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Glycoconjugates in the branchial mucous cells of *Cynoscion guatucupa* (Cuvier, 1830) (Pisces: Sciaenidae)*

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SUMMARY: This study examines the mucous cells from the gills of the stripped weakfish *Cynoscion guatucupa*. The glycoconjugates (GCs) were identified with: (1) oxidizable vicinal diols; (2) sialic acids and some of their chain variants, C7, C8 or C9; (3) sialic acid residues with O-acyl substitution at C7, C8 or C9; (4) carboxyl groups and (5) sulphate groups. The presence of sugar residues in the oligosaccharide side chain of glycoconjugates was investigated by means of a battery of seven biotinylated lectins. One type of mucous cell was identified in the primary and secondary lamellae, the secretory content of which evidenced neutral, sulphated and sialylated glycoconjugates. The distribution pattern of the mucus was identical in the primary and secondary lamellae. PNA had the most staining in the mucous cell content, while UEA-I had the least, as it was completely negative. Con-A showed weak to no staining and WGA showed weak to moderate staining. The reactions to DBA, SBA and RCA-I were moderate. This work clearly demonstrates the heterogeneity of the mucous cell glycoconjugates, which could be involved in various functions, such as lubrication, protection, inhibition of microorganisms and a role in ion regulation and diffusion.

Keywords: Teleost fish, *Cynoscion guatucupa,* gills, mucous cells, histochemistry, lectins.

RESUMEN: GLICOCONJUGADOS EN LAS CÉLULAS MUCOSAS DE BRANQUIAS DE *CYNOSCION GUATUCUPA* (CUVIER, 1830) (PISCES: SCIAENIDAE). – Se estudiaron las células mucosas de las branquias de la pescadilla de red *Cynoscion guatucupa*. Se identificaron glicoconjugados (GCs) con (1) dioles vecinos oxidables, (2) ácidos siálicos y alguna de sus cadenas variables: C7, C8 ó C9, (3) residuos de ácidos siálicos con substitución O-acilo en C7, C8 ó C9, (4) grupos carboxilo y (5) grupos sulfato. En la cadena lateral de los GCs se investigó la presencia de residuos de azúcar por medio de una batería de siete lectinas biotiniladas. En las laminillas primarias y secundarias se identificó un tipo de células mucosas con un contenido secretorio de GCs neutros, sulfatados y sialilados. La distribución del moco fue idéntica en las laminillas primarias y secundarias. Con PNA la tinción fue máxima y con UEA-I completamente negativa. Con A no dio tinción o fue escasa y WGA mostró una tinción débil a moderada. La reacción con DBA, SBA y RCA-I fue moderada. Este trabajo demuestra la heterogeneidad en las células mucosas de los GCs, los que pueden estar involucrados en diversas funciones tales como la lubricación, protección e inhibición de microorganismos y jugar un papel en la regulación y difusión iónica.

Palabras clave: Peces teleósteos, *Cynoscion guatucupa,* branquias, células mucosas, histoquímica, lectinas.

INTRODUCTION

The mucous cells of vertebrates and the composition of the mucus produced by them have been analysed in various studies from both the morphological and histological points of view (Suprasert *et al*., 1986; Park *et al*., 1987). The glycoconjugates (GCs) are the components of the mucosubstances. They are known to have a large variety of functions, from merely mechanical functions, through antimi-

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crobial and antiviral to "osmotic" functions (Allen, 1981). In fish the mucosubstances also have an important role in ion regulation and diffusion (Domeneghini *et al*., 1998).

In fish the mucous cells are unique in their histological characteristics, size and number. Several studies have been carried out on the mucous cells of fish skin. These studies describe the morphology of the different mucous cell types and characterize the glycoconjugates with diverse histochemical techniques, including lectin histochemistry (Genten and Danguy, 1990; Agrawal and Mittal, 1992; Mittal *et al*., 1994, 1995; Fishelson, 1996).

Several works have been published on gills that describe the general morphology of their cells using light and electron microscopy; however, few studies refer to mucous cells or the secretion they produce. From the histochemical point of view different types of mucous cells have been described in the gill epithelium of *Poecilia vivipara* (Sabóia-Moraes *et al.*, 1996). The characterization of the glycoconjugate residues in the gills of *Oncorhynchus mykiss* and *Solea senegalensis* have been studied using lectin histochemistry (Burkhardt – Holm, 1997; Sarasquete *et al*.,1998b). The ultrastructure and the histochemical characterization of the glycoconjugates secreted by the mucous cells located in the gills of *Micropogonias furnieri* were the subject of our previous investigation (Díaz *et al*., 2001; 2004a).

