Volume 238, number 2, 245–248 FEB 06382 October 1988

Six myosin heavy chain isoforms are expressed during chick breast muscle development

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Received 18 August 1988

Two major embryonic myosin heavy chains are expressed in embryonic chick breast muscle until the first week after hatching. Of these, one is already detected in the 8-day-old embryo. The other appears on day 12. Two putative slow embryonic isoforms represent minor components transiently expressed between days 8 and 12. A neonatal heavy chain is expressed at low concentrations on day 8 and increases with development. It is the only isoform two weeks after hatching, and is ultimately replaced by the fast myosin heavy chain in the adult muscle.

Embryogenesis; Muscle development; Myosin heavy chain isoform; (Chick breast muscle)

1. INTRODUCTION

Embryonic, neonatal and adult-fast myosin heavy chain (HC) isoforms have been shown to be sequentially expressed in developing chicken breast muscle [1–4]. The existence of an additional, putative slow embryonic HC was suggested by the work of Benfield et al. [5]. This minor isoform was enriched in slow myosin light chains, reacted with antibodies against adult slow myosin HC, but was distinct from the latter by its peptide cleavage pattern [5]. The existence of two embryonic fast myosin HC transcripts in pectoralis muscle was indicated by S_1-nuclease mapping [6]. In view of the maximally possible number of 31 myosin HC genes in the chicken [7], an even greater HC isoform diversity than demonstrated to date might exist for the sarcomeric myosin heavy chains during myogenesis. However, such a myosin HC diversity has as yet not been detected at the protein level. An improved electrophoretic technique for myosin HC separation has led in our laboratory to the detection of a hitherto unknown fast myosin HC isoform in adult rat muscle [8]. This method was used to readdress the question of myosin HC diversity during breast muscle development in the present study.

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2. MATERIALS AND METHODS

2.1. *Animals, myosin extraction*

Total breast muscle was excised from 8- and 12-day-old chicken embryos (White Leghorn). In the case of older embryos (19 day), young (1-, 6-, 14-day-old) and adult chicken, only the superficial portion of the pectoralis muscle was used. Muscles were frozen and pulverized under liquid N_2. Myosin was extracted in the cold according to Rubinstein and Kelly [9]. The precipitated myosin was dissolved in 40 mM $Na_4P_2O_7$, 1 mM EDTA, pH 8.8, diluted with an equal volume of glycerol and stored at −20°C. All solutions contained 1 mM phenylmethylsulfonyl fluoride. Protein was determined according to Lowry et al. [10].

2.2. *Myosin heavy chain electrophoresis*

Polyacrylamide gel electrophoresis in the presence of SDS was carried out as recently described [8] using a 0.75 mm thick 5–8% gradient separating gel and a 4% stacking gel. Aliquots of the extracts containing 0.15–2.0 μg protein were loaded for electrophoresis after 5 min incubation at 95°C in a final volume of 10 μ lysis buffer (10% glycerol, 5% β-mercaptoethanol, 2.3% SDS, 60 mM Tris-HCl, pH 6.8). Electrophoresis at 120 V and 8–10°C lasted 22 h. Gels were silver-stained according to Oakley et al. [11] and densitometrically evaluated.

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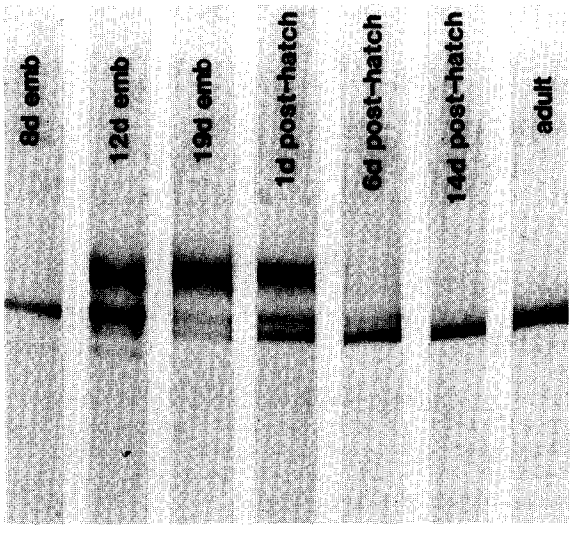

Fig.1. Myosin heavy chain isoforms during embryonic development and maturation of chicken breast muscle as assessed by gradient polyacrylamide (5–8%) gel electrophoresis in the presence of SDS. Age of embryonic (emb) and post-hatched (post-hatch) muscles is given in days (d).

