Physicochemical Studies on the Complexes Troponin C with Troponin T, and Reconstituted Troponin, and Their Interaction with Calcium Ions†

R. S. Mani, William D. McCubbin, and Cyril M. Kay*

ABSTRACT: A complex of troponin C and troponin T in 1:1 mole ratio has been prepared. As well, reconstituted troponin has been produced by combining troponin I, troponin C, and troponin T in equimolar ratios. Solutions of the protein complexes were subjected to physicochemical analysis using sedimentation velocity, circular dichroism, and biological and fluorescence techniques. For the complex, troponin C with troponin T, the sedimentation coefficient increased in the presence of Ca^{2+}. The sedimentation coefficient of reconstituted troponin, although considerably greater than those of the individual constituents, did not appear to be altered significantly by Ca^{2+} ion. Circular dichroism measurements indicated that the Ca^{2+} induced conformational change in TN-C still occurred in both the complex with troponin T and in reconstituted troponin, and in both instances the binding of Ca^{2+} was enhanced. Circular dichroism melting experiments revealed that for both complexes the structures produced in the presence of Ca^{2+} were more resistant to thermal denaturation than those produced in its absence. The complex of troponin C with troponin T had no effect on the Mg^{2+} activated ATPase of synthetic actomyosin, while reconstituted troponin only inhibited this system in the absence of Ca^{2+}. Fluorescence measurements with 8-anilino-1-naphthalenesulfonic acid revealed that the dye was bound in a more hydrophobic environment for both complexes than for any of the individual protein constituents. The fluorescence intensity, as well as the binding of the probe, increased in the presence of Ca^{2+} for both complexes suggesting conformational changes had been induced in the protein molecules, creating a more nonpolar binding environment for the dye. The possible biological significance of a calcium induced conformational change in the troponin C-troponin T complex and in reconstituted troponin is mentioned briefly.

Troponin and tropomyosin are proteins located on the thin filaments of muscle and constitute the system that regulates the interaction between actin and myosin (Ebashi and Endo, 1968). Upon arrival of the nerve impulse at the muscle cell, Ca^{2+} is released from the vesicles of the sarcoplasmic reticulum with the result that the sarcoplasmic Ca^{2+} concentration rises to about 10^{-5} M. At this concentration Ca^{2+} is bound to troponin. This binding of Ca^{2+} induces a structural alteration in troponin which is believed to be propagated *via* tropomyosin to actin (Ebashi *et al.*, 1968; Tonomura *et al.*, 1969). Actin now interacts with myosin and contraction accompanies the high rate of ATP hydrolysis. After stimulation, the free Ca^{2+} concentration in the sarcoplasm is reduced to the resting level once again ($<10^{-7}$ M) and Ca^{2+} troponin. This restores the inhibitory effect of the regulatory protein system and the muscle relaxes.

The active unit of troponin from skeletal muscle is a complex consisting of three protein components present in equimolar amounts, each responsible for a different function (Murray and Kay, 1971; Drabikowski *et al.*, 1971; Wilkinson *et al.*, 1972). The inhibitory protein, TN-I,[1] inhibits the Mg^{2+} activated ATPase of synthetic actomyosin independently of the concentration of free Ca^{2+}. This inhibition is removed in the presence of Ca^{2+} by the second component, TN-C, which binds Ca^{2+} strongly. The third component, TN-T, has a high affinity for tropomyosin and perhaps also for F-actin (Drabikowski *et al.*, 1973).

TN-C binds Ca^{2+} very strongly (Fuchs, 1971), with a binding constant of about 10^6 M^{-1} (Ebashi *et al.*, 1968; Hartshorne and Pyun, 1971). The interaction with Ca^{2+} induces a dramatic conformational change in the TN-C molecule, with no alteration in molecular weight (Murray and Kay, 1972; Van Eerd and Kawasaki, 1972). It has been shown that when TN-C is complexed to TN-I the binding of Ca^{2+} is enhanced and the Ca^{2+} induced conformational change still occurs (McCubbin *et al.*, 1974). As this conformational change occurs over a physiological range of concentration of Ca^{2+} ions it is possibly involved in the ability of TN-C to neutralize the restraining effects imposed on the actomyosin system by TN-I.

