CHANGES IN TROPONIN-T AND MYOSIN ISOZYMES DURING DEVELOPMENT OF NORMAL AND DYSTROPHIC CHICKEN MUSCLES

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Received 22 August 1980

1. Introduction

As reported [1], the type of troponin-T (TN-T) markedly changes during the rapid growth of breast muscle of post-hatched chicken: that is the breast muscles of chick embryo or young post-hatched chicken contains leg-type TN-T (leg TN-T) which is distinguishable from breast-type NT-T (breast TN-T) by the difference in molecular weight [2,3] as a major TN-T component but leg TN-T in the breast muscles is replaced by breast TN-T in 1 week after hatching. However, the relative proportions of three myosin isozymes, FM_1, FM_2 and FM_3 in order of electrophoretic mobility, also change during the growth of breast muscle of post-hatched chicken [4]. In the case of genetically destined dystrophy chicken (New Hamsphire line 413) dystrophy in breast muscle is gradually revealed in 4–5 weeks after hatching. Therefore, the alterations in TN-T and myosin isozymes after hatching seem to be appropriate markers to examine whether there is any difference in the differentiation of the myofibrillar proteins between normal and dystrophic muscle. Here, we demonstrate that the types of TN-T and the patterns of myosin isozymes present in the developing normal and dystrophic chicken skeletal muscles are significantly different from each other by electrophoretic and immunoelectrophoretic investigations.

2. Materials and methods

Breast muscle, m. pectoralis major, of various developmental stages of normal (New Hampshire line 412) and dystrophic (New Hampshire line 413) chicken was used. Myosin was prepared according to [5], except that 5 mM ATP, 5 mM $MgCl_2$ and 2 mM ethylene–glycolbis(aminoethyl ether)–*N,N,N',N,*-tetraacetic acid (EGTA) were present in the extracting solution. Troponin was extracted with 0.4 M LiCl containing 5 mM ethylene–diamine tetraacetic acid (EDTA), 1 mM $NaHCO_3$ and 0.05% NaN_3 for 3–5 h at 0°C from the muscle residue left from myosin extraction, and was partially purified by isoelectric precipitation and ammonium sulfate fractionation methods according to [6]. The antiserum against TN-T from adult chicken breast muscle was obtained as in [7].

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) was performed using 13% acrylamide gel containing 1/37 amount of methylene bisacrylamide with the phosphate buffer system according to [8] or with the Tris–glycine discontinuous system according to [9]. Pyrophosphate–acrylamide gel electrophoresis for myosin isozymes was as in [10].

To quantify the relative proportions of myosin isozymes, the bands for myosin isozymes and the gel pieces without protein of roughly same size was cut out from the same gels which were stained and destained. The staining dye, Coomassie brilliant blue R-250, was extracted from the gel slices with a solution containing 1% SDS and 10 mM Na-phosphate buffer (pH 7.0) for 24 h at 37°C, then the A_{550} of the solution was measured. The relative proportions of myosin isozymes were calculated based on the absorbance values in [11].

3. Results

3.1. *Types of troponin-T*

The electrophoretic pattern of troponin components of developing normal and dystrophic chicken breast muscle are shown in fig.1. As shown in [1], normal chicken breast muscle at 1 week after hatching or later stages contains breast TN-T. In constrast, troponin from the breast muscle of dystrophic chicken of various ages was found to contain two components (T_b,T_1), which might correspond to breast TN-T and leg TN-T as judged by their electrophoretic mobilities. However, since TN-T has been known to be highly sensitive to proteolytic enzymes, the lower molecular weight component of TN-T (T_1) observed in troponin from the dystrophic muscle might be derived from breast TN-T as the result of partial proteolysis during the preparation procedure. Furthermore, tropomyosin which is the almost same molecular weight range as leg TN-T is occasionally contaminated into the troponin preparation, and disturbs the identification of leg TN-T on the electrophoretic gel.