3. RESULTS

At each developmental stage investigated, the myosin HC pattern of the embryonic breast muscle contained several isoforms at variable, stage-specific percentage distributions (fig.1, table 1). Three fast migrating bands were detected in the 8-day-old embryonic muscle. Five HC isoforms were delineated in the 12-day-old embryo. Three of these corresponded to the ones seen in the 8-day-old stage. The major band in the 8-day-old embryonic muscle was still the most prominent isoform on day 12. However, it progressively decreased in the later stages of development and was no longer detectable in chicken older than 6 days. This isoform was tentatively designated as early embryonic HC ($HC_{emb/e}$). The slowest migrating isoform of the 12-day-old embryonic muscle was not detectable on day 8, but became the major band in the later embryonic stages. It was also no longer detectable 6 days after hatching. Therefore, this isoform was tentatively named the late embryonic HC ($HC_{emb/l}$). Immunoblot analyses (not shown) with a monoclonal antibody reacting with embryonic and adult myosin heavy chains [4], gave positive results only with the late ($HC_{emb/l}$), but not with the early embryonic ($HC_{emb/e}$) isoform.

The faint band below the $HC_{emb/e}$ in the 8-day-old embryo displayed the same mobility as the only isoform of the 14-day-old chicken (fig.1). Its amount increased with development until it became the most prominent on day 6 after hatching. It was the only isoform in the pectoralis muscle of the 14-day-old chicken. Immunoblot analysis (not shown) with Bandman's antibody 2E9 [12] identified this band as a neonatal isoform (HC_{neo}). Adult fast pectoralis myosin was present as a single band ($HC_{ad/f}$) with a slightly lower mobility than HC_{neo}. In addition, two minor HC isoforms were transiently expressed in fetal breast muscle (fig.1, lanes 1 and 2). One migrated below the $HC_{emb/l}$ band and was only seen in the 12-day-

Table 1

Densitometrically evaluated percentage distributions of myosin heavy chain isoforms in developing chicken breast muscle

Developmental stage	%$HC_{emb/e}$	%$HC_{emb/l}$	%$HC_{emb/s1}$	%$HC_{emb/s2}$	%HC_{neo}	%$HC_{ad/f}$
Embryonic						
Day 8	99	0	<1	0	<1	0
Day 12	57	17	<1	25	<1	0
Day 19	6	90	0	0	4	0
Post-hatch						
Day 1	15	66	0	0	19	0
Day 6	<1	<1	0	0	99	0
Day 14	0	0	0	0	100	0
Adult	0	0	0	0	0	100

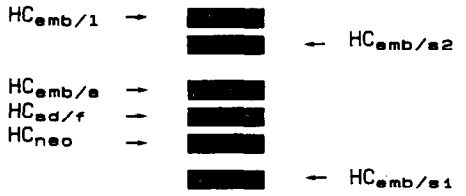

Fig.2. Schematic illustration of the electrophoretic mobilities of myosin heavy chain isoforms found at various developmental stages in chicken breast muscle.

old embryo. The other was the fastest isoform and was visible as a faint band in 8- and 12-day-old embryonic breast muscle. A schematic illustration of the electrophoretic mobilities and a proposed nomenclature for the six myosin HC isoforms is given in fig.2.

