# A NEW MYOFIBRILLARY PROTEIN FROM RABBIT SKELETAL MUSCLE

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UDC 612.744.14

KEY WORDS: skeletal muscle; myofibrils; new protein; metamyosin.

It was reported in 1955 that a protein of the myofibrillary complex had been isolated from mammalian skeletal muscle [4]. On free electrophoresis of extracts of myofibrillary proteins the presence of a fraction differing in electrophoretic mobility from myosin and actomyosin was discovered. This protein fraction was isolated by salt fractionation and was called metamyosin (MM). The content of MM is highest in fetal skeletal muscle and it decreases during postnatal development. No ATPase activity was found in the MM preparations, but it had marked ability to hydrolyze chloline esters [1, 2]. It was found that MM is not an individual protein. During salting out and electrophoresis in agar-agar gel MM was divided into 5-7 protein fractions. In view of the absence of methods of separation and identification of myofibrillary proteins at that time, it was impossible to determine the nature of the MM fractions. These investigations showed as a whole that the French workers [4] had isolated, not an individual protein, but a mixture of unidentified proteins. During the subsequent years various other myofibrillary proteins were discovered (actinins, troponins, and so on), but their relationships to the protein components of MM remained uncertain.

In 1969 Weber and Osborn [8] devised a method of polyacrylamide gel electrophoresis (PGE) of proteins, by which the different myofibrillary proteins could be separated and identified.

The main aim of the present investigation was to study the protein composition of MM preparations by PGE in the presence of sodium dodecylsulfate (SDS).

## EXPERIMENTAL METHOD

The test object was the psoas muscle of newborn and adult rabbits. MM was obtained after purification from contamination with actomyosin twice [5, 6]. The MM preparations were fractionated with a saturated solution of ammonium sulfate. To obtain fractions of myofibrillary proteins the minced muscles were first freed from sarcoplasmic proteins by consecutive extraction with water and 0.05M KCl (twice with each in the course of 15 min). Myofibrillary proteins were then extracted from the separate portions of homogenate in the course of 60 min using different solutions of high ionic strength: Weber (0.6 m KCl, 0.04 M $NaHCO_3$, 0.01 M $Na_2CO_3$), Weber–Edsall (0.6 M KCl, 0.04 M $KHCO_3$, 0.01 M $K_2CO_3$), Bailey (0.5 M KCl, 0.03 M $NaHCO_3$), Gub and Shtraub (0.3 M KCl, 0.09 M $KH_2PO_4$, 0.06 M $K_2HPO_4$), 0.6 M KCl with ATP (2 mg/g muscle), and MM extraction solution (0.3 M KCl, 0.0375 M $KH_2PO_4$, 0.0375 M $Na_2HPO_4$). The extract was centrifuged for 60 min at 20,000 rpm (TsLR-1 centrifuge), the supernatant was diluted 15 times with water, and the residues were collected by centrifugation and dissolved in 0.6 M KCl. The solutions thus obtained were used as preparations of myofibrillary proteins. The myofibrils were isolated [3] and further purified with Triton X-100 [7]. The electrophoretic investigation was conducted in 4.1 and 10% polyacrylamide gel in the presence of SDS [8]. The molecular weight of the proteins was determined from their mobility during PGE, and to plot a calibration curve, mobility of proteins with known molecular weight was calculated: heavy chains of myosin – 200 kiladaltons (kD), α-actinin ($P_{0-25}$ 96 kD, albumin 67 kD, pyruvate kinase 57 kD, actin 42 kD, trypsin 24 kD, and ribonuclease 14 kD. The protein concentration was measured by the microbiuret method.

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Department of Biochemistry, S. M. Kirov Military Medical Academy, Leningrad. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 10, pp. 428-429, October, 1984. Original article submitted October 20, 1983.

0007-4888/84/9810-1351$08.50  

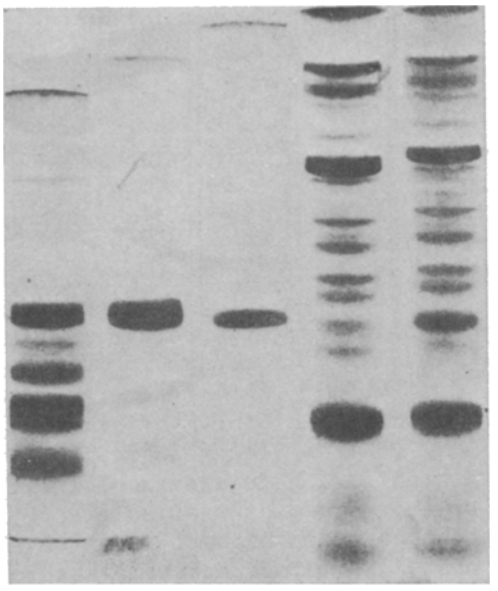


Fig. 1. Electrophoresis in 4.1% polyacrylamide gel in presence of SDS. a) Preparation of metamyosin from adult rabbit skeletal muscles; b, c) protein X with different degrees of purification; d) myofibrils of adult rabbit skeletal muscles and protein X. Arrow indicates band of protein X. [Caption as in Russian original — Publisher.]

