THE POLYMORPHIC FORMS OF TROPOMYOSIN AND TROPONIN I IN DEVELOPING RABBIT SKELETAL MUSCLE

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1. Introduction

In striated muscle the hydrolysis of adenosine triphosphate by the myofibril is regulated by the direct action of Ca^{2+} on the regulatory protein system of the myofibril. This system contains the troponin complex which is composed of equimolar amounts of three proteins, troponin C, troponin I and troponin T, to each of which specific roles can be assigned [1]. The other component of the regulatory protein system is tropomyosin which in some way appears to extend the effect of the periodically localised troponin complex to each actin monomer in the I filament.

It has been shown that two different forms of troponin I are present in rabbit skeletal muscle, one of which is characteristic of fast skeletal muscle and the other being the main form present in slow skeletal muscle [2,3]. Differences also exist in the tropomyosin present in the two muscle types in that the ratio of the molar amounts of the α and β subunits of tropomyosin is 3.8:1 in a fast muscle such as psoas but only 1.1:1 in a slow muscle such as the soleus [4]. In the soleus muscle of the New Zealand Red rabbit the proportion of the fast form of troponin I, which represents about 30% of the total amount present, can be increased to over 70% by replacing the normal innervation with a nerve that normally supplies a fast muscle, the lateral popliteal [3]. At the same time the speed of contraction increases about two-fold, whereas the proportions of α and β tropomyosin are not significantly changed. When the composition of different normal muscles is compared, there appears to be some correlation between muscle speed and the nature of the components of the regulatory system. The cross innervation experiments suggest, however, that the relative amounts of the polymorphic forms of the troponin I and of tropomyosin present in a muscle are not controlled by identical mechanisms.

To throw further light on the factors regulating the amounts of the polymorphic forms of these proteins in a given muscle the changes that occur during development have been investigated. It has been shown that the troponin I characteristic of longissimus dorsi muscle of the adult animal is the only form present in the whole rabbit carcass muscle in significant amounts just before birth and in the longissimus dorsi just after birth. In contrast, the β subunit of tropomyosin represents 70% of the total of the two subunits present in longissimus dorsi in early foetal life and falls to the adult level of about 20% of the total four weeks after birth.

2. Materials and methods

2.1. *Preparation of proteins*

Tropomyosin was prepared from the heart and longissimus dorsi muscles of New Zealand white rabbit before and after birth by the methods of Cummins and Perry [5]. In the case of 20 day old foetuses the whole eviscerated carcasses after removal of the heads and feet were used. When less than 5 g of muscle was available the $(NH_4)_2SO_4$ precipitation steps were omitted to improve the yield. The tropomyosin prepared by this method was sufficiently pure to enable the relative amounts of α and β subunits to be determined. Troponin C was prepared as described by Perry and Cole [6] and desensitized actomyosin as described by Cummins and Perry [5].

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Troponin I was prepared by the affinity chromatographic method of Syska et al. [2] from adult and 21 and 24 day old foetal rabbits. Longissimus dorsi was used for preparations from rabbit after birth, but for the foetuses the whole eviscerated carcasses from which the head and lower legs had been removed (consisting mainly of the longissimus dorsi and containing 2–3 g muscle) were used. The muscle was homogenised in 20 vol. 8 M urea 70 mM Tris-HCl buffer, pH 8.0, 1 mM $CaCl_2$ 15 mM 2-mercaptoethanol, centrifuged 30 min at 12 000 *g* and applied to a 20 g column of Sepharose 4B containing 45 mg of covalently linked troponin C (8 X 1.5 cm). After washing with buffer until the absorbance of the eluate was identical with that of the buffer applied, troponin I was eluted by application of the standard urea buffer to which 10 mM EGTA had been added.

2.2. *Electrophoresis*

Electrophoresis was carried out in 0.1% sodium dodecyl sulphate 100 mM sodium phosphate buffer, pH 7.0 (Weber and Osborn) [7].

