Interaction of Troponin Subunits

THE INTERACTION BETWEEN THE INHIBITORY AND TROPOMYSIN-BINDING SUBUNITS

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The interaction of troponin-I and troponin-T was demonstrated by circular dichroism and gel filtration. Troponin-I gives a negative circular dichroism band between 300 and 260 nm while troponin-T gives two weak positive bands, one at 290 nm and the other at 263 nm. When troponin-I and troponin-T were mixed, the complex produced a strong negative circular dichroism band with a maximum around 280 nm. This band was most intense with a molar ratio of troponin-T to troponin-I of 1:1. The intensity of the band was 2.4 times that expected from the separate components. The interaction was independent of salt concentration from 0.15 to 0.5 M KCl. Gel filtration on Sephadryl S-200 showed that a stable 1:1 complex was formed between troponin-T and troponin-I.

When troponin-C was added to the complex of troponin-T-troponin-I, the reconstituted troponin had a circular dichroism spectrum identical to that of native troponin. The oxidation state of troponin was important in reconstituting troponin. Oxidized troponin-I produced less change in the near ultraviolet circular dichroism when added to troponin-T and troponin-C than did reduced troponin-I. This showed the subunits were not assembled correctly with oxidized troponin-I. When the reconstituted complex was reduced, the circular dichroism was restored to that of native troponin. Troponin reconstituted with oxidized troponin-I did not confer calcium sensitivity on actomyosin ATPase; activity was restored by reducing the complex.

Troponin is a protein complex which regulates contractile activity in vertebrate skeletal muscle (1). The troponin complex consists of three different proteins: troponin-T, the subunit which binds to tropomyosin and has a molecular weight of 30,503; troponin-I, which has a molecular weight of 20,864 and inhibits actomyosin interaction; and troponin-C, the calcium-binding subunit, with a molecular weight of 17,965. The interactions between these three subunits have been studied by many laboratories using a variety of physicochemical techniques (2, 3). The interactions between troponin-T and troponin-C are well documented (1-9); however, direct binding of troponin-I to troponin-T has been excluded (3, 7). It has been difficult to demonstrate interaction, or binding, between troponin-I and troponin-T because they are both sparingly soluble at physiological ionic strength. In addition, it appears that troponin-I and troponin-T tend to aggregate even at very high salt concentrations, as judged by gel filtration experiments (6). Some evidence for possible interaction between troponin-I and troponin-T is suggested by the fact that, under certain conditions, both proteins co-sediment in the analytical ultracentrifuge as well as co-purify (10-12). A unique form of paracrystals which resulted from the addition of troponin-I and troponin-T to tropomyosin also suggests a possible interaction between troponin-I and troponin-T (6, 13, 14). However, none of the aforementioned experiments provides sufficient evidence for a specific complex formation between troponin-I and troponin-T. Recently, Hitchcock (15), in studying the topology of troponin using cross-linking with dimethyl imidoesters, showed that troponin-T and troponin-I must lie within 0.6 nm or less of each other.

In the present study, we have followed the interaction and binding between troponin-I and troponin-T by monitoring their near ultraviolet CD spectra. Near ultraviolet CD spectra, which arise from the aromatic amino acid and cystinyl residues, reflect the tertiary structure of proteins. Near ultraviolet CD spectroscopy can be used as a sensitive tool for detecting conformational changes following interactions between proteins (16). The binding of troponin-I to troponin-T was also studied by gel filtration chromatography. The properties of the troponin-I-troponin-T complex were investigated by the addition of troponin-C to that mixture, thereby reconstituting whole troponin. The physicochemical properties of the reconstituted troponin and native troponin were compared.

EXPERIMENTAL PROCEDURES

Troponin was isolated from rabbit back muscle by the method of Ebashi et al. (17). This material was used to prepare troponin components. For spectroscopic measurements the troponin was purified further by chromatography on a sulfopropyl (SP)-Sephadex C-50 column (10). The Sephadex and troponin were equilibrated in 33 mM sodium chloride, pH 6.0, 1 mM dithiothreitol. Protein was eluted with a gradient consisting of 1 liter of buffer and 1 liter of buffer with 0.2 M KCl added. The second peak eluted from the column contained pure troponin, and these fractions were pooled, dialyzed against 1 mM NaHCO₃, 1 mM 2-mercaptoethanol, and freeze-dried.

