A CIRCULAR DICHROISM STUDY OF THE INTERACTIONS AMONG THE COMPONENTS OF THE BOVINE CARDIAC MUSCLE REGULATORY SYSTEM

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1. Introduction

Calcium-sensitivity is conferred to the interaction between actin and myosin in vertebrate skeletal muscle by the regulatory proteins troponin and tropomyosin [1]. Troponin is a complex of three proteins [2–7]: TN-I, which inhibits the Mg^{2+}-activated actomyosin ATPase; TN-C, a calcium-binding protein; and TN-T, which anchors the troponin complex to tropomyosin.

The components of bovine cardiac troponin have been isolated and studied in this laboratory [8–10]. Physical and chemical characterization and biological activity studies allow functional roles to be assigned to the three cardiac subunits which are analogous to those of skeletal muscle TN-I, TN-C and TN-T. However, differences occur in amino acid composition and in the quantitative behaviour of the subunits from the two muscle types. In this study, combinations of the components of the regulatory system of bovine cardiac muscle were studied by circular dichroism (CD) techniques. The results provide evidence for interprotein interactions among the components and display the effects of binding Ca^{2+} to TN-C. The data offer further evidence that cardiac muscle regulatory activity can be explained, qualitatively, by a model similar to that proposed for the regulation of vertebrate skeletal muscle contraction [11–13].

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Abbreviations: CD, circular dichroism; EGTA, ethylene glycol bis (β-aminoethyl ether)-*N,N'*-tetraacetic acid; $[\theta]_\lambda$, the mean residue ellipticity measured at a wavelength, λ, assuming a mean residue weight of 115; $\Delta[\theta]_{221}$, the difference between the values of $[\theta]_{221}$ of a protein in the plus Ca^{2+} state relative to the minus Ca^{2+} state.

2. Materials and methods

2.1. *Protein preparations*

Troponin was isolated from fresh or fresh-frozen beef hearts using the LiCl extraction procedure of Tsukui and Ebashi [14]. The individual troponin subunits were purified using a combination of ion-exchange and molecular exclusion chromatographic methods as previously detailed [8–10]. Each protein used was judged to be homogeneous by the criterion of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate [15].

2.2. *Protein concentrations*

Concentrations of the troponin subunits and tropomyosin in solution were determined spectrophotometrically using extinction coefficients at 278 nm for 1% protein solutions for cardiac TN-C, TN-I, TN-T and tropomyosin, respectively, of 3.4 [8], 3.7 [9], 3.9 [10] and 3.4 [16]. Various combinations of the components were prepared by mixing the individual proteins in equimolar ratios based upon reported molecular weights for TN-C, TN-I, TN-T and tropomyosin, respectively, of 17700 [8], 22900 [9], 36300 [10] and 66000 [16].

The standard solvent system consisted of 0.5 M KCl, 50 mM tris-HCl, pH 7.5, and 1 mM EGTA. The calculation of free Ca^{2+} ions in this EGTA-containing buffer system was described in an earlier report [8]. In this paper, a 'minus Ca^{2+}' solution refers to the standard buffer system described and a 'plus Ca^{2+}', solution refers to the same buffer to which $CaCl_2$ was added such that the concentration of free Ca^{2+} ions equalled 2×10^{-4} M.

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2.3. *Circular dichroism*

Circular dichroism measurements were performed using a Cary model 6001 circular dichroism attachment to a Cary 60 recording spectropolarimeter as described by Oikawa et al. [17].

Theoretical CD spectra were calculated by taking a linear combination of the contributions from each protein subunit present, assuming no interprotein interactions occur [18–20].

3. Results

Far-ultraviolet CD spectra were recorded for each component of the cardiac muscle regulatory system, tropomyosin, TN-C, TN-I and TN-T. Of these spectra, only that of TN-C was affected by the addition of Ca^{2+}, yielding an increase in the magnitude of $[\theta]_{221}$ by 24 ± 3% (approx. 3400 deg·cm^2/dmole).

Spectra were subsequently measured for each of the combinations TN-C plus TN-T (TN-CT), TN-C plus TN-I (TN-IC), TN-I plus TN-T (TN-IT), tropomyosin plus TN-T, tropomyosin plus TN-I, tropomyosin plus TN-C, and reconstituted troponin (TN-ICT). Of these combinations, those which gave CD spectra which differed significantly from those calculated ($|[\theta]_{221\ \text{calculated}} - [\theta]_{221\ \text{observed}}| > 500$ deg·cm^2/dmole), suggesting the presence of interprotein interactions, included the complexes tropomyosin-TN-T (fig.1), TN-CT (fig.2), TN-IC (fig.3) and reconstituted troponin, TN-ICT (fig.4). The interactions appear to be of two types. In the cases of tropomyosin-TN-T and TN-CT, the observed ellipticity values are less negative than the calculated ones, suggesting a net loss in apparent α-helix content upon interaction. For the complexes TN-IC and TN-ICT, a net gain in apparent α-helix content occurred upon interaction. Similar results have been reported for complexes involving rabbit skeletal muscle regulatory components [18–20].

