LACK OF IDENTITY OF TROPOCALCIN WITH TROPONIN COMPONENTS

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Summary: Tropocalcin, a muscle protein, which has been found to undergo Ca^{++}-dependent changes in fluorescence, ultraviolet absorbance and circular dichroism is not a constituent of troponin complex. It was found that the presence of tropocalcin was not required for the Ca^{++}-dependent modification of actomyosin ATPase mediated by troponin and tropomyosin.

It has recently been shown that troponin prepared by published procedures (Ebashi et al., 1967; Hartshorne and Mueller, 1969; Yasui et al., 1968) usually contains at least four different proteins which can be separated by chromatography on DEAE-Sephadex columns with eluents containing 6M urea (Greaser and Gergely, 1971). Reconstitution experiments have demonstrated that three of these components plus tropomyosin were necessary to establish Ca^{++} control of actomyosin ATPase activity (Greaser and Gergely, 1971). One of the components exhibited strong Ca^{++} binding. This component (TN-C) has a molecular weight of approximately 21,000 and Ca^{++} binding affinity of about 10^6 M^{-1} (Greaser and Gergely, 1970). This Ca^{++} binding component appears to be

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identical with purified troponin A (Hartshorne and Pyun, 1971) and the calcium sensitizing factor of the troponin complex (Greaser and Gergely, 1971; M. C. Schaub, S. V. Perry and W. Häcker, 1972).

Marked Ca^{++}-induced changes in fluorescence, ultraviolet absorption, and circular dichroism of troponin and of a protein separated by Ca^{++} precipitation from troponin ("tropocalcin") have recently been reported (Han and Benson, 1970 and 1971). It was, therefore, of interest to determine whether or not tropocalcin was identical with TN-C or with any other component of troponin.

In comparing various troponin preparations, including that described by Greaser and Gergely (1971), we found that the Ca-induced changes, described by Han and Benson (1970), were observed only with troponin prepared according to the original procedure of Yasui, Fuchs and Briggs (1968) (YFB troponin). We, therefore, compared its SDS-gel electrophoretic band pattern with that of troponin prepared according to Greaser and Gergely (1971) (Fig. 1). The two patterns are identical except that the Yasui, Fuchs and Briggs preparation has an additional slower moving band. The tropocalcin preparation obtained by Ca-precipitation from the YFB type troponin consists chiefly of this slow component whose molecular weight is approximately 47,000, on the basis of its mobility in SDS-polyacrylamide gels in comparison with proteins of known molecular weight.

Troponin activity has been defined in terms of the conferment of a Ca^{++} requirement on actomyosin ATPase or superprecipitation in the presence of tropomyosin. For the following reasons it seems that tropocalcin is not involved in the type of regulation of actin-myosin interaction by the troponin-tropomyosin complex. (1) Purified troponin preparations without the 47,000

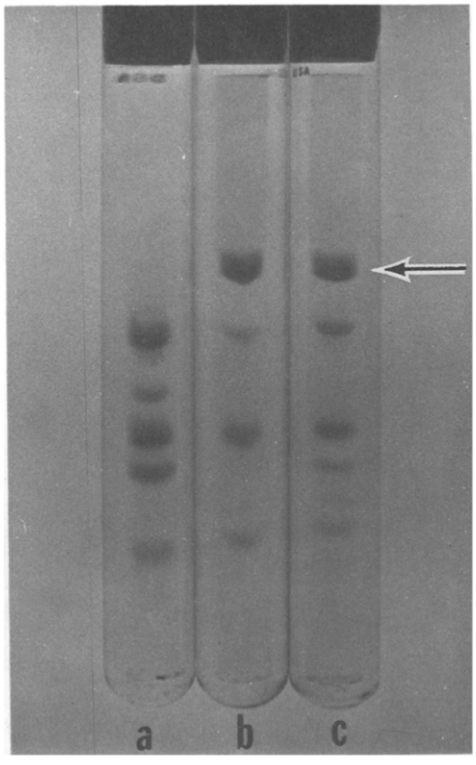

Figure I. Gel electrophoresis of troponin and tropocalcin: Protein samples were subjected to sodium dodecyl sulfate gel electrophoresis by the method of Weber and Osborn (1969). a. Troponin prepared by the method of Greaser and Gergely (1971) - 12 µg; b. Tropocalcin - 4 µg; c. Troponin prepared by the method of Yasui, Fuchs, and Briggs (1968) - 7 µg.

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dalton component do not show the Ca^{++} induced changes in ultraviolet absorbance, fluorescence, and circular dichroism under the conditions described by Han and Benson (1970 and 1971). (2) Tropocalcin, showing characteristic changes on addition of Ca^{++} has no troponin activity in itself or in any combination with other components of troponin.

The relation of tropocalcin to various structural elements of muscle and its possible role in modifying actin-myosin interaction will be the subject of further study.

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