DEVELOPMENTAL CHANGES IN CHICKEN SKELETAL MYOSIN ISOENZYMES

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1. Introduction

There are 5 isoenzymes of myosin in adult chicken skeletal muscles, 3 of these are present in fast-twitch muscle (FM_1, FM_2 and FM_3) and 2 in slow-twitch muscle (SM_1 and SM_2) [1,2]. These isoenzymes are composed of 2 heavy chains (HC) and 4 light chains (LC). There are 3 types of LC in fast-twitch myosin (LC_1^f, LC_2^f and LC_3^f) and 2 types of LC in slow-twitch myosin (LC_1^s and LC_2^s). The LC compositions of the 5 isoenzymes of myosin have been elucidated in [2]. There is some evidence that the HC of fast-twitch and slow-twitch myosins are immunochemically distinct [3,5]. Studies on chick skeletal myosins during late foetal development have revealed some differences in the foetal myosins compared with adult myosins. The Ca^{2+}-activated myosin ATPase activity was shown to increase [6]. This has been attributed to a greater sensitivity of the foetal myosin to sulphydryl oxidation [7]. Foetal fast-twitch myosin contain LC_1^f and LC_2^f, with little or no LC_3^f [5,8,9]. The fast-twitch myosin HCs of day 11 chick embryos have been reported to be indistinguishable from adult fast-twitch myosin HC [5]. However, HC immunochemically related to adult cardiac myosin HC may also be present [10]. Foetal slow-twitch myosin contains LC_1^f and LC_2^f in addition to LC_1^s and LC_2^s [5]. Furthermore, immunochemical evidence suggests the presence of both fast-twitch and slow-twitch myosin HC in foetal slow-twitch myosin [5].

This work seeks to clarify the relationship between the myosins in developing fast-twitch and slow-twitch muscles and the myosin isoenzymes in the mature animal. It will be shown that foetal fast-twitch myosin contains only FM_3, the other 2 isoenzymes appearing successively around the time of hatching. Foetal slow-twitch myosin is predominantly SM_1, which appears to contain fast-twitch myosin LC in addition to slow-twitch myosin LC until 6–12 weeks after hatching.

2. Experimental

The fast-twitch pectoralis and the slow-twitch anterior latissimus dorsi (ALD) muscles of day 11 embryos through to month 5 Leghorn chicks were studied. Myosin was prepared from individual muscles and analysed for isoenzymes by pyrophosphate polyacrylamide gel electrophoresis using methods in [1,2]. The LCs of electrophoretically purified myosin isoenzymes were analysed by slicing the pyrophosphate gels containing the isoenzymes and subjecting the gel slices to SDS–polyacrylamide gel electrophoresis [2].

3. Results and discussion

3.1. *Fast-twitch muscle development*

Myosin from the pectoralis of the day 11 chick embryo showed a single component on pyrophosphate gel electrophoresis. This component co-electrophoresed with FM_3 of adult pectoralis myosin. By day 14 of embryonic life, an additional component which co-electrophoresed with FM_2 became apparent. Trace amounts of a third component which co-electrophoresed with FM_1 was detectable in the day 18 chick embryo. Rapid changes in the distribution of these components occurred after hatching so that by day 7 after hatching their distribution approximates to that seen in the adult. The time-courses of changes in these myosin components are shown in fig.1.

Fast-twitch myosins at various stages of develop-

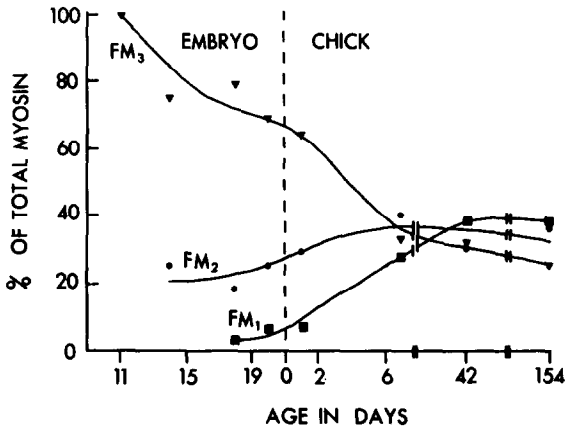

Fig.1. Developmental changes in the distribution of fast-twitch myosin isoenzymes in the chicken pectoralis muscle. The amount of each isoenzyme expressed as % total myosin is plotted against the age of the embryo or chick in days.

