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PARVALBUMIN ISOTYPES IN WHITE MUSCLE FROM THREE TELEOST FISH: CHARACTERIZATION AND THEIR EXPRESSION DURING DEVELOPMENT

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ABSTRACT

Parvalbumin isotypes were isolated by chromatography from trunk white muscle of rainbow trout *(Oncorhynchus mykiss* W.), brown trout (*Salmo trutta* L.), and sea bass *(Dicentrarchus labrax* L.). Five, four and two components were respectively purified and physico-chemically characterized. Expression of the various isotypes was followed in the course of the fish development and, in adult fish, from the anterior to the posterior myotomes. Isotype distribution varies both chronologically and spatially. In trout, parvalbumins occur around hatching; as the fish develop, transitions occur in isotype expression, PA II appearing as the predominant larval form and PA III, IV, or V as the main adult form, as previously observed in *Barbus barbus* (L.). In the sea bass, the developmental expression pattern is more unexpected: the synthesis of both isotypes (PA II and PA V) is delayed and the larval form PA II remains the principal isotype in adult fish. These observations indicate that the polymorphism of parvalbumins in fish constitutes a subtle mechanism modulating the speed and power of muscle contraction. Our results support the view that each isotype plays a specific role in relation to the muscle activity required in fish at a given developmental stage or a given trunk level in the adult.

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INTRODUCTION

Parvalbumins are polymorphic, low-molecular-weight acidic proteins with the ability to bind two calcium ions with high affinity. They are expressed in numerous different populations of cells in vertebrates, where they may participate in some types of cellular Ca^{++}-regulation (14). They are especially abundant in the sarcoplasm of skeletal white muscle fibres (fast-twitch glycolytic fibres) of cold-blooded vertebrates. Fish display two to five parvalbumin isotypes, which are numbered in function of their electrophoretic mobility on gels at alkaline pH; these isotypes are species specific and thus constitute an extremely valuable tool in the study of phylogenic relationships (10). Parvalbumins are involved in Ca^{++} transfer from the myofibrils to the sarcoplasmic reticulum during relaxation (11,12).

The synthesis of parvalbumins has been followed during the prenatal and postnatal development of the chicken (19), rat (3), and rabbit (18). In these higher vertebrates, only one isotype appears around birth, along with the sarcoplasmic reticulum ATPase; its level progressively increases in correlation with the onset of high-frequency neural activity and the differentiation of fast-twitch fibres. A similar phenomenon has been observed during myogenesis in the frog, where at least three different isotypes are expressed (22). More recently we have shown, for the first time in fish, that the three parvalbumin isotypes of *Barbus barbus* (L.) display considerable and unforeseen developmental transitions in their distribution (6). The PA II isotype first occurs 4 days after hatching, steadily augments until the age of 1 month (larval stage), then decreases but remains the predominant form until the fish is about 6 cm long. PA IV appears at 5-10 days and PA III at 2 months (2.4 cm); the synthesis of both increases until the fish reaches 10 cm in length. The typical adult pattern is characterized by a strong predominance of PA IV. On the other hand, in the dorsal white muscle of the adult, the levels of all three parvalbumin isotypes regularly decrease from the first to the last myotomes; in the latter, only PA IV is found in significant amount (15). These observations suggest a specific role for each isotype in relation to muscle activity.

In order to confirm and specify the differential expression of the parvalbumin isotypes, we extended this study to three other teleost fish: two freshwater Salmonidae, the rainbow trout *(Oncorhynchus mykiss* W.) and brown trout (Salmo *trutta* L.), and one marine Serranidae species, the sea bass *(Dicen- trarchus labrax* L.). In the present work we describe the isolation of the various parvalbumin isotypes, the characterization of some of their molecular properties, the chronology of their synthesis from the egg to the adult, and their spatial distribution in the dorso-lateral white muscle.

A preliminary note concerning the development of *D. la- brax* has already been published (8).

MATERIALS AND METHODS

FISH SAMPLES

The numerous specimens of *Oncorhynchus mykiss* (W.) and *Salmo trutta* (L.) were reared under natural conditions at the commercial hatchery MASURE (Maredret, Belgium). Specimens of

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Dicentrarchus labrax (L.) were obtained from the Ecloserie marine SEPIA Exploitation (Montigny-le- Bretonneux, France).

For the purification of parvalbumin isotypes, the trunk dorso-lateral white muscle was dissected free of any pigmented superficial material from large (25-35 cm) and small (7-16 cm) trouts and from a large (50 cm) sea bass.

O. *mykiss* samples were studied from 1.2 cm (day 13 prehatching) to 29 cm, S. trutta samples from 1.8 cm (day 28 post-hatching) to 29 cm, and D. *labrax* samples from 0.14 cm (day 2 pre-hatching) to 45 cm. We distinguished the following successive morphological stages in the development of the fish: an embryonic phase until hatching, an eleutheroembryo- nic phase until all the yolk sac was digested and the fish became free-swimming, a larval phase ending with the acquisition of scales and of fins with rays, a juvenile phase, and the adult phase beginning at sexual maturity. The transition from one stage to another is not exactly determined, however, and varies according to the specimen. First stages were handled under a magnifying lens in a cold solution of 0.01 M Tris— HC1, 0.05 M KC1, 0.01 M DTT, 0.005% (w/v) NaN_3, pH 7.5. Samples of trout up to 3 cm long were constituted by pooling 50 to 5 specimens of each trout species and removing the head, yolk sac, internal organs, and caudal fin; when larger fish were used, the trunk dorso-lateral white muscle was dissected. Samples of D. *labrax* up to 5.5 cm in length were obtained by pooling 50 to 5 specimens. Up to a length of 0.78 cm, whole specimens were used; from 0.8 cm to 5.5 cm, the head and internal organs were discarded. The trunk muscle was dissected from larger specimens. The material was minced and suspended in 10 vol (or less for the first samples) of a solution containing 0.01 M Tris-HCl, 0.05 M KC1, 0.01 M DTT, 0.005% (w/v) NaN_3, 50% (v/v) glycerol, pH 7.5. Samples were kept at 4°C for 24 hr, mixed, and stored at -20°C until use. On the other hand, the whole dorso-lateral muscle from large fish (17 to 34 cm long) was cut, from the head to the tail, into 16 or 10 samples of a same length and treated as described above.

