THE ENZYMIC PROPERTIES OF METAMYOSIN

(UDC 612.744.14 : 577.153.4]-088.1)

V. A. Yur'ev, N. I. Lopatina, Z. N. Zhakhova,
and A. V. Matrosova

Biochemical Laboratory (Head, Docent V. A. Yur'ev), Institute of Obstetrics and Gynecology (Director, Professor M. A. Petrov-Maslakov) of the AMN SSR, Leningrad
(Presented by Active Member AMN SSSR V. M. Karasik)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 58, No. 7, pp. 54-57, July, 1964
Original article submitted April 5, 1963

Metamyosin, obtained like myosin by extraction of muscle tissue with salt solutions of high ionic strength [6-11], differs from myosin by the absence of adenosine triphosphatase (ATPase) activity and inability to combine with actin to form viscous gels, by a difference in its electrophoretic mobility, and so on. Preparations of metamyosin possess very slight deaminase activity. Metamyosin also differs in its physico-chemical properties from the group of readily soluble myofibrillary proteins.

The metamyosin content, highest in the fetus, falls progressively as the animal grows older. The physiological role of metamyosin is uncertain. It has been suggested that thss protein is the precursor of other myofibrillary proteins specific for the muscle tissues of the adult animal. However, this suggestion is unsupported by facts.

In this paper we describe the result of experiments indicating new properties of metamyosin and its possible physiological role.

EXPERIMENTAL METHOD

Adult rabbits were killed by air embolism and fetuses by decapitation. The skeletal muscles were freed from fatty tissue, tendons, and fascia and homogenized by being passed twice through a mincer or cut with scissors, and then ground with quartz sand. Homogenization and all subsequent operations were carried out in the cold with cooled reagents and apparatus. Metamyosin was obtained by the method of Marcaud-Raeber and co-workers [6]. The ATPase activity was determined by the usual method and cholinesterase activity by Hestrin's method [4].

TABLE 1. Metamyosin Content in Skeletal Muscles of Rabbits of Different Ages

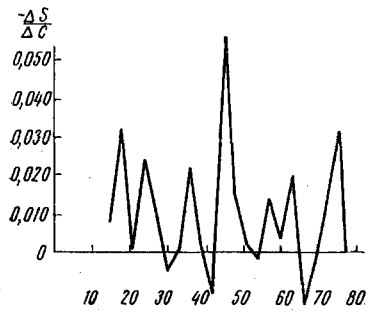
Age	Metamyosin content (in mg nitrogen/g fresh tissue)
Fetuses (28-30 days)	0,42 2,31 1,48
Newborn	0,5 0,22 0,49
Adult	0,16 0,19

Fractional salting out of the metamyosin was carried out by Derrien's method as modified by Oppel' and Serebrennikova [3], with ammonium sulfate in the zone of 12-75% saturation. The results of spectrophotometry at a wavelength of 278 mμ (SF-4 spectrophotometer) was expressed in the form of a salting out curve. The ammonium sulfate concentration was plotted along the axis of abscissas and $\Delta S/\Delta C$ (Δ – change in extinction during a change in the concentration of ammonium sulfate) along the axis of ordinates. Myosin preparations were obtained in the usual way [2].

EXPERIMENTAL RESULTS

The metamyosin content of the rabbit's skeletal muscle showed considerable individual variations, but it was particularly dependent on the age of the animal, The highest content of metamyocin was found in the muscles of the fetuses and newborn animals (Table 1).

Bearing in mind that the content of total and protein nitrogen in the skeletal muscle of the 30-day fetus and the newborn animal is ap-



Salting-out curve of metamyosin. Along the axis of abscissas—concentration of ammonium sulfate in % of saturation; along the axis of ordinates—change in extinction (ΔS) with a change in the concentration of ammonium sulfate.

proximately half that of the adult animal, it must be concluded that the percentage content of metamyosin in the total muscle proteins of the fetuses is several times higher than in the adults. The results given in Table 1, which illustrate the changes in the metamyosin content during ontogenesis, are in good agreement with those reported by Macraud-Raeber and co-workers [6].

Fractional salting out of the metamyosin showed that this protein is not homogeneous. Several peaks may be seen in the salting-our curve (see figure). Intensive absorption of light in the ultraviolet region of the spectrum (255-260 mμ) by solutions of metamyosin demonstrated the presence of large amounts of nucleoproteins in the test preparation. These findings also agreed with those reported in the literature.

The data concerning the enzymic properties of metamyosin were of greatest interest. As mentioned above, according to the findings of Macraud-Raeber and co-workers, metamyosin possesses no ATPase activity. These investigations found only very slight deaminase activity in preparations of metamyosin. Our own experiments confirmed the absence of ATPase activity in metamyosin but demonstrated that this protein possesses considerable acetylcholinesterase activity (Table 2).

The variations in the acetylcholinesterase activity of different metamyosin preparations were evidently associated primarily with differences in their content of enzymically inactive proteins. On the whole, the values of the enzymic activity of the metamyosin obtained from the muscles of the 28-30-day fetuses and newborn animals were of approximately the same order. A much lower acetylcholinesterase activity was found in the metamyosin isolated from the skeletal muscles of the adult animal.

It was important to establish certain properties of this cholinesterase of metamyosin, especially the specificity of this enzyme. For this purpose butyrylcholine and mecholine (acetyl-β-methylcholine) were used, besides acetylcholine, as substrates.