The present study was initiated with a view to establishing whether or not the Ca^{2+} induced conformational change in TN-C would still operate in the presence of TN-T, and to measure the contribution of TN-T when added to TN-IC. In particular, TN-C and TN-T were combined in 1:1 molar ratio allowing an interaction to take place with formation of the complex TN-CT. Also TN-C, TN-I, and TN-T were combined in equimolar ratio to form a reconstituted troponin complex, TN-ICT. Sedimentation velocity, circular dichroism, and fluorescence studies were employed to monitor conformational changes upon formation of TN-CT and TN-ICT, and those induced by Ca^{2+}. Parallel biological activity studies examined the effects of both complexes on the Mg^{2+} activated ATPase activity of synthetic actomyosin. Results from all these techniques indicated formation of the protein complexes TN-CT and TN-ICT and

† From the Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada. *Received May 24, 1974.*

[1] Abbreviations used are: ANS, 8-anilino-1-naphthalenesulfonic acid; ATPase, adenosine triphosphatase; dansyl chloride, 1-dimethylaminonaphthalene-5-sulfonyl chloride; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; SAM, synthetic actomyosin; SDS, sodium dodecyl sulfate; TN-I, inhibitory protein; TN-C, calcium binding protein; TN-T, tropomyosin binding protein; TN-CT, protein complex of TN-C and TN-T; TN-ICT, reconstituted troponin; TM_B, tropomyosin; *Q*, quantum yield.

TABLE I: Sedimentation Coefficients of Troponin Subunits and Their Mixtures.

Species	Concn (mg/ml)	$s_{20,w}$ Values (Svedberg units)	
		$-Ca^{2+}$	$+Ca^{2+}$
TN-C	3.0	1.65	2.0
TN-I	1.0	1.9	1.9
TN-T	1.5	2.1	2.1
TN-CT	1.5–2.5	2.1	2.7
TN-IC	1.3	2.35	2.9
TN-ICT	2.6–3.5	3.0	3.0

in both cases the conformational stability of the complexes was sensitive to Ca^{2+} ions.

Materials and Methods

Isolation and Purification of TN-C, TN-I, and TN-T. TN-C, TN-I, and TN-T were isolated and purified from rabbit skeletal muscle as described previously (McCubbin and Kay, 1973; Mani *et al.*, 1973, 1974). These proteins were all judged to be homogeneous by the criterion of gel electrophoresis in the presence of sodium dodecyl sulfate, as shown in earlier studies (Murray and Kay, 1972; Mani *et al.*, 1973, 1974).

Biological Activity Studies. Assays of ATPase activity were carried out as described earlier (McCubbin and Kay, 1973) using synthetic actomyosin (SAM) prepared by mixing myosin and actin in the weight ratio of 4:1 (Shigekawa and Tonomura, 1972) instead of desensitized actomyosin.

Protein Concentrations. For TN-C, protein concentrations were measured routinely by ultraviolet absorption, employing an $E_{1\text{ cm, }277.5\text{ nm}}(1\%)$ of 2.3 (Murray and Kay, 1972). For TN-I and TN-T, protein concentrations were determined in the ultracentrifuge employing the Rayleigh interference optical system assuming 41 fringes equivalent to 10 mg/ml (Babul and Stellwagen, 1969). In combination with ultraviolet absorption measurements, $E_{\text{cm, }280\text{ nm}}(1\%)$ values of 6.6 (TN-I) and 3.7 (TN-T) were established. TN-CT and TN-ICT protein complexes were formed by mixing the component proteins in equimolar ratios. Prior to mixing, the proteins were dialyzed separately against 0.5 M KCl–1 mM EGTA–50 mM Tris-HCl buffer at pH 8.0.

