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# Type I protein is a slow isoform of troponin T

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Type I protein, a myofibrillar protein thought to be specific to slow-twitch skeletal muscle fibers, was purified. Two-dimensional electrophoresis indicated its identity with the purified slow troponin-$T_{1s}$ isoform. Immunochemical analyses using antibodies raised against type I protein and slow Tn-$T_{1s}$, further substantiated the identity of the two proteins.

Type I protein; Troponin-T slow isoform; Slow-twitch muscle

## 1. INTRODUCTION

A myofibrillar 34 kDa 'type I protein', specific to slow-twitch muscle fibers has previously been described [1]. We have recently shown that this protein is induced in fast-twitch rabbit muscle during fast-to-slow conversion evoked by chronic low-frequency stimulation [2,3]. In order to elucidate its identity and function, we decided to purify this hitherto unknown myofibrillar protein. In the course of this study it became clear that 'type I protein' is a slow isoform of troponin T (Tn-T).

## 2. MATERIALS AND METHODS

### 2.1. *Analytical methods*

One-dimensional polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE) was performed according to [4]. Two-dimensional electrophoresis of acidic proteins was carried out according to [5]. Separation of basic proteins was achieved by non-equilibrium pH gradient electrophoresis (NEPHGE) [6]. For immunoblotting, proteins were separated by SDS-PAGE (15% gel) for 17 h, transferred electrophoretically to a nitrocellulose sheet, and incubated with specific antibodies. Reactive proteins were visualized by incubation with peroxidase-coupled anti-guinea pig antibodies (Sigma) as described [7].

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### 2.2. *Purification of type I protein and of slow troponin-T*

Glycerinated myofibrils [8] from rabbit soleus muscles were washed in 0.2 M KCl and extracted in 0.6 M KI (pH 7.0). The supernatant fraction was dialysed against 0.2 M KCl to precipitate actomyosin. The resulting supernatant fraction was brought to 45% saturated ammonium sulfate (pH 7.0) and the precipitate collected by centrifugation. It was then dissolved in 0.4 M KCl, 20 mM potassium phosphate, 0.1 mM $CaCl_2$ (pH 7.0) and dialysed against the same solution. After centrifugation (100000 × *g*), the supernatant fraction was applied to a hydroxyapatite chromatography column (Bio-Rad). Proteins were eluted with a 0.02–0.4 M potassium phosphate gradient in the above solution. Fractions enriched in the 34 kDa protein were pooled, concentrated by ultrafiltration, dialysed against 10 mM imidazole, 0.1 M NaCl, 6 M urea (pH 7.0), and applied to a Sephacryl S-200 column (Pharmacia). Specific fractions were dialysed against 15 mM imidazole, 15 mM KCl, 0.2 mM dithiothreitol, 6 M urea (pH 7.4) and subjected to anion exchange chromatography on a DE-52 column (Whatman). Proteins were eluted with a 0.015–0.6 M KCl gradient in the above solution. Rechromatography resulted in a 34 kDa protein which was 96% pure. Troponin-T was purified from rabbit soleus muscles according to [9].

### 2.3. *Preparation of antibodies*

Antibodies against type I protein and the highly purified Tn-$T_{1s}$ slow isoform were raised in guinea pigs: 200 μg of the purified 34 kDa protein in complete Freund's adjuvant (CFA) were injected subcutaneously. A booster injection without CFA was applied after 14 days.

## 3. RESULTS AND DISCUSSION

Two-dimensional electrophoresis of myofibrillar extracts from soleus and chronically stimulated

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tibialis anterior (TA) muscles of the rabbit resolved type I protein into three components (fig.1a,c) with isoelectric points of 6.0, 6.1 and 6.2 [1]. Type I protein was undetectable in the unstimulated (fast-twitch) TA (fig.1b). The purified protein also showed three major spots and, in addition, a minor spot with a slightly higher mobility (fig.1d). This minor protein was consistently co-purified during the entire preparation. A separation of this compound was also achieved by NEPHGE, which, however, did not regularly separate the major protein into the three spots seen with two-dimensional electrophoresis in the acid range.

