CHANGES IN THE PHYSICOCHEMICAL AND ENZYMIC PROPERTIES OF MYOSIN DURING ONTOGENY

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During development of the calf fetus two forms of fetal myosin appear. The form of myosin characteristic of the skeletal muscle of the calf fetus at 2.5-6 months of development, not present in the later stages, is salted out with ammonium sulfate in a saturation of up to 25%; it has low ATPase activity and is easily denatured. The cholinesterase activity of the myofibrils is connected with this myosin fraction. The second form of fetal myosin is relatively stable, it has much higher ATPase activity, and it is salted out with ammonium sulfate in saturations of 35-50%. Both forms of myosin are found in the early stages of development of the calf fetus, but only the second form in the later stages. By electrophoresis in polyacrylamide gel marked heterogeneity is found in the region of the heavy components in fetal myosin solutions obtained in the early periods of development.

KEY WORDS: myosin; calf skeletal muscles; ontogeny; ATPase; cholinesterase; electrophoresis of proteins.

The comparative study of myosin from adult skeletal muscle and from fetal muscle [2, 7, 14] has shown significant differences in the biological activity of these proteins as well as certain common physicochemical properties. Structural differences have also been established between the molecules of the adult and embryonic forms of myosin [9-11]. On the basis of differences in the properties of the myosin of calf fetal skeletal muscle at different periods of development the presence of two forms of embryonic myosin corresponding to the early and late stages of intrauterine development has been postulated [4]. Two forms of fetal myosin were found by Obinata [10] in the muscle of chick embryos in the early stages of development. These forms differed in their sedimentation constants, their zones of salting out with ammonium sulfate, and their ATPase activity.

This paper describes a comparative study of fetal myosin from the skeletal muscle of calf fetuses at different periods of intrauterine development.

EXPERIMENTAL METHOD

Myosin was obtained by the method of Trayer and Perry [14]. Fractions of myosin salted out with ammonium sulfate in saturations of 0-25% (fraction P_{0-25}) and 35-50% (fraction P_{35-50}) were obtained [10]. ATPase activity (3.6.1.3) was determined from the increase in inorganic phosphate during incubation for 5 min at 37°C. The incubation mixture contained the following constituents, in mmoles: KCl 150, Na-borate buffer (pH 9.1) 30, $CaCl_2$ 2.5, and ATP-Na_2 2.5. To determine the EDTA-activated activity, the incubation mixture contained: tris-HCl buffer, pH 7.5; 0.5 M KCl, 1 mM EDTA, 2.5 mM ATP. Protein was added in a concentration of 0.1-0.2%. Cholinesterase activity (3.1.1.8) was determined by Hestrin's method [8] after incubation for 30 min at 37°C and pH 7.6. Electrophoresis was carried out in 4% [12] and 10% [15] polyacrylamide gel (PG) in the presence of Na-dodecylsulfate and urea (25°C, 7 mAE, 3 h for 4% PG, 4 h for 10% PG).

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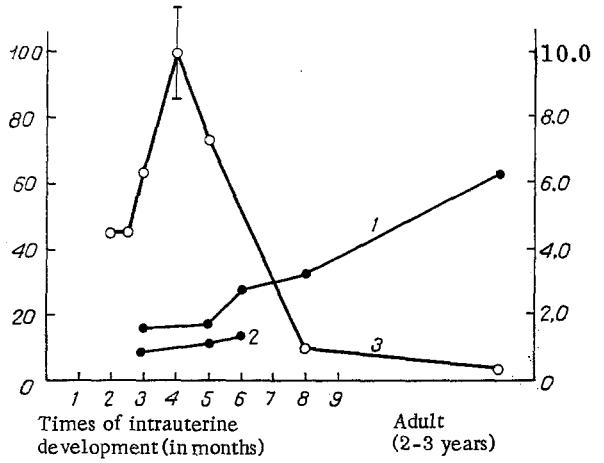

Fig. 1. Age changes in enzyme activity of myosin (results of 8-12 experiments for each age group): 1) Ca-activated ATPase activity of myosin; 2) Ca-activated ATPase activity of myosin fraction P_{0-25}; 3) cholinesterase activity. Ordinate, left: ATPase activity (in μmoles Pi/mg protein/h); ordinate, right: cholinesterase activity (in μmoles acetylcholine/mg protein/h).