Circular Dichroism. The circular dichroism (CD) measurements were made on a Cary Model 6001 circular dichroism attachment to a Cary 60 recording spectropolarimeter in accordance with previously described methodology (Oikawa *et al.*, 1968). The ellipticity melt experiments were all carried out at 220 nm in a water-jacketed cell compartment connected to a Lauda thermoregulator. The protein solution temperature was increased in a stepwise manner from 5 to 70°. The steps in temperature were not equally spaced and in the transition region they were very close together. These melt studies therefore are not true equilibrium melts. The fractional helix f_H, as a function of temperature, was calculated from the relationship

$$f_H = ([\theta]^{t^\circ}_{220\text{nm}} - 4400^\circ)/([\theta]^{5^\circ}_{220\text{nm}} - 4400^\circ)$$

where 4400° was taken as the ellipticity value for a random coil structure (Greenfield and Fasman, 1969) and 5° was the lowest temperature at which the ellipticity could be measured conveniently.

TABLE II: ATPase Measurements of Synthetic Actomyosin.[a]

	ATPase Activity	
	μmol of PO_4 sec^{-1} g of Actomyosin^{-1}	
	EGTA	Ca^{2+}
No Troponin	8.3	8.1
Troponin I	5.3	5.4
Troponin I + C	8.1	8.2
Troponin C + T	8.0	8.1
Troponin I + C + T	2.0	8.4

[a] These measurements were made as described in Materials and Methods. All assay systems contained myosin, 0.60 mg; actin, 0.15 mg; tropomyosin, 0.04 mg; and troponin (TN-I, 0.06 mg; TN-IC, 0.11 mg; TN-CT, 0.14 mg; TN-ICT, 0.48 mg) in 10 ml of reaction mixture.

Fluorescence Measurements. These were made in a Turner Model 210 recording spectrofluorometer, in which the sample compartment was water jacketed and a constant temperature of 20° was maintained by a Lauda thermoregulator. Tryptophan fluorescence of the TN-CT and TN-ICT protein complexes was observed at 355 nm after excitation at 294 nm. The OD_{280} values of the protein complexes were between 0.1 and 0.2. The ammonium salt of ANS was used in the dye binding studies.

Ca^{2+} Concentrations. These were adjusted by means of a Ca^{2+} buffer consisting of Ca^{2+} and 1 mM EGTA in 0.5 M KCl–50 mM Tris-HCl buffer (pH 8.0), assuming a binding constant for EGTA of 2×10^7 M^{-1}, as outlined previously (McCubbin *et al.*, 1974).

Ultracentrifugation. Sedimentation velocity experiments were carried out at 60,000 rpm and 20° in a Beckman Spinco Model E ultracentrifuge, equipped with photoelectric scanner, multiplex accessory, and high intensity light source. Double sector charcoal-filled Epon cells with wide aperture window holders were used.

Results and Discussion

Ultracentrifugation. Sedimentation velocity studies were carried out in 0.5 M KCl–50 mM Tris-HCl–1 mM EGTA (pH 8.0), and in the same buffer containing a known amount of Ca^{2+}. Table I lists the apparent sedimentation coefficients that were obtained for the subunits and the mixtures of the subunits of troponin indicated. The sedimentation patterns of the mixtures were characterized in all cases by symmetrical boundaries and no slower moving peaks could be discerned. The results suggest that TN-C can form a complex with TN-T, and that reconstituted troponin can be formed by mixing equimolar amounts of all three subunits.

Biological Activity Studies. The results of ATPase measurements with various members of the troponin complex present in the assay system are presented in Table II. As can be noted, Troponin I inhibits the Mg^{2+} activated ATPase independently of the calcium concentration. TN-IC is unable to confer Ca^{2+} sensitivity on the actomyosin ATPase, whereas TN-CT has no effect on the ATPase. Reconstituted troponin, TN-ICT, mimics natural troponin by conferring Ca^{2+} sensitivity on the Mg^{2+} activated ATPase of synthetic actomyosin.

