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Glycohistochemical study of the toadfish *Halobatrachus didactylus* (Scheider, 1801) stomach

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SUMMARY: Toadfish *Halobatrachus didactylus* gastric mucosa was studied using conventional and lectin histochemistry. Conventional histochemistry revealed neutral glycoconjugates predominating over acidic ones in the apical zone of both surface epithelial cells and pit cells. The neck cells contained a few neutral glycoconjugates, whereas gastric glands were negative to PAS and AB staining. Lectin histochemistry showed different oligosaccharide expression along the columnar cells. The sub-nuclear cytoplasm was stained with RCA_{120}, SBA, HPA, GSA I-B_4, GSA II, UEA I, LTA, Con A, KOH-sialidase-WGA. The Golgi zone reacted with RCA_{120}, DBA, SBA, HPA, KOH-sialidase-WGA, GSA I-B_4, GSA II, UEA I, LTA, MAL II, SNA, and showed an increase in DBA staining after KOH-sialidase treatment. The granules of the apical zone stained with PNA, UEA I, LTA and showed increased PNA reactivity after KOH-sialidase treatment. The luminal cell coat reacted with PNA, HPA, Con A, KOH-sialidase-WGA, UEA I, LTA, MAL II, SNA and KOH-sialidase-PNA. Pit cells showed a minor expression of lectin-binding sites with respect to columnar cells. Neck cells linked UEA I and LTA and gastric glands reacted with PNA, DBA, SBA, HPA, Con A, GSA I-B_4, KOH-sialidase-WGA and KOH-sialidase-DBA. The results suggest that the stomach of the toadfish *H. didactylus* is characterised by a species-specific glycoconjugate pattern.

Keywords: glycoconjugates, lectin histochemistry, stomach, toadfish.

RESUMEN: EXPRESIÓN GLUCOHISTOQUÍMICA EN EL ESTÓMAGO DEL PEZ SAPO, *HALOBATRACHUS DIDACTYLUS* (SCHNEIDER, 1801). – Se ha estudiado la expresión de residuos glucídicos de glucoconjugados en la mucosa gástrica del pez sapo, *Halobatrachus didactylus* usando histoquímica convencional y de lectinas. La histoquímica clásica reveló la presencia de glicoconjugados neutros predominando sobre los ácidos en la zona apical de la superficie de las células epiteliales y de la criptas gástricas. Las células del cuello contienen algunas glicoproteínas neutras, mientras las glándulas gástricas han sido negativas al PAS y al Azul Alcián (AB). La histoquímica de lectinas mostró diferente expresión de oligosacáridos a lo largo de las células columnares. El citoplasma sub-nuclear reaccionó con las lectinas RCA, SBA, HPA, GSA, I-B4, GSA II, UEA I, LTA, Con A, KOH-sialidasa-WGA. La expresión de RCA_{120}, DBA, SBA, HPA, KOH-sialidasa-WGA, GSA I-B_4, GSA II, UEA I, LTA, MAL II y SNA se localizó en la zona del Golgi, donde se observó un incremento de la reactividad de la lectina DBA después del tratamiento con KOH. Los gránulos de la zona apical reaccionaron con PNA, UEA I, LTA, mostrando un incremento de la reactividad hacia la lectina PNA después del tratamiento con KOH. El borde de las células del lumen reaccionó con PNA, HPA, ConA, KOH-sialidasa-WGA, UEA I, LTA, MAL II, SNA, KOH-sialidasa-PNA. Los sitios de unión de la expresión de las lectinas-glucoconjugados fue menor en las células del cuello de las criptas gastricas que en las células columnares epiteliales. Las células del cuello reaccionaron con la UEA I y LTA, y las glándulas gástricas con PNA, DBA, SBA, HPA, Con A, GSA I-B_4, KOH-sialidasa-WGA y KOH-sialidasa-DBA. Los resultados sugieren que el estómago del pez sapo, *Halobatrachus didactylus*, se caracteriza por un patrón especie-específico de expresión de glicoconjugados.

Palabras clave: glicoconjugados, histoquímica de lectinas, estómago, pez sapo.

INTRODUCTION

The morphology of the stomach in teleosts has been documented for many species and shows a marked diversity in relation to their taxonomy, physical characteristics of the food and feeding habits (Harder, 1975; Kapoor *et al.*, 1975; Martin and Blaber, 1984). In fishes, the gastric mucosa is lined with a simple columnar epithelium and the mucosal epithelium invaginates to form gastric pits at the bottom of which gastric glands open (Kapoor *et al*., 1975; Martin and Blaber, 1984).

In different fish species the gastric mucosal epithelium secretes neutral as well as acidic glycoconjugates (Kapoor *et al*., 1975; Reifel and Travill, 1978). Glycoconjugates secreted by the stomach constitute an important class of macromolecules because they protect the stomach epithelium from auto-digestion caused by HCl and enzymes produced in gastric glands by forming an adherent mucus gel (Ferraris *et al*., 1987; Smith, 1989). Moreover, glycoconjugates play a role in the absorption and transport of macromolecules through the membranes (Reifel and Travil, 1978; Domeneghini *et al.,* 2005; Sarasquete *et al.,* 2001) and dilution in ingested water (Domeneghini *et al*., 1998). The gastric glands of teleosts contain only one cell-type that secretes both pepsinogen and hydrochloric acid (Reifel and Travill, 1978; Rebolledo and Vial, 1979).

Lectins have a specific binding affinity for the sugar residues of glycoconjugates. Due to their specific affinities to a particular sugar, lectins are useful probes for the intracellular localisation of sugar residues (Ihida *et al*., 1991; Danguy *et al*., 1994) and for characterising cellular populations as well as their morpho-functional changes (Spicer and Schulte, 1992; Danguy *et al*., 1994). Lectins have been successfully used to evaluate the composition of the oligosaccharides in the gastric mucosa of some marine fishes such as *Solea senegalensis* (Sarasquete *et al.,* 2001), *Sparus aurata* (Domeneghini *et al*., 1998; Sarasquete *et al.,* 2001), *Umbrina cirrosa* (Parillo *et al.,* 2002; Pedini *et al.,* 2005) and *Cynoscion guatucupa* (Díaz *et al.*, 2008).

