Location of troponin I-binding on troponin T sequence

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1. INTRODUCTION

Interaction between troponin T and troponin I is considered as one of those necessary for the calcium-regulatory mechanism in the thin filament of vertebrate striated muscles [1,2]. Studies using chymotryptic subfragments of troponin T showed that the affinity for troponin I is localized in the region of the C-terminal 101 residues, termed troponin T_2 [3].

Our recent investigations clarified that the troponin T_2 fraction is accompanied with three slightly smaller fragments, troponin $T_2\beta$I, βII and βIII, in addition to the main subfragment, troponin $T_2\alpha$ [4,5]. Their amino acid sequences were determined as follows: troponin $T_2\alpha$ (residues 159–259), troponin $T_2\beta$I (residues 159–242), troponin $T_2\beta$II (residues 159–227) and troponin $T_2\beta$III (residues 159–222) [4,5].

In the present study the binding of the troponin T_2 fragments to troponin I was examined by the use of affinity chromatography and co-sedimentation with actin–tropomyosin. Results indicated that troponin I binds to troponin $T_2\beta$I and βII but not to troponin $T_2\beta$III. This suggests that the region of troponin T represented by residues 223–227 is involved in the interaction with troponin I.

2. MATERIALS AND METHODS

Troponin T, troponin I and tropomyosin were prepared from rabbit skeletal muscle according to the procedures described previously [6]. Chymotryptic subfragments of troponin T were prepared as described before [4,5]. Actin was prepared from the acetone powder of rabbit skeletal muscle by the method of Spudich and Watt [7].

Troponin I was covalently conjugated to Sepharose 4B by the procedures described by Head et al. [8] with slight modification. CNBr-activated Sepharose 4B (10 ml) [9] was mixed with 50 mg of troponin I in the presence of 0.1 M $NaHCO_3$ and 0.4 M NaCl and was shaken gently at 4°C for 20 h. After conjugation, the Sepharose was suspended in 0.2 M Tris–HCl (pH 8.0) for 3 h to block remaining activated groups on the Sepharose and then washed with a solution containing 6 M urea, 20 mM Tris–HCl (pH 7.0), 2 mM $MgCl_2$ and 0.5 mM dithiothreitol to remove unbound protein.

Each of the isolated fragments in a solution containing 20 mM Tris–HCl (pH 7.0), 2 mM $MgCl_2$ and 0.5 mM dithiothreitol, was applied to the column of troponin I–Sepharose 4B previously equilibrated with the same buffer. Subfragments bound to the column were eluted with the above buffer solutions by increasing the concentration of KCl from 0 to 1.0 M and finally eluted with the solution containing 6 M urea. Above procedures were carried out at 20°C. For the pelleting experiments of co-sedimentation, troponin I and troponin $T_2\beta$ (II or III) were mixed with F-actin–tropomyosin. It was centrifuged at 100 000 × g for 2 h at 20°C. The resulting pellets were examined on a 12% SDS acrylamide gel containing 0.1 M Na–phosphate (pH 7.0).

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3. RESULTS AND DISCUSSION

Troponin $T_2\alpha$, troponin $T_2\beta I$, and troponin $T_2\beta II$ were bound to troponin I-Sepharose 4B, even at 1.0 M KCl and eluted only with 6 M urea solution (fig.1a). On the other hand, troponin $T_2\beta III$ was not retained in the column of troponin I–Sepharose 4B in the absence of KCl and passed through the column with slight retardation (fig.1b). Troponin $T_2\beta III$ also was not retained in the column equilibrated with the solutions containing KCl higher than 0.1 M. This was confirmed by the co-sedimentation of the fragments with troponin I–tropomyosin–F-actin in a solution containing 0.1 M KCl, 2 mM $MgCl_2$, 20 mM Tris–HCl (pH 7.0) and 0.5 mM dithiothreitol. Troponin $T_2\beta II$ was found in the pellet, but troponin $T_2\beta III$ could not be detected (fig.2).