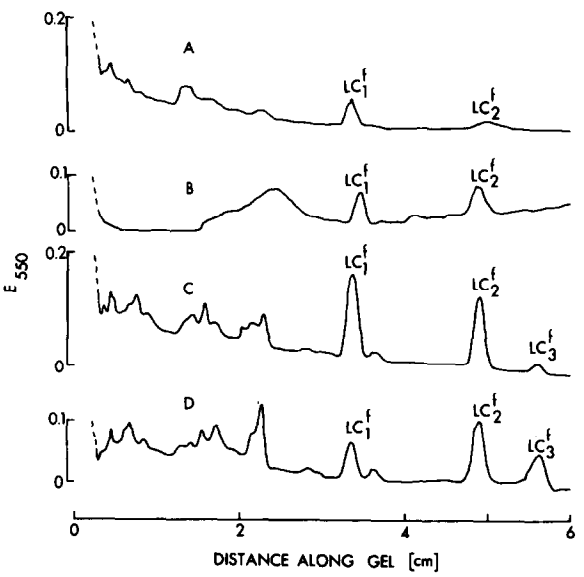

Fig.2. Light chain analysis of partially purified myosins from developing pectoralis muscles. After electrophoresis for 4 h in pyrophosphate gels, myosin bands (isoenzymes not resolved) were cut out for light chain analysis in SDS polyacrylamide gels as in [2]. The figure shows densitometer scans of SDS gels for day 14 embryo (A), day 16 embryo (B), day 1 chick (C) and day 18 chick (D).

ment have been analysed for their LC composition after brief electrophoretic purification by pyrophosphate gel electrophoresis. Myosin from day 14 (fig.2A) and day 16 (fig.2B) day embryos showed LC_1^f and LC_2^f with little or no LC_3^f, in agreement with results in [5,8,9]. Myosin from day 1 chick pectoralis showed principally LC_1^f and LC_2^f; a small amount of LC_3^f was clearly resolved (fig.2C). In pectoralis myosin from day 18 chick (fig.2D), the LC distribution was indistinguishable from that in the adult.

Previous work has shown that the LC compositions of FM_3, FM_2 and FM_1 are $(LC_1^f)_2$ $(LC_2^f)_2$, LC_1^f $(LC_2^f)_2$ LC_3^f and $(LC_2^f)_2$ $(LC_3^f)_2$, respectively [2]. The correlated changes in electrophoretic components of pectoralis myosin and their LC during development are consistent with the suggestion that foetal fast-twitch components are identical to the corresponding adult myosin isoenzymes. In this view, presumptive fast-twitch muscles synthesize FM_3, an isoenzyme present in the adult fast-twitch muscle. Subsequent development leads to the synthesis of FM_2 and FM_1. This interpretation would account for the observations that:

(i) Day 11 embryonic pectoralis myosin reacts with specific anti-fast-twitch myosin antibodies but not with anti-slow-twitch myosin antibodies [5];

(ii) Light meromyosin paracrystals prepared from day 16 embryonic pectoralis myosin is indistinguishable from that of adult pectoralis myosin [9].

However, in view of [10], it remains to be clarified whether FM_3 may contain, in addition to adult fast-twitch myosin HC, another type of HC which is immunochemically related to adult chick cardiac myosin.