The stripped weakfish, *Cynoscion guatucupa* Cuvier (Pisces, Perciformes, Sciaenidae), is a demersal coastal fish of growing commercial value with a wide distribution from 22° 35´S (Rio de Janeiro, Brazil) to approximately 43° S (Argentina) (Cousseau and Perrota, 2000).

The purpose of this work is to describe the morphology of the mucous cells and the distribution and characterization of their glycoconjugates in the gills of *Cynoscion guatucupa*. This study has been carried out using a series of histochemical methods for identifying and visualizing different classes of glycoconjugates. In addition, we applied lectin histochemical methods to survey the distribution of six major classes of carbohydrate moieties: L-fucose, galactose, N-acetyl galactosamine, N-acetyl glucosamine, mannose and sialic acid.

Lectins are carbohydrate-binding proteins that are not enzymes or antibodies. Lectin histochemical studies demonstrated that lectins are useful as biomarkers of specific secretory functions, structural components and alterations of cells and tissues (Chan and Ho, 1999).

MATERIAL AND METHODS

Animals

Live specimens of *Cynoscion guatucupa* (46.4 ± 3.0 cm length; 879.0 ± 181.0 g weight; n=10) were collected from the coast of Mar del Plata, Argentina (38° 05' S, 57° 32' W). Fish were killed by decapitation. The gills were rapidly excised and fixed by immersion in Bouin´s fluid or 10% buffered formalin for light microscope studies.

Histological processing

Samples were routinely processed and embedded in paraffin. Four micron thick histological slides were stained with the following techniques: routine hematoxylin and eosin (H-E), Masson trichrome stain for morphology and Mayer mucicarmin for mucin identification.

Histochemical processing

Sections of tissue were treated with histochemical procedures for the histochemical identification of glycoconjugates (Table 1).

Lectin histochemical processing

Biotinylated lectins were used to identify specific sugar residues of glycoconjugates. The reagent from the Vector-kit is the most sensitive and specific for examining GCs using the biotinylated lectins and the avidin-biotin-peroxidase complex (ABC) (Ellis and Holliday, 1992). Lectin staining methodologies were done according to Gimeno *et al*. (1995). Paraffin sections were deparaffinized with xylene and incubated in 0.3% H_2O_2 in methanol for 30 min at room temperature in order to block endogenous peroxidase activity. They were then hydrated, washed in a phosphate-buffered saline (PBS) 0.01M, pH 7.2 and incubated with biotynylated lectins for 30 min. After that, sections were washed again in PBS and subsequently treated with an ABC kit (Vectastain Elite PK 6200 Vector Laboratories Inc, CA, USA). PBS was then washed again, and the reaction sites were later revealed with diaminobenzidine tetrahydrochloride (DAB) 0.5 mg/mol in tris buffer 0.1 M, pH 7.2, plus 0.02% H_2O_2. Each lectin was used at a 30 μg/ml dilution in PBS, except for PNA, which was applied at a concentration of 10 μg/ml. Table 2 lists the seven lectins

TABLE 1. – Histochemical procedures for visualizing and identifying GCs in mucous cells of *Cynoscion guatucupa* gills. PAS, periodic acid/ Schiff; PA*S, periodic acid / Schiff at low temperature and low pH (oxidation with 0.4 mM periodic acid in 1 M hydrochloric acid at 4°C); Bh, borohydride; PA, periodic acid; TB, toluidine blue; AB, Alcian blue; GCs, glycoconjugates.

