PARVALBUMIN AND MYOSIN EXPRESSION IN THE TELEOST *DICENTRARCHUS LABRAX* (L.) WHITE MUSCLE DURING DEVELOPMENT

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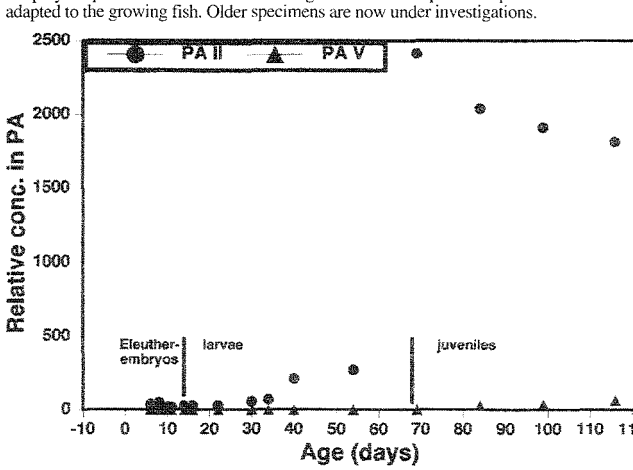
Parvalbumins are Ca^{2+}–binding polymorphic proteins that are abundant in fish white muscle sarcoplasm where they can act as muscle soluble relaxing factor.They are thermostable and display 1 to 5 muscle and species specific isoforms of nearby same low molecular weight (12 KDa). Myosin, the major myofibrillar protein, is a hexameric molecule made of two heavy chains (HC, 200 KDa) and four light chains (LC, 16 to 25 KDa). In terrestrial vertebrates, successive isoforms of HC and LC are expressed during muscle ontogeny.The sequential appearance and disappearance of different isoforms of these proteins in the muscle fibers have been recently observed in various freshwater fishes (FOCANT *et al.*,1992,1994; HURIAUX *et al.*,1994). These isoforms are most probably related to the requirements of the developmental stages of the growing fish.

The sea–bass (*Dicentrarchus labrax* L.) was chosen for this study with in view the availability of the developmental stages of this marine teleost and in order to increase our knowledge on the muscle development of this commercially important species.The specimens (from 3 days before hatching until 115 days post–hatching and adult) were kindly furnished by the "Ecloserie marine SEPIA Exploitation", Montigny–le–Bretonneux, France. Trunk muscle was dissected and muscle fiber membranes were destroyed in a buffered solution containing 50% glycerol.Sarcoplasmic proteins, including parvalbumins, were separated by centrifugation from insoluble myofibrillar material (actomyosin). After heating the sarcoplasmic extract at 80°C for 5 min in order to eliminate most of the proteins, the parvalbumin isoforms [PA II (75%) and PA V (25%) in the adult muscles] were analysed on PAGE in the presence of 10% glycerol at pH 8.6. They were separated according to their negative electric charge; their relative amounts were estimated by densitometry (versus the total sarcoplasmic protein content). The actomyosin complex was dissociated in sodium dodecylsulfate (SDS); the myosin HC and LC were respectively separated on discontinuous high (6% acrylamide, 30% glycerol, pH 8.8) and low (20% acrylamide, pH 8.4) porosity PAGE according to their molecular weight. An unforeseen finding is the very late detection of both parvalbumins and myosin despite the fact that earlier stages contain organized muscle fibers.The sequential apparition of the parvalbumin isoforms (relative amounts of PA II and PA V) during the development is illustrated in the figure.PA II appears first in the 30 days old larvae; its content reaches a maximum at 69 days (transition from larval to juvenile stage) and then slowly decreases. PA II corresponds thus to a "larval" isoform. PA V appears at this 69 days stage and augments very slowly. Myosin HC and LC are not detectable before the age of 40 days.The myosin HC of the larvae cannot be distinguished by their molecular weight from the adult ones. The stoechiometric distribution of the three light chains looks similar to that of adult myosin (LC_1 : 8%, LC_2 : 58%, LC_3 : 34%).

These results are in agreement with the histochemical observations of SCAPOLO *et al.*(1988) showing that myosin ATPase activity cannot be demonstrated in any part of the myotome before 65 days old larvae. According to these authors, the histoimmunological analysis during the different stages of the myotomal development revealed changes in the myosin composition : they suggested the presence of larval forms of myosin (Larval 1 until 28 days and Larval 2 until 20 months), analogous to the embryonic forms found in other vertebrate muscles. These forms without detectable ATPase activity could be very labile, in very low amount or not extracted in our experimental conditions. They histochemically distinguished the definitive adult form by the appearance of characteristic myosin ATPase activity, by 20 months in the trunk muscles. In barbel and trout (FOCANT *et al.*,1992,1994; HURIAUX *et al.*,1994) the "larval" parvalbumin isoform PA II rapidly increased from the hatching. Myosin light chains were also detected very early, the relative proportions of LC_1 and LC_3 quickly changing during the early steps of development. Myosin from embryonic and larval stages contained heavy chain isoforms distinct from adult ones, confirming the existence of different myosins.

Compared with other fish species, the development of the muscle of the sea–bass appears very slow and biochemically less determined. At least in the case of parvalbumins, the polymorphism constitutes a modulating mecanism for speed and power of contraction adapted to the growing fish. Older specimens are now under investigations.



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