Circular Dichroism. Spectra for TN-CT and TN-ICT were measured in 0.5 M KCl-50 mM Tris-HCl-1 mM EGTA at pH 8.0 and in the same buffer containing a known amount of Ca^{2+}. In the far-ultraviolet region the CD spectra of both protein complexes, TN-CT and TN-ICT, possess two negative dichroic peaks located at 221 and 207 nm, typical of α-helix containing proteins. In the case of TN-CT, in the presence of 1 mM EGTA, *i.e.*, the virtual absence of any free Ca^{2+}, $[\theta]_{220\,nm}$ is -9200 ± 300 deg cm^2 dmol^{-1}; while the addition of Ca^{2+} to a final value of 5×10^{-4} M available or free Ca^{2+}, causes an increase to $-10{,}600 \pm 300$ deg cm^2 dmol^{-1}. These figures compare with the calculated values for $[\theta]_{220\,nm}$ in a 1:1 mole complex of TN-C and TN-T, in the absence and presence of Ca^{2+}, of -9000 ± 300 deg cm^2 dmol^{-1} and $-11{,}650 \pm 300$ deg cm^2 dmol^{-1}. It has been shown that the experimentally observed ellipticity values for TN-IC both in the absence and presence of Ca^{2+} are greater than the calculated values and it has been suggested that the interaction between TN-I and TN-C has induced a conformational change with the resulting structure being sensitive to Ca^{2+} ions (McCubbin *et al.*, 1974). For TN-CT, in the absence of Ca^{2+} the virtual agreement between calculated and observed ellipticity values precludes extensive conformational changes accompanying the protein-protein interaction. Van Eerd and Kawasaki (1973) demonstrated, from a disc gel electrophoretic study, that the TN-CT complex was weak in the absence of Ca^{2+}, and required this cation for maximum stability. These CD observations, along with the sedimentation coefficient data in Table I, agree well with this hypothesis. Addition of Ca^{2+} to TN-CT increases the value of $[\theta]_{220\,nm}$, but to a final value which is less than the calculated. This observation also differs from the case of TN-IC where the final experimental value of $[\theta]_{220\,nm}$ was considerably greater than that calculated.

These results taken together suggest that probably the mechanism of interaction between TN-T and TN-C differs considerably from that between TN-I and TN-C. In particular, it is suggested that a lesser charge neutralization accompanies formation of TN-CT. However, the important conclusion to be drawn from these CD observations is that the Ca^{2+} induced conformational change, which occurs in native TN-C, has its counterpart when TN-C is complexed with TN-T.

For reconstituted troponin, or TN-ICT, in the presence of 1 mM EGTA $[\theta]_{220\,nm}$ is $-10{,}000 \pm 300$ deg cm^2 dmol^{-1}, while the addition of Ca^{2+} elicits an increase to $-11{,}000 \pm 300$ deg cm^2 dmol^{-1}. These figures are to be compared with the $[\theta]_{220\,nm}$ calculated values for an equimolar complex of TN-I, TN-C, and TN-T, in the absence and presence of Ca^{2+}, of -9600 ± 300 and $-11{,}400$ deg cm^2 dmol^{-1}. It is again apparent that the secondary structure of TN-ICT is sensitive to the presence of Ca^{2+} ions, and a conformational change, analogous to that induced initially in TN-C can still be invoked by this cation.[2]

The interaction of Ca^{2+} with TN-CT and TN-ICT was studied by circular dichroism in closer detail. In particular, the changes in $[\theta]_{220\,nm}$ as a function of the free Ca^{2+} concentration were monitored and these results are presented in Figure 1. In both instances the half-maximal value of the ellipticity changes occurs at a pCa^{2+} value of 6.7 which yields a binding constant of 2×10^6 M^{-1}. This figure compares well with the value of 2.5×10^6 M^{-1} observed previously for TN-IC (McCubbin *et al.*, 1974), and is considerably higher than the value of 10^5 M^{-1} obtained for TN-C under similar conditions. These data imply that when TN-C is complexed both in TN-CT and TN-ICT the interaction with Ca^{2+} is stronger than in the case of TN-C alone. In substantiation of this conclusion is the study of Potter and Gergely (1972) which has suggested that TN-I has an enhancing effect on the binding of Ca^{2+} by TN-C. As well, Ebashi *et al.* (1974) have concluded, from an electron spin resonance study, that the conformation of TN-C alters upon binding to either TN-I or TN-T, and the conformational changes are more pronounced in the presence of Ca^{2+} ion.

