Comp. Biochem. Physiol. Vol. 107A, No. 1, pp. 187–193, 1994
Printed in Great Britain

0300-9629/94 $6.00 + 0.00

Fish enterocyte hydrolases. Nutrition adaptations

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The activity level of digestive enzymes, especially carbohydrases, in various fish species is to a great extent dependent on the type of feeding and food composition. The most significant interspecies differences have been found for α-amylase, realizing initial and intermediate stages of the carbohydrate hydrolysis. The age dynamics of the enzymatic activity varies in fish of different species and ecological groups. Adaptive changes of the enzymatic spectrum associated with the type of feeding are observed at the start of larval exogenous feeding. It has been revealed that intraspecies variability of the enzymatic activity, especially of α-amylase, is connected with changes in the food composition and intensity of fish feeding.

Key words: Enterocyte hydrolases; α-amylase; Food composition; Fish feeding.

Comp. Biochem. Physiol. 107A, 187–193, 1994.

Introduction

In recent years it has been established that digestive processes on the brush border surface play an important role in the realization of final stages of nutrient depolimerization in fish (Berman and Salienize, 1966; Pegel *et al.*, 1971; Ugolev, 1972; Kuz'mina, 1977; Ugolev *et al.*, 1983; Egorova *et al.*, 1986). The comparison of the activities of enzymes involved in membrane digestion in various fish species demonstrated the correlation between the type of feeding and the level of the enzymatic activity (Pegel *et al.*, 1971; Ugolev *et al.*, 1976; Kuz'mina, 1977) that allowed us to describe some nutritive adaptations (Ugolev *et al.*, 1976; Kuz'mina, 1981a, 1991).

The adaptation of digestive enzymes to the type of fish feeding has been the object of many investigations (review: Vonk, 1937; Barrington, 1957; Phillips, 1969; Kapoor *et al.*, 1975). Although a number of studies did not reveal the correlation between the enzymatic activity and the type of feeding (Chesley, 1934), the results of many investigations supported the phylogenetic adaptive dissociation of enzymes in fish with different spectrums of feeding because carnivorous fish demonstrated a high protease activity while omnivorous and herbivorous fish demonstrated high carbohydrase activity (Vonk, 1927; Turpaev, 1941; Al-Hussaini, 1949; Barrington, 1957; Fish, 1960; Ushiyama *et al.*, 1965; Pegel *et al.*, 1971; Nagayama and Saito, 1969). The question of possible rear-rangements of the enzymatic spectrum as a response to changes in food composition in one fish species was not fully answered for a long time (Barrington, 1957). However, a series of investigations of several fish species has revealed this correlation (Nagase, 1964; Kuz'mina, 1966; Nagayama and Saito, 1969; Shcherbina, 1980).

In most works, the authors studied enzymes involved in the processes of cavital digestion, as well as mucosal enzymes, without considering their role in the processes of membrane digestion. This work presents the results of the studies of nutritive adaptations of enterocyte hydrolases taking part in fish membrane digestion.

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Received 22 April 1992; accepted 12 February 1993

Materials and methods

Fish species

Pike—*Esox lucius* L., roach—*Rutilus rutilus* (L.), ide—*Leuciscus idus* (L.), white bream—*Blicca bjoercna* (L.), bream—*Abramis brama* (L.), safrefish—*Pelecus cultratus* (L.), carp—*Cyprinus carpio* L., crucian—*Carassius carassius* (L.), blue bream—*Abramis ballerus* (L.), burbot—*Lota lota* (L.), perch—*Perca fluviatilis* L., pike perch—*Stizostedion lucioperca* (L.), ruff—*Gymnocephalus cernua* (L.), smelt—*Osmerus eperlanus* (L.).