Halobatrachus didactylus is a Batrachoididae euryhaline species capable of colonising diverse biotopes, from brackish waters of river estuaries such as those of the Guadalquivir (Spain), Mira, Sado, and Tagus (Portugal) to hyper-saline waters of salt ponds (Arias and Drake, 1990). It is a voracious and opportunistic carnivorous predator that feeds on a wide variety of prey including crustaceans as its major prey, as well as molluscs, fish and polychaetes as secondary prey (Cárdenas, 1977). *H. didactylus* is an important component for some fishing communities (Arias, 1976) and has received attention in recent years because of its use as a laboratory animal for multidisciplinary research purposes (see Palazón-Fernandez *et al*., 2001, for references).

The aim of this study was to investigate the glycoconjugate composition of the toadfish *H. didactylus* in the surface epithelial cells and gland cells of gastric mucosa by means of conventional and lectin histochemistry.

MATERIALS AND METHODS

Tissue preparation

Adult toadfish *H. didactylus* were acquired from working fishermen's catches in Cádiz Bay (southwestern Spain) and maintained in running seawater at the Instituto de Ciencias Marinas de Andalucia, CSIC. Five fishes were sacrificed with an overdose (0.3% V/V) of phenoxy-ethanol and their stomachs were quickly removed and fixed in Bouin's fluid for 24 h at room temperature (RT), dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin wax. Serial sections (4 μm thick) were cut and, after de-waxing with xylene and hydration in an ethanol series of descending concentrations, were stained with Masson trichrome for morphology, and by means of conventional histochemical procedures or lectin histochemistry according to Desantis *et al.* (2007).

Conventional histochemistry

Sections were treated with: 1) periodic acid-Schiff (PAS) reaction for neutral glycoconjugates (GCs) (Mc Manus, 1948); 2) Alcian blue at pH 2.5 (AB 2.5) for testing sulphate esters and carboxyl groups in GCs; 3) Alcian blue 8GX at pH 1.0 (AB 1.0) for the characterisation of sulphated GCs (Spicer *et al.,* 1967; Pearse, 1968). In order to reveal cellular combinations of both acidic and neutral glycoconjugates, the AB 2.5/PAS and AB 1.0/PAS staining sequences were performed.

Lectin histochemistry

The lectins used are listed in Table 1. The PNA, DBA, RCA_{120}, SBA, HPA, Con A, WGA, UEA-I

TABLE 1. – Lectins used, their sugar specificities and the inhibitory sugars used in control experiments. Fuc, Fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; GlcNAc, N-acetylglucosamine; Man, mannose; NeuNAc, N-acetyl neuraminic (sialic) acid.

Lectin abbreviation	Source of lectin	Concentration (μg/ml)	Sugar specificity	Inhibitory sugar
MAL II	*Maackia amurensis*	15	Neu5acα2,3Galβ1,4GlcNac	NeuNAc
SNA	*Sambucus nigra*	10	Neu5Acα2,6Gal/GalNAc	NeuNAc
PNA	*Arachis hypogea*	20	Terminal Galβ1,3GalNAc	Galactose
DBA	*Dolichos biflorus*	20	Terminal GalNAcα1,3(LFucα1,2)Galβ1,3/4GlcNAcβ1	GalNAc
RCA_{120}	*Ricinus communis*	15	Terminal Galβ1,4GlcNAc	Galactose
SBA	*Glycine max*	10	Terminal α/βGalNAc	GalNAc
HPA	*Helix pomatia*	15	Terminal αGalNAc	GalNAc
Con A	*Canavalia ensiformis*	15	Terminal and internal αMan>αGlc	Mannose
WGA	*Triticum vulgaris*	15	Terminal and internal βGlcNAc>>NeuNAc	GlcNAc
GSA I-B_4	*Bandeiraea simplicifolia*	15	Terminal αGal	Galactose
GSA II	*Bandeiraea simplicifolia*	15	Terminal D-GlcNAc	GlcNAc
UEA I	*Ulex europaeus*	20	Terminal L-Fucα1,2Galβ1,4GlcNAcβ	Fucose
LTA	*Lotus tetragonolobus*	25	Terminal αL-Fuc	Fucose

lectins were HRP-conjugated (Sigma Chemicals Co., St. Louis, MO, USA), whereas MAL II, SNA, GSA-II, GSA I-B_4 were biotinylated lectins (Vector Laboratories Inc. Burlingame, CA, USA).

Dewaxed and rehydrated tissue sections were immersed in 3% H_2O_2 for 10 min to suppress the endogenous peroxidase activity, rinsed in 0.05 M Tris-HCl buffered saline (TBS) pH 7.4, and incubated in lectin solution at appropriate dilutions (Table 1) for 1 h at RT. After 3 rinses in TBS, the peroxidase activity was visualised by incubation in a solution containing 0.05% 3,3'-diaminobenzidine (DAB) and 0.003% H_2O_2 in 0.05 M TBS (pH 7.6) for 10 min at RT before dehydration and mounting. Tissue sections incubated in biotinylated lectins (MAL II, SNA, GSA I-B_4, and GSA II) were rinsed 3 times with 0.05 M phosphate-buffered saline (PBS) and were incubated in streptavidin/peroxidase complex (Vector Lab. Inc., USA) for 30 min at RT. After washing in PBS, peroxidase was developed in a DAB-H_2O_2 solution as above.

Controls for lectin staining included: (1) substitution of the substrate medium with buffer without lectin; and (2) incubation with each lectin in the presence of its hapten sugar (0.2-0.5 M in TBS).

Enzymatic and chemical treatments

Before staining with MAL II, SNA, PNA, DBA and WGA some sections were incubated at 37°C for 16 h in 0.86 U/mg protein of sialidase (Type V, from *Clostridium perfringens*) (Sigma Chemicals Co.) dissolved in 0.1 M sodium acetate buffer, pH 5.5, containing 10 mM $CaCl_2$. Prior to the neuraminidase treatment, a saponification technique was performed to render the enzyme digestion effective, with 0.5% KOH in 70% ethanol for 15 min at RT (Reid *et al.,* 1978).

As controls of the sialidase digestion procedure, sections were incubated in the enzyme-free buffer solution under the same experimental conditions.