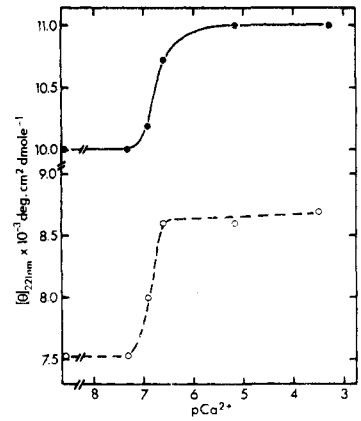

FIGURE 1: The change in ellipticity at 221 nm as a function of the concentration of free Ca^{2+} ions for TN-CT (○) and TN-ICT protein complex (●). Solvent was 0.5 M KCl-50 mM Tris-HCl-1 mM EGTA at pH 8.0, to which was added known aliquots of $CaCl_2$.

Earlier observations on TN-C and TN-IC had shown that the thermal stability of the molecules was enhanced in the presence of Ca^{2+} (McCubbin *et al.*, 1974). It was therefore decided to carry out circular dichroism melt experiments on TN-CT and TN-ICT in the absence or presence of Ca^{2+} to establish whether this is also the case with these systems.

The ellipticity at 220 nm of a dilute solution (1 mg/ml) of TN-T was measured over the temperature range 5–70° in the absence and presence of Ca^{2+}. The ellipticity melt data that were obtained have been used to calculate fraction helix at each temperature by the procedure described in the Materials and Methods section. These results are presented in Figure 2. There is no actual transition temperature, rather a fairly smooth loss in helix content over the temperature range used. It is also apparent from this figure that Ca^{2+} ions have no effect on the rate of loss of secondary structure in TN-T. The melt data do suggest an unstable structure, quite heat labile, since essentially all the α helix has been disrupted by 50°. The inherent susceptibility of TN-T to proteolysis by neutral cathepsins is well known (Drabikowski *et al.*, 1973), and this may well be related to the observed thermal instability.

The melting characteristics of the 1:1 mole protein complex TN-CT were studied in like manner in the absence and presence of Ca^{2+}. As before, the melt data were used to calculate fraction helix at each temperature, and these results are displayed in Figure 3. In both the absence and

[2] The Ca^{2+} induced change in TN-C still operates in the presence of Mg^{2+} (McCubbin and Kay, 1973), while Mg^{2+} has no significant effect on the structures of TN-I and TN-T (Mani *et al.*, 1973; Mani *et al.*, 1974). Furthermore Van Eerd and Kawasaki (1973) have concluded from fluorescence intensity measurements that the propagation of the Ca^{2+} induced conformational change in TN-C to TN-T and TN-I is Ca^{2+} specific and not influenced by Mg^{2+}.

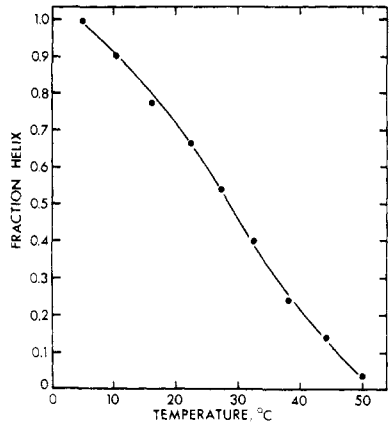

FIGURE 2: Thermal denaturation of TN-T in 0.5 M KCl-50 mM Tris-HCl, at pH 8.0 in the absence or presence of Ca^{2+}. Fraction helix, f_H, as calculated under Materials and Methods, is plotted *vs.* temperature.

presence of Ca^{2+} there does not seem to be actual transition temperatures, rather a progressive loss of secondary structure. It is apparent, though, that the melt data obtained in the presence of Ca^{2+} imply a significantly slower rate of loss of secondary structure than in its absence. The more rigid structure resulting from the conformational change in TN-CT, induced and stabilized by Ca^{2+}, apparently renders the molecule less susceptible to thermal denaturation.

