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Histochemical distribution of digestive enzymes in hake, *Merluccius merluccius* L. 1758

Zvonimir Kozarić[1]*, Snježana Kužir[1], Srebrenka Nejedli[1], Zdravko Petrinec[2], and Emil Srebočan[3]

[1]*Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[2]*Department of Biology and Pathology of Fish and Bees, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[3]*Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

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ABSTRACT

Histochemical localization of non-specific esterase, alkaline and acid phosphatase in the digestive tract of free-living Hake (*Merluccius merluccius* L. 1758) and their contribution to the digestive process were researched. Material originated from the Adriatic Sea (vicinity of the Island of Rab). Histochemical techniques were used for detection of enzymatic activity. Various enzymatic activities had been noticed, in correlation with particular parts of fish digestive tract. Strong esterase activity had been noticed in intestinal epithelial cells. Alkaline phosphatase has a wide distribution and localization in intestine segments, generally in lamina propria, but mostly in brush border of enterocytes. Activity of acid phosphatase has been associated with intestinal epithelial cells, and it is the only enzyme to be detected in gastric glands. Obtained data will have an influence on understanding the digestive processes of investigated fish.

Key words: *Merluccius merluccius,* digestive tract, enzymes, histochemistry

* Contact address:
Prof. Dr. Zvonimir Kozarić, Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, Phone: +385 1 2390 245; Fax: +385 1 2441 390; E-mail: kozaric@vef.hr

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Introduction

A better knowledge of digestive enzyme activities is essential for a deeper understanding of the physiology of fish nutrition. The final stage of digestion in vertebrates is carried out by the intestinal enterocytes expressing brush border enzymes such as disaccharidase, alkaline phosphatase and transpeptidase (MAROUX et al., 1973; SEMENZA 1986; FERRARIS et al., 1992). There are numerous recent reports about enzyme distribution and localization in gastrointestinal tract during the larval period of fish (WALFORD and LAM, 1993; OOZEKI and BAILEY, 1995; BAGLOLE et al., 1998; RIBEIRO et al., 1999; GISBERT et al., 1999). By the time metamorphosis has ended, the digestive system is complete and juveniles are able to assimilate diets in the same way as adults. There are a number of reports on digestive enzyme activities in adult fish (GOEL and SASTRY, 1973; SINHA, 1979; KUPERMAN and KUZ'MINA, 1994; KUZ'MINA and GELMAN, 1997; CAHU et al., 2000; KUZ'MINA et al., 2003). CHAKRABARTI et al. (1995) investigated the digestive enzymes of 11 freshwater fish species in relation to their food habits. In adults the digestive enzymes were present irrespective of the fishes' food habits.

The aim of this study was to determine histochemical localization of non-specific esterases, alkaline and acid phosphatase, in particular parts of the digestive tract of free-living hake and their contribution to intracellular digestive processes.

Materials and methods

Wild, sexually mature hake were caught in the vicinity of the Island of Rab. Fish with a body mass of 250-300 g. were dissected immediately after catching and the samples of esophagus, stomach, and two parts of intestine were fixed in cold (4 °C) formol-calcium. Ten-μm-thick cuts were used for the presentation of enzyme activity. Histochemical techniques for detecting enzymatic activities were taken from LOYDA et al. (1979) for the following enzymes: non-specific esterase (E.C.3.1.1.)-substrate 1-naphthyl-acetate (pH 6.5); alkaline phosphatase (E.C.3.1.3.1)-substrate, sodium β-glycerolphosphate (pH 9.4); acid phosphatase (E.C.3.1.3.2.)-substrate, sodium β-glycerolphosphate (pH 5.5). Enzymatic activity was analyzed visually and described further according to the intensity of the

colour reaction observed, i. e. no enzymatic reaction, weak (barely detectable) reaction, moderate reaction and strong reaction.

Results

Esophagus. Activity of investigated enzymes was weak to moderate. Non-specific esterase was mainly localized in the epithelial cells and in the lamina propria. In epithelial cells activity of enzyme was weak, and moderate in lamina propria. More intensive activity was found in the blood capillaries. Activity of alkaline phosphatase was localized only in lamina propria. Epithelial cells show no enzymatic reaction (Fig. 1). Very strong activity of acid phosphatase was localized in the supranuclear region of epithelial cells, while in lamina propria acid phosphatase is found as particular sites with strong activity (Fig. 2).