RESULTS

The tunica mucosa of the toadfish *H. didactylus* stomach was lined with a simple columnar epithelium and it displayed numerous gastric pits, at the base of which simple tubular gastric glands open (Fig. 1). There is no regional differentiation of the stomach with regard

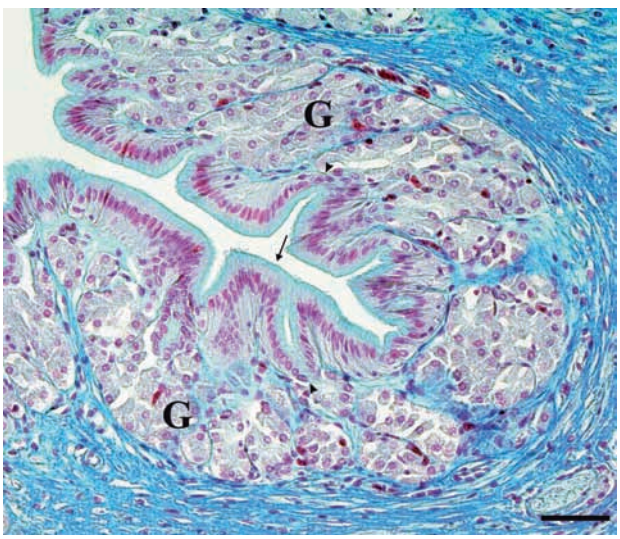

FIG. 1. – Morphology of the gastric mucosa of *Halobatrachus didactylus*. The tunica mucosa is lined with a simple columnar epithelium and shows gastric pits at the base of which gastric glands open. Masson trichrome staining. G, gastric gland; arrow, surface epithelial cells; arrowhead, pit cells. Scale bar: 37 μm.

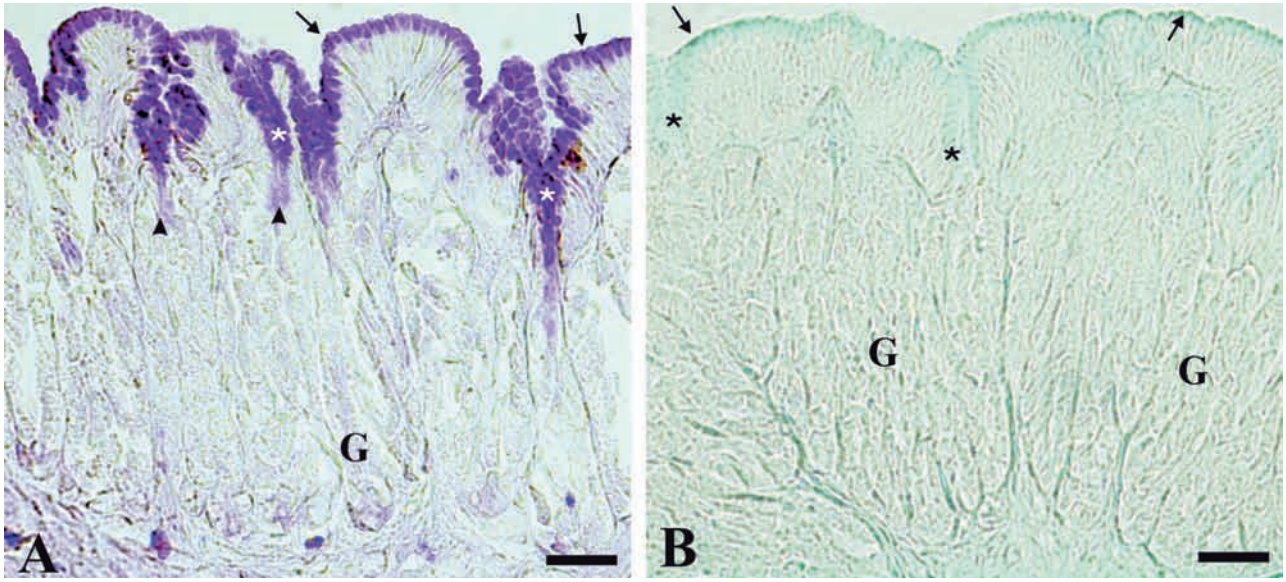

FIG. 2. – Tunica mucosa of *Halobatrachus didactylus* stomach stained with PAS (A) and AB (B) methods. A, PAS reaction intensely marks the apical zone of the surface epithelial cells and the pit cells and very weakly the neck cells. B, AB 2.5 method shows very weak staining in the apical zone of surface epithelial cells and pit cells. G, gastric gland; arrow, surface epithelial cells; arrowhead, neck cells; asterisk, pit cells. Scale bars: 30 μm.

to the surface epithelium or the glandular portion, so we arbitrarily divided it into: initial, middle and terminal. Numerous gastric glands were found throughout the stomach, especially in the initial and middle portions. The conventional and lectin histochemistry did not show any difference between the stomach regions.

Conventional histochemistry

PAS intensely stained the apical zone of the surface epithelial cells and the pit cells and very weakly stained the neck cells (Fig. 2A). AB 2.5 and AB 1.0 very weakly stained the apical zone of surface epithelial cells and pit cells (Fig. 2B).

Lectin histochemistry

The results of lectin histochemistry are summarised in Table 2.

MAL II (Fig. 3A) and SNA (Fig. 3B) showed binding sites for the luminal cell coat and a thin supra-nuclear zone of both the epithelial columnar cells and the pit cells, which were stained moderately and weakly, respectively. These lectins did not stain the

TABLE 2. – Lectin staining pattern of the gastric mucosa of the toadfish *Halobatrachus didactylus*; a, apical zone; g, sub-nuclear granules; lc, luminal cell coat; si, sialidase; sn, supra-nuclear zone; un, sub-nuclear zone; -, negative reaction; ±, faintly visible reaction; +, ++, +++, weak, moderate, strong positive reactions

Lectin	Gastric glands	Neck cells	Pit cells	Surface epithelial cells
MAL II	-	-	+lc/+sn	++lc/++sn
KOH-si-MAL II	-	-	-	-
SNA	-	-	+lc/+sn	++lc/++sn
KOH-si-SNA	-	-	-	-
PNA	++	-	++a/+g	++lc/++a/+g
KOH-si-PNA	++	-	+++a/++g	+++lc/+++a/++g
DBA	++	-	±un	±sn
KOH-si-DBA	+++	-	+un	+sn
RCA_{120}	-	-	±un	++sn/±un
SBA	++	-	++sn/+un	++sn/+un
HPA	+	-	+un	++lc/++sn/+un
Con A	±	-	+un	+++lc/+un
KOH-si-WGA	++	-	±un	+++lc/+sn/±un
GSA $I\text{-}B_4$	++	-	±un	++sn/±un
GSA II	-	-	±un	++sn/+un
UEA I	-	+++	+sn/+un	+++lc/++a/++sn/+un
LTA	-	++	+sn/+un	+++lc/++a/++sn/+un