ISOLATION OF PARVALBUMIN ISOTYPES

The minced muscle was extracted at 4°C with 1.5 vol of 10 mM Tris-HCl, 2% (v/v) glycerol, 1 mM 2-mercaptoethanol, pH 8.7 for 30 min. The suspension was centrifuged at 23,000 *g* for 20 min and the supernatant was fractionated as in Dubois and Gerday (4). The parvalbumin-containing mixture was isolated by precipitation, at 4°C, with $(NH_4)_2SO_4$ between 50 and 90% saturation, centrifuged at 23,000 g for 20 min, partially redissolved in 50 mM NH_4HCO_3, and dialysed against this buffer. The resulting solution was then heated at 60°C for 5 min, centrifuged at 39,100 g for 20 min, concentrated by ultrafiltration on an Amicon YM3 membrane, and chromatographed on a Sephacryl S-100 column (2.5 x 60 cm) equilibrated in 50 mM NH_4HCO_3. The parvalbumin isotypes were separated on a DEAE-cellulose (Whatman DE 52) column (2.5 x 30 cm) equilibrated in 15 mM piperazine-HCl, 1 mM 2-mercaptoethanol buffer (pH 5.7) and eluted with a linear NaCl gradient (400 ml of buffer—400 ml of buffer plus 0.15 M NaCl) at a flow rate of 35 ml h^{-1}. The fractions containing each isotype were pooled, concentrated on an Amicon YM3 membrane, desalted on a Biogel P2 column (1.5 X 45 cm) equilibrated in 50 mM NH_4HCO_3, and lyophilised. The various preparations of O. *mykiss* PA IV or S. *trutta* PA III were pooled and purified by a second chromatography on DEAE-cellulose but with a linear gradient up to 50 mM NaCl.

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CRUDE PARVALBUMIN EXTRACTS

The samples in the glycerol conservative solution were centrifuged at 8,500 g (Beckman Microfuge) for 20 min at 4°C. The parvalbumin-containing supernatant was heated at 80°C for 5 min and again centrifuged. This last supernatant was used for the electrophoretic isotype analysis. Sarcoplasmic protein concentrations were determined before heating by the Bradford (2) method using a solution of bovine serum albumin as the standard.

ELECTROPHORETIC ANALYSES

Vertical-slab polyacrylamide gel (18 x 8 x 0.2 cm) electrophoresis (PAGE) was used under several sets of conditions. Parvalbumin isotypes were separated at pH 8.6 on a 10% acrylamide gel containing 2 M urea or 10% glycerol (v/v) (7). In the study of the temporal and spatial distribution of the isotypes, sample volumes were chosen so that an equivalent amount of sarcoplasmic protein was loaded into each well (the sarcoplasmic protein content was measured before heating; the stoichiometry and the relative concentrations of the isotypes were evaluated from three gels). SDS-PAGE was carried out in 20% acrylamide, pH 8.8, according to Laemmli (17). The marker proteins for estimating the apparent relative molecular mass (M_r) were ribonuclease from bovine pancreas (Boehringer) (13,700), cytochrome-C from equine heart (Calbiochem) (12,270), and parvalbumin IV from carp muscle (11,600). Isoelectrofocusing (IEF) electrophoresis was performed in 7.5% acrylamide gel containing 1.6% ampholytes pH 4-6 (Pharmacia-LKB), 0.4% ampholytes pH 3.5-10 (Pharmacia-LKB), and 8 M urea, at a constant voltage of 100 volts for 20 hr. The pH gradient was determined by cutting 15 sections from an unstained and unloaded part of the gel, soaking each section in deaerated water for 24 hr, and measuring the pH of the extracts.

Protein bands were visualized by staining in 0.25% (w/v) Coomassie Brilliant Blue R-250 or G-250 (IEF) in 45% (v/v) methanol, 10% (v/v) acetic acid and then destaining in 20% (v/v) methanol, 10% (v/v) acetic acid. Densitometry was performed with a Helena Quick-Scan apparatus (Beaumont, Texas) or a Bio-Rad Model GS-670 densitometer combined with the Molecular Analyst gelscan software program.

ULTRAVIOLET SPECTRA

Ultraviolet absorption was measured with a Perkin-Elmer Lambda 5 spectrophotometer, at a spectral bandwidth of 1 nm. The samples were dissolved in 50 mM NH_4HCO_3, at 3 mg • ml^{-1}. The amount of tyrosine residues was evaluated at 280 nm, using 1,230 as the molar extinction coefficient for tyrosine.

TITRATION OF SULFHYDRYL GROUPS

Titration of total sulfhydryl groups was performed with 5.5'- dithio-bis-2-nitrobenzoic acid (DTNB) according to Ellman (5) and to Habeeb (13). The proteins were denatured by a 1 hr incubation at 37°C in 8 M urea. A lysozyme (Sigma) solution was used as the standard.

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RESULTS

IDENTIFICATION OF PARVALBUMINS

Fish parvalbumin isotypes separate well and migrate homogeneously on glycerol-polyacrylamide gels at pH 8.6 (7,16), yet some parvalbumin bands from both trout species appeared diffuse, under these experimental conditions, when the proteinic samples were in solution in the conservative buffer. We obtained a satisfactory and reproducible electrophoretic migration pattern by replacing 10% glycerol with 2 M urea. Therefore all trout parvalbumin samples used in the temporal and spatial study were analysed in the presence of 2 M urea.