Procedures	Interpretation of staining reactions	References
PAS	GCs with oxidizable vicinal diols and/or Glycogen	McManus (1948)
α- amylase/PAS	GCs with oxidizable vicinal diols	Spicer *et al*. (1967)
PA*-S	Sialic acid and some of their chain variants (C7 and/or C9)	Volz *et al*. (1987b)
KOH/PA*-S	GCs with sialic acid residues	Volz *et al*. (1987b)
KOH/PA*/Bh/PA-S	Neutral GCs with oxidizable vicinal diols	Volz *et al*. (1987a)
PA/Bh/KOH/PA-S	Sialic acid residues with O-acyl substitution at C7, C8 or C9 and O-acyl sugars	Reid *et al*. (1973)
AB pH 2.5	GCs with carboxyl groups (sialic acid or uronic acid) and/or with O-sulphate esters	Lev and Spicer (1964)
AB pH 1.0	GCs with O-sulphate esters	Lev and Spicer (1964)
AB pH 0.5	Very sulphated GCs	Lev and Spicer (1964)
TB pH 4.2	GCs with O-sulphate esters	Lison (1953)
TB pH 5.6	GCs with O-sulphate esters and/or carboxyl groups	Lison (1953)

TABLE 2. – Carbohydrate binding specificity of lectins.

Lectin	Abbreviation	Specificity [a,b]
Canavalia ensiformis agglutinin	Con-A	α-D-Man; α-D-Glc
Triticum vulgaris agglutinin	WGA	β-D-GlcNAc; NeuNAc
Dolichos biflorus agglutinin	DBA	α-D-GalNAc
Glycine maxima agglutinin	SBA	α-D-GalNAc; β-D-GalNAc
Arachis hypogaea agglutinin	PNA	β-D-Gal (β1->3) D-GalNAc
Ulex europaeus agglutinin-I	UEA-I	α-L-Fuc
Ricinus communis agglutinin-I	RCA-I	β-Gal

[a]: Goldstein and Hayes (1978). [b]: Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; GlcNAc, N-acetylglucosamine; Man, mannose; NeuNAc, acetyl neuraminic acid (sialic acid).

used in this study as well as their sources and their major sugar specificities.

Controls for lectin staining included exposing the specimens to substrates without lectin and incubating samples using lectins that had been pre-incubated with the corresponding haptene sugar inhibitor. The control sections were negative.

RESULTS

Histology

The gill arch structure of *Cynoscion guatucupa* is similar to that of other teleosts in that it has primary and secondary filaments. These filaments are made up of a cartilaginous support, a vascular system and a multilayered epithelium. The external layer consists mainly of pavement cells with mitochondria-rich cells and mucous cells spread among them. The secondary filaments occur as a two-cell layer epithelium with mucous and mitochondria-rich cells. Mitochondria-rich cells are acidophilic and they are generally found in marine fish and rarely in freshwater fish. The mucous cells characteristically appear depressed on the surface of the primary or secondary epithelium. They are big, rounded cells with secretion globules that displace the nucleus towards the eccentric zone. Mucous discharge is performed by exocytosis. Hematoxylin-eosin or trichrome coloured preparations showed no colour in the mucous cell content.

Histochemistry

The histochemical procedures for visualizing and identifying glycoconjugates in the mucous cells of primary as well as secondary lamellae are summa-