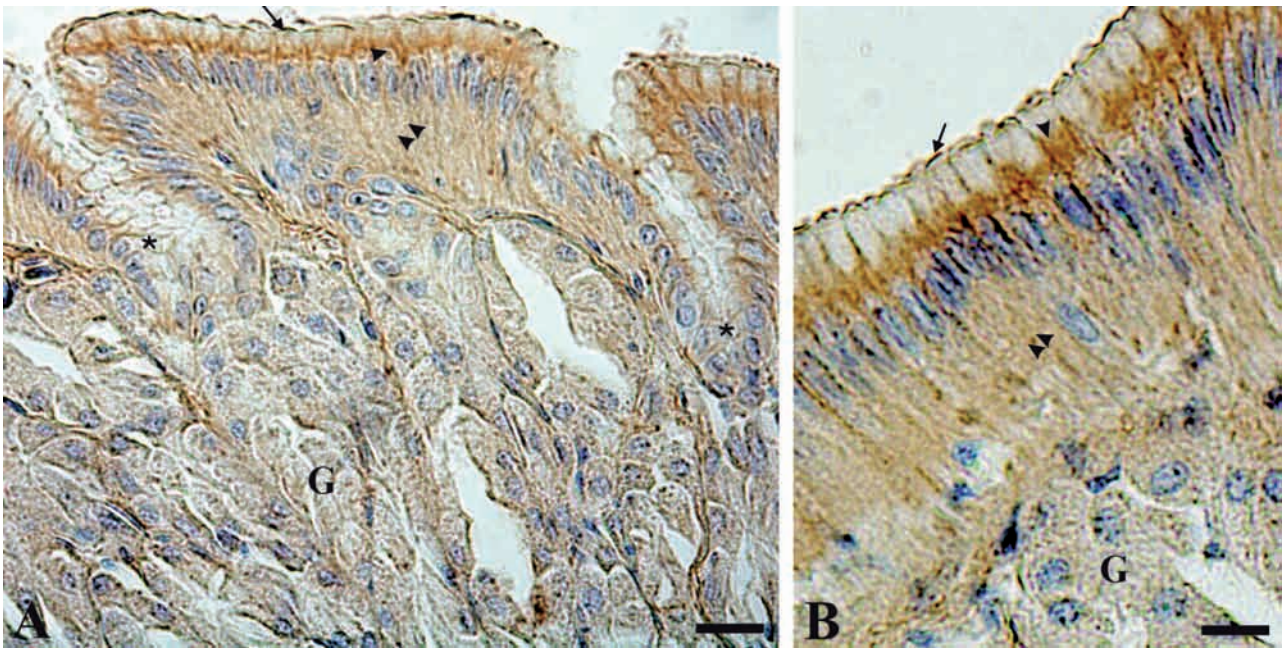

FIG. 3. – MAL II (A) and SNA (B) binding pattern of *Halobatrachus didactylus* gastric mucosa. These two lectins display similar binding patterns as revealed by the reactivity for the luminal cell coat and the supra-nuclear zone of the epithelial columnar cells and the pit cells. Gastric glands show a negative reaction. G, gastric gland; arrow, luminal cell coat of surface epithelial cells; arrowhead, supra-nuclear zone; double arrowhead, sub-nuclear cytoplasm; asterisk, pit cells. Scale bars: A, 16 μm; B, 9 μm.

neck cells or the gastric glands. Saponification followed by neuraminic acid cleavage (KOH-sialidase) abolished the reactivity.

PNA displayed a moderate reaction for the luminal cell coat and the apical zone of both the surface epithelial cells and the pit cells. Weak staining was observed at the level of the sub-nuclear granules of the lining epithelium and pits (Fig. 4A). Gastric glands were moderately stained. KOH-sialidase pre-treatment increased the staining in the above-men-

