P. C. S. and Hodges, R. S. (1982) J. Biol. Chem. 255, 3757). Our results suggest that a Ca²⁺-induced structural change in the TnI-binding region of TnC could be transmitted to TnT by affecting the TnT-binding region of TnI as part of the chain of events in the regulation of muscle contraction.

Contraction of vertebrate striated muscle is regulated by the troponin-tropomyosin complex located in the thin filaments (1). Troponin is a protein composed of three subunits (2, 3). TnC is the Ca²⁺-binding moiety, and TnT has been shown to bind to tropomyosin. TnI inhibits actomyosin ATPase, but it should not be assumed that with the troponin complex inhibition of activation of myosin ATPase by actin is solely attributable to TnI. TnI binds to actin filaments and to actin-tropomyosin filaments (4, 5), inhibiting actin-activated actomyosin ATPase activity. Ca²⁺ initiates the contraction process by binding to TnC and inducing changes in its secondary and tertiary structure (6-11). These changes are somehow transmitted from TnC to the other troponin subunits, to tropomyosin, and to actin, resulting in activation of contractile ATPase and tension development of muscle (1, 12). With a view toward elucidating the molecular mechanisms involved in the transmission of the contraction signal, we have attempted to clarify the role of TnI in thin-filament regulation through the study of conformational changes in TnI in response to Ca²⁺-binding by TnC.

TnI from rabbit white skeletal muscle is a basic protein consisting of 178 amino acids, \( M_r = 20,700 \) (13). Although TnI occurs in muscle as a ternary complex with TnC and TnT, it does form binary complexes with TnT (14) and with TnC (15). A fragment of TnI (residues 96-116) produced by cyanogen bromide cleavage binds both to actin, with substantial inhibitory activity, and to TnC (16). Another fragment containing residues 1-20 also binds to TnC (16). Evidence for the functional importance of the cysteine thiol groups of TnI came from the work of Horwitz et al. (14) showing that a biologically active troponin complex can only be formed if the sulfhydryl groups of TnI are kept fully reduced. Subsequently, Chong and Hodges (17) suggested on the basis of sulphydryl modification studies that Cys 48 and 64 are in a region which is a binding site for TnT, while Cys 133 is exposed to solvent in both binary and ternary complexes. Studies of lysine reactivities also suggest that the portion of TnI consisting of residues 40-98 contains a binding site for TnT (18).

The fluorescent reagent N-(1-pyrene)maleimide has been shown by Lehrer and his colleagues (19-21) to be a useful probe of sulphydryl proximity and conformational change by virtue of an emission peak corresponding to excited dimers (excimers). Preliminary studies showing disulfide formation in TnI whose Cys 133 had been blocked with iodoacetamide labeled TnI; DABMA, 4-dimethylaminophenylazophenyl-4'-maleimide; IAEDANS, N'-iodoacetyl-N'-[(5-sulfo-1-naphthyl)dithylenedia- mine; CM-DAB-TnI, troponin I labeled with iodoacetamide and DABMA; HPLC, high-performance liquid chromatography; DAB-TnI, TnI labeled with DABMA in the ternary complex using conditions for labeling Cys 133; IAEDANS-DAB-TnI, troponin I labeled with IAEDANS and DABMA; NTA, nitriloacetic acid; IAANS, 2-(4'-iodoacetamido)aminino)napththalene-1-sulfonic acid; MBS, 2-(N-morpholino)ethanesulfonic acid; HEPES, 4-(2-hydroxyethyl)1-piperazineethanesulfonic acid; Bicine, N,N-bis(2-hydroxyethyl)glycine; SDS, sodium dodecyl sulfate; PMSF, phenylmethanesulfonfluoride.
Ca2+-sensitive Pyrene Excimer in the TnI-TnC Complex

suggested that Cys 48 and 64 are close together and that the pyrene label would be a useful structural probe of TnI. In these studies, we have labeled Cys 48 and 64 with the pyrene label. The spectrum is indicative of a pyrene excimer. The spectrum of labeled TnI complexed with TnC shows Ca2+-sensitive changes. The range of effective Ca2+ concentrations and the lack of a Mg2+ effect on the excimer suggest that the low-affinity Ca2+-binding sites of TnC (22) are responsible for the change.

