acta histochemica 113 (2011) 353–357

Contents lists available at ScienceDirect

acta histochemica

journal homepage: www.elsevier.de/acthis

Histochemical studies on mucin-rich cells in the digestive tract of a teleost, the Buenos Aires tetra (*Hyphessobrycon anisitsi*)

Ingvar Leiv Leknes

Faculty of Engineering and Science, Sogn og Fjordane University College, Box 133, N-6851 Sogndal, Norway

ARTICLE INFO

Article history:
Received 16 April 2009
Received in revised form
11 January 2010
Accepted 17 January 2010

Keywords:
Mucin
Mucus cells
Digestive tract
Buenos Aires tetra
Teleost

ABSTRACT

Types of mucus cells and mucins in the epithelial cell layer throughout the digestive tract of the Buenos Aires tetra (*Hyphessobrycon anisitsi*) are described and compared. The mucin was produced in three different cell types: in sac-like cells in the esophagus, in surface epithelial cells in the stomach and in goblet cells in the caeca and intestine. Nearly the entire esophageal epithelial cell layer consisted of mucus cells, filled by both neutral mucin and non-sulfated acidic mucin. The gastric mucin occurred in the distal area of the surface epithelial cells only and contained mainly neutral proteoglycans rich in glucosamine and some galactosamine and sialic acid. The goblet cells contained mainly non-sulfated acidic mucin in the caeca and sulfated acidic mucin throughout the entire intestine. Much glucosamine and some galactosamine and sialic acid occurred regularly in these cells in both the caeca and intestine. The observation that goblet cells often displayed colors ranging between blue and purple–magenta when alcian blue staining was followed by periodic acid-Schiff (PAS), or between blue and red–brown when the alcian blue was followed by neutral red, may reflect different ages or stages of development and differentiation for these cells. The highly variable affinities to wheat germ agglutinin (WGA-lectin) seen in these cells in the present study strengthens this view. However, such results may also suggest a true cellular heterogeneity reflecting various roles in lubrication, immunological defence, digestion and absorption.

Introduction

Recently, the amounts and composition of mucin throughout the digestive tract in teleosts have been investigated by means of various histochemical and biochemical techniques in species of phylogenetically old teleost orders such as: Cyprinoformes, Anguilloformes and Clupeidoformes, but also in evolutionary much younger bony fish orders such as: Atheriniformes, Pleuronectiformes and Perciformes (Domeneghini et al., 1998, 2005; Morrison and Wright, 1999; Arellano et al., 2001; Nakamura et al., 2001; Pedini et al., 2001; Sarasquete et al., 2001; Díaz et al., 2003, 2006, 2008a, b; Cinar and Senol, 2006; Carrassón et al., 2006; Santos et al., 2007). However, such techniques have, as far as we know, not been used so far to study the mucin in the alimentary canal of species in the order Characidiformes, which is one of the oldest and most specialized teleost orders, consisting mainly of carnivorous freshwater species.

The aim of the present study was to demonstrate and compare types of mucus cells and mucins in the epithelial cell layer throughout the esophagus, stomach, caeca and intestine of a characid, the Buenos Aires tetra (*Hyphessobrycon anisitsi*) by histochemical techniques, and to compare the results with those for the corresponding cell layers and mucins in species from other stomach-containing teleosts.

Material and methods

Twenty-eight specimens of the Buenos Aires tetra (*Hyphessobrycon anisitsi* Eigenmann, 1907), 1–3 years old, body mass about 2.5 g, maintained in a well aerated aquarium at 21–25 °C were used in this study. They were killed with an overdose of chlorobutanol and the digestive tract was fixed at 4 °C for a week in 4% formaldehyde, prepared from paraformaldehyde 24 h before use, in phosphate buffer (pH 7.4); alternatively fixation was done in a solution composed of 100% ethanol, 37% formaldehyde and 100% acetic acid in a volume ratio 85:10:5 (Leknes, 1980, 1986, 2005; Reite, 1996). After cleansing in buffer or ethanol depending on the fixative, the tissues were dehydrated in ethanol, treated with xylene, embedded in paraffin wax and sectioned.