Biology of fish

The pike and the pike perch are typical predators. They prey on other fish species and their young. The burbot and the perch are predator-facultative benthophages. The ruff is a benthophage-facultative predator. The food of these fish species consists not only of fish, but also bottom-dwelling invertebrates. Roach, ide, bream, carp and crucian are typical benthophages. Oligochaetes and larvae of chironomids are predominant in the food of the bream. One can find also the larvae of Trichoptera, Copepoda, Cladocera and Mollusca in the food. Ground and detritus are always present in the food lump. The feeding spectrum of the white bream is similar to that of the bream; however, oligochaetes are absent, while Copepoda, Cladocera and Mollusca are present in great amounts. The food of the roach and ide contains a great amount of Mollusca and macrophytes. The latter are most frequent in the food of the crucian 'carp'. The carp differs from other benthophages in that it feeds on pellet food in winter. Blue bream is a typical planktophage. Cladocera, Copepoda and Rotifers are predominant in the food of this species. It also contains Ostracoda, larvae of chironomids and other benthic forms. Safrefish and smelt are predator-facultative planktophages. They feed on juvenile fish and plankton. Typical facultative predators feed during the whole year. Typical benthos and plankton eaters do not feed in the winter period. In spring (May) and autumn (September, October) they feed less intensively than in summer. All the fish species, with the exception of the burbot, spawn in May; the burbot spawns in the winter which is also its most active feeding period.

Preparations tested

We studied small intestinal segments and homogenates of the preliminary rinsed intestinal mucosa. The intestine was washed and the desorption was carried out (we used desorbents). The fish intestine was excised at 0–4°C and rinsed in 20 ml Ringer solution for poikilothermic animals (pH 7.4) in order to expel cavital enzymes. Then the intestine was cut into segments at 0–4°C As a rule, mid-intestinal segments were used. In order to resolve some problems, we used intact intestine or intestines divided into equal segments: proximal, middle and distal parts. In further experiments we used everted intestinal segments that were put on glass rods or mucosal homogenates. The latter were prepared in Ringer solution for poikilothermic animals (10%). Desorbents were obtained by putting the rods with intestinal segments into tubes containing Ringer solution, which were treated in Schuttel apparatus for various time periods (30–120 min).

Enzyme assays

The activity of α-amylase (EC 3.2.1.1) was assayed by a decrease in starch by the modified method of Smith and Roe (Ugolev, 1969), sucrase (EC 3.2.1.48) by an increase in hexose by the modified method of Nelson (Ugolev and Iezuitova, 1969), enzymes of the maltase group (EC 3.2.1.20) by an increase in glucose by the glucoso-oxidase method (Ugolev and Iezuitova, 1969), that of alkaline phosphatase by an increase in *p*-nitrophenol concentration (at 405 nm). Common proteolytic activity was assayed by an incease in the formed tyrosine in the modified method of Anson (1938) and the activity of di-, tri- and tetrapeptidases (EC 3.4.3.13) by the increase in the formed glycine (Ugolev and Timofeeva, 1969). The hydrolysis rate was expressed in μmol/min/g wet tissue. The α-amylase activity was expressed in mg/(g min). Soluble starch (0.1% and 1.8 g/l), 58.4 mM sucrose and maltose solutions, 0.6 mM sodium *p*-nitrophenyl phosphate, casein (1%), di-, tri and tetrapeptides (20 mM) solutions prepared in Ringer solution for poikilothermic animals (pH 7.4) were used as substrates. The incubation of enzymatically active preparation and the substrates was carried out at 20°C for 10–30 min in a specially designed chamber with a thermostatic device. In some experiments intestines obtained from several animals were utilized. The experimental data were statistically analyzed using Student's *t*-test.