The coexistence of two classes of TN-T in the dystrophic chicken breast muscle was established by the immunodiffusion combined with SDS–PAGE. In this method, the proteins were directly dissolved from quick frozen breast muscle of dystrophic or normal chicken with a SDS solution at 95°C to minimize the degradation of troponin by cellular proteolytic enzymes, and the extracts were subjected to the immunodiffusion combined with SDS–PAGE. As shown in fig.2, A,B, the SDS extracts from 1 month as well as 1 year dystrophic chicken formed two immunoprecipitin lines, one major and one minor, against anti-TN-T antibody, while the extract from breast muscle of normal chicken of the same ages gave only one immunoprecipitin line, which is due to breast TN-T, against anti-TN-T antibody. Since the mobility of tropomyosin is close to that of leg TN-T

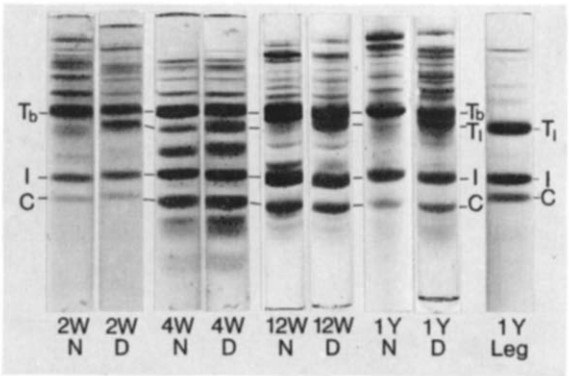

Fig.1. SDS–PAGE of troponin components from breast muscle of normal (N) and dystrophy (D) chicken at 2 weeks (2W), 4 weeks (4W), 12 weeks (12W) and 1 year (1Y) after hatching. The pattern of troponin components from leg muscle (1Y Leg) is indicated for comparison. The electrode buffers used were the phosphate buffer in 2W N, D and 1Y (Leg) or the Tris–glycine buffer in the other cases. Because of the difference in the electrode buffer, the mobilities of troponin components were slightly different between the gels. T_b, breast TN-T; T_1, leg TN-T; I, troponin-I; C, troponin-C.

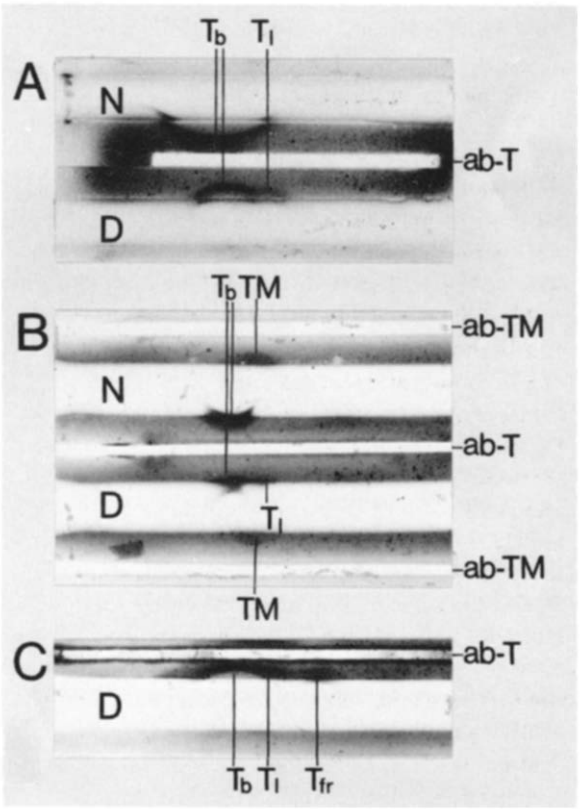

Fig.2. Immunodiffusion combined with SDS–PAGE of the whole SDS extracts from the breast muscle of normal (N) and dystrophy (D) chicken at 4 weeks (A) or 1 year (B) after hatching, and troponin from the breast muscle of dystrophy chicken at 12 weeks after hatching. ab-T, antiserum against tropomyosin. Immunoprecipitin lines derived from breast TN-T (T_b), leg TN-T (T_1), tropomyosin (TM) and the proteolytic fragment of TN-T (T_{fr}) were marked, respectively, in the figures.

but considerably higher than that of breast TN-T, it was possible to identify the type of TN-T by comparing the positions of immunoprecipitin lines of TN-T formed by anti-TN-T antibody with that of tropomyosin formed by anti-tropomyosin antibody [1]. As judged by the positions of immunoprecipitin lines, the major line formed with the extract of dystrophic muscle was identified to be derived from breast TN-T and the other one from leg TN-T. When troponin prepared from dystrophic muscle was tested by the same method, an additional lower molecular weight component having a common antigenicity with TN-T (T_{fr} in fig.2C), probably a proteolytic product of TN-T, was occasionally detected. However, such proteolytic fragment was scarcely detected in the cases of the SDS extracts from the muscles, irrespective of normal or dystrophic chicken (see fig.2A,B), suggesting that the direct SDS extraction should be effective to avoid proteolytic degradation.