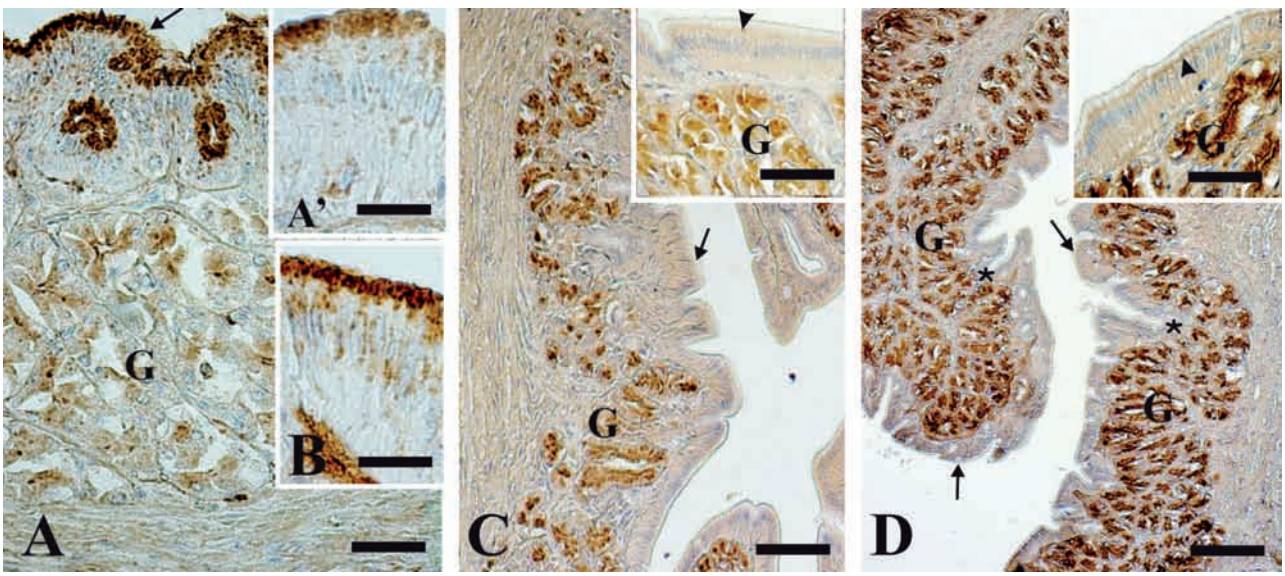

FIG. 4. – PNA (A, A'), KOH-sialidase-PNA (B), DBA (C), and KOH-sialidase-DBA (D) reactivity of the gastric mucosa of *Halobatrachus didactylus*. A, PNA moderately stains the luminal cell coat, the apical zone of the surface epithelial cells and the pit cells as well as the gastric glands. Scale bar: 52 μm. A' and B show the staining of the surface epithelial cells with PNA and KOH-sialidase-PNA staining, respectively. Note the increased PNA staining after KOH-sialidase pre-treatment. Scale bars: A' and B, 15 μm. C, DBA shows a very faint reaction with a thin supra-nuclear zone of the surface epithelial cells and with the sub-nuclear cytoplasm of the pit cells, whereas it moderately stains the gastric glands. Scale bar: 90 μm; scale bar of inset: 50 μm. D, after KOH-sialidase treatment the DBA staining shows increased reactivity (compared with C). Scale bar: 180 μm; scale bar of inset: 50 μm. G, gastric gland; arrow, surface epithelial cells; arrowhead, supra-nuclear zone; asterisk, epithelial pit.

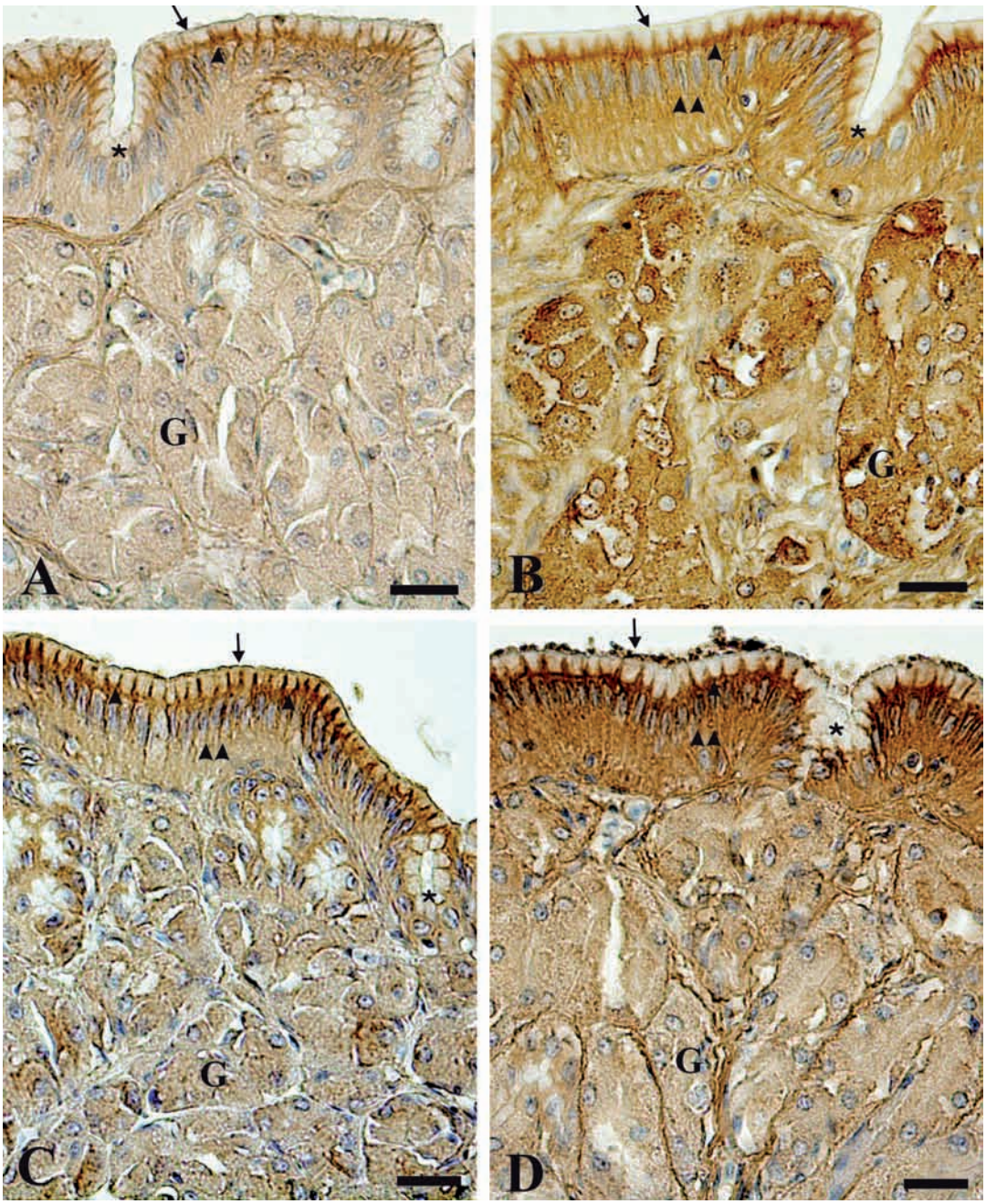

FIG. 5. – RCA_{120} (A), SBA (B), HPA (C) and Con A (D) binding pattern in the gastric mucosa of *Halobatrachus didactylus*. A, RCA_{120} moderately stains a thin supra-nuclear zone in the surface epithelial cells, and very weakly stains the sub-nuclear cytoplasm of both surface and pit cells. Scale bar: 22 μm. B, SBA weakly stains a thin supra-nuclear zone and the sub-nuclear cytoplasm of both lining epithelium and pit cells, whereas it moderately stains the gastric glands. Scale bar: 17 μm. C, HPA binding sites are located in the luminal cell coat, thin supra-nuclear zone and sub-nuclear cytoplasm of surface epithelial cells, in the sub-nuclear cytoplasm of pits cells and in the gastric glands. Scale bar: 22 μm. D, Con A strongly stains the luminal coat and weakly stains the sub-nuclear zone of both the columnar epithelial cells and pit cells. The gastric glands show faintly visible staining. Scale bar: 22 μm. G, gastric gland; arrow, surface epithelial cells; arrowhead, supra-nuclear zone; double arrowhead, sub-nuclear cytoplasm; asterisk, pit cells.

tioned structures of columnar epithelial cells and in the pit cells, whereas it did not affect the gastric gland staining intensity (Fig. 4B).