Changes in isoenzyme distribution in developing fast-twitch muscle correlate well with changes in contractile properties. For reasons explained in [2], these isoenzymes are expected to differ in actin-activated ATPase activity, with FM_3 having the lowest while FM_1 having the highest activity. The actin-activated ATPase activity of myosin is correlated with the speed of muscle contraction [11]. The progressive appearance of isoenzymes of higher actin-activated ATPase activity should lead to an increase in muscle speed during development. This has indeed been reported to occur in chicken fast-twitch muscle [12,13]. This correlation between myosin isoenzymes and muscle speed strongly supports the hypothesis

that variation in isoenzyme distribution provides a mechanism for modulating the speed and power of muscle fibres [2].

3.2. *Slow-twitch muscle development*

Myosin from the ALD of a day 14 chick embryo showed 2 electrophoretic components which co-migrate with SM_1 and SM_2 of the adult muscle. The component co-migrating with SM_1 formed ~80% of the total myosin. In contrast with the developing pectoralis, little change in electrophoretic pattern of myosin occurred around the time of hatching. The proportion of the myosin component co-migrating with SM_1 decreased progressively later in post-hatched life, the time-course of this change is shown in fig.3.

Analysis of the LCs from day 1 chick ALD myosin (SM_1 and SM_2) after brief electrophoresis in pyrophosphate gels showed LC_1^s and LC_2^s (fig.4a); in addition, another component which has a mobility consistent with LC_1^f was also present in significant amounts. This finding of fast-twitch LC in developing slow-twitch muscle agrees well with previous work which reported the presence of both LC_1^f and LC_2^f [5]. In fig.4B, only SM_1 of day 18 chick ALD muscle was analysed for LC. The LC_1^f was again present, to an extent approximately equal to LC_1^s. Similar analyses of both SM_1 and SM_2 from older chicks showed that just detectable quantities of LC_1^f were present in both components at week 6 but absent at week 12. Since SM_1 was the principle component during the period when LC_1^f was present in significant amounts, most of the LC_1^f during development was associated with this component.

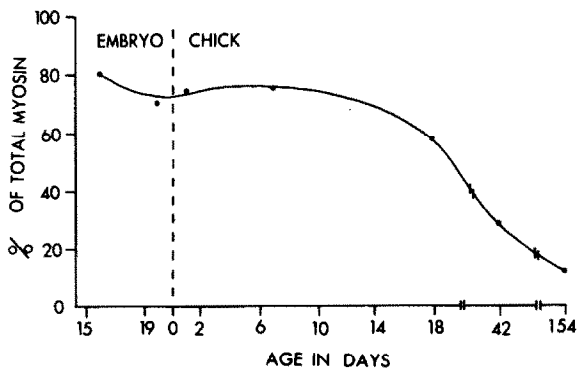

Fig.3. Developmental changes in the distribution of slow-twitch myosin isoenzymes in the ALD muscle. Two components which co-migrate with SM_1 and SM_2 of adult ALD were present throughout the period studied. The amount of SM_1 expressed as a percentage of total myosin is plotted against the age of the embryo or chick in days.

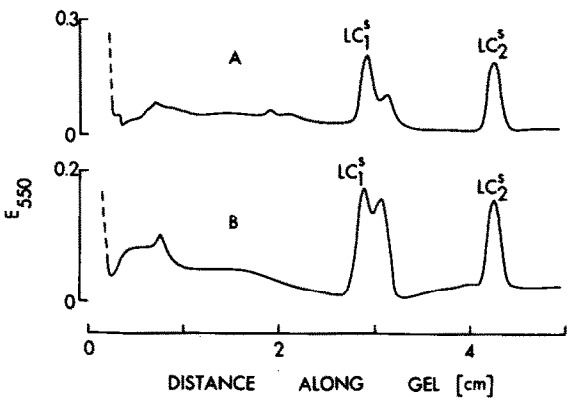

Fig.4. Light chain analysis of myosins from developing ALD muscles analysed as in fig.2. (A) Light chains from day 1 chick ALD myosin (SM_1 and SM_2). The peak near LC_1^s has the same mobility as LC_1^f. (B) Light chains from SM_1 (obtained after 16 h pyrophosphate gel electrophoresis) of day 18 chick ALD.