EXPERIMENTAL PROCEDURES

Protein Preparation—Troponin was prepared using the procedure of Greser and Gergely (3), followed by chromatography on an Affi-Gel blue column (23). Troponin subunits were isolated as previously described (3). Purified proteins were stored in 6 M urea at −10 °C.

Protein concentrations were determined by absorbance at 280 nm, subtracting the absorption at 320 nm to correct for light scattering. The following absorbance values, A (1% 280 nm, 1 cm), were used: TnT, 4.58; TnI, 3.97; and TnC, 1.59. The protein concentration of pyrene-labeled TnI was determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA) with unlabeled TnI as the standard.

Protein Labeling—In order to label TnI with pyrene at Cys 48 and 64, it was first necessary to block Cys 133; this was done by reacting the troponin complex with iodoacetamide according to the procedures of Glauert and Hunt (24). After quenching with dithiothreitol, CM-TnI was isolated (3). The sulfhydryl content of the CM-TnI, determined by the Ellman procedure (25), was 2.0 ± 0.1 mol/mol. CM-TnI was rechromatographed on a Sephadex G-25 column equilibrated with 0.5 M KCl, 25 mM MES, pH 6.0. Solid GdmCl was added to a final concentration of 5 M to the pooled fractions containing CM-TnI (1–3 mg/ml), followed by addition of N-(1-pyrene)maleimide dissolved in dimethyl formamide (1 mg/ml) in a 20:1 molar ratio. The mixture was stirred for 4 h at room temperature; dithiothreitol was then added to quench unreacted label and the solution was stirred overnight at 4 °C. The reaction mixture (containing TnI, KCl, and MES) was applied to a Sephadex G-25 column using 0.5 M KCl, 25 mM MES, pH 6.0, as the eluant, and the labeled protein was dialyzed exhaustively against the same buffer to remove any remaining unreacted label.

Previous work has shown that the spectral properties of pyrene-labeled proteins are influenced by the state of the succinimidio moiety (19–21, 24). At pH < 6.0, the ring remains intact (pyr-TnI), giving rise to characteristic emission peaks. At higher pH, the ring opens by hydrolysis or aminolysis (24) and there is a red shift of about 10 nm in the emission peaks (19–21). Pyr-TnI was converted to an open-ring derivative (pyr-TnI-d) by addition of solid GdmCl to 5 M and adjusting the pH to 8.0 with bicarbonate buffer. After allowing the solution to stand at room temperature for 24 h, this solution was stored at −10 °C. Aliquots were dialyzed as needed against the desired buffer.

The degree of labeling, determined spectrophotometrically using a value of ε = 2.3 × 104 M−1 cm−1 at 345 nm (20) and assuming the same extinction coefficient for pyr-TnI and pyrII-TnI, was 3.9 ± 0.1 mol of pyrene/mole of TnI.

Other types of modified TnI were prepared for analysis and verification of the locations of the pyrene labels: 1) CM-TnI (carboxymethylated at Cys 133) was allowed to react with the chromophore DABMA (cf. Ref. 26) under the conditions used for making pyr-TnI to yield the product CM-DAB-TnI (21). TnI labeled in the ternary complex with DABMA using conditions for labeling Cys 133 (17) and will be referred to as DAB-TnI. DABMA, dissolved in dimethyl formamide (1 mg/ml), was added to a 10:1 molar ratio to the troponin complex, and after 4 h at room temperature, the reaction was quenched with dithiothreitol. Labeled TnI was isolated by the procedure described above (17). After quenching the reaction with dithiothreitol.