DBA gave a faintly visible staining of the thin supra-nuclear cytoplasm of the surface epithelial cells and the sub-nuclear cytoplasm in the pit cells, whereas it moderately stained the gastric glands (Fig. 4C). After KOH-sialidase treatment, the lectin DBA revealed cryptic binding sites at all the above-mentioned sites (Fig. 4D).

RCA_{120} moderately stained a small supra-nuclear zone in the surface epithelial cells, and very weakly

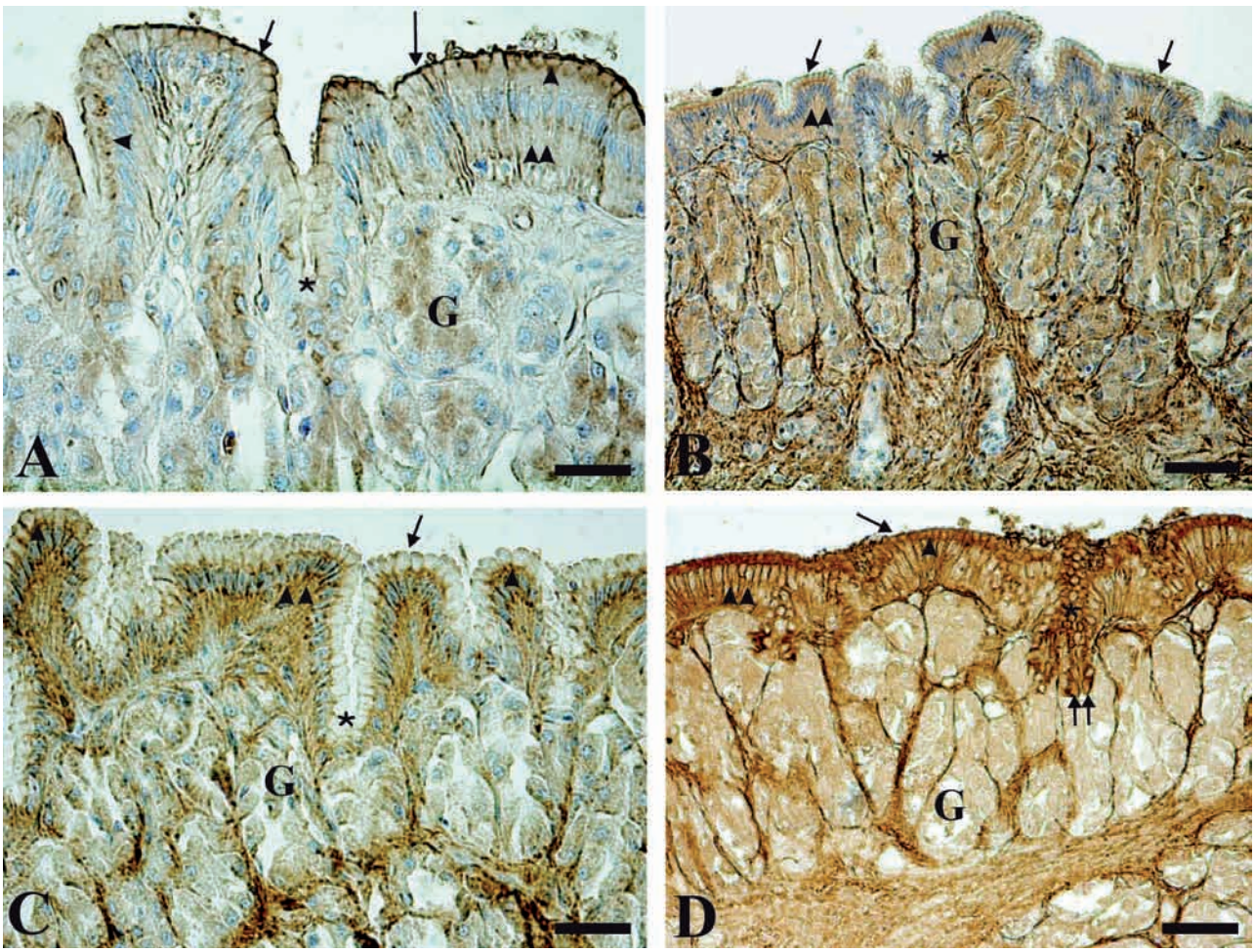

FIG. 6. – KOH-sialidase/WGA (A), GSA I-B_4 (B), GSA II (C), and UEA I (D) labelling of the gastric mucosa of *Halobatrachus didactylus*. A, WGA binding sites are present in the luminal surface, supra-nuclear and sub-nuclear cytoplasm of surface epithelial cells as well as in the sub-nuclear cytoplasm of the pit cells and the gastric glands. Scale bar: 22 μm. B, GSA I-B_4 shows moderate staining in a thin supra-nuclear cytoplasm of epithelial columnar cells and gastric glands, whereas it gives a faintly visible reaction in the sub-nuclear cytoplasm of surface epithelial cells and pit cells. Scale bar: 46 μm. C, GSA II moderately stains the supra-nuclear cytoplasm and weakly stains the sub-nuclear cytoplasm of the surface epithelial cells. Scale bar: 30 μm. D, UEA I strongly stains the luminal coat of columnar epithelial cells and the neck cells, moderately stains the apical and supra-nuclear zones of the surface epithelial cells, and weakly stains the sub-nuclear cytoplasm of lining epithelial cells and pit cells. Scale bar: 46 μm. G, gastric gland; arrow, surface epithelial cells; arrowhead, supra-nuclear zone; double arrow, neck cells; double arrowhead, sub-nuclear zone; asterisk, pit cells.

stained the sub-nuclear cytoplasm of both surface and pit cells, whereas it did not bind neck cells or gastric glands (Fig. 5A).

SBA showed a moderate and a weak reaction for a thin supra-nuclear zone and the sub-nuclear cytoplasm of both lining epithelium and pit cells. Gastric glands were moderately stained (Fig. 5B).

HPA gave a moderate staining of the luminal cell coat and of a thin supra-nuclear zone of surface epithelial cells, which were also weakly stained in their sub-nuclear cytoplasm. HPA weakly reacted with the sub-nuclear cytoplasm of pit cells and with the gastric glands (Fig. 5C).

Con A showed a strong reaction for the luminal coat of the columnar epithelial cells, which were weakly stained in the sub-nuclear zone. The same reaction was seen in the sub-nuclear cytoplasm of pit cells, whereas the gastric gland showed faintly visible staining (Fig. 5D).