As seen in fig.2, 3 and 4, those complexes involving TN-C undergo a conformational change upon binding Ca^{2+}. For TN-ICT, $[\theta]_{221}$ increases in magnitude by 10 ± 2% (approx. 1200 deg·cm^2/dmole). These results suggest that the conformational change seen in

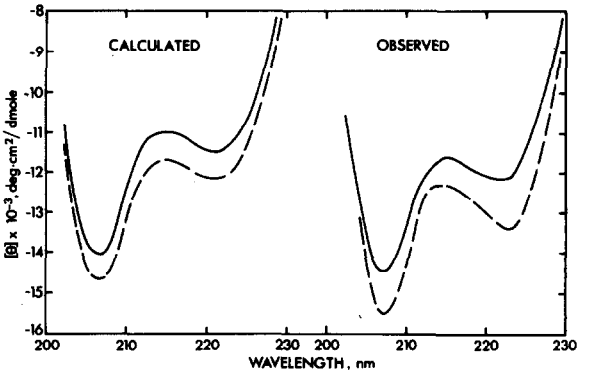

Fig.2. Calculated and observed CD spectra of an equimolar mixture of TN-T and TN-C. (——) 'Minus Ca^{2+}'; (– – –) 'plus Ca^{2+}'. Solvent conditions are described in the text.

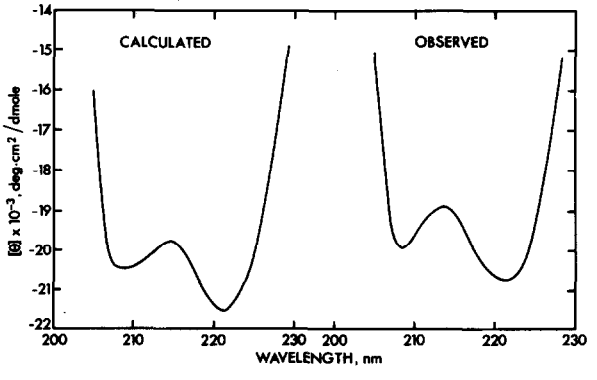

Fig.1. Calculated and observed CD spectra of an equimolar mixture of tropomyosin and TN-T. Solvent conditions are described in the text.

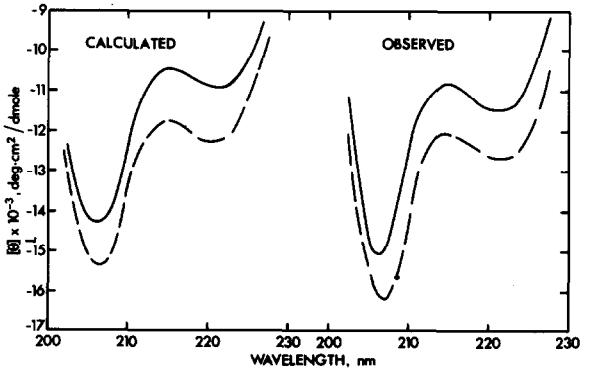

Fig.3. Calculated and observed CD spectra of an equimolar mixture of TN-I and TN-C. (——) 'Minus Ca^{2+}'; (– – –) 'plus Ca^{2+}'. Solvent conditions are described in the text.

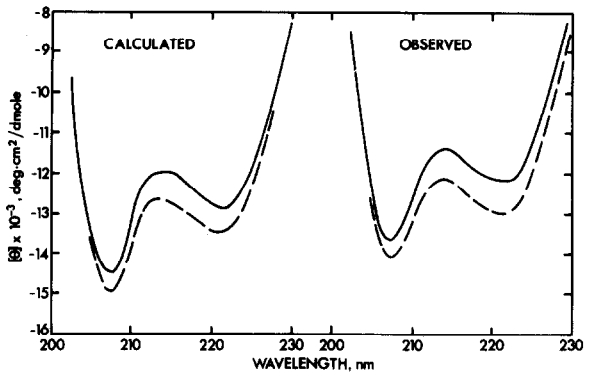

Fig.4. Calculated and observed CD spectra of equimolar mixture of TN-I, TN-C and TN-T (reconstituted troponin). (——) 'Minus Ca^{2+}'; (– – –) 'plus Ca^{2+}'. Solvent conditions are described in the text.

isolated cardiac TN-C in solution [8] persists in those complexes containing TN-C, including reconstituted troponin.

A comparison of the calculated and observed spectra for TN-ICT in the minus and plus Ca^{2+} states (fig.4) reveals that the change in $[\theta]_{221}$ for the observed spectra upon the addition of Ca^{2+} (10 ± 2%, average ± s.d. for 5 trials) is significantly greater than that predicted (6 ± 1%) by simply taking into account a dilution of the Ca^{2+}-effect on TN-C due to the presence of TN-I and TN-T. For the complexes TN-IC and TN-CT, the differences between the calculated and observed $[\theta]_{221}$ values were not significant. A potentiation of the effect of Ca^{2+} on isolated TN-C appears to result in the presence of both of the remaining troponin components.

4. Conclusions

This study suggests that, in the cardiac muscle regulatory system, interactions occur between TN-T and tropomyosin, TN-I and TN-C, and TN-C and TN-T. Those complexes which contain TN-C, including reconstituted troponin, undergo a conformational change upon binding Ca^{2+}. These results parallel CD results from skeletal muscle regulatory proteins [18–20], and fit the proposed regulatory model for skeletal muscle contraction [11–13].

The observation that, in the reconstituted troponin complex, the effect of binding Ca^{2+} to TN-C is amplified may be interpreted as a magnification of the direct effect of Ca^{2+}-binding on TN-C. However, it may, alternatively, represent a situation in which the direct effect on TN-C (i.e. that seen on isolated TN-C in solution) leads to changes in the conformation of one or both of the remaining troponin subunits due to the nature of the subunit interactions. By extrapolation, such a series of events could result in a shifting of tropomyosin away from a blocking position on the f-actin strands, allowing myosin to interact with actin. The net result would be an actively contracting muscle system.

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