Characterization of Labeled Protein—Proteolytic fragments of the DAB-TnI and CM-DAB-TnI preparations were analyzed to verify the locations of the pyrene labels. The labeled TnI preparations were dialyzed against 50 mM NaHCO3 and subjected to limited proteolysis by trypsin (1:1, w/w) in the presence of dithiothreitol, Worthington Biochemicals, 1:50, w/w for 4 h at 37 °C. The digests were stopped by adding a 100-fold molar excess of PMSF and the reaction mixtures were lyophilized. The peptides were dissolved in 0.1% trifluoroacetic acid and analyzed by HPLC (Beckman Instruments) using a C18 Bondapak reversed-phase column (Waters Associates) with a linear gradient of 0–7% acetonitrile over 30 min. The eluent was monitored simultaneously at 230 and 355 nm. DAB-labeled peaks were collected, freeze-dried, and subsequently hydrolyzed in 6 n HCl in vacuo at 110 °C for 20 h. Amino acid analysis was carried out on a Beckman Model 119-CL amino acid analyzer.

The ellipticities of the dichroic moments of DAB-TnI were carried out on labeled and unlabeled TnI (0.2 mg/ml of protein concentration) in solutions containing 0.15 M KCl, 25 mM Pipes buffer (pH 6.8), and 0.5 mM dithiothreitol. Scans were taken between 240 and 200 nm at 25 °C using a path length of 1 mm on a modified Cary Model 60 instrument (Aviv Circular Dichroism Spectropolarimeter, Model 60 DS).

The biological activity of pyr-TnI and reconstituted troponin containing pyr-TnI were measured by determining the actomyosin ATPase activity in the presence of tropomyosin and either Ca2+ or EGTA. One-m1 samples containing 0.2 mg of myosin subfragment 1 (kindly donated by Dr. R. Lu, Department of Muscle Research, Boston Biomedical Research Institute), 0.04 mg of F-actin, 0.02 mg of tropomyosin, 0.006 mg of TnC, 0.01 mg of TnI, and 0.006 mg of labeled or unlabeled TnI were incubated for 5 min at 25 °C in a solution containing 10 mM Tris-HCl, pH 7.5, 1 mM ATP, 1 mM MgCl2, 30 mM KCl, and either 1 mM EDTA or 0.1 mM CaCl2. The reaction was started by the addition of the ATP and stopped by the addition of 2 ml of 2% SDS, and the liberated phosphate was determined by the method of Fiske and SubbaRow (27).

Fluorescence Measurements—Fluorescence emission and excitation spectra were obtained using a Spex Fluorolog 2 spectrofluorometer in the ratio mode to correct for lamp variations. Corrections were made for solvent scattering and the wavelength dependence of the response of the instrument. Slit widths of 12.5 nm (2.25 nm bandwidths) were used for both excitation and emission.

Ca2+-titrations of pyr-TnI-TnC complexes were performed using a Perkin-Elmer MFP-4 spectrofluorometer with slits set for bandwidths of 2 nm (excitation) and 8 nm (emission). The proteins were dissolved in 0.1 M KCl, 50 mM HEPES, pH 7.30, 2 mM EGTA, 2 mM NTA. The concentration of pyr-TnI was 1–3 μM, and 4–10 molar ratios of TnC:TnI were used. CaCl2 (0.1 mM) was added using a Hamilton microsyringe, corrections were made for dilution, the pH was monitored during titrations, and the program of Prigozhin and Sayce (28) was used to calculate free Ca2+ concentrations.

The logarithms of the binding constants used were as follows: H+ + EGTA2−, 9.46; H+ + HEGTA2−, 8.85; H+ + H2EGTA2−, 2.68; H+ + H2EGTA3−, 2.00; Ca2+ + EGTA2−, 11.00; Ca2+ + HEGTA2−, 5.33; H+ + NTA2−, 10.33; H+ + HNTA2−, 2.94; H+ + H3NTA, 0.66; and Ca2+ + NTA2−, 7.61 (29).

 Stoichiometric titrations of the pyr-TnI-TnC complex with Ca2+ were carried out after exhaustively dialyzing the complex (15–20 μM) against 0.1 M KCl, 25 mM HEPES, 2 mM EDTA, pH 7.5, followed by dialysis against 0.1 M KCl, 25 mM HEPES, 1 mM EDTA. Microfiltration analysis of CaCl2 (1 mM) were added and excimer fluorescence was monitored.

