CHANGES IN THE COMPOSITION OF THE MYOFIBRILLAR FRACTION DURING DEVELOPMENT OF THE RABBIT

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1. Introduction

In several recent papers the difference between the protein composition of adult and foetal myofibrils has been pointed out [1–3]. These differences were related to the changes in specific activity of myofibrillar Mg^{2+}-stimulated ATPase, which reflects the actin–myosin interaction, and to its inhibition in the absence of Ca^{2+} ions, which reflects the functioning of the regulatory proteins [1].

It has been suggested that during development the light-chain composition of myosin is altered, however the nature of these changes is controversial. Adult skeletal myosin contains three light chains LC_1, LC_2 and LC_3*, in a molar ratio of LC_2 to $LC_1 + LC_3$ of 1:1 [4]. Dow and Stracher [5], Sréter et al. [6] and John [7] have suggested that foetal myosin is either devoid of LC_3 or contains it only in trace amounts. On the other hand, Pelloni-Mueller et al. [2,3] have reported recently that foetal myosin is enriched with respect to both LC_2 and LC_3, compared to the adult form. Some controversy also exists as to the appearance and functioning of the troponin complex during development [8,9].

In view of this, a detailed study was undertaken on the composition of foetal myofibrils, isolated at different stages of development. Application of urea–polyacrylamide gel electrophoresis performed at acidic and alkaline pH, in addition to SDS–gel electrophoresis enabled us to characterize the proteins and especially to distinguish between the light chains of myosin and the components of troponin.

* LC_1, LC_2 and LC_3 light chains have also been termed as A_1, DTNB and A_2 light chains, respectively, in certain papers herein referenced

2. Materials and methods

The back and leg muscles of foetal rabbits were removed and immediately frozen in dry ice and stored at –70°C. The period of gestation that was studied was from 19 days until birth.

2.1. *Preparation of proteins*

The myofibrillar fraction was prepared essentially according to Perry and Żydowo [10]. An additional washing with 0.2% Triton X-100 was included in order to reduce contamination by sarcoplasmic reticulum, mitochondria and ribosomes [11]. The myofibrillar fraction will be referred to as myofibrils even though some contamination by non-contractile proteins may have been present.

Myosin was obtained by the method of Perry [12]. Regulatory proteins (troponin and tropomyosin) were obtained from an alcohol–ether dried muscle powder as described previously [13]. Separation of the troponin components (TN-C, TN-I and TN-T) was performed on a DEAE–Sephadex A-50 column [13].

2.2. *Gel-electrophoresis*

SDS–Polyacrylamide gel electrophoresis was carried out in 0.1% SDS, 100 mM sodium phosphate buffer, pH 7.5 and 12% acrylamide [14]. Urea–polyacryl-

amide electrophoresis was performed with 7% acrylamide gels in the presence of 6 M urea and either 20 mM Tris–80 mM glycine, at pH 8.6 [15] or in 5% acetic acid, pH 3.2 [16].

2.3. *Protein content*

Protein content was determined according to Lowry et al. [17] with bovine serum albumin as standard.

3. Results

SDS–Gel electrophoresis of foetal myofibrils isolated at the earlier stages of development (i.e., 19 days gestation) showed an increased content of proteins in the range 15 000–19 000 daltons (fig.1), in agreement with the results of Pelloni-Mueller et al. [2,3]. At later stages of development these bands diminished. Included in this range of molecular weights would be the myosin light chains, LC_2 and LC_3 and TN-C. Since it seemed unlikely that these proteins appeared in much higher amounts at early stages of development our attention was focused on the possible presence of histones. These proteins could have been present as a result of contamination of the myofibrils with nucleoproteins. Indeed SDS–gel electrophoresis (fig.1) showed that the mobility of the main fraction of commercial preparations of calf thymus histones corresponded to the mobility

