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Acid and alkaline phosphatase localization in the digestive tract mucosa of the *Hemisorubim platyrhynchos*

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ABSTRACT

This cytochemical study investigated the acid and alkaline phosphatase of the digestive tract of *Hemisorubim platyrhynchos*. Acid phosphatase was detected in the lining epithelium throughout the digestive tract, whereas alkaline phosphatase was only observed in the intestine. In the esophagus, an acid phosphatase reaction occurred in the apical cytoplasm of the epithelial cells and was related to epithelial protection and freeing of superficial cells for sloughing. Similar results were also observed in epithelial cells of gastric epithelium. In the gastric glands, acid phosphatase occurred in lysosomes of the oxynticopeptic cells acting in the macromolecule degradation for use as an energy source, whereas in the vesiculotubular system, its presence could be related to secretion processes. Furthermore, acid phosphatase in the intestine occurred in microvilli and lysosomes of the enterocytes and was correlated to absorption and intracellular digestion. However, no difference was reported among the regions of the intestine. However, alkaline phosphatase reaction revealed a large number of reaction dots in the anterior intestine, with the number decreasing toward the posterior intestine. This enzyme has been related to several functions, highlighting its role in the nutrient absorption primarily in the anterior intestine but also being essential in pH regulation because this is a carnivorous species with many gastric glands with secretions that could damage the intestine.

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1. Introduction

The characterization and localization of the enzymes may help to elucidate the physiology of the organs of the digestive tract of fish and to resolve several nutritional and health problems in fish feeding. Several researchers have studied acid and alkaline phosphatase in fish digestive tract (Sastry, 1975; Chakravorty and Sinha,

1982; Harpaz and Uni, 1999; Arellano et al., 2001; Kozarić et al., 2004; Kuz'mina, 2008; Mir and Channa, 2010). Acid phosphatase in mucosa of the digestive tract could play a role in the freeing of superficial cells for sloughing (Hopwood et al., 1978) and in protection against harmful agents (Kent et al., 1966), as well as being correlated to the absorption and transport of metabolites (Mir and Channa, 2010), which serves as a marker of lysosomes (Kuz'mina, 2008). The absorption of nutrients by pinocytosis and subsequent intracellular digestion by the lysosomes enzymes in the enterocytes in fish has been discussed, and may be important to complement the luminal digestion (Govoni et al., 1986; Murray et al., 1996).

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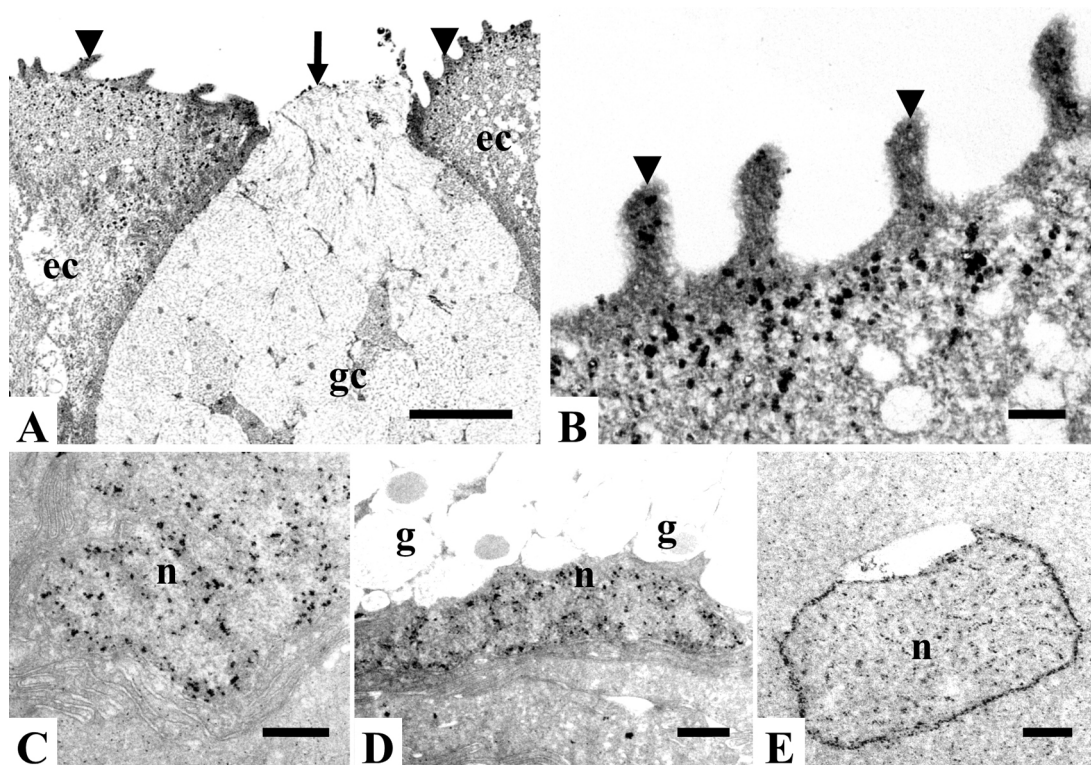