The melting profiles of reconstituted troponin TN-ICT in the absence and presence of Ca^{2+} are shown in Figure 4. Both S-shaped curves are rather similar, but again it is apparent that Ca^{2+} produces a stabilizing effect on the secondary structure.

In all the circular dichroism melt experiments reported in this study no significant turbidity developed in the protein solutions over the temperature range examined. Also good reversibility of the heating effect was noted: samples heated to approximately 70°, when recooled to 27°, gave CD spectra identical within 3–5% of those initially observed.

Fluorescence Studies. The tryptophan fluorescence properties of TN-CT and TN-ICT were monitored in the absence and presence of Ca^{2+}. In both systems, addition of Ca^{2+} did not induce any significant change in their fluorescence properties, suggesting that the tryptophan moieties in TN-I and TN-T are essentially unchanged upon complex

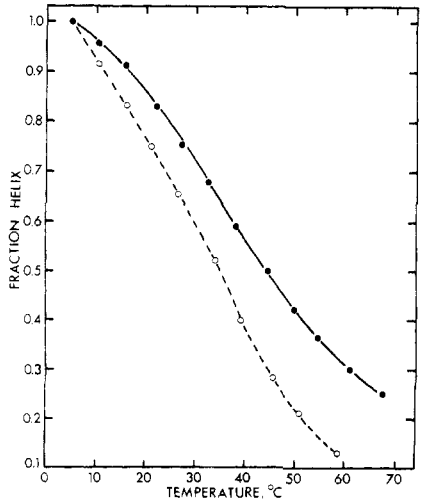

FIGURE 3: Thermal denaturation of TN-CT in 0.5 M KCl-50 mM Tris-HCl-1 mM EGTA at pH 8.0 (○) and in 0.5 M KCl, 0 mM Tris-HCl, 1 mM EGTA, and 5×10^{-4} M free Ca^{2+} ion at pH 8.0 (●). Fraction helix plotted *vs.* temperature.

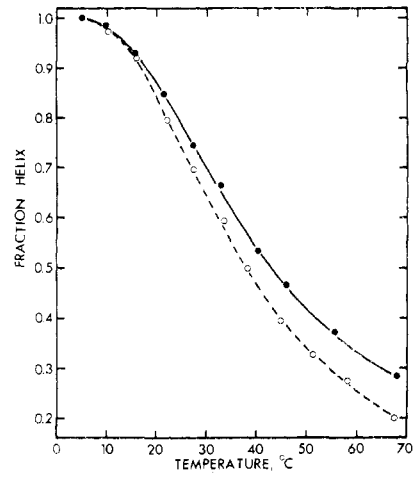

FIGURE 4: Thermal denaturation of TN-ICT in 0.5 M KCl-50 mM Tris-HCl-1 mM EGTA at pH 8.0 (○) and in 0.5 M KCl, 50 mM Tris-HCl, 1 mM EGTA, and 5×10^{-4} M free Ca^{2+} ion at pH 8.0 (●). Fraction helix plotted *vs.* temperature.

formation with TN-C. It was therefore decided to try an alternative fluorescence technique using the dye ANS as a sensitive hydrophobic probe of muscle protein conformation (Duke *et al.*, 1966; Cheung and Morales, 1969; McCubbin *et al.*, 1974).

ANS is almost nonfluorescent in water (Q, 0.004) and has its emission maximum at 515 nm, when excited at 380 nm (Weber and Lawrence, 1954). In the presence of ANS, the emission maxima for the proteins TN-C, TN-I, and TN-T occurred at 505, 495, and 505 nm, respectively, upon excitation at 380 nm. However, for TN-CT and TN-ICT, the emission maximum occurred at 500 and 490 nm, respectively. The observed blue shift in emission maximum upon the formation of the protein complexes from their individual protein constituents suggests a more nonpolar environment in the neighborhood of the dye binding site (Stryer, 1965). Addition of Ca^{2+} to TN-CT produced a further blue shift with the λ emission maximum decreasing from 500 to 490 nm, implying that the ANS binding site on TN-CT, in the presence of Ca^{2+}, is even more hydrophobic. Ca^{2+} addition caused a nearly 50% increase in the fluorescence intensity of TN-CT (Figure 5). The binding constant for Ca^{2+}, as evaluated from the half-maximal value of the transition curve (Figure 5), was approximately 3.2×10^6 M^{-1}, in good agreement with the value of 2×10^6 M^{-1} obtained from circular dichroism measurements. Figure 5 also includes the Ca^{2+} titration with TN-ICT. In this case, the increase in fluorescence intensity was of the order of 30%. However, addition of Ca^{2+} did not cause any apparent shift in the emission maximum from 490 nm. The binding constant for Ca^{2+}, obtained from the transition curve (Figure 5), was approximately 3.2×10^6 M^{-1} again in good agreement with the CD ascertained value of 2×10^6 M^{-1}. These findings are in general agreement with the observations of Van Eerd and Kawasaki (1973), who have used dansyl chloride as a probe in their conformational studies of troponin subunit interactions.