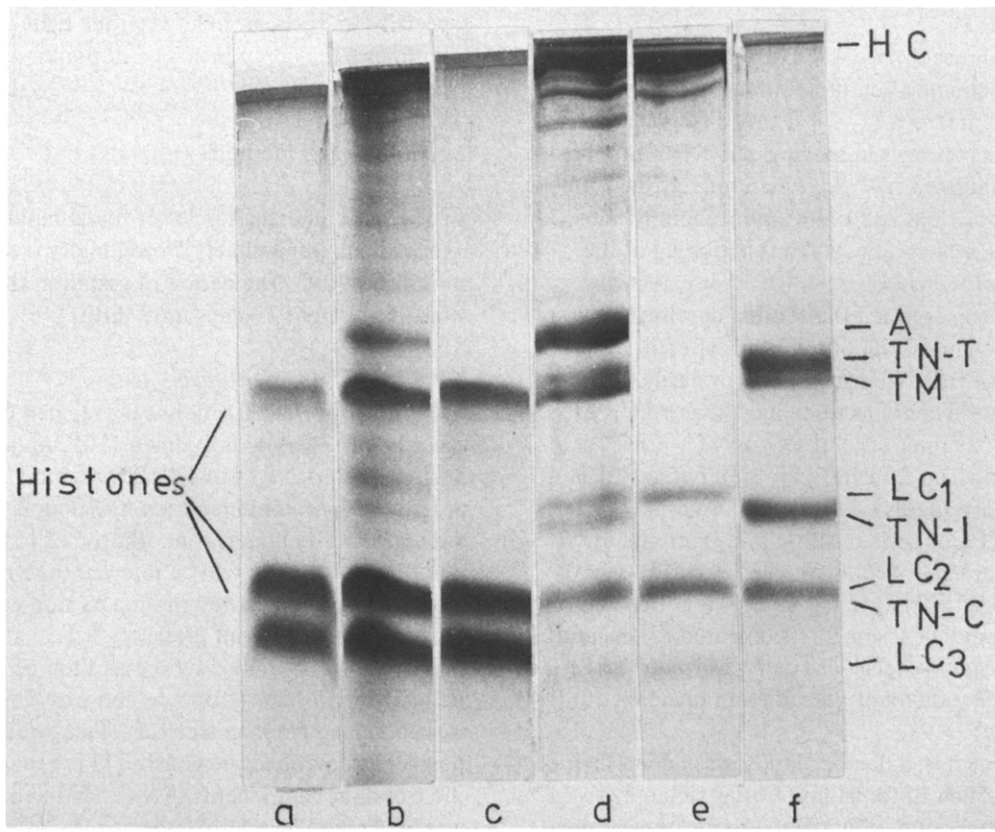

Fig.1. SDS–Polyacrylamide gel electrophoresis, at pH 7.5, of myofibrils from rabbit foetuses. (a) Commercial preparation of calf thymus histones (25 μg). (b) Myofibrils from 19 day-old foetus (100 μg). (c) 0.25 M HCl extract of alcohol–ether dried myofibrils shown in (b) (30 μg). (d) Myofibrils from adult rabbit muscle (100 μg). (e) Myosin from adult rabbit muscle (70 μg). (f) Troponin (20 μg) and tropomyosin (7.5 μg) from adult rabbit muscle. HC – myosin heavy chain, LC_1, LC_2 and LC_3 – myosin light chains, A – actin, TM – tropomyosin, TN-T, TN-I and TN-C – the tropomyosin binding, inhibitory and Ca^{2+}-binding components of troponin.

of the low-molecular-weight proteins present in myofibrils. To distinguish between the myosin light chains, TN-C and histones, urea–gel electrophoresis of myofibrils was performed, at pH 3.2 [16]. Among the myofibrillar proteins only the basic components, TN-I and TN-T, migrated under these conditions, but these did not interfere with the identification of the histones. The commercial preparation of histones was separated, at pH 3.2, into three fractions (fig.2). Myofibrils contained two bands with similar mobilities to those of the histones. These were noticably higher in the preparations obtained from early foetal muscle. Further support for the presence of histones in the myofibrils from young foetuses was obtained by the specific extraction of these proteins with 0.25 M HCl. This was achieved by extracting an alcohol–ether powder of myofibrils with 1 M KCl to remove the regulatory proteins [13] followed by extraction of the residue with 0.25 M HCl.