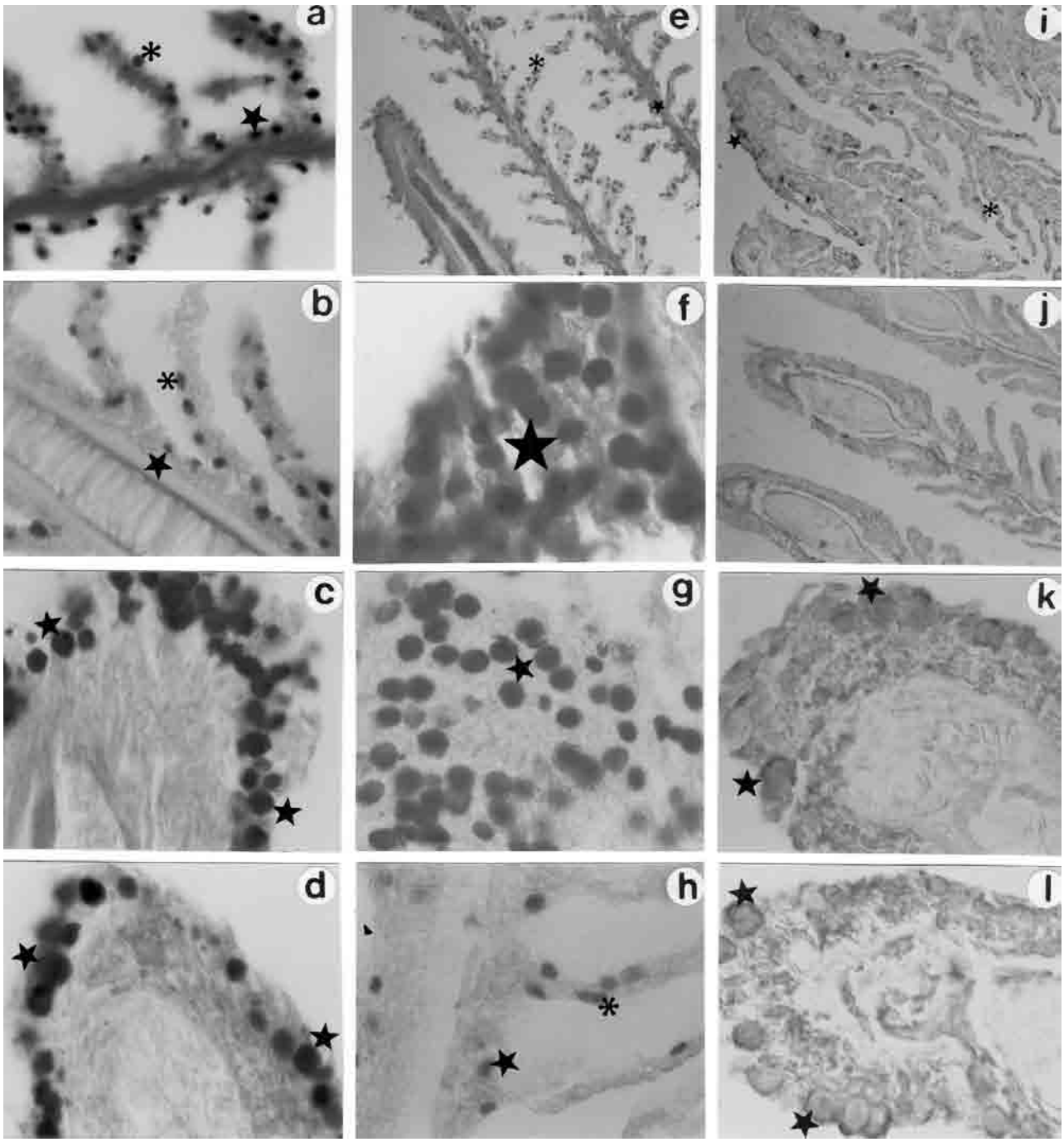

FIG. 1. – Histochemical and lectin-histochemical staining of mucous cells from primary lamellae (star) and secondary lamellae (asterisk) of *Cynoscion guatucupa* gills; a, PAS x 132; **b**, PA*S x 132; **c**, PA/Bh/KOH/PAS x 132; **d**, KOH/PA*/Bh/PAS x 132; **e**, AB pH 2.5 x 33; **f**, AB pH 2.5 x 132; **g**, AB pH 1.0 x 132; **h**, AB pH 0.5 x 132; **i**, PNA x 33; **j**, UEA-I X 33; **k**, SBA x 132; **l**, WGA x 132.

rized in Tables 1 and 3. No histochemical differences were detected between the mucous cells of the primary and secondary lamellae. The secretory contents of the mucous cells reacted positively to mixed neutral and acidic glycoconjugates. The histochemical properties of the contents of the mucous cells revealed by the PAS method indicated that glycoconjugates with oxidizable vicinal diols were present (Fig. 1a). The colouration disappeared after acetylation and recovered after saponification. Control sections subjected to α-amylase were positive to the PAS reaction after this treatment. A positive reaction to PA*S revealed the presence of sialic acids and some of their side chain variants (Fig. 1b). The reaction with the PA/Bh/KOH/PAS method indicated that there were sialic acid residues with O-

TABLE 3. – Histochemical staining properties of GCs in mucous cells of *Cynoscion guatucupa* gills. M, magenta; T, turquoise; m, metachromasia. The staining intensity is subjectively described from +++ (very strong) to + (weak).

Procedures	Reaction shades
PA-S	+++ M
α- amylase/PA-S	+++ M
PA*-S	++ M
KOH/PA*-S	++ M
KOH/PA*/Bh/PA-S	+++ M
PA/Bh/KOH/PA-S	+++ M
AB pH 2.5	+++ T
AB pH 1.0	++ T
AB pH 0.5	++ T
TB pH 4.2	++ m
TB pH 5.6	++ m

TABLE 4. – Lectin binding in the mucous cells of gills from *Cynoscion guatucupa*. Staining intensity: - negative, + weak, ++ moderate, +++ strong. When two symbols are employed, for example +/-, they indicate variability.

