CHANGE IN FRACTIONAL COMPOSITION AND CHOLINESTERASE ACTIVITY OF LIGHT MEROMYOSIN DURING INDIVIDUAL DEVELOPMENT

M. D. Printsev

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The properties of light meromyosin (LMM) obtained by the enzymic hydrolysis of myosin from 28-day fetuses, and neonatal, young (12 days), and adult rabbits were studied. The cholinesterase activity of LMM was highest in the fetuses and newborn animals, after which it decreased. LMM of adult rabbits does not hydrolyze acetylcholine. Electrophoresis showed marked changes in the fractional composition of LMM during individual development.

Some of the properties of the myosin of vertebrate striated muscles belong to its subunits – heavy and light meromyosin. The ATPase activity of myosin and its ability to combine with actin are linked with heavy meromyosin. According to some workers, cholinesterase activity is a property of light meromyosin (LMM) [2, 13]. In the course of development, simultaneously with a change in the character of the motor response of the skeletal musculature, the properties of its myosin also change [4, 5, 8, 9, 11, 12]. This phenomenon is evidently linked with changes in the structure and properties of individual meromyosin.

To confirm this last hypothesis experimentally, age differences in the cholinesterase activity and fractional composition of LMM were studied.

EXPERIMENTAL METHOD

Myosin was isolated from the skeletal musculature of 28-day rabbit fetuses, and of neonatal, young (12 days), and adult rabbits by extraction for 8 min with Weber's solution containing ATP (2 mg per gram muscles) followed by reprecipitation and purification of actomyosin [1]. To obtain LMM the myosin was hydrolyzed with trypsin by Szent-Györgyi's method [10]. Twice reprecipitated (recrystallized in the case of adult animals) LMM was used in the work. To determine the cholinesterase activity the LMM solution was incubated with acetylcholine (AC) at pH 7.8. The concentration of AC in the samples was measured by Hestrin's method [7]. Protein nitrogen was determined by the micro-Kjeldahl method.

LMM was fractionated by microelectrophoresis in agar gel, after which the fractions were investigated with a densitometer (Carl Zeiss, Jena). Cholinesterase was identified in the LMM fractions after electrophoresis by Grabar's method [6] in the writer's modification: after the end of electrophoresis the strips of agar were incubated for 3 h at 20°C in medium of the following composition: 0.11 M Na-phosphate buffer (pH 7.8), 10^{-3} M sodium azide, 10^{-3} M EDTA (disodium salt), and 3.5×10^{-3} M acetylthiocholine iodide (Chemapol, Czechslovakia). The gel was then washed with water and placed in a freshly prepared 4.5×10^{-3} M solution of iron ferrocyanide. The presence of cholinesterase activity was shown by a blue coloration of the protein bands. Gel treated for 30 min with 10^{-3} M neostigmine solution and incubated in medium with inhibitor was used as the control.

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TABLE 1. Content of Electrophoretic Fractions of LMM (in %)

Group of rabbits	Electrophoretic fractions		
	1+2	3+4	5
Fetuses	44,2	48,0	7,8
Newborn	48,0	46,8	5,25
12-day	54,5	38,0	7,5
Adult	61,2	26,3	12,5

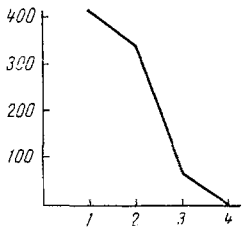

Fig. 1. Change in cholinesterase activity of LMM in individual development. Ordinate, cholinesterase activity (in μg acetylcholine/mg protein/h). Abscissa, age groups of animals: 1) fetuses, 2) newborn, 3) 12-day, 4) adult. Mean value for two to six experiments are shown.

EXPERIMENTAL RESULTS AND DISCUSSION

The results showed that crystalline IMM obtained from purified myosin of an adult rabbit does not hydrolyze AC. LMM from fetal, neonatal, and young rabbits possesses cholinesterase activity, and the younger the animal the higher the activity (Fig. 1). Changes of a similar character in cholinesterase activity during individual development have been found for preparations of myosin and actomyosin [3, 14].

It was found that LMMof animals of different ages is not an individual protein. During fractionation by electrophoresis in agar gel, the LMM from rabbits of all age groups was found to contain five components with similar electrophoretic mobility. The fractions designated Nos. 1 and 2 migrated during electrophoresis toward the cathode, and fractions Nos. 3, 4, and 5 toward the anode. Since fractions 1 and 2, and also 3 and 4, were not completely separated from each other on electrophoresis or densitometry, they are included together in Table 1.

The results in Table 1 show that about 50% of LMM from the fetuses and newborn rabbits corresponds to fractions 1 and 2, and approximately the same amount to fractions 3 and 4. Fraction 5 accounts for less than 10%. With age, appreciable changes took place in the fractional composition of LMM: an increase in the proportion of components 1, 2, and 5 and a decrease in the combined content of components 3 and 4.

The cholinesterase activity of the individual LMM fractions was studied. Enzyme electrophoresis showed that two fractions (3, 4) of LMM from fetuses and newborn and 12-day rabbits possess enzymic activity. The electrophoretic fractions of LMM from the adult animal were completely without enzyme activity. These results do not accord with the view that LMM is identical with cholinesterase. The differences in the enzymic properties of fractions 3 and 4 of LMM from the young and old animals can evidently be attributed to differences in their ability to form complexes with the cholinesterase of the myofibrils.

The results thus show that substantial changes take place during individual development in the properties of the LMM fragment of the myosin molecule.

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