KOH-sialidase-WGA treatment (performed to highlight βGlcNAc, but not sialic acid) displayed a strong positive reaction for the luminal surface epithelial cells, which were weakly stained in the supra-nuclear zone. The lectin moderately stained the gastric glands (Fig. 6A).

GSA I-B_4 showed a moderate staining of a thin supra-nuclear cytoplasm of epithelial columnar cells and gastric glands, whereas it gave a faintly visible reaction for the sub-nuclear cytoplasm of surface epithelial cells and pit cells (Fig. 6B).

GSA II displayed a moderate and a weak reaction for the supra-nuclear and the sub-nuclear cytoplasm

of the surface epithelial cells, respectively (Fig. 6C). The lectin did not label the neck cells or the gastric glands.

UEA I strongly stained the luminal coat of columnar epithelial cells and the neck cells, moderately stained the apical and supra-nuclear zones of the surface epithelial cells, and weakly stained the sub-nuclear cytoplasm of lining epithelial cells and pit cells (Fig. 6D).

LTA showed a similar UEA I staining pattern except for the negative reaction with neck cells.

DISCUSSION

This study investigates the glycoconjugate pattern of the surface epithelial cells and gastric glands of the toadfish *H. didactylus*.

The stomach is a component of the digestive tract which is lacking in some families of Teleostei. Three different types of stomach have been found in Teleostei: a straight stomach, a siphon-shaped stomach and a stomach with a gastric caecum (Harder, 1975). The toadfish *H. didactylus* contains a short sac-shaped type of stomach which is lined with a single layer of columnar epithelial cells and numerous gastric pits placed between the surface lining epithelium and the tubular gastric glands.

Conventional histochemistry showed intense staining with PAS and very weak affinity for AB 2.5 and AB 1.0 at the level of the apical zone of surface epithelial cells and pit cells. Thus, conventional histochemistry revealed that the lining epithelium of the gastric mucosa of the toadfish *H. didactylus* produces a mixture of neutral and acidic glycoconjugates with the neutral glycoproteins predominating over acidic, sialylated and sulphated ones. Significant variety in glycoconjugate composition has been described in many teleost species (Jirge, 1970; Kapoor *et al*., 1975; Reifel and Trevil, 1978). A large quantity of neutral glycoconjugates, together with small amounts of acidic mucins, are secreted by the gastric mucosa of sea bream (*S. aurata*) and senegal sole (*S. senegalensis*) (Sarasquete *et al*., 2001), two fish species with different feeding habits. High quantities of both neutral and acidic glycoconjugates have been found in the epithelial cells lining stomach of species with similar (i.e. *Umbrina cirrosa*) (Pedini *et al.*, 2001) or different (i.e. *Anguilla anguilla*) (Domeneghini *et al.*, 2005) feeding habits, behaviour and biology with respect to *H. didactylus*. The neutral and acidic glycoconjugates produced by gastric epithelial cells may serve to form a visco-elastic gel acting as a physical barrier to protect the underlying mucosa against HCl and against injury by enzymes produced in gastric glands (Ferraris *et al.,* 1987; Smith, 1989). Neutral mucins on the surface of gastric epithelial cells have been related to the absorption of easily digestible substances such as disaccharides and short fatty acids (Grau *et al.,* 1992). Sulphated glycoconjugates may form a complex with pepsin stabilising or buffering the enzyme (Spicer and Schulte, 1992).

Lectin histochemistry showed different oligosaccharides in the surface epithelial cells, displaying the presence of well-defined cellular areas such as the sub-nuclear cytoplasm, a thin supra-nuclear area, the apical cytoplasm and the luminal cell coat of the surface. The sub-nuclear cytoplasm reacted with RCA_{120}, SBA, HPA, GSA I-B_4, GSA II, UEA I, LTA and Con A. This finding indicates the presence of asialoglycoconjugates terminating with lactosamine, N-acetylgalactosamine, α-galactose, β-N-acetylglucosamine, and α-L-fucose, as well as glycans with terminal/internal α-D-mannose. Con A binding sites indicate the presence of N-linked glycans from high α-mannose, through an intermediate/hybrid, to a small bi-antennary complex type, irrespective of bisection (Goldstein and Hayes, 1978; Debray *et al.,* 1981). In this zone scattered granules containing glycans terminating with galactose(β1-3)N-acetylgalactosamine (PNA reactivity) and sialic acid linked to galactose(β1-3)-N-acetylgalactosamine (increased PNA staining after KOH-sialidase treatment) were observed. The PNA reactivity indicates that this granular material contains O-linked glycans (Spicer and Schulte, 1992). Ultrastructural studies in the stomach of *Anarhichas lupus* (Hellberg and Bjerkås, 2000) and *S. senegalensis* (Arellano *et al.,* 2001) show the presence of a granular endoplasmic reticulum in the basal region of surface mucous cells. This can explain the lectin staining in the basal cytoplasm of the columnar epithelial cells in the gastric mucosa of toadfish *H. didactylus*.

In this study a thin supra-nuclear cytoplasm of the epithelial columnar cells showed the presence of glycans terminating with N-acetylgalactosamine (DBA, SBA, HPA), lactosamine (RCA_{120}), galactose (GSA I B_4), N-acetylglucosamine (GSA II), α-L-fucose (UEA I, LTA) and sialoglycoconjugates terminating with sialic acid(α2-3)galactose(β1-4)N-

acetylglucosamine and sialic acid(α2-6)galactose/N-acetylgalactosamine, as well as with sialic acid linked to N-acetylgalactosamine (MAL II, SNA, and an increase in DBA staining after KOH-sialidase treatments). DBA and HPA labelling indicates the presence of O-linked oligosaccharides (Spicer and Schulte, 1992). This zone probably corresponds to the Golgi apparatus since this structure is distinctly present in the supra-nuclear region of surface mucous cells of other teleost gastric mucosa (Gargiulo *et al.,* 1997; Arellano *et al*., 2001).