	Con-A	WGA	DBA	SBA	PNA	UEA-I	RCA-I
Mucous cells	+/-	+/++	++	++	+++	-	++

acyl substitution at C7, C8 or C9 (Fig. 1c). A positive reaction with KOH/PA*S confirmed the presence of sialic acid residues. Neutral glycoconjugates with oxidizable vicinal diols were revealed using the KOH/PA*/Bh/PAS method (Fig. 1d).

A sequence of procedures utilizing alcian blue at different pH levels (Fig.1e-h) showed the presence of glycoconjugates with carboxylic groups and O-sulphate esters.

The toluidine blue method can be used to analyse metachromasia in terms of pH. Metachromasia was observed in both pH treatments, thus confirming the presence of carboxylated and sulphated glycoconjugates.

Lectin histochemistry analysis

We used seven kinds of biotinylated lectins in order to examine glycoconjugate expression patterns in the mucous cells from the gills of *Cynoscion guatucupa*. The resulting staining patterns are summarized in Table 4.

Primary and secondary lamellae showed the same distribution pattern as lectins of the mucous cells. PNA had the maximum reactivity in the mucous cell content (Fig.1i) while UEA-I had the minimum reactivity, as it was completely negative (Fig.1 j).

Con-A proved to be a weak to negative dye. Both DBA and SBA (Fig. 1k) showed a similar distribution pattern. They moderately stained the content of mucous cells. The WGA lectin showed a weak to moderate staining (Fig. 1l); the RCA-I lectin moderately stained the mucous cells.

DISCUSSION

Histochemical methods have proved to be valuable tools for localizing and characterizing gill cells. The histochemical techniques used allowed us to characterize the mucous cells. The main components of mucus are high molecular weight glycoproteins with numerous carbohydrate chains O-glycosidically linked to a protein core (Burkhardt-Holm, 1997).

In the contents of mucous cells from the primary and secondary lamellae from the gills of *Cynoscion guatucupa* there are neutral glycoconjugates; glycoconjugates with sialic acids and some of their side chain variants (C7, C8 or C9); and carboxylated and sulphated glycoconjugates.

As the mucous cells were intensely stained with PAS and α-amylase/PAS, they must contain neutral hexoses. These results could suggest the absence of glycogen as evidenced in other species whose mucous cells are also stained with this reaction (Sarasquete *et al*.,1989,1998a, b; Díaz *et al*., 2001).

The presence of substituted and non-substituted sialic acids at C7, C8 and C9 was confirmed by the sequence of techniques carried out (PA*S, PA/Bh/KOH/PAS, KOH/PA*S). It has been proposed that sialic acids cause GC molecules to extend because of their negative charge and that they also play a role in hydrating the immediate environment (Mittal *et al*., 1994).

The alcian blue sequences showed the secretion of sialo and sulphated glycoconjugates. These produce an increase in the secretion viscosity, which is probably related to the desalinization of ingested seawater and to a higher protective role (Loretz, 1995; Suprasert *et al*.,1986, 1987). Sialic acid residues together with sulphated groups are responsible for the negative charge of the GCs and may mask receptor sites for viruses and mycoplasma species (Zimmer *et al*., 1992). The results obtained in this work suggest that the GCs elaborated and released by the mucous cells in the gills of *C. guatucupa* are heterogeneous in their cytochemical composition and they coincide with the histochemical characteristics found in the gill mucous cells of *Micropogonias furnieri* (Díaz *et al*., 2001, 2004a).

Care must be taken with comparisons concerning histochemical analyses of fish because in some cases, identical species under different conditions have shown differences in the type of GCs produced (Solanki and Benjamin, 1982). In addition, the biosynthesis of GCs includes modifications of the secretory protein, and different stainings can represent the different cell stages. Initially, PAS negative mucous cells only contain proteins. PAS positivity is mainly related to the production of glycoproteins, the alcian blue staining coincides with the carboxylation stage, and the conjugation with sulphated groups with the detection of sulphated glycoproteins (Arellano *et al*., 1999). Moreover, in some fresh-water fish (*Monopterus cuchia* and *Pungitius pungitius*), the epidermal mucous cell sulphated proteins predominate in the mucus composition, whereas in marine fish (*Blennius tentacularis* and *B. sanguinolentus)*, GCs with sialic acid prevail (Whitear and Mittal, 1984). Likewise, in the fresh-water fish *Odontesthes bonariensis* (Díaz *et al.,* 2004b), sulphated GCs predominate in their gills. It has been postulated that sulphated GCs deter the proliferation of pathogenic micro-organisms in fresh-water fish which are more likely to become infected in this type of environment (Whitear and Mittal, 1984; Mittal *et al*., 1994).