During electrophoresis of myofibrils in 6 M urea, at pH 8.6 [15] the basic proteins (i.e., histones TN-I and TN-T) did not move from the origin, whereas the acidic proteins, actin, tropomyosin, light chains of myosin and TN-C migrated towards the anode. Under these conditions, in the absence of calcium ions, the fastest migrating band corresponded to TN-C (fig.3A). This band was only faint in myofibrils from 19 day-old foetuses. Its intensity increased during development. Myosin light chains were well resolved, although the LC_2 component was masked by actin and tropomyosin and it was therefore not possible to determine the stoichiometry of this light chain during development. The LC_1 and LC_3 components did not co-migrate with other proteins. The LC_3 subunit was virtually absent in muscle from 19 and 22 day-old foetuses and it appeared only at later stages of development.

In order to show the presence of TN-I, which in the presence of calcium ions forms a complex with TN-C [15], urea–gel electrophoresis was performed also in the presence of calcium. Under these conditions the TN-C band disappeared in all cases and a new band appeared which corresponded to the TN-I–TN-C complex (fig.3B). Similar observations concerning the parallel appearance of all troponin components during early foetal life were obtained by analysis of urea gels of the 1 M KCl extract from alcohol–ether dried myofibrils.

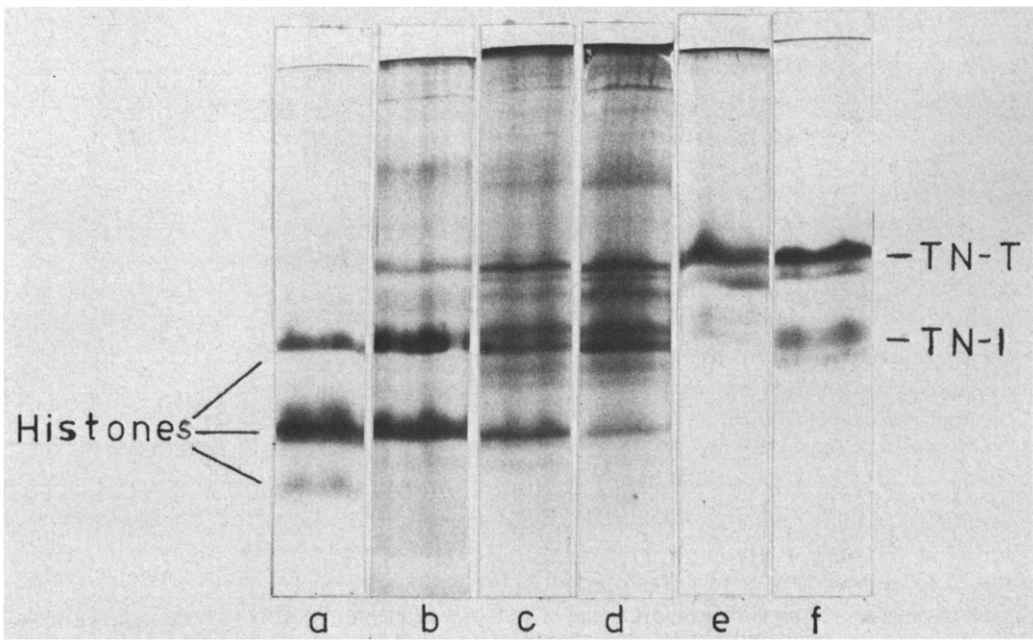

Fig.2. Urea–polyacrylamide gel electrophoresis, at pH 3.2, of myofibrils from rabbit foetuses. (a) Commercial preparation of calf thymus histones (20 μg). (b) Myofibrils from 19 day-old, (c) 22 day-old, (d) 26 day-old foetuses (100 μg). (e) Myofibrils from adult muscle (100 μg). (f) Troponin components TN-I and TN-T (each 20 μg).