The ratio of α and β subunits in tropomyosin preparations was determined by densitometric scanning of the bands obtained after electrophoresis on 10% (w/v) acrylamide, 0.14% bisacrylamide gels containing 6 M urea 82.5 mM Tris–400 mM boric acid, pH 7.0 [5]. After staining the urea gels in 0.25% Coomassie Brilliant Blue R (George T. Gurr Ltd., High Wycombe, Bucks., England) in 50% (w/v) trichloroacetic acid and destaining in 1:2:12 (v/v/v) acetic acid:methanol: water, the gels were scanned using a Gilford model 2400 recording spectrophotometer fitted with a Gilford model 2410 gel scanning attachment. Each preparation of tropomyosin was run on at least three gels and the results of the densitometric analyses averaged.

The forms of troponin I present were identified by electrophoresis on 8% acrylamide, 6 M urea, 25 mM Tris–80 mM glycine, pH 8.6 in the presence of at least 1.5 times molar excess of troponin C from white skeletal muscle of the rabbit [2,3]. Under these conditions troponin I migrates as the troponin I–troponin C complex which has a mobility characteristic for each of the forms of troponin I. The relative amounts of the proteins on the bands were determined by staining and densitometric scanning as described above.

3. Results and discussion

Up to about four days before birth there was about twice as much β as α subunit in tropomyosin present in longissimus dorsi muscle of the rabbit. To our knowledge this is the first report of tropomyosin from isolated rabbit striated muscle containing the β subunit as the major component. In the few days before birth the relative amounts of α tropomyosin rapidly increased but thereafter changed rather slowly until the α:β ratio reached the adult value of 3.8:1 at about thirty days after parturition (figs. 1 and 2). The data

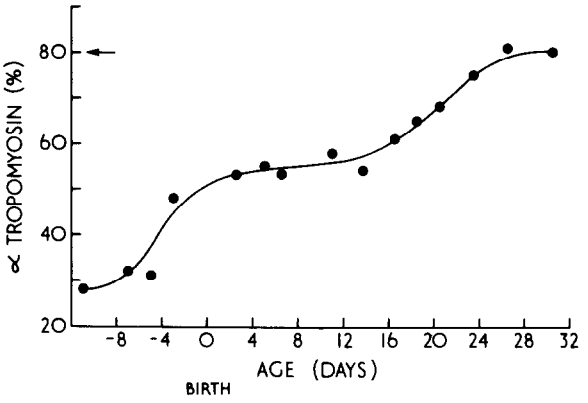

Fig.1. Change in proportions of α and β tropomyosin of longissimus dorsi muscle of the rabbit during development. α and β tropomyosin separated by electrophoresis and relative amounts determined as described in Materials and methods. Gestation period taken as 31 days. Arrow on ordinate indicates adult value.

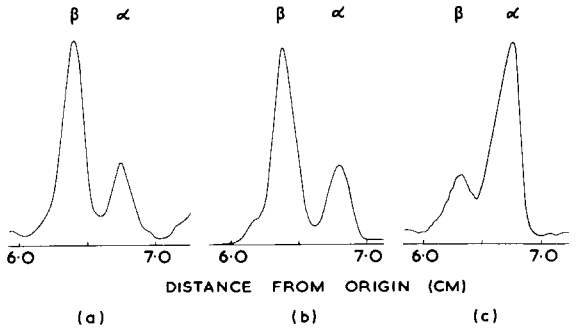

Fig.2. Densitometric scans of electropherograms of tropomyosin isolated from rabbit muscle at different stages of development. Gels were 11 cm long and run for 1½ times the period required for bromophenol blue to migrate off the gel. (a) From whole eviscerated carcass of 20 day old foetus (see Materials and methods). (b) From longissimus dorsi of 24 day old foetus. (c) From longissimus dorsi 30 days after birth.