Figure 1. *2 M urea-PAGE of sarcoplasmic extracts heated to 80°C, from Cyprinus carpio (Cc), Oncorhynchus mykiss (Om), Salmo trutta (St), and Dicentrarchus labrax (Dl).*

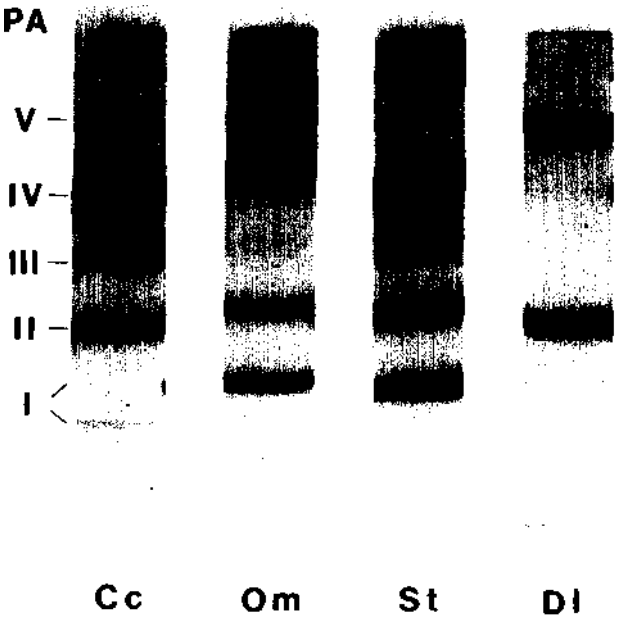

Figure 1 shows the parvalbumin electrophoretic patterns obtained with the three fish species. An *Oncorhynchus mykiss* specimen 10 cm long exhibits five bands, named PA I, PA II, PA III (only a trace), PA IV, and PA V by comparison with carp *(Cyprinus carpio L.)* isotypes. A *Salmo trutta* specimen of same size contains PA I, PA II, PA III, and PA IV. Corresponding isotypes have the same mobility in both trout species. In *Dicentrarchus labrax* (25 cm long), only PA II and PA V are seen and they migrate somewhat further than the corresponding trout bands. The presence of reducing agents (3 mM dithiothreitol or 2-mercaptoethanol) in the sample causes an abnormal migration of PA IV and PA V from O. *mykiss* and PA III and PA IV from S. *trutta* on glycerolpolyacrylamide gels: these isotypes, surprisingly, migrate as faster, diffuse bands, PA V to the level of PA IV and PA IV to the level of PA III, hindering any stoichiometric estimate.

ISOLATION OF PARVALBUMINS

In both trout species, the relative amount of each isotype varies considerably with fish size; PA II could be isolated in a pure state and in sufficient amount from small specimens only and PA IV and V from large ones only. The purification of O. mykiss PA IV and S. trutta PA III required a

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second chromatography on DEAE-cellulose with a less concentrated NaCl gradient. In both fish, the yield in PA III was very poor. Chromatographic isolation of the two D. labrax parvalbumins was easier. Figure 2 illustrates the electrophoretic pattern of the purified isotypes.

CHARACTERIZATION OF PARVALBUMINS

The apparent relative molecular mass (Mr) and the isoelectric point (pI) of the various parvalbumins, determined respectively by SDS-PAGE and IEF-PAGE, are presented in Table 1. The results are very similar for the two trout species: corresponding isotypes have identical molecular masses and isoelectric points, except for a small pI difference between the two PA IV; PA III, IV, and V have the same molecular mass (11,800), differing from those of PA I (11,600) and PA II (12,000). Both parvalbumin isotypes of D. labrax appear more characteristic of this fish.

Figure 2. *Glycerol-PAGE of the various parvalbumin isotypes purified from Oncorhynchus mykiss (Om), Salmo trutta (St), and Dicentrarchus labrax (Dl). Cc corresponds to a sarcoplasmic extract from Cyprinus carpio heated to 80°C.*