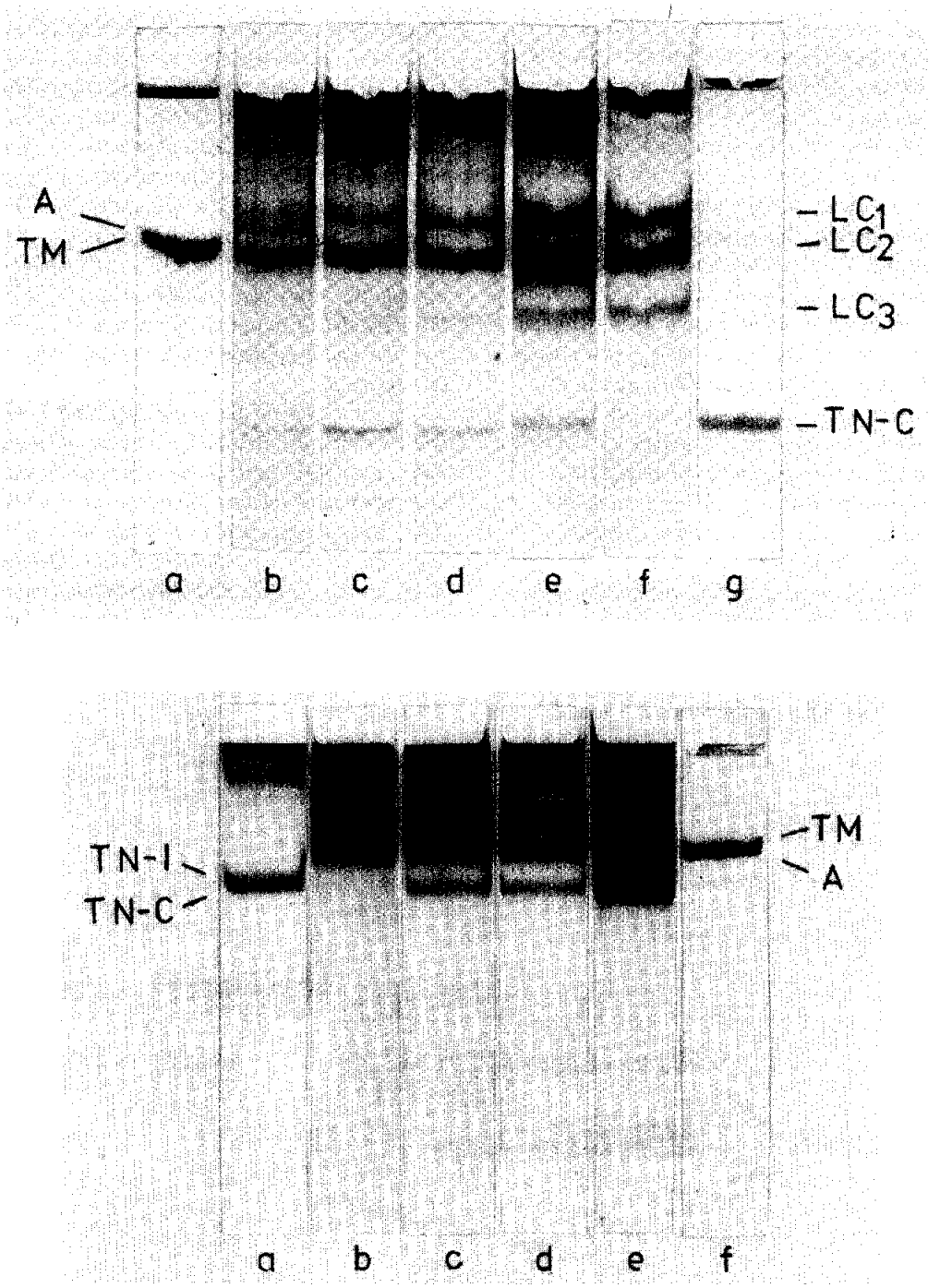

Fig.3. Urea–polyacrylamide gel electrophoresis, at pH 8.6, of myofibrils from rabbit foetuses. (A) In the presence of 5 mM EGTA: (a) tropomyosin (TM) and actin (A) (each 10 μg); (b) myofibrils from 19 day-old; (c) 22 day-old, (d) 26 day-old foetuses (100 μg); (e) myofibrils from adult rabbit-muscle (100 μg); (f) Myosin from adult rabbit-muscle (70 μg); (g) troponin component TN-C (10 μg). (B) In the presence of 5 mM $CaCl_2$: (a) troponin components TN-I and TN-C (each 10 μg); (b–e) the same as (b–e) in (A); (f) Tropomyosin (TM) and actin (A) (each 10 μg). LC_1, LC_2 and LC_3 – myosin light chains.

4. Discussion

This work clearly indicates that myofibrils isolated from rabbit foetuses are heavily contaminated by histones which interfere with the quantitation of the myosin light chains. Previous reports [6,7,18] have suggested that foetal-muscle myosin has the same light chain composition as adult myosin, the only difference being the reduced amount of LC_3. Taking into account the possibility that LC_3 could be lost during purification of myosin we analysed the proportion of LC_1 : LC_3 on electrophoregrams of whole myofibrils separated under conditions in which histones do not interfere with light chains. The data obtained indicate that LC_3 is synthesized later than LC_1. Recent studies [19,20] have pointed out the presence in adult rabbit skeletal-muscle myosin of two homo-dimers: one containing LC_2 and LC_1 and the other containing LC_2 and LC_3. The present results strongly suggest the sole existance of the homo-dimer containing LC_1 at early stages of myogenesis. Synthesis of a homo-dimer containing LC_3 develops subsequently to give rise to the characteristic isozyme pattern of adult muscle. This idea is supported by the recent observation of Takahashi [21], who also found a lack of