Stopped-flow fluorescence experiments were performed on a Di- onex Model 13000 spectrofluorometer. In one set of experiments, pyr-TnI-TnC, dissolved in Mg2+-buffer (0.1 M KCl, 25 mM HEPES, pH 7.5, 5 mM MgCl2, 1 mM EGTA), was mixed with an equal volume of Ca2+-buffer (0.1 M KCl, 25 mM HEPES, pH 7.5, 0.6 mM CaCl2, 5 mM MgCl2). In another set of experiments, pyr-TnI-TnC, dissolved in Ca2+-free buffer (0.1 M KCl, 25 mM HEPES, pH 7.5, 1 mM EGTA), was mixed with an equal volume of Ca2+-buffer (0.1 M KCl, 25 mM HEPES, pH 7.5, 0.6 mM CaCl2). Excimer fluorescence was monitored using a Corning 9-72 filter to cut off monomer fluorescence.

RESULTS

Separation and Analysis of the Labeled Peptides—Since we assumed, following Chong and Hodges (17), that the native thiol reactive in the ternary complex is Cys 133, we wished to confirm that the sites of reaction of TnI with a maleimide compound, after blocking the reactive thiol in the ternary complex, are restricted to Cys 48 and Cys 64.

According to the amino acid sequence of TnI (13), Cys 48 and 64 belong to the same tryptic peptide (41–65) while Cys 133 belongs to a different tryptic fragment (residues 132–137). The HPLC elution profile of DAB-TnI tryptic peptides monitored at 538 nm shows a single peak corresponding to 36% acetonitrile concentration (Fig. 1A). In the case of CM-DAB-
produced at pH 6.0, exhibits the fluorescence peaks at 376, the latter case, the Ca²⁺-dependent release of ATPase incorporated into the ternary troponin complex (see Table 3). The fluorescence maxima of the pyrI-TnI, whose Cys 133 is blocked, is labeled with DABMA, the DAB-labeled fractions contain Cys 48 and 64, suggesting that the stoichiometric titration which shows that very little change occurs on adding 2 Ca²⁺/mol of TnC and that most of the fluorescence change occurs between 2 and 4 mol of Ca²⁺/mol of TnC (Fig. 5).

Pyrene-labeled TnI retains its ability to inhibit the hydrolysis of ATP by actomyosin both alone and when it is incorporated into the ternary troponin complex (see Table I). In the latter case, the Ca²⁺-dependent release of ATPase inhibition is similar to that of unmodified troponin. These results suggest that the presence of the pyrene labels on TnI did not induce changes in the protein that grossly affect its secondary structure or biological activity.

Fluorescence Properties of the TnI Derivatives—PyrI-TnI, produced at pH 6.0, exhibits the fluorescence peaks at 376, 396, and 416 nm, characteristic of the pyrene monomer, and an additional broad peak at 475 nm, characteristic of the pyrene excimer (Fig. 2). The fluorescence maxima of the pyrI-TnI monomer, formed at pH 8.5, are shifted to 386, 406, and 426 nm and that of the excimer, to 487 nm. The excimer:monomer ratio for pyrI derivative is considerably higher than for pyrI-TnI, as is the case with the corresponding pyrene derivatives of tropomyosin (19). In what follows, data are shown only for pyrI-TnI; similar results were obtained with pyrI-TnI.

Effects of TnC and TnT on pyrI-TnI Fluorescence—Addition of TnC in the absence of Ca²⁺ to pyrI-TnI had little effect on monomer and excimer peaks. Addition of Ca²⁺ to this complex caused a 25% decrease and a 5-nm blue shift of the excimer peak with a corresponding increase in the monomer peak (Fig. 3). Addition of Mg²⁺ in the absence of Ca²⁺ had no effect on excimer fluorescence, although there was a slight (5%) decrease in monomer fluorescence (not shown). Ca²⁺ titration of the pyrI-TnI-TnC complex in the presence of an EGTA/NTA buffer system shows that the decrease in excimer occurs in the Ca²⁺ concentration range corresponding to the dissociation constant of Ca²⁺ from the low affinity sites of TnC (pCa ≈ 6.22, Fig. 4). These results are confirmed by the stoichiometric titration which shows that very little change occurs on adding 2 Ca²⁺/mol of TnC and that most of the fluorescence change occurs between 2 and 4 mol of Ca²⁺/mol of TnC (Fig. 5).