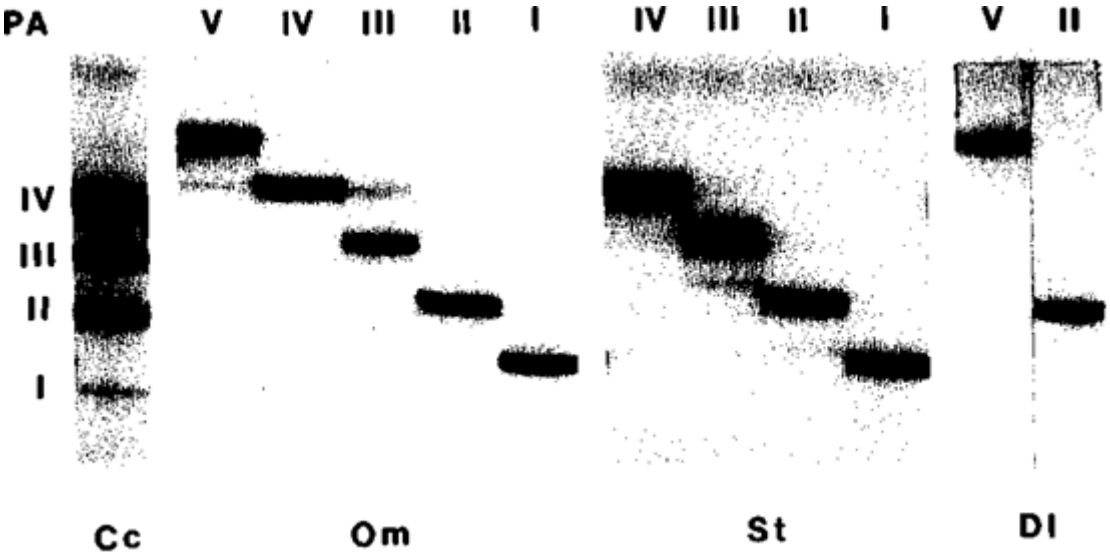

Figure 3 shows the ultraviolet absorption spectra of the main isotypes found in the three fish. Each spectrum shows the absorption band typical of phenylalanine residues, which illustrates the high phenylalanine-to-tyrosine ratio characteristic of parvalbumins. The amount of tyrosine could be estimated from these spectra: in each fish species, PA I and II contain one tyrosine residue but PA III, IV and V are devoid of this amino acid (Table 1). The low absorbance at 290 nm indicates a lack of tryptophan in all parvalbumins.

While PA I and PA II everywhere contain only one cysteine residue, three residues were titrated in D. labrax PA V and four in PA IV and V from O. mykiss and S. trutta (Table 1). To our knowledge such a very high amount of cysteine has never been observed in fish other than trout; it might explain the abnormal migration of these parvalbumins, in glycerol-PAGE experiments, in the presence of reducing agents. The cysteine content of trout PA III could not be measured due to an insufficient amount of protein.

Table 1. *Physico-chemical properties of the parvalbumin isotypes*

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				Estimated number of residues		
Species	PA	M_r*	pI +	Tyr	Trp	1/2 Cys
O. *mykiss*	V	11,800	5.20 ± 0.17	0	0	4
	IV	11,800	5.01 ± 0.12	0	0	4
	III	11,800	4.94 ± 0.11	-	-	-
	II	12,000	4.88 ± 0.10	1	0	1
	I	11,600	4.54 ± 0.10	1	0	1
S. *trutta*	IV	11,800	5.07 ± 0.11	0	0	4
	III	11,800	4.95 ± 0.10	0	0	-
	II	12,000	4.89 ± 0.09	1	0	1
	I	11,600	4.54 ± 0.09	1	0	1
D. labrax	V	12,100	4.99 ± 0.05	0	0	3
	II	11,600	4.56 ± 0.03	1	0	1

* Average of 3-6 scans rounded to the nearest hundred; SD ≤ 70.
+ Average of 4-6 scans

Developmental Evolution *of Parvalbumin Isotypes*

By computing the densitometer traces of parvalbumin electro- phoretograms, it is possible to evaluate the amount of each isotype synthesized for a same total sarcoplasmic protein content. This was done for fish trunk muscle from the egg to the adult stage. Early development was expressed in days until 4 months post-hatching (Figs 4A, 5A and 6A). Because the size of specimens of the same age becomes more variable after this time, the evolution of the concentration of the various isotypes was followed in older fish as a function of their body length until they reach the adult stage (Figs 4B, 5B and 6B). On the other hand, figures 4C, 5C and 6C recapitulate how isotype stoichiometry varies according to the size of the fish, clearly identifying the main isotype present at the different developmental stages independently of the total parvalbumin concentration. In both trouts three parvalbumin contents were determined: PA I, PA II and PA IV + V in O. *mykiss,* PA I, PA II and PA III + IV in S. *trutta.* The physicochemical properties presented in Table 1 suggest that PA III, IV and V could be electrophoretically different forms of the same isotype; the amount of O. *mykiss* PA III was not included in the measurements because it appears extremely low and constant in all samples.

In O. *mykiss,* all three isotypes occur around hatching (1.7 cm) (Fig. 4A). The synthesis of PA II rapidly augments during the whole eleutheroembryo stage (the transition to the larval stage occurs around 48 days or 2.3 cm), then remains stable until 4 months after hatching (the transition to the juvenile stage is less clear-cut and located around 2 months or 2.5-2.9 cm). Over this 4-month interval PA I and PA IV + V increase more slowly but regularly. In the course of ulterior growth (Fig. 4B), PA II quickly diminishes, disappearing at the adult stage (25-29 cm). The PA 1 and PA IV + V contents increase in parallel in fish up to 9 cm long, after which they evolve differently, tending towards their adult levels, with PA IV + V strongly predominating in the established adult pattern (74.5% PA IV + V, 25% PA I and 0.5% PA II at 29 cm). PA II appears as the principal eleutheroem- bryonic and larval form and PA IV + V as the main adult form (Fig. 4C).

Figure 3*. Ultraviolet absorption spectra (1 cm light path) of parvalbumin isotypes (3 mg • ml $^{-1}$) in 50 mM NH_4HCO_3; A: Oncorhynchus mykiss; B: Salmo trutta; C: Dicentrarchus labrax.*

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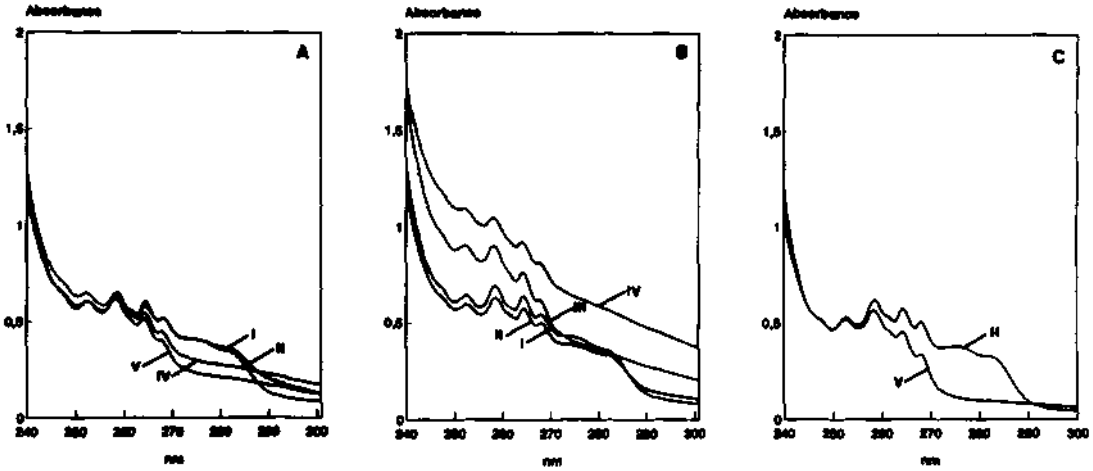