Troponin-T, troponin-I, and troponin-C were prepared from whole troponin (about 600 mg) by chromatography on DEAE-cellulose using a modification of the method of Margossian and Cohen (8), with the modification that 0.5 mM EGTA was included in all chromatography buffers and the ammonium sulfate fractionation of the elution products was omitted. For best results the tubes were pooled as indicated in Fig. 1. Troponin-I- and troponin-T-containing fractions were pooled.
were dialyzed against 5 liters of 0.5 M KCl, 1 mM NaHCO₃, and 10 mM 2-mercaptoethanol, followed by dialysis against 1 mM NaHCO₃, and 10 mM 2-mercaptoethanol on a continuous flow exchange basis at a rate of about 12 liters/24 h, to a final sample conductivity of ≤0.05 mmho. At this point troponin-I fractions invariably produced insoluble material (containing some troponin-I and a series of higher molecular weight proteins as judged by SDS-acrylamide gel electrophoresis) which was removed by centrifugation at 10,000 × g for 30 min.

Tropomin-C was made up to 50 mM 2-mercaptoethanol and dialyzed against successive changes of 2 liters of 1 mM NaHCO₃, 1 mM 2-mercaptoethanol, and 0.05 mM CaCl₂ to a sample conductivity of ≤0.05 mmho. Finally all proteins were dialyzed for 28 h against deionized distilled water with a continuous flow of N₂ gas and freeze-dried. The products were characterized by SDS-gel electrophoresis, ultraviolet absorption, and circular dichroism spectroscopy and bioassay as discussed below.

Myosin was prepared as described by Moomaerts (18) and was stored at −20°C in 0.5 M KCl, 0.01 M potassium phosphate (pH 7.0), and 50% glycerol (v/v). Before use, it was dialyzed against the same buffer without glycerol and kept on ice for up to 1 week. Actin was prepared as described by Hitchcock et al. (19), and used within 2 weeks of preparation. Tropomyosin was prepared by Bailey's method modified by Hartshorne and Mueller (11).

ATPase activity was measured by pH-stat as described by Hitchcock et al. (19). For measuring the effect of troponin on actomyosin ATPase activity, the components were added together at high ionic strength. Tropomin-C was dissolved in 0.5 M KCl, 0.01 M potassium phosphate (pH 7.0), 2 mM dithiothreitol. Troponin-T and troponin-I were dissolved in 6 M urea and 0.01 M potassium phosphate, pH 7.0. Tropomin-T was then dialyzed against 0.5 M KCl and 0.01 M potassium phosphate, pH 7.0, which resulted in a slightly turbid solution. Part of the troponin-I in urea was reduced by adding dithiothreitol to 0.1 M, left on ice for 2 to 3 h, and dialyzed overnight against 0.5 M KCl, 0.01 M potassium phosphate (pH 7.0), and 2 mM dithiothreitol. Another part of the troponin-I in urea was oxidized by dialyzing for about 24 h against 0.5 M KCl and 0.01 M potassium phosphate, pH 7.0, with a steady stream of oxygen bubbling through the dialysis solution. The reduced sample was slightly turbid, while the oxidized sample had a heavy precipitate. The suspensions of troponin-I were centrifuged and the supernatant was used in the ATPase assay.

For the ATPase assay, equimolar mixtures of the three troponin components were added to tropomyosin. Actin was then added, followed by myosin. The amount of myosin used in an assay was 0.75 mg and the proportions of myosin, actin, tropomyosin, and troponin were 6:2:1:3.

SDS-polyacrylamide gel electrophoresis was performed on 2-mm slabs by modifying the method of Laemmli (20) for tube gels. Densitometric tracings of gels were obtained by using Beckman ACTA MVI spectrophotometer equipped with a Beckman model 2 gel scanner. No dithiothreitol or 2-mercaptoethanol were added to troponin-C or oxidized troponin-I in order to observe aggregation due to the formation of disulfide bonds.

Protein concentrations were determined by absorption at 280 nm. The following extinction coefficients were used: for troponin-T, ε₁₇₅, 280 nm = 5.04 (21); for troponin-I, ε₁₇₅, 280 nm = 5.96 (21); and for troponin-C, ε₁₇₅, 280 nm = 1.41 (7). For the determination of molar concentrations, the following molecular weights were used: troponin-T, M, = 30,503 (22), troponin-I, M, = 20,864 (23), and troponin-C, M, = 17,965 (24).