Stopped-flow experiments were performed to study the kinetics of the response of pyrene-TnI excimer to Ca²⁺-binding by TnC. When pyrI-TnI-TnC was rapidly mixed with...
buffer containing Ca\(^{2+}\), a biphasic decrease in excimer fluorescence was observed (Fig. 6). About 80% of the total fluorescence change occurred within the mixing time of the instrument (2 ms) indicating a rate constant \(k_1 > 350 \text{ s}^{-1}\). The remaining change was a slower process with a rate constant \(k_2 = 11 \text{ s}^{-1}\). When pyr-Tnl-TnC in Mg\(^{2+}\) buffer was mixed with Ca\(^{2+}\) buffer, a similar biphasic change was found (\(k_1 > 300 \text{ s}^{-1}\), \(k_2 = 9 \text{ s}^{-1}\), data not shown).

Addition of Tnl to pyr-Tnl (10 mol/mol) had no effect on pyrene fluorescence. However, addition of Tnl to the pyr-Tnl-TnC complex, produced a 20% drop in monomer fluorescence, while having little change in excimer. This suggests that the binding of Tnl to the complex in some way alters the local milieu of one or both of the pyrene fluorophors without significantly affecting the distance between them or their positions relative to one another. Changes in excimer fluorescence of the ternary complex induced by Ca\(^{2+}\) were similar to those of the pyr-Tnl-TnC complex.

**DISCUSSION**

The presence of an excimer component in the fluorescence spectrum of Tnl labeled at Cys 48 and 64 shows that these residues are close to each other. The increased yield of excimer with pyr-Tnl compared with the case of pyr-Tnl probably results from opening of the succinimidio ring in the former (21, 24). The opening of the ring would reduce the constraints that might interfere with stacking interactions of the pyrene moieties (cf. Ref. 21).

Binding of Ca\(^{2+}\) by the low-affinity sites of the TnC induces a strong decrease in excimer fluorescence, suggesting that the pyrene molecules are pulled further apart by a conformational change affecting the region of TnI that contains Cys 48 and 64. Little, if any, effect is seen upon Mg\(^{2+}\)- or Ca\(^{2+}\)-binding to the high-affinity sites. The structural changes observed here are consistent with earlier studies showing that inhibition of actomyosin ATPase in reconstituted myofibrils is reversed as Ca\(^{2+}\) is bound to the low-affinity sites of TnC (12).

Addition of TnlT to pyr-Tnl had no effect on the fluorescence spectrum. It is possible that TnlT could not bind to Tnl because the two very bulky, hydrophobic pyrene groups directly block the TnlT binding site or because the presence of the labels induces a substantially reduced affinity of Tnl for TnlT.

Our kinetic studies of the response of pyr-Tnl-TnC to Ca\(^{2+}\)-binding indicate a biphasic change in conformation. The large, rapid conformational change in pyr-Tnl induced by Ca\(^{2+}\) binding is nearly complete within the dead time of the instrument (\(k > 350 \text{ s}^{-1}\)), as is the structural change in TnC resulting from binding of Ca\(^{2+}\) to the low-affinity sites (31). This induced structural change in Tnl is sufficiently rapid to be involved in the regulatory mechanism. The basis for the slow change (\(k = 11 \text{ s}^{-1}\)) is not clear. The slow change could result from Ca\(^{2+}\) binding to the high-affinity sites, although no change in excimer was observed in the Ca\(^{2+}\) titration experiments in the pCa region corresponding to the high-affinity sites (Fig. 3). We cannot, however, exclude the possibility that the high-affinity sites are responsible for some structural change when Ca\(^{2+}\) is bound to the low-affinity sites. Alternatively, the excimer change results only from binding of Ca\(^{2+}\) to the low-affinity sites, but the ensuing conformational change itself is biphasic.

Robertson et al. (30) have shown in modeling studies that complete Mg\(^{2+}\)-Ca\(^{2+}\) exchange at the high-affinity sites of TnC, at which Mg\(^{2+}\) is bound in resting muscle, would be too slow to occur within the time that the peak of muscle twitch tension is reached. Hence, they suggest that Ca\(^{2+}\) binding at

**FIG. 5. Stoichiometric titration of Pyr-Tnl-TnC with Ca\(^{2+}\).** Excimer fluorescence monitored at 480 nm; excitation was at 345 nm. Conditions: 0.1 M KCl, 25 mM HEPES, pH 7.5, 1 \(\mu\)M EGTA. [Pyr-Tnl-TnC] = 15 \(\mu\)M.

