Presence of the tropomyosin $\beta$-chain in dystrophic chicken breast muscle

SHINICHI TAKEDA and YOSHIAKI NONOMURA
Department of Pharmacology, Faculty of Medicine, University of Tokyo, Hongo, Tokyo 113, Japan

ABSTRACT
The presence of the $\beta$-chain of tropomyosin in the breast muscle of adult dystrophic chickens (line 134), albeit in a small amount, was clearly detected together with the $\alpha$-chain by two-dimensional gel electrophoresis with urea-SDS and SDS. Only the $\alpha$-chain was present in the control (line 412) muscle. This is the first report to show a definite difference in protein level between the dystrophic and control muscle. Together with the results obtained from growing muscle, our evidence suggests that the appearance of $\beta$-chain in dystrophic muscle is closely related to the developmental and regenerative process of the muscle.

KEY WORDS $\beta$-tropomyosin / dystrophic chicken muscle / growing muscle

Since the first introduction of chickens with hereditary muscular dystrophy (dystrophic chickens) in 1956 (1), much research has been done in the morphological field comparing dystrophic chicken muscle with normal controls. On the other hand, a biochemical approach to contractile proteins using dystrophic chickens has rarely been made.

When we preliminarily checked the proteins in the breast muscle, the most damaged of all the muscles in dystrophic chickens, some difference was noticed in the tropomyosin molecules between dystrophic and control muscles (12). The present study aimed at confirming this observation using a more progressive technique, two-dimensional gel electrophoresis with SDS and urea-SDS. Such a change in protein level may throw light on the genesis of muscular dystrophy. The change in tropomyosin molecules was also examined in growing muscle until nearly 100 days after hatching.

The breast muscles (pectoral and supracoracoid) were isolated from control (line 412) and dystrophic (line 413) adult chickens. Almost the same procedure was carried out in growing muscles, but in younger chickens separation of pectoral and supracoracoid muscles was impossible, so that both muscles had to be isolated together. To prepare whole muscle extract, small amount of breast muscle was separated and homogenized in a solution containing 1% SDS, 50 mM Tris-HCl (pH 7.0), and 0.5% $\beta$-mercaptoethanol. Native tropomyosin and tropomyosin-rich fractions were prepared from both pectoral and supracoracoid muscles with a slight modification of the method of Ebashi et al. (4). Details of SDS and urea-SDS gel electrophoresis are described in the figure legends.

Sender (11), using urea-SDS polyacrylamide gel electrophoresis (urea-SDS-PAGE), found that tropomyosin migrated more slowly than in the usual SDS-PAGE. Carmon et al. (2) utilized this property to identify the presence of tropomyosin molecules in abundant proteins on two-dimensional slab gel with urea-SDS and SDS. We also used this two-dimensional slab gel technique to identify tropomyosin. Whole muscle extract was first applied on the urea-SDS-PAGE and then the sliced gel was applied on the slab SDS-PAGE. As shown Fig. 1, almost all the proteins appear aligned diagonally in the second dimension, whereas tropomyosin was retarded in the first gel and then acquired a

Biomedical Research 1, 176-179 (1980)
position outside the diagonal line. Another spot separated from the diagonal line was detected around the 43,000 dalton actin band in the second step, SDS-PAGE. This spot will be discussed later. The sample prepared from the control muscle showed only an α-chain spot around the 33,000 dalton tropomyosin band in the second step (Fig. 1a), but the sample from the dystrophic muscle had another minor spot which was slightly more retarded than the α-chain spot both in the first and second gel steps (Fig. 1b). From the profiles of the standard rabbit tropomyosin with both α and β chains (Fig. 1c), the position of the minor spot around 33,000 daltons in dystrophic muscle was identified as the β-chain of tropomyosin. To confirm the presence of tropomyosin β-chain in dystrophic breast muscle, further tests were carried out using a preparation of native tropomyosin and tropomyosin-rich fractions.

Tropomyosin-rich fractions prepared from control and dystrophic muscles were compared with rabbit skeletal tropomyosin by the usual SDS-PAGE. As shown in Fig. 2, the presence of the β-chain is clearly demonstrated in the dystrophic muscle, but it is not visible in the control; the α-chain is observed in both cases. The ratio of α-chain to β-chain in rabbit back muscle is reported to be about 3 to 1 (3); judging from Fig. 2, this ratio in the dystrophic breast muscle is about 10 to 1. This finding was confirmed on two-dimensional gel pattern with native tropomyosin preparations. The α-chain spot alone can be seen in the special position of the two-dimensional gel of the control (Fig. 3a), while the β-chain spot can also be detected with α-chain in the two-dimensional gel of the dystrophic sample (Fig. 3b). In this case the retarded spot around 43,000 daltons in the SDS-PAGE is also visible.