Circular dichroism spectra were recorded with a modified Beckman CD spectrophotometer (25) coupled with a Hewlett-Packard 5480 signal analyzer (26). Low noise CD spectra were obtained by averaging either 32 or 64 scans. The CD instrument was calibrated with an aqueous solution of D-10 camphorsulfonic acid, Δε = 2.20 M⁻¹ cm⁻¹ (27). Absorption measurements were performed on a Beckman ACTA VI spectrophotometer. In some experiments, a slight turbidity was observed upon mixing troponin-I with troponin-T; in these cases, solutions were clarified by centrifugation at 40,000 × g for 20 min at 4°C. For all gel loading studies, gel filtration chromatography was performed on Sephacryl S-200, Sephadex G-100, or Bio-Gel A-0.5m columns. Buffers for spectral measurements containing reduced dithiothreitol were prepared daily, and the amount of oxidized dithiothreitol was determined from the absorption peak using ε = 273 M⁻¹ cm⁻¹ (28).

**RESULTS**

The elution profile of the separated troponin subunits on the DEAE-cellulose column is shown in Fig. 1 and is similar to that obtained by Margossian and Cohen (8). Tubes were pooled as indicated on the abscissa and, typically, the most pure components were obtained only in the numbered fractions as judged by SDS-acrylamide gel electrophoresis. 1, troponin-I; 2, troponin-T; 3, troponin-C.

![Fig. 1. Example of DEAE-cellulose chromatography of rabbit skeletal (back) muscle troponin essentially according to the method of Margossian and Cohen (8). Usually 600 to 1200 mg of whole troponin is equilibrated with chromatography buffer (6 M freshly deionized urea, 0.5 M EGTA, 0.05 M Tris-HCl (pH 7.9 at 4°C), 1.0 mM dithiothreitol by dialysis against two changes of 1.1 each and applied to a column (2.5 cm × 43 cm) previously equilibrated with chromatography buffer without dithiothreitol. The column is then washed with 0.7 liters of chromatography buffer to elute troponin-I. A linear 0 to 0.18 M KCl salt gradient in 960 ml is then applied to elute tropomin-T. Finally, 360 ml of buffer with 0.20 M KCl is applied to elute troponin-C. All solutions are made with chromatography buffer and the column is run at 3-5°C at a flow rate of 40 ml/h. Tubes are pooled as indicated on the abscissa and typically the most pure components are obtained only in the numbered fractions as judged by SDS-acrylamide gel electrophoresis. 1, troponin-I; 2, troponin-T; 3, troponin-C.](http://www.jbc.org/)

![Fig. 2. SDS-gel electrophoresis of troponin and its components. The stacking gel contained 4.5% acrylamide; the running gel contained 13% acrylamide. Columns 1 and 6, 12 µg of troponin; Column 2, purified troponin-T; Column 3 and 4, two different preparations of purified troponin-I; Column 5, purified troponin-C.](http://www.jbc.org/)
Near Ultraviolet CD of Troponin-I—The near ultraviolet CD spectrum of troponin-I is shown in Fig. 3. The negative CD maximum which peaks at around 280 nm arises from tryptophan and tyrosine residues. Weak phenylalanine bands are observed at 260 and 265 nm. The overall wavelength profile of the near ultraviolet CD spectrum of troponin-I is similar to that reported recently by Wu and Yang (30).

Near Ultraviolet CD of Troponin-T—The near ultraviolet CD spectrum of troponin-T also is illustrated in Fig. 3. A relatively weak positive band is seen at 290 nm with two positive bands at 257 and 264 nm, and this CD spectrum is entirely different from that reported by Wu and Yang (30). Wu and Yang have shown that troponin-T possesses a weak negative CD band in the near ultraviolet which is similar to that observed for troponin-I (see Fig. 3 in Ref. 30). Nevertheless, our experiments reproduced repeatedly the spectrum shown in Fig. 3. We have observed the same spectrum for troponin-T in measurements from six different preparations. We emphasize that the CD intensity of troponin-T in the near ultraviolet is very weak and only by signal averaging using multiple scanning did we reproduce the CD fine structure shown in Fig. 3. Note also that the molar concentration of troponin-T in Fig. 3 is 1½ times that of troponin-I.