Because Tn-T displayed similar electrophoretic properties, we compared type I protein with the slow isoform of Tn-T purified from rabbit soleus muscle. Analysis of purified slow Tn-T by NEPHGE showed a major spot with a mobility similar to type I protein (fig.2b). The additional faint spot corresponded to the minor compound of the purified type I protein (fig.2a) also seen in SDS-PAGE (not shown, but compare fig.1c,d and fig.3). In fact, two isoforms of slow Tn-T, named Tn-$T_{1s}$ and Tn-$T_{2s}$, have previously been described in rabbit muscle by Schachat et al. [10], who used a purification protocol similar to that in the present study. Obviously, Tn-$T_{1s}$ and Tn-$T_{2s}$ were co-purified with our procedure. The identity of both the major and minor compounds with Tn-$T_{1s}$ and Tn-$T_{2s}$ was confirmed by co-electrophoresis of purified type I protein with slow Tn-T (fig.2c). The fact that small amounts of an additional protein which could be identified as the slow isoform of Tn-I, co-purified with type I protein (fig.2a), substantiates the assumption that type I protein is a component of the troponin system.

The identity of type I protein with slow Tn-T was further substantiated by immunoblot analyses. Antibodies raised against type I protein strongly reacted with type I protein and the two slow Tn-T isoforms (fig.3A). In addition, this antibody reacted with the slow isoform of Tn-I (not shown). The antibody against the purified Tn-$T_{1s}$ isoform strongly reacted with 'type I protein' (fig.3B), but not with slow Tn-I. Therefore, type I protein seems to be identical with Tn-$T_{1s}$. The two antibodies

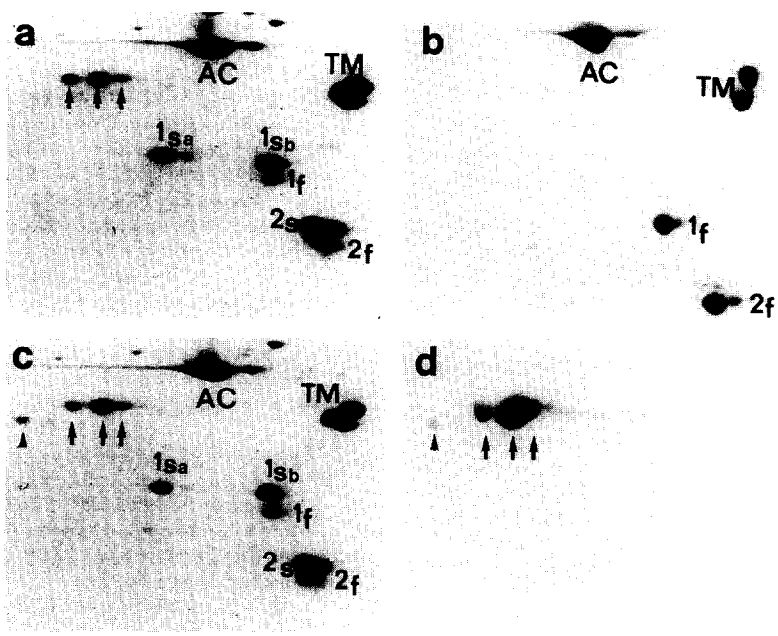


Fig.1. Two-dimensional electrophoresis of myofibrillar extracts from rabbit muscles, (a) soleus, (b) normal tibialis, (c) 60 d stimulated (10 Hz, 12 h/d) tibialis, and (d) of purified 'type I protein'. Coomassie blue staining. Arrows indicate type I proteins 1, 2, 3; arrowheads mark the minor protein which co-purifies with 'type I protein'. AC, actin; 1f, 2f, fast myosin light chains; $1s_a$, $1s_b$, 2s, slow myosin light chains; TM, tropomyosin.