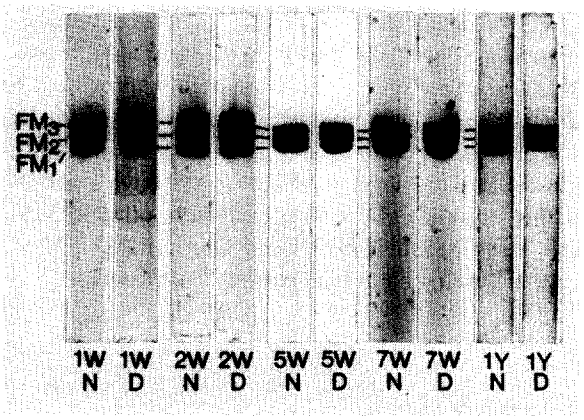

Fig.3. Pyrophosphate–acrylamide gel electrophoresis of myosin isozymes from the breast muscle of normal (N) and dystrophy (D) chickens. FM_1, FM_2 and FM_3 three myosin isozymes in fast muscle; 1W–7W and 1Y, ages in weeks and in year after hatching.

3.2. *Patterns of myosin isozymes*

When the patterns of myosin isozymes in developing skeletal muscle of normal and dystrophic chicken were compared, remarkable differences were not observed up to 3 weeks after hatching: in both normal and dystrophic muscles, FM_1 was present in only small amount at 1 week after hatching but the amount of FM_1 became larger than those of the other isozymes at 2 weeks after hatching. However, at 5–7 weeks after hatching when muscular dystrophy was revealed, the pattern of myosin isozymes in dystrophic muscle differed from that in normal muscle, as shown in fig.3. In the normal breast muscle at 5–7 weeks, the relative proportion of FM_1 is largest, while in dystrophic muscle, FM_1 is present in less amount than FM_2 and FM_3 (fig.4). The difference in the isozyme patterns between normal and dystrophic muscles was quite remarkable in ~1 year after hatching. The relative proportion of three myosin isozymes in dystrophic muscle is like that in the normal muscle at younger developmental stages (fig.3,fig.4).

4. Discussion

The types of TN-T and tropomyosin change co-ordinately during the development of the breast muscle of normal chicken [1]. Breast muscle of dystrophic chicken contains α- and β-tropomyosin as embryonic breast muscle, while the breast muscle of normal chicken contains only α-tropomyosin [2]. The alteration in TN-T in the dystrophic muscle may be also correlated with that in tropomyosin ([2], this work). Furthermore, this study shows that the alteration in myosin occurs at the similar ages. But the change in actin has not been detected in the dystrophic muscle, as examined by isoelectric focusing electrophoresis.

Myosin from the dystrophic muscle was deficient in one of light chains (LC_3) as examined by SDS–PAGE (not shown [3,14]). The deficiency in LC_3 may be closely related to the decrease in the relative proportion of FM_1, because FM_1 is a myosin isozyme which contains LC_3 [10].

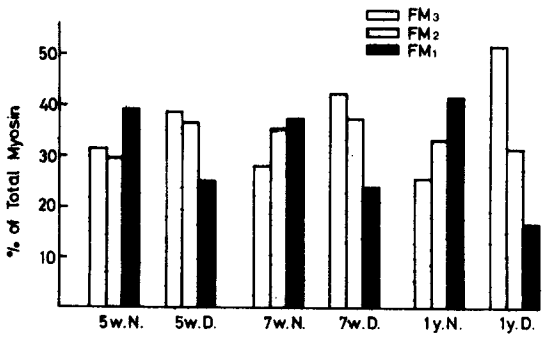

Fig.4. The relative proportions of three myosin isozymes in normal and dystrophic muscle. The proportions were quantified as described in section 2. Symbols are as in fig.3.

As judged by the type of TN-T and the pattern of myosin isozymes, the muscle in the dystrophic state seems to be restored to the state of immature muscle. The changes in the muscle proteins in the dystrophic muscle could be the result of an increase in immature muscle cells in a regenerating state, although a switch in the expression of one set of genes to another within the same fiber could be possible as postulated in the case of developing normal chicken breast muscle [1,15].

Acknowledgements

We thank Professor T. Masaki for his support and helpful discussions. This work was supported by grants from the Japanese Ministry of Education and from National Center for Nervous, Mental and Muscular Disorder (NCNMMD) of the Ministry of Health and Welfare, Japan.

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