CD Mixtures of Troponin-I and Troponin-T—When troponin-T was added to an equimolar solution of troponin-I, the near ultraviolet CD spectra changed drastically, as shown in Fig. 4. Calculated from the area between 255 and 300 nm, the rotatory strength of the mixture of troponin-T-troponin-I is twice the sum of troponin-I' and troponin-T analyzed separately. This result suggests an interaction between troponin-T and troponin-I. Since troponin-T does not exhibit any optical activity between 270 and 280 nm, we can follow the titration of troponin-T with a fixed amount of troponin-I by monitoring the changes in CD intensity at 280 nm. The results of such titrations are shown in Fig. 5. Each of the preparations of troponin-T and troponin-I produced a maximum change at 280 nm when the stoichiometry of troponin-I to troponin-T was 1:1.

Effects of Calcium and Ionic Strength on the Interaction between Troponin-I and Troponin-T—Addition of 2 mM EGTA to troponin-I and troponin-T solutions prior to their mixing had no effect on the interaction between the two proteins, as judged by the near ultraviolet CD spectra. Similarly, no special changes were observed upon addition of 2 mM CaCl2 to troponin-I, to troponin-T, or to the mixture of both proteins.

Because troponin-T is only sparingly soluble at physiological ionic strength (6), 0.5 M KCl was always added to the buffers used. When a mixture of troponin-I and troponin-T with 0.5 M KCl was brought to a final concentration of 0.15 M KCl either by dilution or dialysis, about 20% of the protein mixture precipitated. When the precipitate was removed by centrifugation, the CD spectrum of this solution (15 μM troponin-T-troponin-I) was similar to that observed with 0.5 M KCl. The ratio of rotatory strength to absorption at 280 nm was also identical to that observed with 0.5 M KCl. In another experiment, we monitored the interaction between troponin-I and troponin-T when each of the proteins was solubilized at a lower salt concentration prior to mixing. Sufficient troponin-I for CD and absorption measurements could be solubilized by using buffers containing 0.15 M KCl. With troponin-T, however, the minimum salt concentration needed for analysis was 0.25 M KCl. When a sample of lyophilized troponin-I was dissolved in a buffer containing 0.15 M KCl and then mixed with a sample of troponin-T which was dissolved in a buffer containing 0.25 M KCl, giving a final concentration of 15 μM troponin-T, 15 μM troponin-I, and 0.2 M KCl, the CD spectrum of the mixture was similar to that observed at high ionic strength (Fig. 4). Therefore, as judged by CD measurements, the interactions between troponin-T and troponin-I (at a protein concentration of ~15 μM) are independent of salt concentration between 0.15 and 0.5 M KCl.

Effects of Dithiothreitol on the Interaction between Troponin-I and Troponin-T—Using the intensity of the CD band at 280 nm as an indicator of the interaction between troponin-
I and troponin-T, we found that only 20% or less of some early preparations of troponin-I reacted with troponin-T. In these preparations, the dialysis of troponin-I was performed without nitrogen bubbled through the dialysis solution. Since troponin-I contains 3 cysteine residues, these are prone to oxidation. Indeed, upon addition of freshly made diithiothreitol, the magnitude of the CD changes increased and was comparable to that shown in Fig. 4. Subsequently, nitrogen was bubbled through the dialyzing solutions of troponin-I (see "Experimental Procedures"), and diithiothreitol was added to freshly made solutions of troponin-I. As troponin-T contains no cysteine residues, dithiothreitol would only reduce troponin-I. Even though 1 to 2 mM diithiothreitol was used in all buffers, we found that it is of the utmost importance to use only freshly made diithiothreitol solutions, especially with buffers of pH higher than 7. Although aqueous solutions of reduced diithiothreitol are stable in air oxidation (28), the introduction of buffers of pH higher than 7 in the presence of oxygen will greatly increase the rate of oxidation of diithiothreitol and thus diminish its efficiency as a reducing agent. For example, 2 mM dithiothreitol in 50 mM Tris-HCl, pH 8.5, at room temperature, in air, has a half-life of 65 min. The use of so-called "ultrapure" buffers, or addition of EDTA, did not appear to affect the rate of oxidation of diithiothreitol.

**Interaction of Troponin-T with Troponin-I**

Reconstitution of Troponin from Its Three Protein Components—The CD spectrum of the reconstituted troponin formed when troponin-C was added to a complex of troponin-T and troponin-I was similar to that of native troponin. As shown in Fig. 7B, the wavelength profile of the reconstituted troponin is close to that of native troponin, but the CD intensity below 280 nm for the reconstituted troponin is somewhat different. This difference is due to a small amount of excess troponin-C which was present in this particular mixture. An expected, troponin-C with its unusual CD spectrum (Fig. 7A) contributes to the optical activity only below 280 nm (31). When this mixture was chromatographed on a Sephacryl S-200 column, the excess troponin-C was separated from the reconstituted troponin (Fig. 6) and the CD profile of the reconstituted troponin was identical to that of native troponin. Within our range of experimental error, native troponin, the reconstituted troponin, the troponin-T-troponin-I complex, and troponin-T by itself, all eluted at approximately similar volumes (see Fig. 6). The elution profile of native and reconstituted troponin was independent of salt concentration (from 0.05 M KCl to 1.0 M KCl) and independent of protein concentration (0.5 to 90 mg/ml). In all cases, the troponin eluted as a single band similar to that shown in Fig. 6 for troponin-T and troponin-I.