**FIG. 6. Stopped-flow fluorescence studies of Pyr-Tnl-TnC.** A, mixing of Pyr-Tnl-TnC, dissolved in 0.1 M KCl, 25 mM HEPES, pH 7.5, 0.5 mM EGTA, with the same buffer; B, mixing of Pyr-Tnl-TnC, dissolved in 0.1 M KCl, 25 mM HEPES, pH 7.5, 0.5 mM EGTA, with 0.1 M KCl, 25 mM HEPES, pH 7.5, 0.5 mM EGTA, 0.6 mM Ca\(^{2+}\). Initial [Pyr-Tnl] = 2.8 \(\mu\)M; [TnC] = 14 \(\mu\)M. Data were obtained at 1-ms intervals for the first 100 ms, followed by 10-ms intervals for 5 s.
the low-affinity sites is the key event for activation. Our stopped-flow experiments showing rapid changes in TnI upon Ca\(^{2+}\) binding to the low-affinity sites of TnC and detecting no difference in excimer fluorescence between the Mg\(_2\)-Ca\(^{2+}\)-TnC-TnI and Ca\(^{2+}\)-TnC-TnI suggest that the events reflected in the induced pyrene excimer changes are associated with the process that in vivo leads to activation.

In this study TnI has been specifically labeled, and a conformational change is induced in the labeled region of TnI by Ca\(^{2+}\) binding to TnC. Previous work has suggested other Ca\(^{2+}\) sensitive conformational changes in TnI. Johnson et al. (31), in stopped-flow studies on the interaction of IAANS-labeled TnI in the ternary complex with Ca\(^{2+}\), found a biphasic Ca\(^{2+}\) induced change with \(k_1 = 110 \text{ s}^{-1}\) and \(k_2 = 3 \text{ s}^{-1}\). They report that the IAANS label is primarily on Cys 48 (31, 32); however, it is not clear from their report how the TnI was labeled, nor is the specificity of the labeling stated, and hence it is difficult to reconcile their results with ours. Nishio and Iio (33) reacted troponin with IAANS under conditions in which Cys 313 should have been labeled. They observed a single rate constant of \(k > 630 \text{ s}^{-1}\) for the IAANS fluorescence change upon Ca\(^{2+}\) binding to TnC. Thus, their results also show a rapid conformational change in TnI, although they cannot be directly compared with our results since their label probes a different region of TnI.

The domain of TnI containing residues 48 and 64 has been suggested as a region interacting with TnT based on studies of amino acid reactivities (17, 18). Cys 48 and 64 are accessible to reaction with iodoacetamide in purified TnI, but are unreactive in TnI-TnT and whole troponin complexes (17). The lysine modification studies of Hitchcock show that Lys 40 and 65 have reduced reactivities in TnI-TnT and Tn complexes as compared with TnI. Furthermore, the reactivities of Lys 40 and 65 in the ternary complexes are Ca\(^{2+}\)-sensitive, suggesting that a Ca\(^{2+}\)-induced structural change occurs in the TnT-binding region of TnI. Our results using the pyrene probe clearly show that a rapid conformational change occurs in the putative TnT binding site of TnI in response to Ca\(^{2+}\) binding to the low-affinity sites of TnC. This change could be transmitted to TnC and hence to tropomyosin as part of the regulatory mechanism of the troponin-tropomyosin complex. Experiments to study this possibility are in progress.

Acknowledgments—We are grateful to Dr. S. S. Lehrer for helpful discussions on the use of pyrene labels and to Dr. R. C. Lu for performing amino acid analyses.