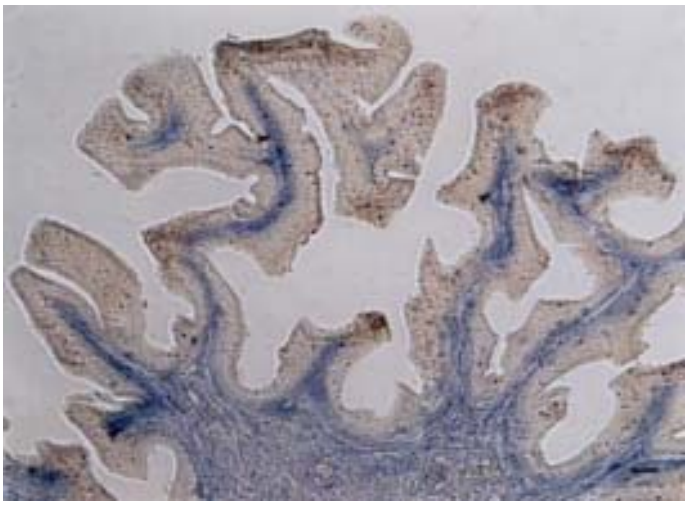

Fig. 1. Esophagus, alkaline phosphatase. Very strong activity of enzyme is observed in lamina propria (arrowheads). Scale bar = 50μm.

Stomach. Epithelial cells of stomach mucosa showed no activity of non-specific esterase, but moderate activity was observed in connective lamina propria (Fig. 3). There was no enzymatic activity in gastric glandular cells. More intensive enzyme activity was found in the blood capillaries

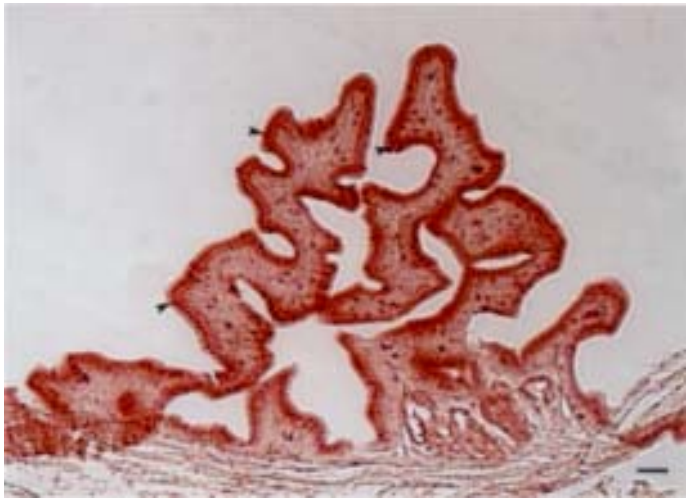

Fig. 2. Esophagus, acid phosphatase. Activity of enzyme is localized in supranuclear region of epithelial cells (arrowheads). Scale bar = 50 μm.

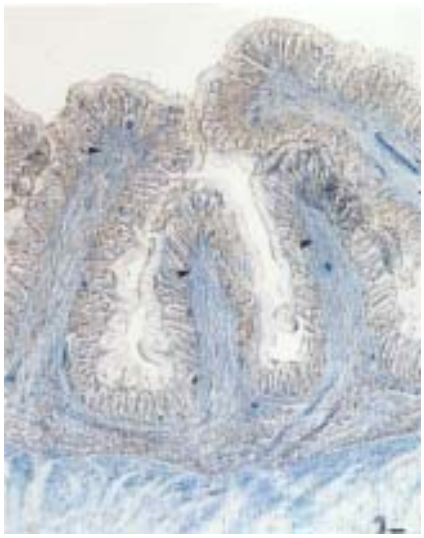

Fig. 3. Stomach, non-specific esterase. Enzyme activity is observed in lamina propria (arrowheads). Scale bar = 50 μm.

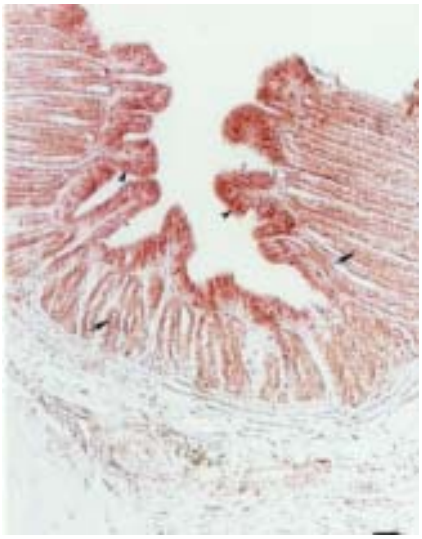

Fig. 4. Stomach, acid phosphatase. Activity of enzyme is observed in supranuclear region of epithelial cells (arrowheads) and in cytoplasm of glandular cells (arrows). Scale bar = 50 μm.