The addition of a partially oxidized solution of troponin-T to equimolar solutions of troponin-T and troponin-C resulted in a spectrum entirely different from that observed for native troponin (Fig. 8). The relatively small increase in CD intensity at 280 nm for this mixture suggests that only 15 to 20% of the troponin-I reacted with troponin-T and troponin-C. The addition of 1.0 mM dithiothreitol to this mixture greatly en-

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**Fig. 6.** Gel filtration elution profile of troponin components chromatographed on Sephacryl S-200. V0, void volume; T + I, complex of troponin-T-troponin-I; I, troponin-I; C, troponin-C. Native troponin as well as troponin-T was eluted around 40 ml where the complex of troponin-T-troponin-I elutes. For reference, monomeric bovine serum albumin was eluted at 52 ml. Column size was 1.5 x 60 cm. Flow rate, 65 ml/h. Fractions of 2.0 ml were collected. The column was equilibrated with 20 mM phosphate buffer (pH 7), 1 mM dithiothreitol, 0.5 M KCl. Similar profiles were obtained using 20 mM Tris-HCl buffer, pH 8.5, with 2.0 mM dithiothreitol and 0.5 M KCl.

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**Fig. 7.** A, CD spectrum of troponin-C. The path length was 1.0 cm; A280 = 0.084. B, ———, CD spectrum of native troponin. The path length was 1.0 cm; A280 = 0.7. ———, CD spectrum of reconstituted troponin. The path length was 1.0 cm. For troponin-T, A280 = 0.5; for troponin-I, A280 = 0.42; for troponin-C, A280 = 0.084. All other conditions were the same as described in Fig. 3. BL, base-line.
Interation of Troponin-T with Troponin-I

**Table I**

The effect of reconstituted troponin on actomyosin ATPase activity

<table>
<thead>
<tr>
<th>Troponin components</th>
<th>ATPase activity</th>
<th>Calcium sensitivity</th>
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<tbody>
<tr>
<td></td>
<td>EGTA + Ca²⁺</td>
<td>µmol ATP/mg actomyosin/min</td>
</tr>
<tr>
<td>1. None</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>2. TnC, troponin-C; TnT, troponin-T; TnC, reduced troponin-I; TnT, oxidized troponin-I; troponin-I was reduced or oxidized as described under &quot;Experimental Procedures.&quot; The single cysteine residue of TnC was reduced as shown by the presence of the monomer only in SDS-gel electrophoresis. In Assay 4, the troponin components were mixed in 0.5 M KCl, 0.01 M potassium phosphate, pH 7.0, then incubated with 0.1 M dithiothreitol at 0°C for 2 h and dialyzed against 0.5 M KCl, 0.01 M potassium phosphate, pH 7.0, and 2 mM dithiothreitol.</td>
<td></td>
<td>62.8</td>
</tr>
<tr>
<td>3. TnIo + TnC + TnT</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>4. (TnIo + TnC + TnT) dithiothreitol</td>
<td>0.18</td>
<td></td>
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Calcium sensitivity = [1 - EGTA: ATPase/Ca²⁺ ATPase] 100.

**DISCUSSION**

In past studies of the interaction among the three components of troponin, attention was directed mainly to the interaction between troponin-C and troponin-I, and between troponin-C and troponin-T (1-9). The limited solubility of troponin-I and troponin-T made it difficult to study their mutual interaction. Thus, it is generally accepted that there is no direct binding between troponin-I and troponin-T. The major experimental evidence against interaction and binding between troponin-I and troponin-T stems from the work of Van Eerd and Kawasaki (7), who used the criteria of standard disc gel electrophoresis to test for direct binding among the three components of troponin. They found that troponin-I did not enter the gel and attributed this fact to the insolubility of troponin T. They also found that troponin-I, in the absence of Ca²⁺, did enter the gel, but showed "a broad distorted band." Since Van Eerd and Kawasaki’s mixture of troponin-I and troponin-T gave the same electrophoretic pattern as troponin-I by itself, the logical conclusion was that it was unlikely that these two proteins interact strongly. It should be noted that the above experiments were performed at very low ionic strength, where both troponin-I and troponin-T are not very soluble (6). Thus, standard disc electrophoresis, which calls for a low ionic strength buffer system, is not the method of choice for detecting binding between troponin-I and troponin-T. On the other hand, optical activity and gel filtration measurements have been useful for monitoring the interaction and binding between these two proteins.