Dewaxed sections (4 μm) were treated with Heidenhain's azan, 0.5% toluidine blue (pH 1, 1.3 or 3.5), alcian blue (pH 2.5, 1, 0.5 or 0.2), alcian blue (pH 2.5) followed by neutral red and/or eosin, periodic acid-Schiff (PAS), alcian blue (pH 2.5) followed by PAS or high iron diamine followed by alcian blue (pH 2.5) (Gray, 1954; Grimstone and Skaer, 1972; Culling, 1974; Pearse, 1980).

For lectin histochemistry, dewaxed sections were exposed to 3% hydrogen peroxide for 10 min to inhibit endogenous peroxidase

E-mail address: ingvar.leknes@hisf.no

0065-1281/$ - see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.acthis.2010.01.010

activity and then incubated for 60 min at room temperature with peroxidase-conjugated wheat germ agglutinin (WGA-lectin) or *Dolichos biflorus* agglutinin (DBA-lectin) (Sigma-Aldrich, St. Louis, MO, USA) in 0.1 M Tris buffered saline, pH 7.4 (Scillitani et al., 2008). The peroxidase activity was then visualized with 0.005% 3-3′-diaminobenzidine (DAB) (Sigma-Aldrich) in 0.05 M Tris buffered saline containing 0.01% hydrogen peroxide for 10 min in the dark at room temperature (Graham and Karnovsky, 1966). Controls were performed by incubating the sections in Tris buffered saline without WGA or DBA.

Some of the dewaxed sections were incubated in 0.1 U/ml neuraminidase Type *V* from *Clostridium perfrigens* (Sigma-Aldrich) in 0.05 M acetate buffer, pH 5.5, containing 0.1% calcium chloride at 37 °C for 30, 90 min, 20 or 22 h, before the hydrogen peroxide treatment and lectin histochemistry described above (Leathem and Atkins, 1983; Scillitani et al., 2008).

Results

The epithelial cell layers throughout the entire digestive tract of the Buenos Aires tetra (*Hyphessobrycon anisitsi*) were markedly better preserved in ethanol–formaldehyde fixative than in traditional buffered formaldehyde fixative (Fig. 1b). The intestinal wall was very thin and delicate consisting mainly of the epithelial cell layer, i.e. the connective tissue was poorly developed and the circular and longitudinal muscle layers were very thin. The staining results described below were, however, somewhat more distinct in tissue fixed in buffered formaldehyde than in tissue fixed in ethanol–formaldehyde.

The esophageal epithelial cell layer was mainly composed of sac-shaped mucus cells (Fig. 1a). In the stomach, the surface epithelial cell layer consisted of columnar cells (Fig. 1b, c). There occurred numerous gastric glands beneath the surface epithelium in the anterior half of the stomach (Fig. 1b, c). Goblet cells were regularly seen in the epithelial cell layers in the caeca and intestine and were most common in the intestine near the caeca (Fig. 1d, e).

The esophageal mucins displayed no affinity to toluidine blue, but were colored intensely by alcian blue at pH 2.5 or periodic acid-Schiff (PAS) solutions (Table 1, Fig. 1a). When the pH level of the alcian blue solution was reduced, these cells were correspondingly less intensely colored, and nearly lacked any color at pH 0.2. When the alcian blue pH 2.5 was followed by PAS, these cells often displayed various colors ranging between blue and purple–magenta, whereas they were mainly light brown when the alcian blue staining was followed by neutral red. When treated with high iron diamine followed by alcian blue (pH 2.5), most of the esophageal mucus cells displayed an intense blue color, whereas some cells displayed a strong purple–brown color (Table 1, Fig. 2a). The latter cell type was most frequently seen near the pharynx. The WGA-lectin affinity in the esophageal mucus was moderate, both in the normal and in neuraminidase digested tissue. The DBA-lectin displayed no affinity to the esophageal mucus cells in either the normal or the neuraminidase-pretreated tissue (Table 1).