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Figure 4. *Evolution of the concentration of each parvalbumin isotype in Oncorhynchus mykiss in the course of development A: relative content until 4 months; B: relative content from 6 to 29 cm; C: stoichiometry according to the body length. The last measurement in A corresponds with the first one in B. ■, PA I; ▲, PA II; ▼, PA IV + V*

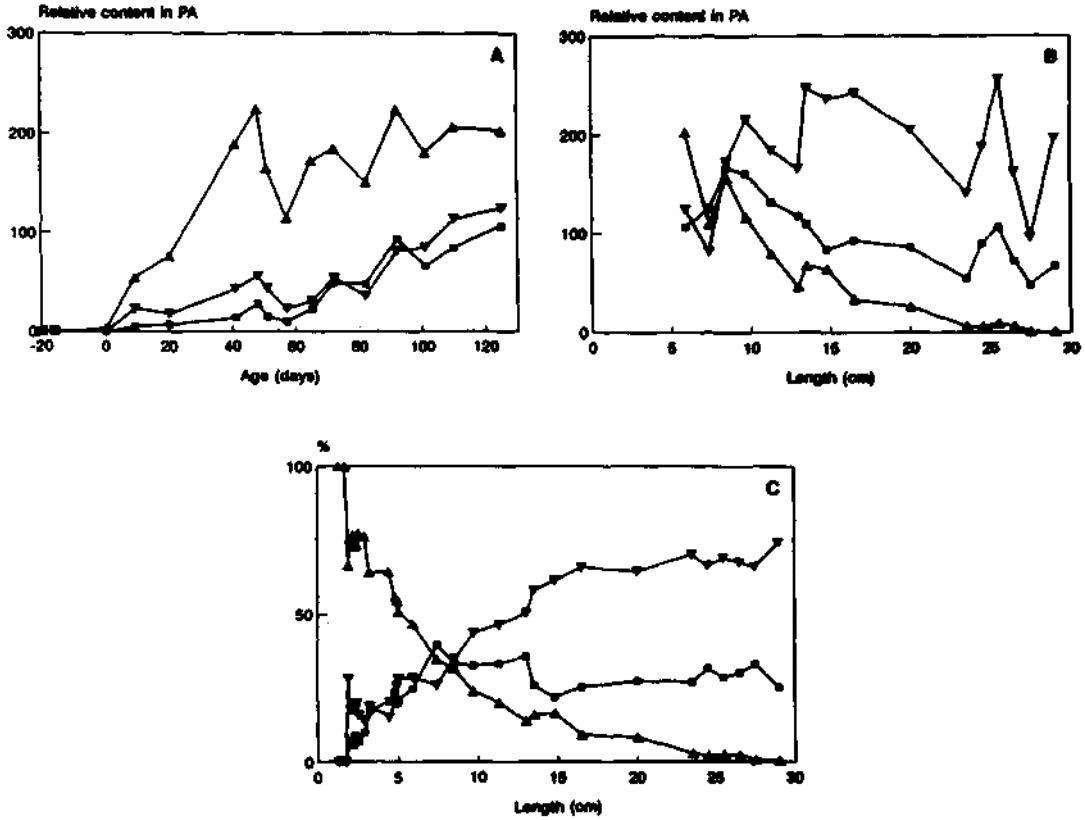

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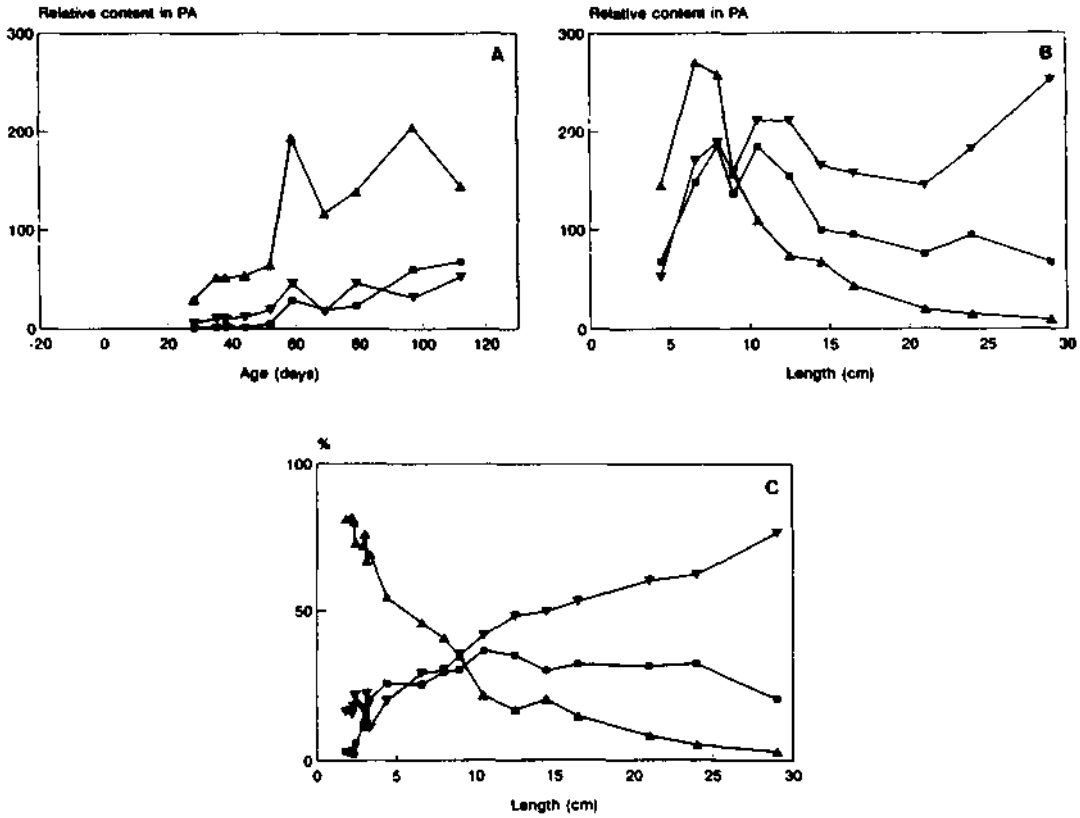
Figure 5. *Evolution of the concentration of each parvalbumin isotype in Salmo trutta in the course of development. A: relative content until 4 months; B: relative content from 4.4 to 29 cm; C: stoichiometry according to the body length. The last measurement in A corresponds with the first one in B. ■, PA I; ▲, PA II; ▼, PA III + IV.*