Addition of ANS to TN-CT and TN-ICT produced quenching of the tryptophan fluorescence at 355 nm when excited at 294 nm and the extent of this quenching became greater as the level of ANS was increased. However, the protein-ANS fluorescence at 550 and 490 nm in the case of TN-CT and TN-ICT, respectively, was found to increase as the ANS level was increased. For any particular concentration of protein (TN-CT or TN-ICT) and dye, the pro-

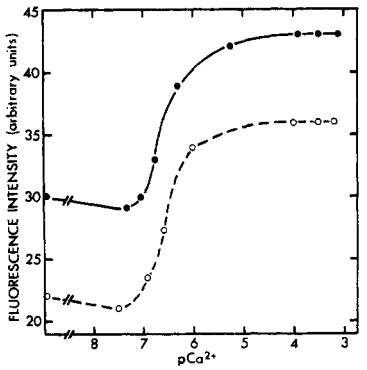

FIGURE 5: The change in the fluorescence intensity of TN-CT-ANS (●) and TN-ICT-ANS (○) complex as a function of the free Ca^{2+} concentration, pCa^{2+} The excitation was at 294 nm. The intensity of fluorescence is uncorrected and given in arbitrary units. Solvent was 0.5 M KCl-50 mM Tris-HCl-1 mM EGTA at pH 8.0, to which was added known aliquots of $CaCl_2$.

tein–ANS fluorescence was greater in the presence of Ca^{2+}. For this reason, the binding of ANS to TN-CT and TN-ICT was studied in the absence and presence of Ca^{2+}. The intrinsic dissociation constant, K, was determined as described previously (Cheung and Morales, 1969; McCubbin *et al.*, 1974). K values of $2.4 \pm 0.2 \times 10^{-5}$ and $1.9 \pm 0.2 \times 10^{-5}$ M were obtained for TN-CT in the absence and presence of Ca^{2+}, respectively, suggesting that the ANS dye indeed binds more strongly to TN-CT in the presence of Ca^{2+}. In the case of TN-ICT, K values of $1.2 \pm 0.1 \times 10^{-5}$ and $0.7 \pm 0.1 \times 10^{-5}$ M, in the absence and presence of Ca^{2+}, respectively, lead to a similar conclusion.

Native troponin interacts with tropomyosin and actin to regulate muscle contraction. A key observation in this regulation was the discovery that the precise location of tropomyosin on the actin helix depends on the state of troponin with respect to the absence or presence of Ca^{2+} ions (Huxley, 1972; Haselgrove, 1972). A possible mechanism to account for this observation has recently been proposed (Margossian and Cohen, 1973). TN-I is believed to attach the complex to actin and the position of tropomyosin is thus determined by its steric effect. In the absence of Ca^{2+}, TN-I would bind strongly to actin and also to TN-C. In the presence of the cation, the intrinsic subunit interactions of troponin are strengthened, and the binding to actin is decreased. As a consequence of this hypothesis, it is also proposed that each troponin subunit has at least two critical binding sites: TN-T binds both to TM_B and to TN-C; TN-C binds also to TN-I; and TN-I binds to actin.