LC_3 in foetal muscle and the inability of LC_3 isolated from adult muscle myosin to bind to foetal myosin. Weeds and Taylor [20] have shown the V_{max} of the actin-stimulated ATPase activity of subfragment-1 containing LC_3 to be higher than that of the subfragment-1 with LC_1. From these results it could be inferred that actin-stimulated ATPase activity of myosin containing LC_1 might be lower than that of the LC_3 counterpart. This hypothesis is consistent with our findings since foetal myosin was found to have a lower proportion of the LC_3 component and unpublished results from our laboratory indicate that the level of actin activation of ATPase activity increases during myogenesis. This also supports the contention that the ratio of LC_3 to LC_1 reflects, to some extent, the level of ATPase activity.

Urea–gel electrophoresis of myofibrils performed in the presence and in the absence of calcium ions was found to be useful for following the appearance of the troponin components, TN-C and TN-I, during development. All the troponin components seem to be synthetized simultaneously, but later than actin and tropomyosin, so that the ratio of the former proteins to tropomyosin increases during the early stage of development. Moreover, the results of Amphlett et al. [22] and our unpublished data show that at early stages of development β chains of tropomyosin predominate. All these observations suggest that there are two kinds of thin filaments: those which appear at a very early stage of development and are characteristic of undifferentiated cells, and the others, synthesized later [23], which are destined for incorporation into myofibrils. The former kind of thin filaments seem to be devoid of troponin and are composed only of F-actin and the $\beta\beta$ dimers of tropomyosin.

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