## EXPERIMENTAL RESULTS

The results of electrophoresis of MM preparations from adult rabbit skeletal muscles in 4.1% polyacrylamide gel are shown in Fig. 1. The MM was found to consist of 6-9 protein fractions differing in molecular weight and content. The composition and relative proportions of individual fractions, incidentally, were not identical in different MM preparations, as also was observed on fractionation of MM by other methods. Some of the MM fractions were identical in moelcular weight with already known myofibrillary component proteins of tropomyosin, with troponins, etc.

Meanwhile the principal component of MM (protein X) which, according to the results of densitometry, accounted for 35-45% of the protein material of the preparations, differed in molecular weights from myofibrillary proteins so far identified. To isolate protein X the method of fractional salting out with a saturated solution of ammonium sulfate was used. It was found that protein X is salted out over a wide range of concentrations (40-70% saturation), but is precipitated in virtually homogeneous form within the range of 41 and 46% saturation (Fig. 1). The molecular weight of protein X (according to the results of PGE in 10% gel) was 78-79 kD.

The next step was to discover whether this protein is extracted only by MM extraction solution, and whether it is myofibrillary in origin. For this purpose, myofibrils were isolated from rabbit skeletal muscles. Myofibrillary proteins were extracted from the same portion of muscle by different solutions with high ionic strength. The resulting preparations were tested electrophoretically separately and mixed with protein X. The results of these experiments showed (Fig. 1) that this protein is in fact present in myofibrils and is extracted from muscles by all the solutions used. It is extracted in relatively large quantities (a stronger band on the gel after electrophoresis) by solutions with weakly acid pH (6.5-6.8) — (the solution of Gub and Shtraub, 0.06 M KCl with ATP, and MM extraction solution).

We thus isolated from MM preparations a protein which differs in its molecular weight from other myofibrillary proteins so far known.

This protein was obtained from MM preparations of the skeletal muscle of newborn rabbits. On electrophoresis of purified proteins X, isolated from muscles of rabbits of different ages, and also mixtures of them, no difference in mobility was found, evidence of equality of their

molecular weights. The content of protein X in functionally immature muscles was much lower than in muscles of the adult rabbits. In other words, its concentration increases during postnatal ontogeny and during formation of the contractile reaction of the muscle fiber. The yield of MM from adult rabbit muscles was 0.5-0.6 mg/g tissue, and from newborn muscles about twice as high. However, protein X accounted for 35-45% and 3-6% of MM preparation of adult and newborn animals respectively. It must be pointed out that on the whole, as regards their fractional composition, MM preparations from muscles of newborn and adult animals differ significantly from each other.

The functional properties of protein X and its participation in the act of muscular contraction are still unknown. According to our own data, the protein possess neither ATPase, nor creatine kinase, nor cholinesterase activity. Preliminary experiments showed that in the presence of purified preparations of protein X the molecular weight of F-actin is reduced by more than half (the molecular weight was measured by intensity of light scattering). This is a problem which requires further study.

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# WATER-SOLUBLE PROTEINS OF THE SUBESOPHAGEAL GANGLION COMPLEX OF *Helix pomatia* IN THE EARLY STAGES OF LEARNING


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UDC 612.822+612.8.015+577.352

KEY WORDS: water-soluble proteins; *Helix pomatia;* conditioned reflex


It was shown previously by ultra microbiochemical methods that the formation of a defensive conditioned reflex in *Helix pomatia* is accompanied by an increase in the concentration of acid water-soluble protein in the command neurons in the arc of this reflex. Unconnected stimulation and reinforcing procedures gave a substantially smaller effect [4, 11].

Since the phenomenon of formation of a new protein "phenotype" was fixed in the final stage of the reflex, when a 100% level of learning had been achieved, and it was absent in the homogenate of the whole ganglion, it was postulated that in the early stages of learning the increase in content of acid proteins reflects a general reaction of the nervous system of the snail and is observed in all cells with equal or nearly equal intensity, but later this level of protein metabolism is fixed only in a very limited number of nerve cells.

This hypothesis is supported by the results of an investigation [5] which showed a marked increase in the content of acid proteins in total homogenate of the ganglion in rela-


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(Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 10, pp. 429-431, October, 1984. Original article submitted October 25, 1983.

0007-4888/84/9810-1353$08.50