The results of the present investigation are well in accord with these latter proposals. The Ca^{2+} induced conformational change in TN-C has now been shown to occur in two other complexed states, *viz.*, TN-CT and TN-ICT. It is of interest to mention that preliminary CD observations suggest the conformational change to occur also in the reconstituted troponin-tropomyosin system. The interaction of TN-C with Ca^{2+} ions is enhanced in the presence of the other troponin subunits. Moreover, CD melt experiments have shown that the structures of TN-CT and TN-ICT have increased thermal stability in the presence of Ca^{2+} ions, which implies a strengthening of protein–protein interactions. In the light of all these observations it is certainly possible that the Ca^{2+} induced conformational change, though it originates in the TN-C moiety, is transmitted throughout the whole troponin complex, and thus may well be the biological trigger which controls muscle relaxation.

Acknowledgments

The authors are indebted to A. Keri, V. Ledsham, and K. Oikawa for their technical expertise. They are also grateful to the Medical Research Council of Canada, the Muscular Dystrophy Association of Canada, and the Alberta Heart Foundation for their generous financial support.

References

Babul, J., and Stellwagen, E. (1969), *Anal. Biochem. 28,* 216.

Cheung, H. C., and Morales, M. F. (1969), *Biochemistry 8,* 2177.

Drabikowski, W., Dabrowska, R., and Barylko, B. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 12,* 148.

Drabikowski, W., Dabrowska, R., and Barylko, B. (1973), *Acta Biochim. Polon. 20,* 181.

Duke, J. A., McKay, R., and Botts, J. (1966), *Biochim. Biophys. Acta 126,* 600.

Ebashi, S., and Endo, M. (1968), *Progr. Biophys. Mol. Biol. 18,* 123.

Ebashi, S., Kodama, A., and Ebashi, F. (1968), *J. Biochem. (Tokyo) 64,* 465.

Ebashi, S., Ohniski, S., Abe, S., and Maruyoma, K. (1974), *J. Biochem. (Tokyo) 75,* 211.

Fuchs, F. (1971), *Biochim. Biophys. Acta 245,* 221.

Greenfield, N. J., and Fasman, G. D. (1969), *Biochemistry 8,* 4108.

Hartshorne, D. J., and Pyun, H. Y. (1971), *Biochim. Biophys. Acta 229,* 698.

Haselgrove, J. C. (1972), *Cold Spring Harbor Symp. Quant. Biol. 37,* 341.

Huxley, H. E. (1972), *Cold Spring Harbor Symp. Quant. Biol. 37,* 361.

Mani, R. S., McCubbin, W. D., and Kay, C. M. (1973), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 29,* 243.

Mani, R. S., McCubbin, W. D., and Kay, C. M. (1974), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 38,* 357.

Margossian, S. S., and Cohen, C. (1973), *J. Mol. Biol. 81,* 409.

McCubbin, W. D., and Kay, C. M. (1973), *Biochemistry 12,* 4232.

McCubbin, W. D., Mani, R. S., and Kay, C. M. (1974), *Biochemistry 13,* 2689.

Murray, A. C., and Kay, C. M. (1971), *Biochem. Biophys. Res. Commun. 44,* 237.

Murray, A. C., and Kay, C. M. (1972), *Biochemistry 11,* 2622.

Oikawa, K., Kay, C. M., and McCubbin, W. D. (1968), *Biochim. Biophys. Acta 168,* 164.

Potter, J., and Gergely, J. (1972), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 31,* 501.

Shigekawa, M., and Tonomura, Y. (1972), *J. Biochem. (Tokoyo) 72,* 957.

Stryer, L. (1965), *J. Mol. Biol. 13,* 482.

Tonomura, Y., Watanabe, S., and Morales, M. F. (1969), *Biochemistry 8,* 2171.

Van Eerd, J. P., and Kawasaki, Y. (1972), *Biochem. Biophys. Res. Commun. 47,* 859.

Van Eerd, J. P., and Kawasaki, Y. (1973), *Biochemistry, 12,* 4972.

Weber, G., and Lawrence, D. J. R. (1954), *Biochem. J. 56,* 31.

Wilkinson, J. M., Perry, S. V., Cole, H. A., and Trayer, I. P. (1972), *Biochem. J. 127,* 215.