Fig. 1. Acid phosphatase reaction in the esophagus of *H. platyrhynchos*. (A and B) Esophageal epithelial cell showing small dots of reaction near microridges. (C) Nucleus of epithelial cell with small dots of reaction. (D) Nucleus of goblet cell, exhibiting small electron-dense dots. (E) Club cell nucleus, showing small electron-dense dots. Legends: ec-epithelial cell; gc-goblet cell; n-nucleus; g-granules; arrow-opening of goblet cell; arrowhead-microridge. Bars: (A) 2 μ m; (B) 200 nm; (C and D) 500 nm; (E) 200 nm.

Alkaline phosphatase is a widely distributed enzyme in different organs., [Silva et al. \(2010\)](#) reported that in fish intestine this is the predominant enzyme of the brush border of enterocytes, being expressed by active and mature enterocytes ([Zambonino-Infante and Cahu, 2001](#)). According to [Harpaz and Uni \(1999\)](#), the anterior region of the intestine in tilapia hybrids showed high activity levels of alkaline phosphatase, which was related with protein degradation. This enzyme is found in cell membranes where active transport takes place and plays a role in nutrient absorption ([Roubaty and Portmann, 1988](#); [Dupuis et al., 1991](#); [Gawlicka et al., 1995](#); [Morales and Almeida, 2014](#)). According to [Lallès \(2010\)](#), alkaline phosphatase acts as a regulator of fat absorption and also participates of regulation of surface pH and bicarbonate secretion. Further, [Bates et al. \(2007\)](#) showed that the intestinal alkaline phosphatase, located in the brush border of *Danio rerio*, could promote mucosal tolerance to resident gut bacteria. In this sense, [Lallès \(2010\)](#) concluded that alkaline phosphatase has a pivotal role in intestinal homeostasis.

The studied species belongs to the Pimelodidae family and Siluriformes order, which includes large catfishes and is distributed throughout Neotropical regions. Fishes of this family are highly appreciated for aquaculture because of the quality and flavor of their meat and the absence of intramuscular bones. *Hemisorubim platyrhynchos*, popularly called “jurupoca” in Brazil, is a migratory species with nocturnal carnivorous habit. The population size of this species has been reduced mainly due to building of hydroelectric dams that interrupt the migration flow required for reproduction ([Bressan et al., 2009](#)). Previous studies have described the morphology of the digestive tract of *H. platyrhynchos* ([Faccioli et al., 2014, 2015](#)). Thus, the current study analyzed the cytochemistry of the acid and alkaline phosphatase of the digestive tract of *H. platyrhynchos* to understand the morphophysiology of the carnivorous catfish digestive tract.

2. Materials and methods

2.1. Animals

The fishes of this study were obtained from Pirai Pisciculture in Terenos, MS Brazil. Fifteen adult specimens (37.4 ± 3.4 cm total body length) were fasted for one day to empty the digestive tract and were later anesthetized and euthanized with an overdose of benzocaine. Digestive tracts were dissected and fragments of the esophagus, stomach (cardiac, fundic and pyloric regions) and intestine (anterior, middle and posterior) were fixed in 1% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 1 h, at 4 °C and they were intended for acid and alkaline phosphatase detection.

2.2. Detection of acid phosphatase

The fixed fragments of each organ were rinsed in 0.1 M sodium cacodylate buffer, pH 7.2, and then incubated at 37 °C for 1 h in 25 mg of cytidine-5'-monophosphate, 12 mL distilled water, 10 mL 0.05 M acetate buffer (pH 5.0) and 3 mL 1% lead nitrate ([Pino et al., 1981](#)). After incubation, the samples were once again fixed in 2.5% glutaraldehyde, in 0.1 M sodium cacodylate buffer, pH 7.2; they were then post-fixed in 1% osmium tetroxide in the same buffer for 2 h, in the dark. Samples were again rinsed several times in 0.1 M sodium cacodylate buffer and block-stained with an aqueous solution of 2% uranyl acetate for 2 h. Samples were then dehydrated in a graded acetone series and embedded in Araldite resin. Ultra-thin sections (60–80 nm) were mounted on copper grids and analyzed without post-staining. Controls were incubated in medium without substrate. The photodocumentation was performed with a Philips® CM100 transmission electron microscope (Netherlands).

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