In the stomach, the surface epithelial cells were strongly colored by PAS towards the lumen (Fig. 1c). The WGA-lectin and the DBA-lectin displayed a strong and weak affinity, respectively, to this cell layer with the color reaction after DBA being

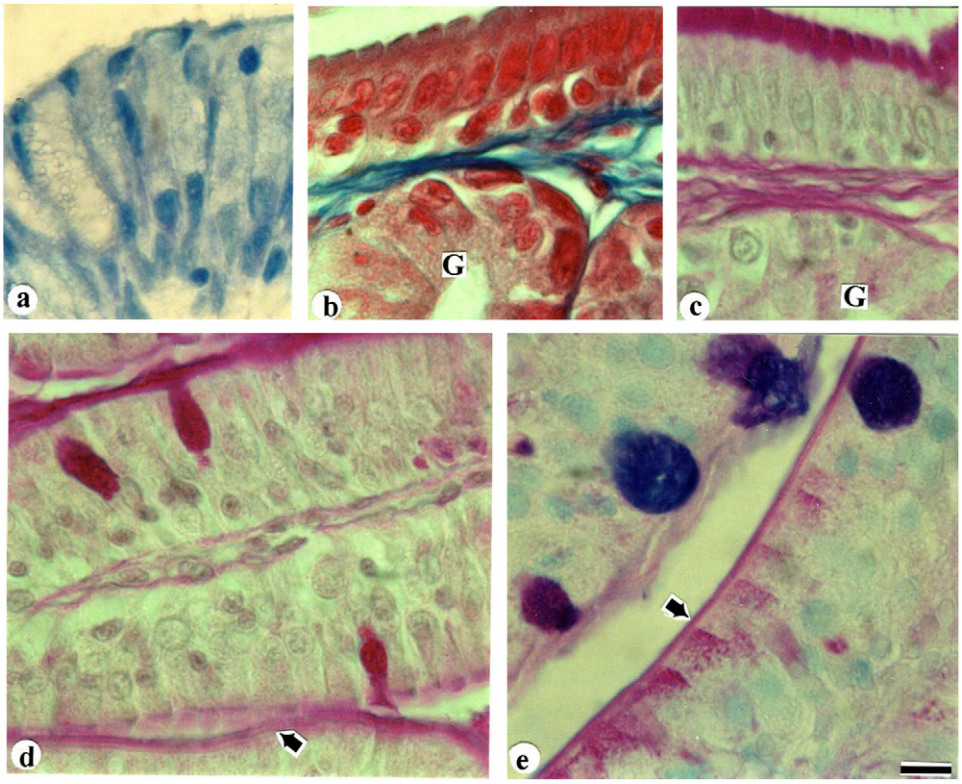

Fig. 1. (a) Esophageal epithelial cell layer composed of sac-shaped cells packed by mucin droplets (stained by toluidine blue at pH 3.5). (b) Surface gastric epithelial cell layer with gastric gland (G) below (fixed in ethanol–formaldehyde, stained with Heidenhain's Azan). (c) Surface gastric epithelial cell layer with gastric gland (G) below (stained with periodic acid-Schiff (PAS)). The most luminal part of the surface epithelial cell layer displays a positive PAS reaction, i.e. a strong purple–magenta color. (d, e) Epithelial cell layers in the pyloric caeca (d), stained with PAS, and (e), stained with alcian blue pH 2.5 followed by PAS. The goblet cells in (d) display a positive PAS reaction, whereas these cells display (e) various colors ranging between blue and purple–magenta. The brush border (arrows) and granular materials in the distal part of the epithelial cells display a positive PAS reaction in both (d) and (e). Scale bar: 20 μm.