4. DISCUSSION

Our results show a pronounced myosin HC polymorphism in developing chick breast muscle. To our knowledge, we demonstrate for the first time six electrophoretically distinct myosin HC isoforms during chicken fast-twitch muscle development. The presence of five myosin HC isoforms in 12-day-old embryonic chicken breast muscle may be explained by taking into consideration its cellular heterogeneity at this stage [13,14]. Primary myotubes are predominant until day 8. The secondary myotubes formed between days 8 and 12, originate from another myoblast population [15]. Interestingly, the onset of secondary myotube formation coincides with the appearance of two additional heavy chains. It is noteworthy that these differences between the myosin HC patterns on day 8 and day 12 agree with previously shown dissimilar immunochemical reactivities of early and late embryonic breast muscle myosins [16].

Two minor HC isoforms disappear between days 12 and 19. During the same time period, the pectoralis muscle looses its transiently expressed slow myosin light chain complement (not shown). Thus, these two minor heavy chains may represent slow myosin HC isoforms. Therefore, they have tentatively been designated $HC_{emb/s1}$ and $HC_{emb/s2}$ (fig.2). The repression of these two isoforms on day 19 may be brought about by neural influence because it coincides with the transition from embryonic to adult patterns of innervation and changes in contractile properties [17].

A second transition in the developmental program of myosin HC expression occurs during the first week after hatching with a suppression of $HC_{emb/e}$ and $HC_{emb/1}$. By this time, HC_{neo} which is expressed at low concentration already at the earliest time point investigated, becomes the predominant isoform. Similar to the metabolic maturation of fast-twitch muscle [18], this transition coincides with an increase in muscular activity. The final developmental transition is the exchange of HC_{neo} with $HC_{ad/f}$ in the adult muscle such that a total of six myosin HC isoforms is expressed during the development of chick breast muscle.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 156. The authors express their gratitude to Dr Susan Lowey and Dr Everett Bandman for generously supplying the antibodies 12C5 [4] and 2E9 [12].

REFERENCES

[1] Bandman, E., Matsuda, R. and Strohman, R.C. (1982) Dev. Biol. 93, 508–518.
[2] Lowey, S., Benfield, P.A., LeBlanc, D.D. and Waller, G.S. (1983) J. Muscle Res. Cell. Motil. 4, 695–716.
[3] Bader, D., Masaki, T. and Fischman, D.A. (1982) J. Cell Biol. 95, 763–770.
[4] Winkelmann, D.A., Lowey, S. and Press, J.L. (1983) Cell 34, 295–306.
[5] Benfield, P.A., Lowey, S., LeBlanc, D.D. and Waller, G.S. (1983) J. Muscle Res. Cell Motil. 4, 717–738.
[6] Umeda, P.K., Kavinsky, C.J., Sinha, A.M., Hsu, H.-J., Jakovcic, S. and Rabinowitz, M. (1983) J. Biol. Chem. 258, 5206–5214.
[7] Robbins, J., Horan, T., Gulick, J. and Kropp, K. (1986) J. Biol. Chem. 261, 6606–6612.
[8] Bär, A. and Pette, D. (1988) FEBS Lett. 235, 153–155.
[9] Rubinstein, N.A. and Kelly, A.M. (1978) Dev. Biol. 62, 473–485.
[10] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[11] Oakley, B.R., Kirsch, D.R. and Morris, N.R. (1980) Anal. Biochem. 105, 361–363.
[12] Bandman, E.R. (1985) Science 227, 780–782.
[13] Rubinstein, N.A. and Kelly, A.M. (1981) J. Cell Biol. 90, 128–144.

[14] Stockdale, F.E. and Miller, J.B. (1987) Dev. Biol. 123, 1–9.
[15] Crow, M.T. and Stockdale, F.E. (1986) Dev. Biol. 113, 238–254.
[16] Crow, M.T. (1987) Am. Zool. 27, 1043–1053.
[17] Gordon, T. and Vrbová, G. (1975) Pflügers Arch. 360, 199–218.
[18] Bass, A., Lusch, G. and Pette, D. (1970) Eur. J. Biochem. 13, 289–292.