The histochemical composition of the mucous secretion in the respiratory tract is different for the various species of mammals. Thus, sulphated GCs are abundant in rabbits, dogs and monkeys and are absent in hamsters (Kennedy *et al.,* 1978). Differences have also been found in the composition of the mucosubstances of cells from the respiratory tract of different reptilian species (Tesik, 1984; Pastor *et al.,* 1987).

Using histochemical techniques, four types of mucous cells with basic characteristics and differences according to their location in the gill epithelium were identified in the euryhaline fish *Poecilia vivipara* (Sabóia-Moraes *et al*., 1996). In the present study we have identified just one type of mucous cell in the primary and secondary lamellae of *Cynoscion guatucupa*. The general characteristics of their mucous cells are similar to those described for the gills of *Scomber australasicus* (Perera, 1993), and *Micropogonias furnieri* (Díaz *et al.,* 2004a), and the skin epidermis of other teleosts (Agrawal and Mittal, 1992). No histochemical differences were reported in the mucous cell population of *Micropogonias furnieri* (Díaz *et al*., 2001; 2004a).

Lectins are proteins or glycoproteins that specifically recognize and label different carbohydrate moieties. They have been confirmed to be good tools for characterizing carbohydrates that form the oligosaccharide chains of glycoproteins (Danguy *et al*., 1994). Lectin histochemistry makes it easier to identify sugar residues. In this study the terminal sugars predominantly found were: *ß*-D-Gal (1-3)-GalNAc, *a*-D-GalNAc, *ß*-D-GalNAc, *ß*-D-GlcNAc and N-acetyl-neuraminic acid.

The reaction intensity varied with the different lectins, in particular, PNA binds strongly to mucous cells, while other lectins (DBA, SBA, WGA and RCA-I) bind to them moderately, poorly (Con-A) or not at all (UEA-I).

Con-A negative staining allows us to infer that *a*-D-Glc and Man residues are almost unrecognizable in mucous cells. These results coincide with the negative Con-A staining demonstrated by Sarasquete *et al*., (1998b), in the mucous cells of the gills from *Solea senegalensis*. Genten and Danguy (1990), found that mucous cells in the skin of various teleosts were stained moderate to strongly with Con-A, which indicates the presence of terminal *a*-mannosyl residues. Con-A, like other Man-binding lectins, was recently employed to visualize glycoproteins containing N-linked oligosaccharides, since O-linked oligosaccharides contain less than 1% Man (Spicer and Schulte, 1992). Mannose is specific to serous cells in human bronchioles (Rogers *et al*., 1993). A Con-A binding protein, that has been suggested to protect fish against bacterial infection, was detected in the skin mucus carp (Lemaitre *et al*., 1996), and it can be assumed that it was secreted by a serous cell.

Mucous cells showed PNA positivity, which is probably due to the presence of O-linked GCs, as PNA preferentially binds O-linked oligosaccharides (Pajak and Danguy, 1993).

WGA exhibits a strong affinity to sialic acid at its terminal position. Its binding pattern therefore indicates the localization of this carbohydrate in the mucous cell. By using the PA/Bh/KOH/PAS reaction it was determined that the positivity obtained with WGA is due to sialic acids with O-acyl substituents at C7, C8 or C9.

Sialic acids have been found in a variety of fish through conventional histochemical methods and chemical analyses (Genten and Danguy, 1990).

Results obtained for adult *Sparus aurata* and *Solea senegalensis* by conventional mucin histochemistry and lectins suggest that WGA reactivity in

epidermal goblet cells is due to GlcNAc and sialic acid residues (Sarasquete *et al*., 1998a ,b).