Adult ALD contains SM_1 and SM_2 but no fast-twitch myosin isoenzymes [1,2] and little or no fast-twitch myosin light chains [2,5]. Yet adult ALD myosin has been reported to react with specific antibodies against fast-twitch myosin HC, albeit less strongly than its reaction with antibodies against slow-twitch myosin HC [4,5]. These observations raise the possibility that HC of SM_1, the minor component of adult ALD myosin, may be responsible for its reactivity with anti-fast-twitch myosin antibodies. This possibility is strongly supported by the correlation of the isoenzyme pattern during development with the immunochemical studies of others: foetal ALD myosin, containing predominantly SM_1, reacts more strongly with anti-fast-twitch myosin antibodies than with anti-slow-twitch myosin antibodies [5]. Thus, the HC of foetal and adult SM_1 may be the same as those of FM_3, which migrates just ahead of SM_1 in pyrophosphate gels. This hypothesis implies that adult SM_1 is a hybrid myosin isoenzyme with slow-twitch LC but fast-twitch HC. According to this hypothesis, developing ALD contains small quantities of slow-twitch isoenzyme SM_2 (which is composed of slow-twitch HC and principally slow-twitch LC) and

large quantities of fast-twitch HC which are associated with both fast-twitch and slow-twitch LC in SM_1. In other words, foetal SM_1 is heterogeneous with respect to the type of LC. The developmental change in ALD consists in an increase in the proportion of slow-twitch HC at the expense of reduced fast-twitch HC, while fast-twitch LC is eventually replaced by slow-twitch LC.

It is of interest to correlate the myosin isoenzyme changes in the ALD with histochemical and physiological data. When stained for Ca^{2+}-activated ATPase activity, adult ALD fibres are uniformly pale (type I fibres) while adult fast-twitch fibres are uniformly dark (type II fibres). In day 1 chicks fast-twitch fibres are also uniformly type II while ALD fibres are predominantly type II [14]. The presence of fast-twitch LC and/or HC is presumably responsible for the type II behaviour of developing ALD. The speed of contraction of ALD in the day 18 embryo is clearly slower than that of fast-twitch muscle [12,13] as would be expected from the difference in their myosin isoenzymes. The time-course of subsequent speed change in ALD reflect the time-course of changes in myosin isoenzymes; little speed change occurs up to about 3 weeks [13], thereafter the speed of ALD becomes progressively reduced over a period of months [14]. The excellent correlation between myosin isoenzymes and muscle speed is apparently contradicted by the finding of a sudden and marked change in muscle speed occurring between the day 16 and the day 18 of incubation [12,13]. Prior to this time, both types of muscle gave a slow contracture in response to stimulation. Since this change is accompanied only by a minimal change in isoenzyme distribution, but a marked rise in tetanic tension, it is probably related to changes in the mechanism of excitation or excitation–contraction coupling.

4. Conclusion

Myosin isoenzyme analysis of developing chicken fast-twitch and slow-twitch muscles reported here suggest that from the day 11 embryo through to maturity these muscles do not synthesize a foetal myosin as earlier chick embryos apparently do [15,16]. Rather, these muscles are already expressing some of the myosin genes which are expressed in the adult animal. The pattern of myosin gene expression is clearly distinct in the two types of muscle in day 14 embryos. Changes in the pattern of expression of these genes occur in both types of muscle during subsequent development. These changes are correlated well with changes in contractile properties. The myosin changes may be brought about by nerves which are known to influence the contractile properties of regenerating chicken muscles [12] and the expression of mammalian myosin genes [17].

Acknowledgements

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