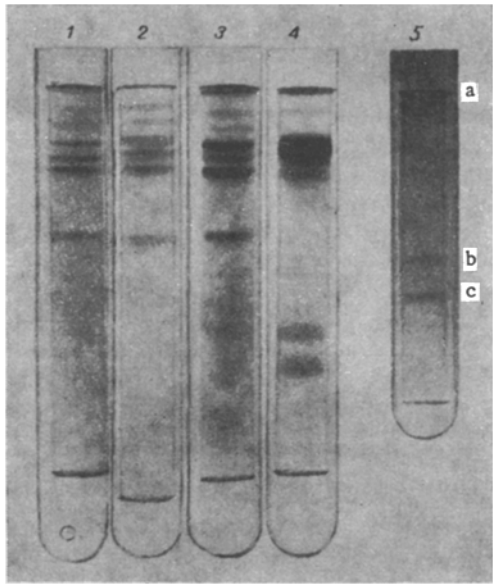

Fig. 2. Electrophoresis of myosin in polyacrylamide gel. In 4% PG: 1) myosin from 3-month fetuses; 2) fraction P_{0-25} of myosin from 3-month fetuses; 3) myosin from 4-month fetuses; 4) myosin from 6-month fetuses. In 10% PG: 5) myosin from 8.5-month fetuses; a) heavy chain of myosin; b and c) light chain.

EXPERIMENTAL RESULTS AND DISCUSSION

Solutions of myosin from the skeletal muscle of 2.5-6-month fetuses contained two forms of myosin that differed in their salting-out behavior (fractions P_{0-25} and P_{35-50}). The relative proportion of the P_{0-25} fraction decreased with age. This component was completely absent in the solution of fetal myosin from fetuses aged 8-8.5 months and myosin of the adult type. The P_{0-25} fraction of calf fetal myosin may perhaps correspond to the form of chick embryonic myosin with a sedimentation constant of 3S isolated by Obinata [10]. As regards the P_{35-50} fraction, in this case the opposite relationship was observed. The content of this fraction was least in myosin solutions from fetuses in the early periods of development and it was the larger fraction in myosin solutions from fetuses aged 8-8.5 months and adult animals. In all cases an inconstant amount of protein was precipitated by ammonium sulfate in 25-36% saturation and this could be attributed either to the presence of a mixture of the P_{0-25} and P_{35-50} fractions and also, perhaps, to some contamination with actomyosin.

In the course of development of the calf fetus an increase was observed in the Ca-activated (Fig. 1) and EDTA-activated ATPase activity of the skeletal muscular myosin. Fetal myosin at the age of 2.5-4 months had low (8-20 μmoles Pi/mg protein/h) Ca-activated ATPase activity; the myosin activity from fetuses aged 5-6 months was more than doubled. By the end of the intrauterine period of development and after birth a further increase in activity was observed. Changes in the EDTA-activated myosin ATPase activity were similar in character. The ATPase activity of myosin fraction P_{0-25} from fetuses aged 3-6 months still remained low (8-13 μmoles Pi/mg protein/h).

A distinguishing feature of the myosin P_{0-25} fraction was its marked cholinesterase (CE) activity. Despite the wide variations in CE activity in the different samples of myosin, the activity of this enzyme was always high in the P_{0-25} fractions of fetal myosin at 3-5 months of development (4.6-12.25 μmoles acetylcholine/mg protein/h). The activity fell sharply in myosin P_{0-25} fraction in 6-month fetuses (Fig. 1). So far as the P_{35-50} fraction is concerned, the CE activity in all the samples of myosin was either very low (0.37-0.61 units of activity) or absent altogether.

Previous investigations showed [5, 6, 13] that CE activity discovered in myosin solutions is not a property of the myosin but is attributable to the presence of CE, closely bound with it as an impurity. Meanwhile, other workers [1, 3] give evidence of the cholinesterase properties of myosin molecules. The myosin of mature bovine skeletal muscle evidently does not possess cholinesterase properties. The wide scatter of the Ca activity values in preparations of fetal myosin is evidence in support of the view that this en-

zyme is present as an impurity. CE activity has been shown to be bound chiefly with the P_{0-25} fraction of fetal myosin.

CE activity also was found in the present experiments in myofibrils from the skeletal muscle of calf fetuses after washing 6-8 times. Further washing the myofibrils with a 1% solution of Triton X-100 led to loss of the cholinesterase properties of the myofibrils.

The presence of cholinesterase activity closely bound with the myofibrils in fetal skeletal muscle is thus a distinguishing feature of functionally immature tissue with a tonic type of response. As the skeletal muscle matures and response of tetanic type is formed the cholinesterase properties of the myosin preparations isolated from the skeletal muscles are lost.

By electrophoresis in polyacrylamide gel some age differences were discovered in the fractional composition of the myosin solutions (Fig. 2). Myosin from younger fetuses was distinguished by the greater variety of the assortment of subunits in the region of heavy components. Besides the large and small myosin subunits, a second heavy component and several unidentified fractions of fairly high molecular weight were found on electrophoresis. Among these fractions there were probably proteins that determined the length of the myosin filaments and CE. The high lability of the fetal myosin in the early stages of development and its well-marked tendency to undergo denaturation must also be noted. The P_{0-25} fraction of fetal myosin is evidently weakly bound with the myofibrils and is distinguished by its ready solubility and the ease of its extraction by brief treatment (15 min) of the muscle homogenate with Perry's solution.

The changes observed in the composition and properties of myosin in ontogeny are undoubtedly connected with the formation of the contractile response of the skeletal muscle.

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