Results

Data concerning activities of the studied enzymes are indicative of great differences in the levels of enzymatic activity between species (Fig. 1). For example, the activity of α-amylase

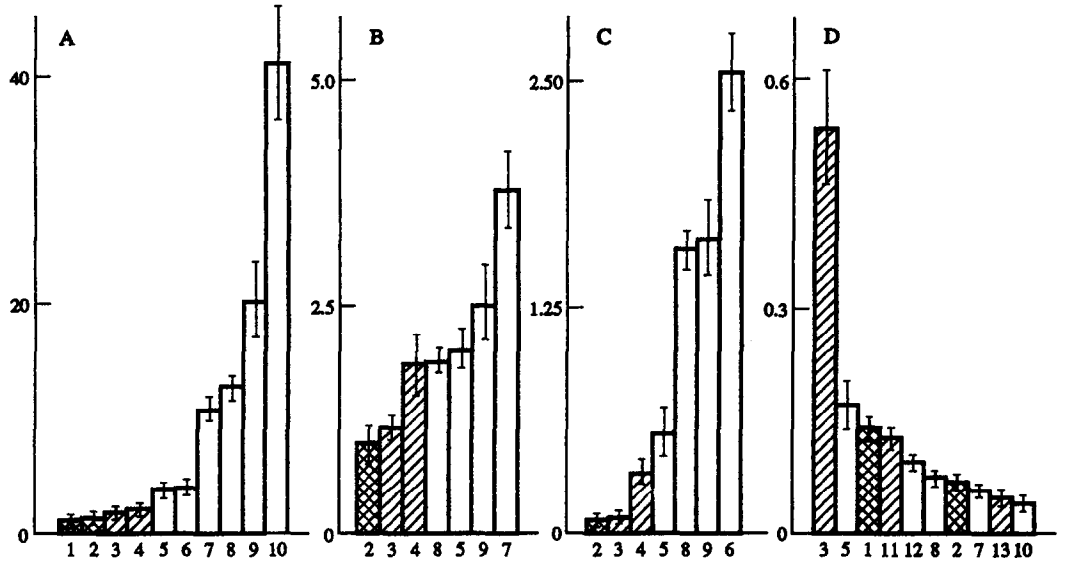

Fig. 1. The activity of some carbohydrases and alkaline phosphatase in the mucosa of the medial part of the intestine in some fish species. Non-hatched bars—typical benthophages (□); cross-hatched bars—typical predators (■); hatched bars—predators—1-pike perch, 2—pike, 3—burbot, 4—perch, 5—bream, 6—white bream, 7—crucian, 8—roach, 9—ide, 10—carp, 11—safrefish, 12—blue bream, 13—smelt.

in the mid-intestine was 0.9 ± 0.1 mg/min for the pike perch and 41.4 ± 5.3 mg/min for the carp. In other fish species intermediate values of the enzymatic activity were estimated. The minimal maltase and sucrase activities were seen in the pike (3.8 ± 0.9 and 0.3 ± 0.1 μmol/min), while the maximal ones in the crucian (15.4 ± 1.4 μmol/min) and in the white bream (10.1 ± 1.6 μmol/min). The minimal alkaline phosphatase activity level was found in the carp (0.07 ± 0.01 μmol/min), while the maximal level was found in the burbot (0.59 ± 0.06 μmol/min).

We pay attention to the correlation between the carbohydrase activity level and fish reference to any ecological groups, as well as lack of this correlation in the case of alkaline phosphatase. Thus, the carbohydrase activity increases as follows: typical predators→predator-facultative bentho- and planktophages→typical benthophages and planktophages. The most significant differences have been revealed in the studies of α-amylase. The level of the maximal individual enzymatic activity (for the carp) is 70–100-fold higher than the minimal one (for the pike). The variability range of disaccharidases activity is several fold lower: the maxima are 22.5-fold higher than minima for sucrase (the white bream-pike) and 4.1-fold higher for maltase (crucian-pike). For typical planktophage blue bream and predator-facultative planktophage safrefish similar data have been obtained (in summer the activity of α-amylase in the desorbents was 31.0 ± 9.3 and 6.6 ± 0.9 mg/min, respectively). As a rule this tendency is being preserved in fish during the whole year.

The comparison of the activity of carbohydrases involved in the starch hydrolysis carried out in the desorbents and homogenates of the intestinal mucosa in different fish species allowed us to demonstrate that the most significant interspecies differences are characteristic of the first fraction, the least significant of the three (Table 1). Thus, the activity of the easily desorbed α-amylase in the carp is 45.1-fold higher than in the pike. The activity of starch hydrolyzing enzymes (α- and γ-amylase) in homogenates in the carp is only 20.3-fold higher than in the pike. So the adaptation of enzymes of the carbohydrase chain to polysaccharide content being realized is mainly due to absorbed enzymes of the pancreatic origin.