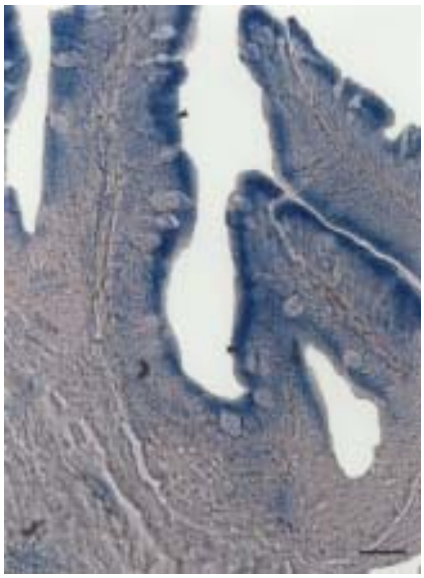

Fig. 5. Intestine, non-specific esterase. Enzyme activity is localized in cytoplasm and brush border of epithelial cells (arrowheads). Scale bar = 50 µm.

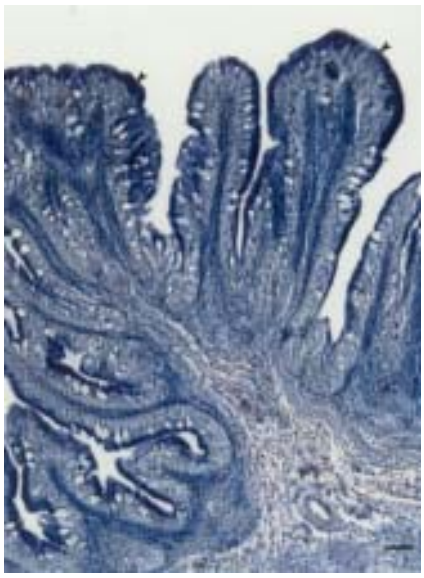

Fig. 6. Intestine, alkaline phosphatase. Very strong activity in brush border, moderate activity in cytoplasm of epithelial cells is observed (arrowheads). Scale bar = 50 µm.

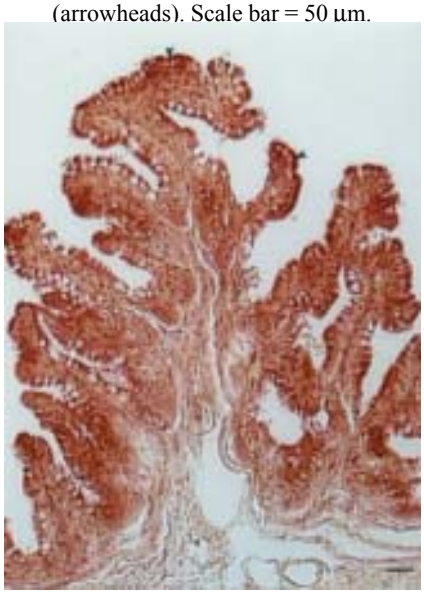

Fig. 7. Intestine, acid phosphatase. Very strong activity in epithelial cells (arrowheads), moderate activity in lamina propria is observed. Scale bar = 50 µm.

of lamina propria. The distribution of alkaline phosphatase was similar those in esterase. However, activity of acid phosphatase was very strong in the supranuclear region of epithelial cells, and moderate in gastric glandular cells. Activity of acid phosphatase was observed as granular reaction, different in size and dispersed in connective tissue (Fig. 4).

Intestine. Intestine was the part of the digestive tract showing the strongest activities of investigated enzymes. Activity of esterase was mainly localized both in cytoplasm and brush border of epithelial cells. Intraepithelial goblet cells showed no enzymatic activity (Fig. 5). Particular sites of enzymatic activity were observed in connective lamina propria. Activity of alkaline phosphatase was very strong in brush border, while its activity was of moderate intensity in cytoplasm of epithelial cells and in lamina propria (Fig. 6). Slightly weaker reaction was noticed at the same places in posterior part of intestine. Enzyme activity in lamina propria of the anterior intestine was lower in comparison with the posterior intestinal region. The highest activity of acid phosphatase was localized in cytoplasm of the supranuclear part of epithelial cells. Goblet cells showed no enzymatic activity, but dispersed granular enzymatic activity was observed in the connective tissue of lamina propria (Fig. 7).

Discussion

Gastrointestinal enzyme activities are in close correlation with digestion and resorption of food. Many papers have been published on the digestive physiology of fish larvae (WALFORD and LAM, 1993; OOZEKI and BAILEY, 1995; GISBERT et al., 1999; BAGLOLE et al., 1998; RIBEIRO et al., 1999) and pointed to a correlation between enzyme activities and maturation of digestive tract. In adults, digestive enzyme activities are in correlation with feeding habits (GOEL and SASTRY, 1973; SINHA, 1979; CHAKRAVORTY and SINHA, 1982). Recent studies have shown diet-related digestive enzyme activities (DIAZ et al., 1997; CAHU et al., 2000). In our investigations the activity of all enzymes express various degrees of intensity, in particular parts of the digestive tract.