The results given in Table 3 show that the cholinesterase of metamyosin can cause hydrolysis of all the choline esters that we tested, but hydrolyzed acetylcholine most intensively of all. The ratio between the rates of hydrolysis of butyrylcholine and mecholine was different for the metamyosins isolated from the skeletal muscles of the newborn and adult animals.

The discovery of a considerable cholinesterase activity in metamyosin suggests a possible physiological role of this protein. In the first place, however, it must be stressed that metamyosin is not an individual protein, but a fairly complex fraction of the myofibrillary proteins. This is shown both by data in the literature [6-11] and by our own findings. It is evident that the metamyosins isolated from the muscles of fetuses and adult animals are different. Probably only certain components of this protein fraction possess cholinesterase activity.

The maximal content of metamyosin and, at the same time, its highest enzyme (cholinesterase) activity were found in the muscle tissue of fetuses and newborn animals, i.e., in functionally immature muscle. Parallel with the formation of the typical tetanic reaction of skeletal muscle, its metamyosin content and cholinesterase activity fell sharply.

TABLE 2. A TPase and Acetylcholinesterase Activity of Metamyosin Preparations

Age of animals from whose skeletal muscles metamyosin was obtained	ATPase activity Q_p	Cholinesterase activity (in μg acetylcholine hydrolyzed by 1 mg protein per h)
Fetuses (28-30 days)	0	1 013
	0	1 344
Newborn	0	932
	—	1 539
Adult	50	308
	35	214

TABLE 3. Specificity of Cholinesterase Action of Metamyosin (activity of enzyme expressed as μg substrate hydrolyzed by 1 mg protein per h)

Age of animals from whose skeletal muscles metamyosin was obtained	Substrate		
	acetylcholine	butyrylcholine	mecholine
Fetuses and newborn	1 344	1 138	392
	1 056	1 424	341
Adults	308	53	83
	214	63	121

In his investigations of the cholinergic structure of the muscle fiber, A. G. Ginetsinskii [1] showed that embryonic muscle and muscles with a tonic type of reaction are especially rich in choline-receptive substance, in which acetylcholine and cholinesterase are concentrated. We may ask ourselves whether metamyosin, which also is present in large quantities in embryonic muscle tissue, and which has so marked a cholinesterase action, may be the choline-receptive substance described by A.G. Ginetsinskii.

Meanwhile, it should be remembered that, according to Varga and co-workers [5, 12, 13, 14], the cholinesterase of the aneural part of the striated muscle is in fact the basic contractile protein of muscles—myosin—which, according to these workers, possess appreciable cholinesterase activity; the carrier of this activity is not the myosin molecule itself, but only one of its components—L-meromyosin. However, I. I. Ivanov and N. N. Mirovich (cited in [2]) could not confirm Varga's results in respect to the cholinesterase activity of myosin.

We carried out special experiments to determine the cholinesterase properties of myosin isolated from the skeketal muscles of the adult rabbit. We found that, in the course of purification of the myosin preparations, its ability to hydrolyze acetylcholine fell sharply, and finally disappeared completely, whereas its ATPase activity increased. It must also be noted that the cholinesterase activity of the preparation of metamyosin isolated from embryonic muscle tissue was many times greater than the activity of crystalline L-meromyosin obtained in Varga's laboratory [13].

Hence, the problem of the nature of the choline-receptive substance of the muscle fiber cannot yet be regarded as finally solved.

It is of considerable interest to determine the causes of the unusually wide spectrum of enzymic action of the cholinesterase of metamyosin. Our findings in relation to the specificity of this enzyme do not allow it to be grouped with the true cholinesterase, but with the pseudocholinesterases. Perhaps metamyosin contains an enzyme with an unusual type of action or a mixture of several cholinesterases. The experimental investigation of this problem will be our next task.

LITERATURE CITED

1. A. G. Ginetsinskii, Fiziol. zh. SSSR, 33, 4, 413 (1947).
2. I. I. Ivanov and V. A. Yur'ev, The Biochemistry and Pathobiochemistry of Muscles [in Russian], Leningrad (1961).
3. V. V. Oppel' and T. P. Serebrenikova, Doklady Akad. Nauk SSSR, 122, 2, 271 (1958).
4. S. Hestrin, J. biol. Chem., Vol. 180, p. 879 (1949).
5. T. Kovacs, A. Kover, and G. Balogh, J. cell. comp. Physiol., Vol. 57, p. 63 (1961).
6. L. Marcaud-Raeber, G. Schapira, and J. Dreyfus, C. Bull. Soc. Chim. biol., (Paris), Vol. 41, p. 283 (1959).
7. L. Marcaud-Raeber, Ibid., p. 297.
8. Idem, Ibid., p. 315.
9. L. Raeber, G. Schapira, and J. C. Dreyfus, C. R. Acad. Sci. (Paris), Vol. 241, p. 1000 (1955).
10. G. Schapira, L. Marcaud-Raeber, and J. Dreyfus, J. Bull. Soc. Chim. biol., (Prais), Vol. 39, p. 1059 (1957).
11. G. Schapira, J. Dreyfus, and L. Marcaud-Raeber, Sem. Hop. Path. Biol., Vol. 8, p. 657 (1960).
12. E. Bapra, et al., Zhurnal obshchei biologii, 20, No. 1, 3 (1959).
13. E. Varga, T. König, and E. Kiss, et al., Acta physiol. Acad. Sci. hung., t. 7, 171 (1955).
14. E. Varga, A. Köver, T. Kovacs, et al., Ibid. 11, 243 (1957).