Table 1. The activity of enzymes providing the hydrolysis of starch in different brush border zones of enterocytes in some fish species (mg/min)

Species	1	2	3
Pike	0.95 ± 0.24	0.11 ± 0.06	0.30 ± 0.10
Burbot	1.47 ± 0.13	0.12 ± 0.02	0.97 ± 0.20
Perch	3.96 ± 0.47	0.23 ± 0.10	1.17 ± 0.01
Bream	7.04 ± 3.71	2.50 ± 0.33	1.67 ± 0.19
Carp	42.82 ± 6.87	33.9 ± 5.30	6.40 ± 1.43

1—the fraction of the easily desorbed α-amylase. 2—the fraction of hardly desorbed α-amylase. 3—homogenate (the fraction of non-desorbed α-amylase as well as γ-amylase).

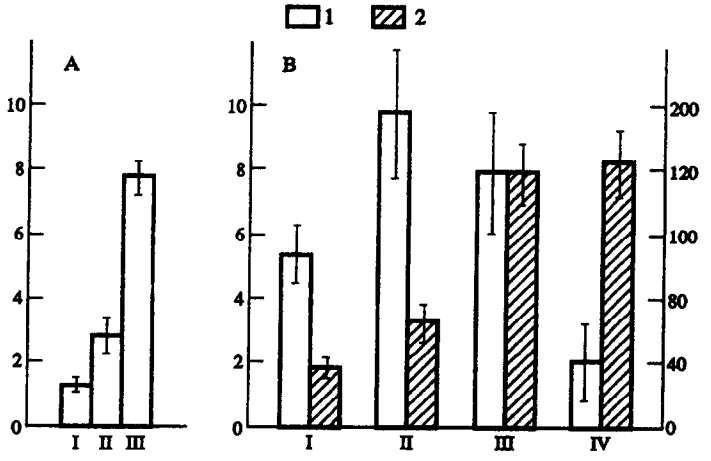

Fig. 2. Common proteolytic activity (A) and activity of peptidases (B) in the mucosa of the mid-intestine in some fish species. Vertical line—enzymatic activity, μmol/min, horizontal line—A: fish species (I—carp, II—bream, III—pike), B: substrates (I—triglycilglycine, II—diglycilglycine, III—glycilglycine, IV—glycil-L-valine), I–III—left scale, IV—right scale, hatched bars—bream, non-hatched bars—pike. $n = 6$.

The level of common proteolytic activity (Fig. 2) in the pike is several fold higher than in the bream and the carp (7.8 ± 0.4, 2.0 ± 0.5 and 1.3 ± 0.1 μmol/min, respectively). However, interspecies differences in the protease level are less than those in the carbohydrases: in the pike the activity is 6-fold higher than in the carp and 2.7-fold higher than in the bream.

The activity of mucosal tetra- and tripeptidases is different in the pike and the bream (Fig. 2B). Thus, the activity of enzymes hydrolyzing triglycilglycine in the pike was 5.3 ± 0.9, in the bream was 1.9 ± 0.4 μmol/min, that of enzyme hydrolyzing diglycilglycine was 9.8 ± 2.3 and 3.3 ± 0.6 μmol/min, respectively. The activity of enzymes hydrolyzing glycilglycine in these fish species is similar—8.0 ± 1.9 and 8.0 ± 2.0 μmol/min. The data demonstrate that the activity of tetra- and tripeptidases in the intestinal mucosa in predatory fish is higher than in benthophages by a factor of 2.7 and 2.9. This corresponds to interspecies differences in the activity of enzymes providing initial stages of the protein hydrolysis.