REFERENCES

1. Ebashi, S., Endo, M., and Ohtsuki, I. (1969) Q. Rev. Biophys. 2, 351-384
2. Greaser, M. L., Yamaguchi, M., Brekke, C., Potter, J., and Gergely, J. (1973) Cold Spring Harbor Symp. Quant. Biol. 37, 235-244
3. Greaser, M. L., and Gergely, J. (1973) J. Biol. Chem. 248, 2125-2133
4. Potter, J. D., and Gergely, J. (1974) Biochemistry 13, 2697-2703
5. Hitchcock, S. E. (1975) Eur. J. Biochem. 52, 255-263
6. Murray, A. C., and Kay, C. M. (1972) Biochemistry 11, 2622-2627
7. van Eerd, J.-P., and Kawasaki, Y. (1972) Biochem. Biophys. Res. Commun. 47, 859-863
8. Potter, J. D., Seidel, J. C., Leavis, P., Lehrer, S. S., and Gergely, J. (1976) J. Biol. Chem. 251, 7551-7556
9. Seamon, K. B., Hartshorne, D. J., and Bothner-By, A. A. (1977) Biochemistry 16, 4039-4046
10. Levine, B. A., Mercola, D. M., Coffman, D., and Thornton, J. M. (1977) J. Mol. Biol. 115, 743-769
11. Nagy, B., and Gergely, J. (1979) J. Biol. Chem. 254, 12732-12737
12. Bremel, R. D., and Weber, A. (1972) Nat. New Biol. 238, 97-101
13. Wilkinson, J. M. and Grand, R. J. A. (1975) Biochem. J. 149, 493-496
14. Horwitz, J., Bullard, B., and Mercola, D. (1979) J. Biol. Chem. 254, 350-355
15. Perry, S. V., Cole, H., Head, J. F., and Wilson, F. J. (1972) Cold Spring Harbor Symp. Quant. Biol. 37, 251-262
16. Syska, H., Wilkinson, J. M., Grand, R. J. A., and Perry, S. V. (1976) Biochem. J. 153, 375-387
17. Chong, P. C. S., and Hodges, R. S. (1982) J. Biol. Chem. 257, 2549-2555
18. Hitchcock-De Gregori, S. E. (1982) J. Biol. Chem. 257, 7372-7380
19. Betcher-Lange, S., L. S. and Lehrer, S. S. (1978) J. Biol. Chem. 253, 3757-3760
20. Graceffa, P., and Lehrer, S. S. (1980) J. Biol. Chem. 255, 11296-11300
21. Lott, S. S., Graceffa, P., and Betteridge, D. (1981) Ann. N. Y. Acad. Sci. 368, 285-299
22. Potter, J. D., and Gergely, J. (1975) J. Biol. Chem. 250, 4628-4633
23. Reidler, E., Liu, J., Mercola, M., and Horwitz, J. (1980) Biochim. Biophys. Acta 623, 243-256
24. Wu, C.-W., Yarbrough, L. R., and Wu, F. Y.-H. (1976) Biochemistry 15, 2863-2868
25. Ellman, G. L. (1959) Arch. Biochem. Biophys. 82, 70-77
26. Chang, J.-Y., Knecht, R., and Braun, D. G. (1983) Biochem. J. 211, 161-171
27. Fiske, C. H., and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375-400
28. Perrin, D. D., and Sayee, I. G. (1967) Talanta 14, 833-842
29. Silen, L. G., and Martell, A. E. (1964) Stability Constants of Metal Ion Complexes, 2nd Ed., Special Publication No. 17, The Chemical Society, Burlington House, London
30. Robertson, S. P., Johnson, J. D., and Potter, J. D. (1981) Biochem. J. 193, 559-569
31. Johnson, J. D., Charlton, S. C., and Potter, J. D. (1979) J. Biol. Chem. 254, 3497-3502
32. Potter, J. D., Robertson, S. P., Collins, J. H., and Johnson, J. D. (1980) in Calcium-binding Proteins: Structure and Function (Siegel, F. L., Carafoli, E., Kretsinger, R. H., MacLennan, D. H., and Wasserman, R. H., eds) pp. 279-288, Elsevier/North-Holland, New York
33. Nishio, T., and Iio, T. (1983) J. Biochem. (Tokyo) 94, 745-754
34. Wu, C.-S. C., and Yang, J. T. (1976) Biochemistry 15, 3007-3013