Recently Roy et al. (10) reported the presence of a tropomyosin β-chain in chicken breast muscle in the embryonic stage and even several days after hatching. Since we considered the presence of β-chain in the dystrophic breast muscle to be related to a developmental problem, the change in the profile of tropomyosin with growth was examined both in the control and dystrophic muscles after hatching. The β-chain could be detected on the 27th day after hatching in the dystrophic muscle, but in the control it had already disappeared on the 10th day. The presence of the β-chain became, however, uncertain on the 40th day in the dystrophic muscle. This state continued for some time.

---

Fig. 1 Two-dimensional gel electrophoresis of whole extract of chicken breast muscle and rabbit tropomyosin. Proteins were separated in the first dimension on 13% polyacrylamide gel containing 0.1% SDS and 7 M urea, and in the second dimension on 13.5% polyacrylamide mini slab gel containing 0.1% SDS with the Tris-glycine discontinuous system according to Laemmli (7). a) Whole extract from the control supracoracoid muscle. b) Whole extract from the dystrophic supracoracoid muscle. c) Tropomyosin from rabbit back muscle. The α-chain and β-chain are indicated by arrows. A unique protein (unidentified) is indicated by a double arrow. A indicates the position of actin on SDS-PAGE.
time. After about the 100th day, the β-chain gradually reappeared in the breast muscle of the dystrophic chicken and afterwards the β-chain remains in the breast muscle throughout life. During this period, the control breast muscle retains only the α-chain of tropomyosin.

This report is the first clear presentation of the change at the protein level occurring in dystrophic muscle. As to tropomyosin in the dystrophic breast muscle, Irish et al. (5) reported an additional protein band near the tropomyosin band on SDS-PAGE of crude thin filament fraction, but they did not identify this band. On the other hand, John (6) reported that SDS-PAGE of myofibrils prepared from dystrophic murine muscle did not differ from that of the control. The amount of the β-chain in the dystrophic breast muscle is very small compared to that of the α-chain and its existence might be overlooked unless the two-dimensional gel technique is used.

In this study we found another protein that had the same mobility as actin on SDS-PAGE, but was retarded on urea-SDS-PAGE, as shown in Figs. 1a, 1b and 3b. The mobility of actin and 10 S-α-actinin (8, 9) was tested by urea-SDS-PAGE, because both proteins have the same mobility on SDS-PAGE. Neither actin itself nor 10 S-α-actinin was retarded on urea-SDS-PAGE, and we concluded that the other

![Fig. 2 SDS gel electrophoresis of a tropomyosin-rich fraction of chicken breast muscle and rabbit skeletal tropomyosin. Proteins were applied on 13.5 % polyacrylamide mini slab gel containing 0.1% SDS according to reference 7. a) Rabbit tropomyosin. b) Tropomyosin from the control breast muscle. c) Tropomyosin-rich fraction from the dystrophic breast muscle. The α-chain and β-chain are indicated by arrows.](image)

![Fig. 3 Two-dimensional gel electrophoresis of native tropomyosin from chicken breast muscle. Gel treatment was similar to that in Fig. 1. a) Native tropomyosin from the control breast muscle. b) Native tropomyosin from the dystrophic breast muscle. The α-chain and β-chain are indicated by arrows. Double arrow indicates an unidentified protein.](image)
The protein found in this study is neither actin nor 10 S-α-actinin. Identification of this unique protein is urgently required.

According to Roy et al. (10), the presence of the β-chain in breast muscle means the existence of the embryonic form of tropomyosin and, thus, the survival of the β-chain suggests a delayed development in growing dystrophic muscle. The reappearance of the β-chain after the 100th day might indicate the beginning of a regenerative process in the dystrophic breast muscle. Hereafter, the differentiation of contractile proteins should be an effective means for approaching the genesis of muscular dystrophy, if combined with morphological studies. Obinata (personal communication) is now pursuing some changes in troponin and myosin light chains in dystrophic breast muscle along a similar line.

We sincerely thank Professor S. Ebashi for his warmful discussion and encouragement and we also thank Miss Y. Tsunashima for her helpful assistance. This work was supported by grants from the Ministry of Education, Science and Culture, Japan, the Ministry of Health and Welfare, Japan, the Muscular Dystrophy Association of America, Inc. and the Iatrochemical Foundation.

Received for publication 28 February 1980

REFERENCES