Troponin-I and troponin-T each possess a characteristic near ultraviolet CD spectrum. These spectra reflect the tertiary structure of each protein which arises from contributions of aromatic amino acids to the optical activity (16). The changes in the near ultraviolet CD spectrum upon mixing troponin-I and troponin-T point to a direct interaction between them. At present, however, it is not possible to determine if, upon combining the two proteins, conformational changes have occurred in either protein or in both of them. While the observed changes in the optical activity in the near ultraviolet
implicate specifically the aromatic amino acids, it is also possible that residues other than the aromatic are involved in the interaction and that only the microenvironment of some aromatic amino acid residues have changed upon interaction, giving rise to a different CD spectrum (16). Nevertheless, the changes in the CD spectrum point to a direct interaction between the two proteins, and the observation that there is a well defined stoichiometry in the changes observed proves the interaction is specific. Furthermore, the reconstitution experiments show that the troponin-T-troponin-I interaction largely accounts for the negative near ultraviolet CD band of native troponin. This result suggests that the troponin-T-troponin-I interaction is a characteristic of native troponin.

It is evident from the gel filtration experiments that a stable, 1:1 complex resulted when troponin-I and troponin-T were combined. As with the CD results obtained when troponin-I and troponin-T were titrated, excess troponin-I, when combined with troponin-T, separated from the complex on the column. While our results with gel filtration chromatography point to a formation of a stable complex, this complex may be a multimer of equimolar contributions from troponin-T and troponin-I. Troponin-T and the complex of troponin-T-troponin-I elute anomalously on the gel filtration column (Fig. 6). In the case of troponin-T (M, = 30,503), if it is globular, it should elute at a volume larger than 52 ml where serum albumin (M, = 68,000) elutes. This suggests either that troponin-T is a multimer, or that the protein is not globular. Furthermore, native troponin elutes at about the same volume as the troponin-T-troponin-I complex. The elution volume is inconsistent with troponin being globular and having a molecular weight of about 70,000. That troponin elutes anomalously on gel filtration column can also be seen from the earlier work of Drabikowski et al. (32), where troponin was eluted about 15 ml before bovine serum albumin and estimation of molecular weight based on gel filtration was in error (see Fig. 2 in Ref. 32). Recent evidence has been found that troponin can undergo self-association, which may explain its anomalous elution properties (33). While the shape and association properties of native troponin and its subunits need to be elucidated, this does not affect the conclusion about the interaction between troponin-T and troponin-I.

In studying the hydrodynamic and optical properties of troponin-C and troponin-C-troponin-I complexes, Kay and his collaborators (4, 31) showed that calcium induces conformational changes in these proteins. In contrast, however, the interaction and binding between troponin-T and troponin-I is calcium-independent, as judged by CD and gel filtration chromatography.

Greaser and Gergely (34) were the first to reconstitute active troponin from its three protein components. They found that recovery of activity could be achieved only when the three proteins were mixed first in the presence of urea. Later, Eisenberg and Kielley (21) succeeded in reconstituting troponin without using urea. Our reconstitution experiments also did not need urea. Our experiments show that one of the critical elements in the correct assembly of troponin from its subunits is the state of oxidation of the cysteine residues in troponin-I. The CD and troponin activity measurements show that troponin-I must have reduced —SH groups to form a troponin complex with the conformation and activity of native troponin.

Several models have been proposed to explain the mode of regulation of troponin (15, 19, 35). Since direct interaction between troponin-I and troponin-T was excluded, no direct binding or interactions are shown between troponin-I and troponin-T. In her recent work, Hitchcock (15) has suggested that troponin-I and troponin-T are close to each other (0.6 nm or less apart), in the native troponin complex. Our results show direct interaction and binding between troponin-I and troponin-T. The intricate, accurate topographical relations among the troponin subunits must await x-ray analysis and the solution of the three-dimensional structure of this complex protein.

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