The morphological stages of the post-hatching development of S. *trutta* are chronologically similar to those observed in O. *mykiss.* The temporal evolution of the three parvalbumin isotypes is also similar (Fig. 5). PA II remains the predominant form in young specimens up to 8 cm long and PA III + IV predominates in the older ones (77% PA III + IV, 20% PA I and 3% PA II in adults 29 cm long).

In *D. labrax,* where the larval stage begins on day 15 posthatching or at 0.75 cm, PA II tardily appears in 29-day-old larvae (1.1 cm), reaching a maximum level at 68 days or 2.8 cm, i.e. within the period of transition from the larval to the juvenile stage. It then slowly decreases (Fig. 6A). PA V is still near the method's limit of detection at 68 days and afterwards increases very slowly. Although PA II occurs, as in the other fish, in the larvae, it remains the major isotype in adult fish (70% at 45 cm), contrary to what happens in both trout species (Fig. 6 B and C).

The total parvalbumin content peaks in all three fish species when the fish reach a length of 8-10 cm during the juvenile period.

Spatial Distribution of Parvalbumin Isotypes

The antero-posterior variation of the parvalbumin isotype contents in the dorso-lateral white muscle is similar in both trout species (Fig. 7A-D) and presents comparable features in D. *labrax* (Fig. 7 E, and F). From the first to the last myotomes, amounts of all isotypes, expressed with respect to the sarcoplasmic protein content, decrease regularly in O. *mykiss* (Fig. 7A) and S.

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trutta (Fig. 7C); in the myotomes near the tail, only PA IV + V and PA III + IV were detected in significant amounts. In D. *labrax* (Fig. 7E), the PA V content remains low and fairly constant, contrary to the PA II level, which markedly decreases from head to tail, even though this latter isotype remains the major form in all myotomes and, as seen above, throughout the development of this fish species. Percentages of the various isotypes also depend on the myotomal location: the relative abundance of the "adult" parvalbumin (PA IV + V in O. *mykiss,* PA III + IV in S. *trutta* and PA V in *D. labrax)* increases regularly from the head to the tail, at the expense of PA I and PA II (Fig. 7 B, D, and F).

Figure 6. *Evolution of the concentration of each parvalbumin isotype in Dicentrarchus labrax in the course of development. A: relative content until 4 months; B: relative content from 5.5 to 45 cm; C: stoichiometry according to the body length. The last measurement in A corresponds with the first one in B. ▲, PA II; ▼, PA V.*