Distribution of esterase in hake varied in esophagus, stomach and intestine. Esterase activity is higher in intestinal epithelial cells than in esophageal and gastric ones. Low activity was observed in lamina propria

in esophagus, stomach and posterior part of intestine. Correlation has been made in several fish species between the presence of this enzyme with lipid digestion (CHAKRABARTI et al., 1995) and lipid absorption (GAWLICKA et al., 1996). Further, this enzyme may be of particular importance because fish utilize lipids as their main nutritional source rather than carbohydrates and proteins (CHAKRABARTI et al., 1995). Differential distribution of esterase activity could reflect differences in function on particular parts of post-gastric regions. The intestine is generally associated with lipid absorption based on the appearance of lipid-containing vacuoles (KJORSVIK et al., 1991; SENGER et al., 1994; SARASQUETE et al., 1995; DIAZ et al., 1997). The low esterase activity in epithelial cells of esophagus and stomach leads us to conclude that hydrolysis of carboxylic esters (by non-specific esterase) occurs to a small degree in these regions of the gut of hake. However, GAWLICKA et al. (1995) detected lipase activity in multi-cellular glandular glands of stomach, suggesting the presence of non-pancreatic lipase activity in the digestive tract of Siberian sturgeon. OOZEKI and BAILEY (1995) have reported similar results in *Theragra chalcogramma*.

Alkaline phosphatase has a wide distribution and localization within gut segments. In hake, alkaline phosphatase is generally found in lamina propria. However, in both investigated intestine segments the highest enzyme activity was found in brush border of enterocytes. According to LOYDA et al. (1979) this enzyme was found primarily in cell membranes where active transport takes place and where this enzyme contributes to absorptive processes (COUSIN et al., 1987), because it is considered to be a general marker of nutrient absorption (SENGER et al., 1989). Small peptides and amino acids resulting from the action of digestive enzymes are transported with the collaboration of alkaline phosphatase and ATP-ase through intestinal enterocytes membrane (GAWLICKA et al., 1995; BAGLOLE et al., 1998). The activity and distribution of alkaline phosphatase in particular parts of gut in hake is in correlation with findings in other fish species (GOEL and SASTRY, 1973; SINHA, 1979; CHAKRAVORTY and SINHA, 1982).

Activity of acid phosphatase in investigate parts of hake gut is associated with epithelial cells. The enzyme is localized in supranuclear parts of enterocytes, but particular globular sites with enzyme activity are observed in lamina propria where they presented macrophages. This finding

is in correlation with RODE and FRANK (1967), but not with SINHA (1979), and CHAKRAVORTY and SINHA (1982), who found acid phosphatase in brush border. Acid phosphatase is one of the marker enzymes for lysosomes, but its activity was also founded outside lysosomes (CHI-WEI and FISHMAN, 1972). From all investigated enzymes, acid phosphatase is the only one which is founded in gastric glands of hake, its activity probably being connected with processes of secretion.

However, alkaline and acid phosphatase were stronger in the posterior region of the intestine, their activity appearing to be correlated with the presence of dense vesicle, containing proteins, in the posterior intestine enterocytes, indicating pinocytotic processes (BAGLOLE et al., 1998; RIBEIRO et al., 1999).

In summary, the activity of investigated enzymes indicates the site and intensity of intracellular digestion processes and transport of nutritives in a particular part of hake gut. The main absorption processes take place in the anterior part of hake intestine.

Acknowledgments

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KOZARIĆ, Z., S. KUŽIR, S. NEJEDLI, Z. PETRINEC, E. SREBOČAN: Histokemijska raspodjela probavnih enzima u oslića, *Merluccius merluccius* L. 1758. Vet. arhiv 74, 299-308, 2004.

SAŽETAK

Istražena je histokemijska lokalizacija nespecifičnih esteraza, lužnatih i kiselih fosfataza u probavnom sustavu slobodnoživućeg oslića (*Merluccius merluccius* L. 1758) te njihova uloga u probavnim procesima. Materijal potječe iz Jadranskog mora (okolica otoka Raba). Za utvrđivanje enzimskog djelovanja korištena je histokemijske metoda. Utvrđena je različita enzimska aktivnost u korelaciji s određenim dijelom ribljeg probavnog sustava. Jaka aktivnost esteraza uočena je u epitelnim stanicama crijeva. Lužnata fosfataza je imala široku rasprostranjenost u crijevima, općenito u lamina propria, ali u najvećoj mjeri u četkastom porubu enterocita. Aktivnost kisele fosfataze je povezana sa crijevnim epitelnim stanicama, a to je ujedno jedini enzim utvrđen u želučanim žlijezdama. Aktivnost istraživanih enzima u pojedinim dijelovima probavila ukazuje na jačinu apsorpcijskih procesa, koji su u oslića naizraženiji u prednjem dijelu crijeva.

Ključne riječi: oslić, probavni sustav, histokemija enzima