The only difference between peptides $T_2\beta II$ and $T_2\beta III$ is at the C-terminal region, Lys–Arg–Ala–Lys–Tyr, residues 223–227 of troponin T [5]. Both fragments showed no significant difference in the secondary structure studied by circular dichoism (Tanokura et al. unpublished). Thus it is very probable that the residues 223–227 interact directly with troponin I. This region is rich in basic residues, three of five, and might interact with the oppositely charged region in troponin I. The pres-

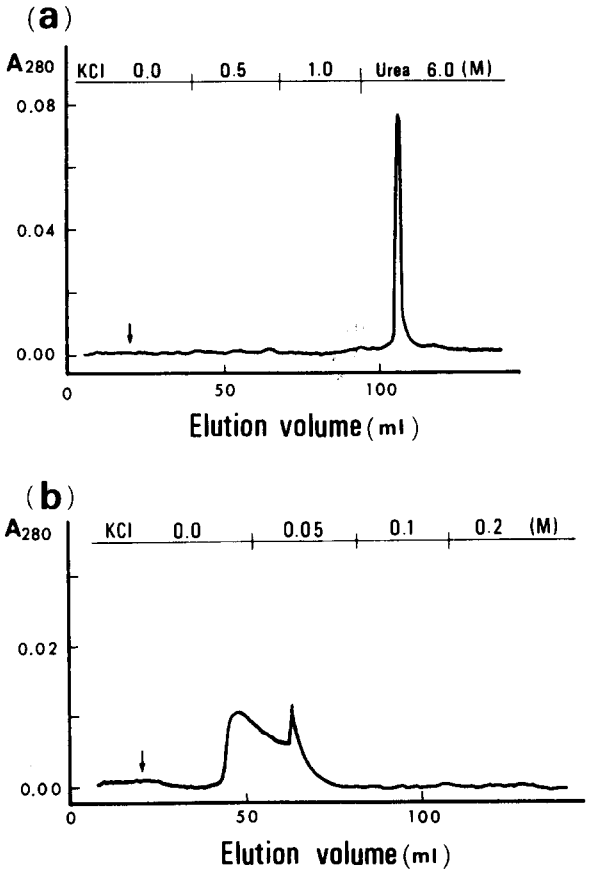

Fig.1. Elution profile from a column of troponin I–Sepharose 4B (1 × 7 cm): (a) troponin $T_2\beta II$; (b) troponin $T_2\beta III$. Flow rate was 20 ml/h. The protein was applied at the arrow indicated in the figure. Absorbance was monitored using an ultraviolet flow cell at 280 nm wave length.

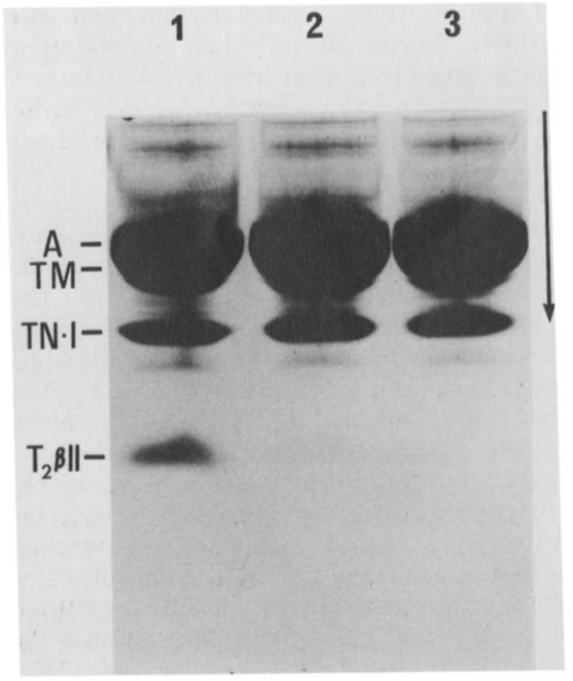

Fig.2. SDS gel electrophoretic pattern of the pellets formed by the centrifugation of the mixture of troponin $T_2\beta II$ or βIII and troponin I-tropomyosin-actin. (1) F-actin, tropomyosin, troponin I, troponin $T_2\beta II$. (2) F-actin, tropomyosin, troponin I, troponin $T_2\beta III$. (3) F-actin, tropomyosin, troponin I. Protein concentrations: F-actin, 1.00 mg/ml; tropomyosin, 0.23 mg/ml; troponin I, 0.77 mg/ml; troponin $T_2\beta II$, 0.33 mg/ml; troponin $T_2\beta III$, 0.33 mg/ml. After centrifugation, the pellets were treated for electrophoresis.

ent study is also in accord with the finding of Hitchcock and Zimmerman [10] that, among lysyl residues of troponin T, Lys-223 was most suppressed from acetylation when troponin T was complexed with troponin I. These discussions strongly suggest that rather restricted region containing residues 223–227 of troponin T is the main binding site for troponin I.

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