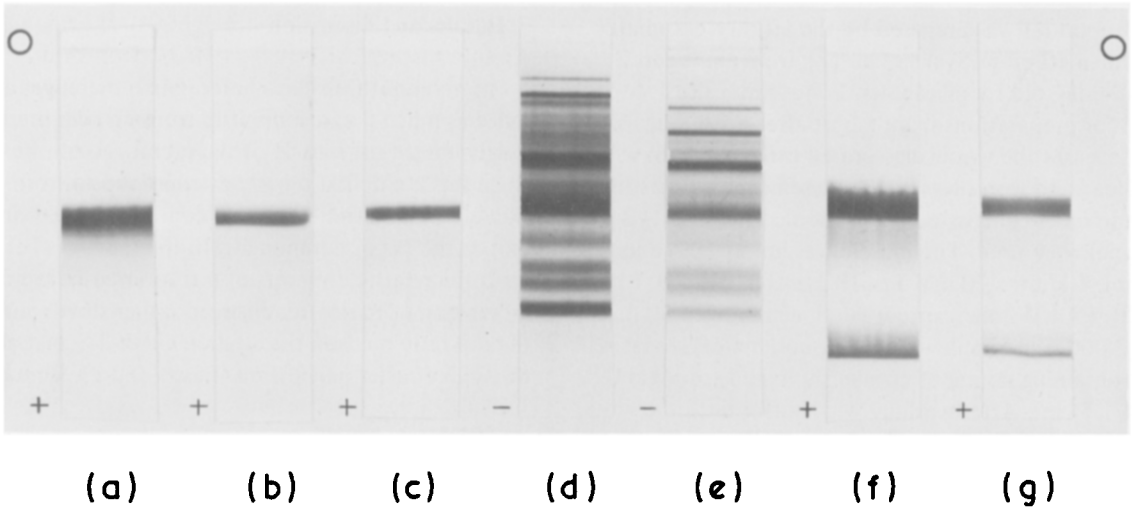

Fig.3. Polyacrylamide gel electrophoresis of troponin I isolated from rabbit foetal muscle. Troponin I isolated by affinity chromatography from whole carcass of 24 day old rabbit foetus. Electrophoresis conditions: (a)–(c) 10% (w/v) acrylamide, 0.1% (w/v) sodium dodecyl sulphate, 100 mM sodium phosphate buffer, pH 7.0; (d)–(e) 15% (w/v) acrylamide, 6 M urea, 5% acetic acid, pH 3.2; (f)–(g) 8% (w/v) acrylamide, 6 M urea, 25 mM Tris–80 mM glycine pH 8.6. 0, Origin. (a) 25 μg rabbit foetal troponin I. (b) 10 μg adult rabbit longissimus dorsi troponin I. (c) 5 μg rabbit foetal troponin I and 5 μg adult longissimus dorsi troponin I. (d) 50 μg rabbit foetal troponin I after cyanogen bromide digestion. (e) 30 μg adult rabbit longissimus dorsi troponin I after cyanogen bromide digestion. (f) 25 μg rabbit foetal troponin I + 30 μg adult rabbit troponin C. (g) 20 μg adult rabbit longissimus dorsi troponin I + 25 μg adult rabbit troponin C.

suggest that the α:β ratio did not increase steadily with age after birth but exhibited at least two phases of change in the first four weeks.

Over the same period the composition of cardiac tropomyosin did not change. One preparation from the heart of a 26 day old foetus and two from 24 day old foetuses consisted only of α tropomyosin as has been reported for adult rabbit cardiac tropomyosin [4,5].

Troponin I isolated from the whole carcass muscles (see Materials and methods) of 21 and 24 day old rabbit foetuses migrated as a single component when examined by electrophoresis in sodium dodecyl sulphate at pH 7.0 using sample loadings of up to 50 μg (fig.3). When electrophoresis was carried out in 6 M urea, 25 mM Tris–80 mM glycine, pH 8.6, in the presence of troponin C from rabbit white skeletal muscle and Ca^{2+}, troponin I from foetal whole carcass muscle migrated as a complex of identical mobility to that obtained with the troponin I–troponin C complex from adult rabbit longissimus dorsi muscle (fig.3). The troponin I isolated from foetal muscle had an identical electrophoretic mobility in sodium dode-

Table 1
Amino acid analysis of rabbit foetal troponin I

	Foetal troponin I	Adult white troponin I[a]
Asp	16.5	15
Thr	4.6	3
Ser	11.6	10
Glu	30.5	33
Pro	6.3	5
Gly	9.2	8
Ala	14.9	14
Val	7.7	7
Met	7.3	9
Ile	6.1	5
Leu	18.2	17
Tyr	2.3	2
Phe	2.8	3
His	3.6	4
Lys	22.9	24
Arg	14.3	16

Amino acid analyses carried out as described by Wilkinson et al., [12] Values are the average of 2 preparations of troponin I isolated from the whole carcass of the 24 day old foetus. Expressed as residues per 21 000 g.