The absence of interspecies differences in the activity level of glycilglycinedipeptidase appears to be caused by the fact that the homogenates, besides the enzymes involves in membrane digestion, contained intracellular dipeptidases. Attention should be drawn to the activity level of glycilvalinedipeptidase that is 3.8-fold higher in the bream as compared to that of the pike. Reverse correlation between protein content in the food and the activity level of this enzyme in fish appears to depend on high valine content in food proteins as well as in tissue proteins in the bream (review: Kuz'mina, 1981b). These proteins can enter the alimentary fish canal as a result of excretion and be hydrolyzed together with protein components of food.

Also, species differences were found in fish in the early juvenile stages of development. Thus, the level of common amylolytic activities on the G stage is similar in the pike and perch, but it

Table 2. The activity of some enzymes in juvenile (G stage) and adult fish of various species

	The level of enzymatic activity, μmol/min			
	Common amylolytic activity		Alkaline phosphatase	
	Juvenile (G stage)	Adult fish	Juvenile (G stage)	Adult fish
Pike*	3.30 ± 0.25 (5)	0.46 ± 0.02 (12)	0.04 ± 0.002 (5)	0.10 ± 0.001 (12)
Perch	2.90 ± 0.49 (6)	1.30 ± 0.13 (10)	0.29 ± 0.045 (6)	0.33 ± 0.01 (10)
Roach	15.50 ± 3.93 (6)	4.28 ± 0.99 (8)	0.18 ± 0.026 (6)	0.11 ± 0.03 (8)
Roach*	5.13 ± 0.90 (6)		0.19 ± 0.02 (6)	
Blue bream	10.80 ± 1.99 (6)	7.09 ± 2.70	0.23 ± 0.06 (6)	0.13 ± 0.01 (6)
Blue bream*	7.32 ± 1.60 (6)		0.29 ± 0.07 (6)	

*D_1 stage of the pike development according to I. P. Shamardina, that is similar to the G stage of the carp according to V. V. Vasnetsov and perch according to S. G. Kryzhanovski (see: Kuz'mina, 1986). The number of fish studies in indicated in parentheses. The intestines of juveniles were joined together (10–60 examples in the sample).

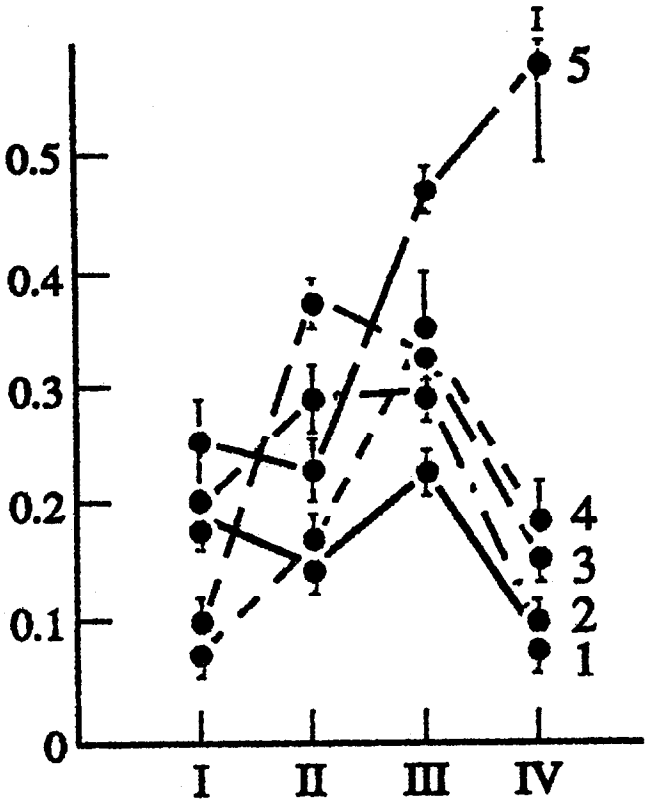

Fig. 3. Season dynamics of alkaline phosphatase of the intestinal mucosa of some fish species. Vertical line—enzymatic activity, temperature of the incubation at 20°C μmol/min. I—winter, II—spring, III—summer, IV—autumn. A: 1—pike, 2—roach, 3—pike perch, 4—bream, 5—burbot. $n = 10$.

is 3–5-fold higher in the blue bream and roach as compared to these species (Table 2). These data demonstrate that not only the greater activity of carbohydrases in the species as they grow go on to the plankton and benthos feeding, but also to the various age dynamics of the enzymatic activity.