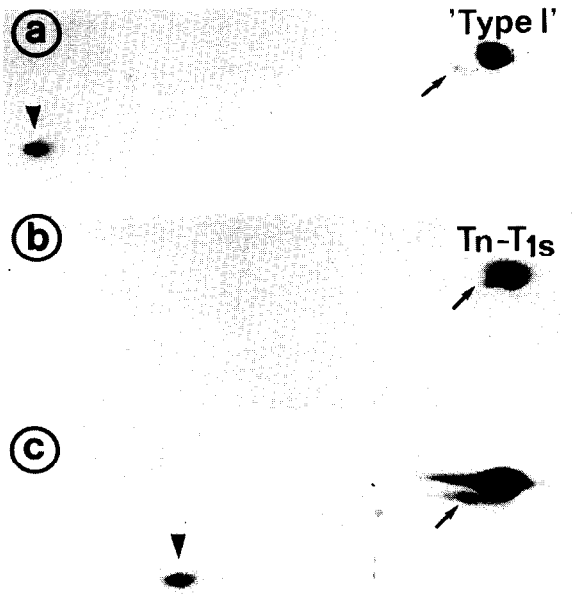


Fig.2. Two-dimensional (NEPHGE) electrophoresis of purified 'type I protein' (a), slow troponin-T (b), and (c) co-electrophoresis of a and b. Arrow marks putative Tn-$T_{2s}$ and arrowheads putative slow Tn-I.

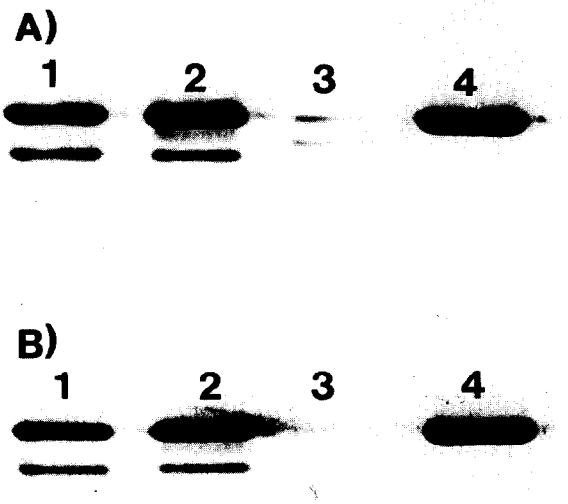


Fig.3. Immunoblots of myofibrillar extracts (40 $\mu$g of protein), purified 'type I protein' (1.7 $\mu$g) and purified troponin-$T_{1s}$ (1.7 $\mu$g). Proteins were transferred after SDS-PAGE to a nitrocellulose sheet and reacted (A) with antibodies against 'type I protein' and (B) with antibodies against Tn-$T_{1s}$. Lanes: 1, rabbit soleus; 2, purified 'type I protein'; 3, rabbit extensor digitorum longus; 4, purified Tn-$T_{1s}$.

also reacted with a myofibrillar extract from rabbit soleus muscle (fig.3, lane 1). Only faint reactions occurred with extracts from fast-twitch muscle, most probably due to small percentages of slow-twitch fibers (fig.3, lane 3). Finally, both antibodies reacted with the minor, slightly faster moving protein (see above). The co-purification of this protein and its positive reaction with the anti-slow Tn-$T_{1s}$ antibody suggests its identity with the slow Tn-$T_{2s}$ isoform. This antibody also reacted with Tn-$T_{2s}$ in Tn-T preparations from slow-twitch muscle (not shown).

A still unresolved question concerns the nature of the three Tn-$T_{1s}$ forms which, due to differences in their p*I* values, were separated by two-dimensional electrophoresis. It remains to be seen whether they represent true isoforms or post-translational modifications.

In summary, 'type I protein' which was thought to be an unknown myofibrillar protein specific of slow-twitch (type I) muscle fibers [1], is identical with the major slow isoform of Tn-T, i.e. Tn-$T_{1s}$.

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