[a] From sequence data of Wilkinson & Grand [13]

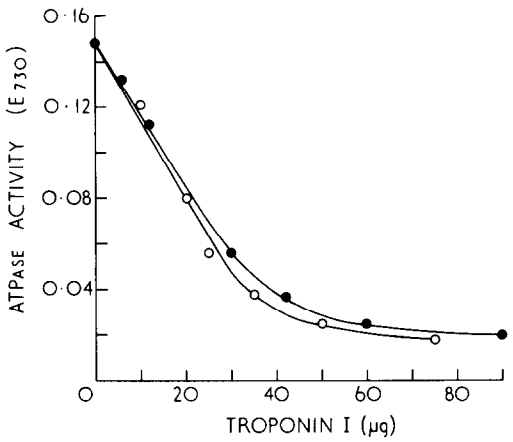

Fig.4. Inhibitory activity of troponin I isolated from foetal and adult rabbit fast white muscle on the Mg^{2+}-stimulated ATPase of desensitized actomyosin. Desensitized actomyosin prepared as described in Materials and methods, 750 μg desensitized actomyosin incubated with 25 mM Tris-HCl, pH 7.6, 2.5 mM ATP, 2.5 mM $MgCl_2$, 140 μg tropomyosin, total vol. 2 ml. Incubated 5 min at 25°C. Full circles are for foetal muscle, empty circles for adult muscle.

cyl sulphate, possessed a similar amino acid composition (table 1) and a virtually identical inhibitory activity on the Mg^{2+}-stimulated ATPase of desensitized actomyosin (fig.4) to the protein isolated from longissimus dorsi muscle of the adult animal. The cyanogen bromide digest of the troponin I isolated from foetal and adult muscle also appeared very similar on electrophoresis in 6 M urea, pH 3.2 (fig.3.). The only differences in band pattern were in the region where undigested and partially digested protein migrated. When allowance is made for variations in the extent of digestion obtained with cyanogen bromide the results strongly suggest that the two preparations were very similar, if not identical, in primary structure.

The findings reported here, taken with the results of the cross innervation studies reported elsewhere [3] indicate that different mechanisms are involved in the control of the expression of the genes regulating the synthesis in skeletal muscle of the two forms of troponin I on one hand and α and β tropomyosin on the other. Whereas the proportions of the fast and slow muscle forms of troponin I present in adult rabbit soleus muscle are changed by increasing the speed of contraction, there is no corresponding change in troponin I composition during development of a fast skeletal muscle from the foetal to the adult form despite the increase in speed of contraction that occurs during this period (see [8] for review). It appears that in the case of longissimus dorsi at least, which represents the major carcass muscle, the polymorph of troponin I corresponding to the properties of the adult form of muscle is laid down at a very early stage in development.

In contrast, development produces a marked increase in the relative amount of the α subunit of tropomyosin. Although this change parallels the increase in speed that accompanies development in the longissimus dorsi, in the soleus muscle, for example, a similar change can not be induced by increasing the speed by cross innervation [3]. The fact that β tropomyosin appears to be the main form present in longissimus dorsi muscle of the foetal rabbit indicates that the dimer β_2 must exist in rabbit foetal muscle.

The rapid changes in tropomyosin composition that take place just before birth correlate with those which occur in the ATPase activity of the sarcoplasmic reticulum [9,10], but occur before the rapid changes that occur in creatine kinase and adenylic deaminase immediately after birth in the rabbit [11].

Acknowledgements

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