Sialic acids have been used to estimate the degree of skin mucification in different species (Harris *et al*., 1973). They are the terminal components of many N and O-glycosidic glycoproteins; they also bind to water or regulate water transport into these glycoproteins. Thus, sialic acids could influence the fluidity and/or rheological properties of mucus (Meyer *et al*., 2001*)*. Therefore, sialic acid residues influence the conformation of glycoconjugates, which are important for correctly arranging the glycoconjugate molecules in cell membranes and for maintaining the activity of glycoprotein enzymes. In addition, sialic acids are essential components of receptors. For example, the capacity of viruses and toxins to infect cells is exclusively dependent on the presence of sialic acids in the cell membranes (Pajak and Danguy, 1993).

Our results with Gal binding lectins suggest that mucous cells have Gal moieties. The presence of β-GalNAc is doubtful because both lectin DBA and SBA show identical staining patterns. These two lectins may be considered specific for GalNac but DBA binds preferentially to α-GalNAc, whereas SBA does not show anomeric specificity. Moreover, N-acetyl galactosamine sequences are present in every mucous cell in the gills, and the galactose binding lectin RCA-I shows a moderate staining intensity in these cells. In addition, the terminal oligosaccharide Gal(*β*1->3)GalNAc, termed the T-antigen, is the specific binding site for PNA lectin (Sáez *et al*., 2000).

Epithelium binding cells for WGA, DBA and SBA have been detected in mucous cells from the gills of *Cyprinus carpio* (Hidalgo *et al*., 1987). A common feature of the mucous cells of the gills from the rainbow trout (*Oncorhynchus mykiss*) is the presence of PNA and DBA lectin binding sites that bind solely to terminal sugars (Burkhardt-Holm, 1997).

Variations in the intensity of PNA and SBA binding in the gill mucous cells of stripped weakfish could indicate different degrees of terminal glycosilation, probably caused by a maturation stage or a functional status of the cell (Burkhardt-Holm, 1997).

UEA-I did not localize any Fuc residues in the mucous cells. This lectin binds preferentially to Fuc with an *a*(1,2) linkage and to the outer region of the oligosaccharide chain (Sugii *et al*., 1982).

Mucous cells from the gills of *Micropogonias furnieri* and those from the epidermis of *Brachydanio rerio* are not stained by UEA-I; however, *Kryptopterus bicirrhis, Pangasius micronemus* and *Acanthoppthalmus kuhlii* exhibit weak to moderate binding (Genten and Danguy, 1990).

In vertebrates, mucus glycoconjugates take part in lubrication, controlling infections and preventing dehydration (Mittal *et al*., 1994). The mucus may act as an important diffusion barrier allowing ion absorption without direct contact between sea water and the epithelial cells (Shephard, 1982). Moreover, glycoconjugates containing O-sulphate esters have a lubricant role (Park *et al*., 1987; Mittal *et al*., 1994, 1995). A protection activity against bacterial and viral invasion has been associated with glycoconjugates containing sialic acid residues (Suprasert *et al*., 1987). In fish, glycoconjugates with oxidizable vicinal diols could also control the acidity of the mucous secretion (Mittal *et al*., 1995; Díaz *et al*., 2001). In addition, GCs in the mucous cells, especially their acidic forms, have been related to the desalinization of ingested seawater (Loretz, 1995). At this point, it is interesting to emphasize that the only type of mucous cell that is found in the gills of *Cynoscion guatucupa* has a more diversified secretory content in relation to its mucosubstances than the content found in the various types of skin mucous cells of other teleosts. This fact could be related to the distinct and additional function that the gill mucus fulfils compared to skin mucus. For instance, we can assume that gill mucus is involved in gas exchange, ion and osmoregulation, as well as binding and uptake of xenobiotics (Randall *et al*., 1996).

Fish mucosubstances have a large variety of functions, from mechanical, antimicrobial and antiviral functions to osmotic ones; therefore they can link and transport different ions. This is especially true for marine fish. Shepard (1982), noted that GCs influence ion movement across the epitelial cells of the branchia. The gill epithelium synthesizes different mucosubstances. The combination of GCs possibly enables the gills to respond quickly to changes in the environmental conditions. The various components of GCs found in *C. guatucupa* epithelial secretory cells, especially their acidic forms (sulphated and sialoglycoconjugates), may be related to the gills having the general osmoregulatory role of regulating the transfer of ions and fluids.

In conclusion, the high heterogeneity occurring in glycoconjugates from gills of *Cynoscion guatucupa* would contribute to covering the various functional roles and avoiding interference in the gas and ion exchange process. Further work is required to characterize the functional significance of the mucous cells in gills from the stripped weakfish.

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