The apical zones of surface epithelial cells were full of O-glycans with galactosyl, sialylgalactosyl and fucosyl residues as evidenced by PNA, KOH-sialidase-PNA, UEA I and LTA. O-linked oligosaccharides (mucin-type glycans) are typical secretory moieties. Ultrastructural investigations have shown the presence of secretory granules in the apical zone of surface epithelial cells in teleost gastric mucosa (Arellano *et al.,* 2001; Carassón *et al.,* 2006). It is noteworthy that a major function of O-glycosylation is the ability of mucins to form a gel (Pajak and Danguy, 1993), which acts as a protective barrier for the under-lying mucosa against injury by HCl and enzymes produced in gastric glands (Ferraris *et al.,* 1987; Smith, 1989; Domeneghini *et al.,* 1998). O-glycans terminating with galactosyl and sialylgalactosyl have been evidenced in secretory granules of the epithelium lining the *U. cirrosa* gastric mucosa (Pedini *et al*., 2005) by means of PNA and KOH-sialidase-PNA.

The luminal cell coat of the lining epithelium showed glycans ending with sialyl- and/or asialyloligosaccharides. The sialoglycoconjugates terminate with sialic acid(α2-3)galactose(β1-4)N-acetylglucosamine, sialic acid(α2-6)galactose/N-acetylgalactosamine, and sialic acid linked to galactose(β1-3)-N-acetylgalactosamine. The presence of sialomucins can provide a negative charge to the luminal surface protecting the mucosa from the acidic gastric juice (Scocco *et al.,* 1996). Asialoglycans containing terminal/internal α-D-mannose, N-acetylglucosamine and terminal galactose(β1-3)-N-acetylgalactosamine, α-N-acetylgalactosamine and α-L-fucose residues are expressed in other teleost fishes such as *S. aurata* (Domeneghini *et al.,* 1998), *U. cirrosa* (Pedini *et al.,* 2005), *Cynoscion guatucupa* (Díaz *et al.*, 2008). The presence of fucosylated glycoproteins has been related to a protective role for the luminal surface of the gastric mucosa. It has been established that disorders in the mucous barrier of the stomach are caused by the lack of the main component αL-Fuc in patients with ulcer associated with *Helicobacter pylori* (Rustamova and Khamraev, 2005).

Conventional histochemistry in the pit cells of *H. didactylus* gastric mucosa showed a staining pattern similar to that of surface epithelial cells with strong PAS staining in the apical zone and very weak staining with AB 2.5, AB 1.0, indicating the presence of a higher amount of neutral glycoconjugates than acidic ones. Lectin histochemistry revealed differences in the staining pattern between the surface epithelium and pits, except for MAL II, SNA, and SBA. The sub-nuclear cytoplasm reactivity indicates the lack of glycans terminating with sialic acid linked to galactose or to N-acetylgalactosamine and with β-galactose. The probable Golgi zone shows oligosaccharides terminating with sialoglycoconjugates as well as asialoglycans terminating with N-acetylgalactosamine and α-L-fucose. The apical zone contains granules characterised by asialo- and sialoglycans terminating with galactose(β1-3)N-acetylgalactosamine and sialic acid linked to galactose(β1-3)-N-acetylgalactosamine. The luminal cell surface contains sialoglycoconjugates ending with sialic acid(α2-3)galactose(β1-4)N-acetylglucosamine and sialic acid(α2-6)galactose/N-acetylgalactosamine.

The cytoplasm of the neck cells, was stained with PAS, UEA I and LTA. This indicates that the neck cells of toadfish *H. didactylus* gastric mucosa produce chiefly glycoconjugates containing hexoses with vicinal hydroxyls terminating with α-L-fucose residues. The neck cells have been found in the gastric mucosa of a few species of teleosts. As in *H. didactylus*, the neck cells of *Anarhichas lupus* (Hellberg and Bjerkas, 2000) contain PAS-positive and AB-negative granules, whereas in *Seriola dumerili* they express neutral mucosubstances (Grau *et al.,* 1992). This histochemical study shows fucosylated-neutral glycoconjugates for the first time in the gastric neck cells of a teleost species. The presence of α-L-fucose residues in gastric neck cells has been demonstrated by means of lectin histochemistry in other vertebrates such as amphibians (Liquori *et al.,* 2007) and mammals (Sommer *et al.,* 2001; Jiang *et al.,* 2004).

Gastric glands were un-reactive with conventional histochemistry methods, whereas lectin histochemistry showed the presence of glycoconjugates. It worth underlining that a discrepancy between conventional and lectin histochemistry has already been observed in the sacciform cells of the toadfish *H.*

didactylus oesophagus epithelium (Desantis *et al.,* 2007). Lectin reactivity indicates that *H. didactylus* gastric glands contain O-linked glycans terminating with β-galactose, N-acetylgalactosamine, α-galactose and sialic acid linked to N-acetylgalactosamine, as well as N-linked oligosaccharides with internal N-acetylglucosamine.

Gastric glands containing both N- and O-linked glycans have also been found in *S. aurata* (Sarasquete *et al.,* 2001) and *U. cirrosa* (Pedini *et al.,* 2005) The presence of glycans terminating with N-acetylglucosamine seems a common finding in the gastric glands of marine teleosts (Sarasquete *et al.,* 2001; Pedini *et al.,* 2005; Díaz *et al.*, 2008), whereas sialic acid linked to N-acetylgalactosamine is also expressed in *U. cirrosa* gastric glands (Pedini *et al.,* 2005). Secretion of neutral glycoconjugates containing sugar residues has been observed in the gastric glands of fish species which differ in feeding habits, habitat and biology (Gutiérrez *et al.*, 1986; Domeneghini *et al.,* 1998; Sarasquete *et al.,* 2001; Pedini *et al.,* 2005; Díaz *et al.*, 2008). Neutral glycoconjugates could represent evidence of the absorption and transport of macromolecules through the membranes (Reifel and Travill, 1978; Sarasquete *et al.*, 2001; Pedini *et al.*, 2005), whereas acid glycoconjugates prevent damage to the gut epithelium, acting as a lubricant for the stomach contents and as a buffer for the highly acidic gastric juices (Ferraris *et al.*, 1987).

In conclusion, the lectin-binding pattern indicates that the gastric mucosa of the toadfish *H. didactylus* expresses species-specific glycoproteins with some carbohydrate sequences not previously detected in other teleost species. The composition of glycoconjugates in different piscine species is likely to be related to different feeding habits, environmental characteristics and taxonomic positions. As in other fishes, muco-substances produced in the *H. didactylus* stomach may protect the gastric mucosa against the acidic contents of the fish stomach and may assist the absorption of macromolecules.

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