A common proteolytic activity at early stages of the larvae development in the pike and bream intestines has not been observed. In the pike at the age of 1 month the activity of casein hydrolyzing enzymes was found to be 2.0 ± 0.6 μmol/min. In the bream a minimal activity of the same enzymes was detected. These data demonstrate that the activity of the enzymes providing initial stages of protein hydrolysis in predatory fish in all the stages of the onthogenesis is significantly higher than in benthophages.

At the same time it has been demonstrated that the change of the contents of main energetic food components may lead to the change of enzyme activity. Thus, the replacement of the natural food of benthophages containing 2–4% of carbohydrates by more carbohydrate-rich pellet food (up to 40%) leads to an increase of mucosal α-amylase activity from 33.9 ± 4.7 to 384.4 ± 39.1 μmol/min. These data indicate that adaptive rearrangements associated with the change of food composition involve the process of membrane digestion too.

It is known that fish, to a great extent, depend on the temperature of the environment. The intensity of feeding in fish of various species is different at various times of the year. In order to estimate the effect of the feeding behavior on the level of enzyme activity and exclude the effect of the temperature factor we compared the activities of membrane alkaline phosphatase in standard conditions: temperature 20°C (Fig. 3). The nature of changes of the enzymatic activity during the whole year in various fish species and groups is different.

So, alkaline phosphatase activity in different fish species and ecological groups varies and to a great extent depend on the intensity of fish feeding. In typical benthophages, which do not feed in the winter period, the maximal enzymatic activity is observed in summer. In typical and facultative predators, which feed during the whole year, the maximal activity may be absent or be observed in other seasons of the year, including winter (burbot).

Discussion

The data presented demonstrate that the level of membrane enzyme activity in various fish species, to a great extent, depends on the peculiarities of the feeding behavior and the presence of nutrients. The data obtained agree with the conclusions made by other investigators concerning the fact that the level of intestine carbohydrases in predatory fish is lower than in omnivorous and herbivores while, on the contrary, the level of proteases is higher (Vonk, 1927; Turpaev, 1941; Al-Hussaini, 1949; Barrington, 1957; Fish, 1960; Ushiyama *et al.*, 1965; Pegel *et al.*, 1971; Nagayama and Saito, 1969). At the same time, our data allow us to draw conclusions about the existence of finer adaptations to the food composition than had been earlier suggested.

The most significant changes in the level of the enzymatic activity effected by the feeding spectrum have been demonstrated for realizing initial stages of carbohydrate and protein hydrolysis.

The presence of adaptations is shown also by the nature of age rearrangemets of the spectrum of enzymes providing membrane digestion in fish. Those results agree with the data concerning a significant decrease in the activity level of the brush border of γ-amylase when the age of the horse-mackerel increases from 0+ to 2+ (Ugolev *et al.*, 1976) and the increase of it when the age of the carp increases from 0+ to 1+ (Gredin, 1975).

Intraspecies differences of the activity of enzymes taking part in the processes of membrane digestion and connected with the change in the feeding spectrum have not been earlier investigated. However, the data obtained in the carp studies confirm the possibility of adaptive changes in the spectrum of membrane enzymes when the food composition in one fish species changes, as well as cavital enzymes (Kuz'mina, 1966).

The data concerning the season dynamics of the enzymatic activity confirm the dependence of the studied characteristics on the intensity of fish feeding—a decrease in the substrate quantity or its absence is accompanied by the decrease in the activity of enzymes (Berman and Salienize, 1966).

Thus, the enzymes providing the processes of membrane digestion in fish are adapted to the spectrum and intensity of fish feeding as well as to the food composition.

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