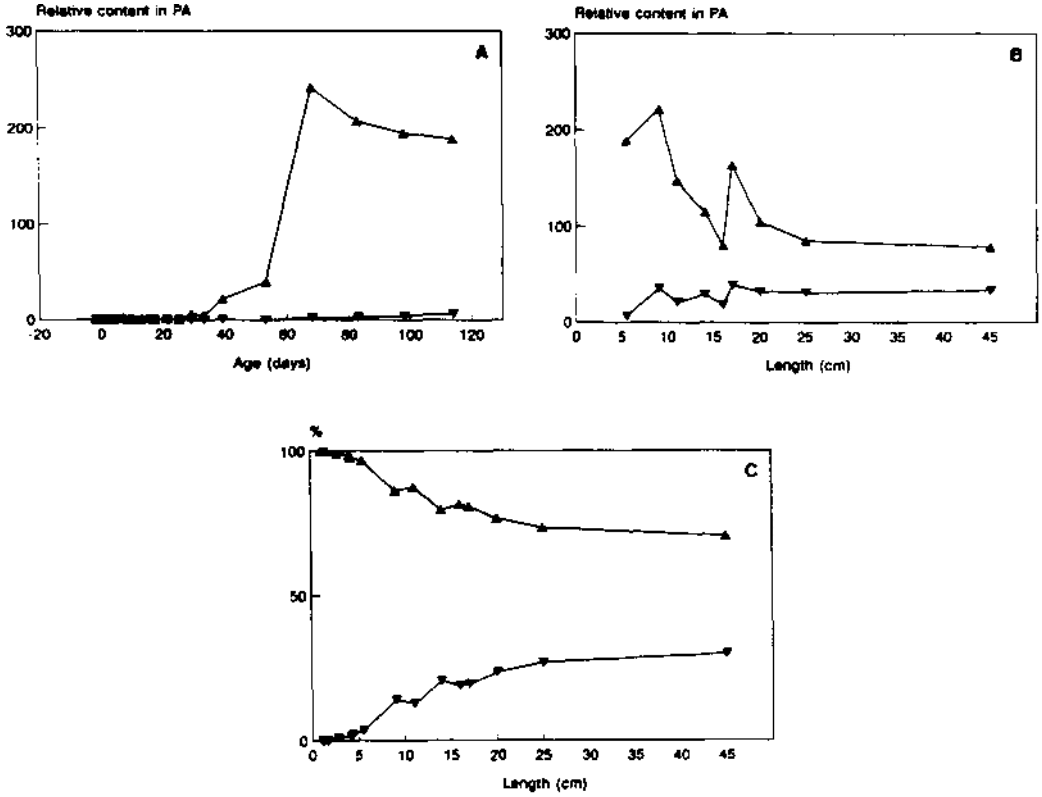

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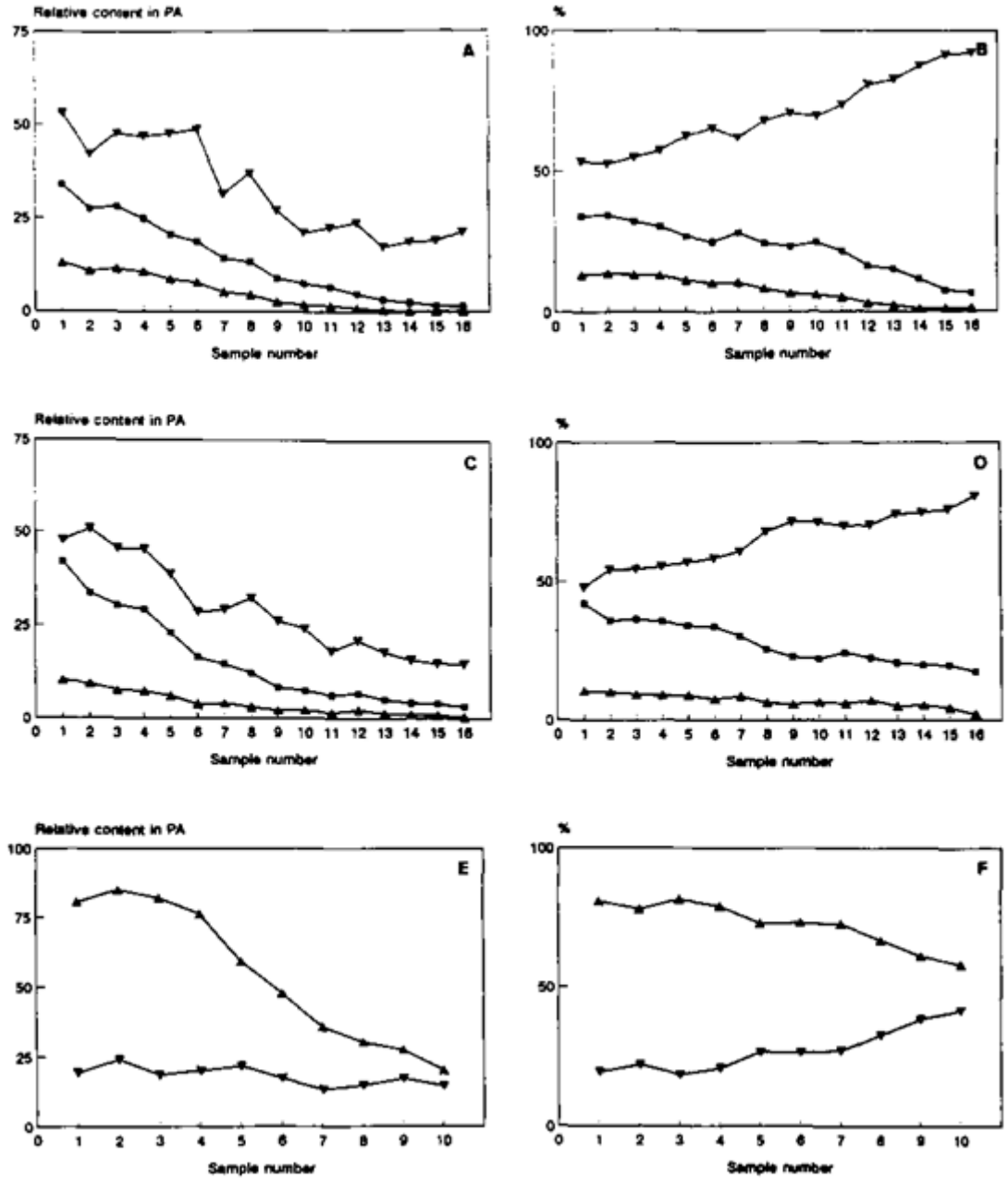
Figure 7. *Variation of the concentration of each parvalbumin isotype in the dorso-lateral muscle according to the antero-posterior location. A, C and E: relative content taking into account that the total parvalbumin content, as estimated by densitometry, is sample 1 = 100. B, D and F: stoichiometry. A and B: Oncorhynchus mykiss; 16 samples 1 cm long; ■, PA I; ▲, PA II; ▼, PA IV + V. C and D: Salmo trutta; 16 samples 1 cm long; ■, PA I; ▲, PA II; ▼, PA III + IV. E and F: Dicentrarchus labrax; 10 samples 1 cm long; ▲, PA II; ▼, PA V.*



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DISCUSSION

Numerous fish parvalbumin isotypes have been isolated in a pure state and chemically characterized (10). No such study, however, has ever focused on the parvalbumins of Salmonidae and Serranidae. We therefore decided to purify the various isotypes of two species of freshwater Salmonidae and one species of marine Serranidae and to further compare their synthesis in the course of the development of these commercially very important teleosts.

The rainbow trout (O. *mykiss)* and the brown trout (S. *trutta)* exhibit many parvalbumin components (five and four, respectively) under non-denaturing conditions. This complicates the chromatographic purification of these components. In the sea bass (D. *labrax),* on the other hand, only two isotypes are distinguished. All the isotypes isolated present physico-chemical properties characteristic of parvalbumins: a relative molecular mass around 12,000, an acidic isoelectric point ranging from 4.5 to 5.2 under the conditions used for 8 M urea-PAGE, typical ultraviolet spectra in which the absorption bands of phenylalanine residues are distinctly visible, due to the fact that tryptophan is absent in all cases and only one tyrosine residue, at most, is present.

Until a few years ago, the rainbow trout *Oncorhynchus mykiss (W.)* was called *Salmo gairdneri* (R.) and classified in the same genus *Salmo* as brown trout (S. *trutta* L.) and Atlantic salmon (S. *salar* L.). Recent osteological and biochemical studies, however, have shown this fish to be more closely related to Pacific salmons (genus *Oncorhynchus)* (23). The fact that the corresponding parvalbumin isotypes of the two trout species are so similar confirms their close relationship: identical electrophoretic mobilities at alkaline pH, identical M_r, pI, and tyrosine and cysteine residue contents, yet O. *mykiss* can be distinguished from S. *trutta* by the presence of PA V, the almost total absence of PA III, and a small difference in the pI of PA IV. The biochemical difference is more marked between the isotypes of either Salmonidae species and the corresponding forms of *D. labrax* (Serranidae). A previous work comparing five Serranidae species showed that whilst all specimens possess two isotypes, the electrophoretic mobility of these isotypes tends to differ among these species, making it possible to identify each species but not to establish phyletic relations in this teleost family (9).

The similarity of the two trout species also appears in the course of development. Parvalbumins occur around the period of hatching, when the fish begins to respond to outer stimuli. At this time myofibrils appear in great number (1) and cells show the features typical of white fibres (20,24). This delayed onset of parvalbumin synthesis, not occurring until the start of activity, has previously been observed in barbel (6), frog (22), and higher vertebrates (3,18,19). It suggests that parvalbumin expression is under the positive control of fast-type motor-neuron activity (18). As we described for *Barbus bar- bus,* the different isotypes are not synthesized synchronously during trout development, PA II being the principal eleuther- oembryonic and larval form and PA III, IV, or V the essential adult form. PA I seems less characteristic of a particular growth stage and constitutes a minor adult form. The PA II content peaks at about the same stage of development in both trout species, i.e. during the phase of transition from the eleutheroembryo to the larva. This period corresponds with changes in locomotor behaviour;

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seizing exogenous food requires fast and frequent movements. The contractile characteristics of the propulsive musculature evolve with growth, a fact probably linked to changes in parvalbumin expression. So far, however, no connection between white muscle movements and isotype distribution has been found. In D. *labrax,* a marine fish contrary to trouts and barbel, PA II is again the larval isotype, although it remains the predominant form in juveniles and adults. It is noteworthy that between the larval and adult isotypes of all three fish studied here, similar differences were observed in the cysteine and tyrosine contents.

An unexpected finding is the very late appearance of both parvalbumins in D. *labrax* (during the larval period for PA II and from the beginning of the juvenile period for PA V), even though earlier stages exhibit well-organized myofibrils (25) and active muscles. This tallies with the histochemical observations of Scapolo *et al.* (21), showing no myosin ATPase activity in any part of the myotome in 65-day larvae, and with our recent work in which myosin remained undetectable by PAGE until day 40 (8). It is interesting to note that the myosin isoforms we observed at this age were indistinguishable from the adult ones. By comparison with other species, the development of white muscle in *D. labrax* thus appears very slow and more difficult to characterize biochemically (the PA V content remains low and constant).

In numerous growing teleosts, the white muscle has a so- called mosaic appearance; it consists of a mixture of large fibres (hypertrophic growth) and new small fibres (hyperplastic growth). In the fish investigated here, which grow more or less continuously throughout their lifespan, hyperplasia continues along with hypertrophic growth, well into the post-larval period to the adult stage (25,26). As the distribution of the parvalbumin isotypes varies greatly during development, chiefly in trout, it does not seem plausible that the synthesis of a particular isotype is linked to a fibre of given size and age, but rather to the expression of different genes within a same cell. This hypothesis should be confirmed by immunohistochemistry.

Trouts and sea bass are intensively reared in Europe due to their commercial value. This work shows that parvalbumin isotypes constitute an excellent biochemical tool for determining the degree of maturity of these fish. It proves, furthermore, that parvalbumin isotype synthesis is subject to temporal transitions and varies spatially in a continuous way within the muscle of adult fish. This latter fact makes it necessary to pay special attention when choosing the location of samples taken for comparative analyses.

In conclusion, all these results confirm our previous work and raise the question of the peculiar physiological role of each isotype, a subject already approached in barbel (6,15). Parvalbumins seem to act as soluble relaxing factors, especially in the white muscles of cold-blooded vertebrates (12); their expression is assumed to be controlled by motor unit activity. Their polymorphism in fish may constitute a subtle mechanism for modulating the speed and power of contraction. The presence and distribution of the various isotypes might thus be adapted in relation to the swimming style, to provide the specific activity of a muscle or fibre according to the developmental stage or the precise location within the trunk muscle.

Work is in progress to compare larval and adult parvalbumins from this physiological standpoint.

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