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Histochemical Analysis of Glycoconjugates in the Skin of a Catfish (*Arius Tenuispinis, Day*)

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Summary

A histochemical study using conventional carbohydrate histochemistry (periodic-acid staining including diastase controls, alcian blue staining at pH 1 and 2.5) as well as using a battery of 14 fluorescein isothiocyanate (FITC)-labelled lectins to identify glycoconjugates present in 10 different areas of the skin of a catfish (*Arius tenuispinis*) was carried out. The lectins used were: mannose-binding lectins (Con A, LCA and PSA), galactose-binding lectins (PNA, RCA), *N*-acetylgalactosamine-binding lectins (DBA, SBA, SJA and GSL I), *N*-acetylglucosamine-binding lectins (WGA and WGAs), fucose-binding lectins (UEA) and lectins which bind to complex carbohydrate configurations (PHA E, PHA L). Conventional glycoconjugate staining (PAS staining, alcian blue at pH 1 and 2.5) showed that the mucous goblet cells contain a considerable amount of glycoconjugates in all locations of the skin, whereas the other unicellular gland type, the club cells, lacked these glycoconjugates. The glycoproteins found in goblet cells are neutral and therefore stain magenta when subjected to PAS staining. Alcian blue staining indicating acid glycoproteins was distinctly positive at pH 1, but gave only a comparable staining at pH 2.5. The mucus of the goblet cells therefore also contains acid glycoproteins rich in sulphate groups. Using FITC-labelled lectins, the carbohydrate composition of the glycoproteins of goblet cells could be more fully characterized. A distinct staining of the mucus of goblet cells was found with the mannose-binding lectins LCA and PSA; the galactosamine-binding lectins DBA, SBA and GLS I; the glucosamine-binding lectin WGA; and PHA E which stains glycoproteins with complex carbohydrate configurations. No reaction occurred with the fucose-binding lectin UEA and the sialic acid-specific lectin SNA. In addition, the galactose-binding lectins PNA and RCA showed only a weak or completely negative staining of the mucus in the goblet cells. The specificity of the lectin staining could be proved by inhibiting binding of the lectins by competitive inhibition with the corresponding sugars. From these data, we can conclude that the mucus produced by the epidermal goblet cells of *A. tenuispinis* is rich in mannose, *N*-acetylgalactosamine and *N*-acetylglucosamine residues.

Introduction

We have demonstrated that there are four types of Ariid catfish in the northern Arabian Gulf (Al-Hassan et al., 1988). The most abundant species is *Arius bilineatus*, whose secretions have been the subject of our previous studies (Al-Hassan et al., 1987a,b; Al-Lahham et al.,1987). This species has been consistently misidentified by area fish taxonomists as *Arius thalassinus* (Al-Hassan et al., 1988). Consequently, all of our previous studies have

utilized the incorrect nomenclature designating *A. bilineatus* as *A. thalassinus* in the publications that referred to single fish species only. *Arius thalassinus* is actually quite rare in Kuwaiti waters.

In general, fish skin comprises three layers; the epidermis, dermis and hypodermis, with an aqueous mucous layer covering the epidermal surface. The hypodermis, as the innermost layer, is closest to the striated muscle underneath the skin. The stratum superficiale, the uppermost layer of the epidermis, shows microridges that contain mucus and antibacterial substances secreted to the surface from mucous goblet cells located in the intermediate stratum of the epidermis (Mittal and Whitear, 1979). The dermis is mainly composed of dense connective tissue with a large amount of collagen fibres, and the hypodermis consists of loosely organized collagen fibres and rich supply of vessels.

Besides normal epithelial cells, fish epidermis contains various types of unicellular glands (Mittal et al., 1994). There has been some confusion in the literature about the identity and nomenclature of these secretory cells, but fine structural studies (Whitear, 1981; Whitear and Mittal, 1983) clearly showed that the epidermis of *A. bilineatus*, *Val.* contains two different types of glandular cells, namely goblet (mucous) cells and club cells. The number of goblet cells varies in the different areas of the catfish skin. The composition of the mucus, which they produce, may vary depending on their location. The slipperiness of the mucus is considered to be a result of the presence of high molecular weight gel-forming macromolecules and it is assumed that the predominant gel-forming macromolecules in mucus are glycoproteins.

A specific cell population, the club cells, is found in the epidermis of a number of fishes, including the catfish *A. bilineatus*, *Val.* (Whitear and Mittal, 1983). The club cell contents are largely proteinaceous, with comparatively little carbohydrate components. Their functions are not well defined, but some protective roles have been suggested (Cameron and Endean, 1973; Al-Hassan et al., 1985). Several studies also provided evidence that preparations from the skin secretions of *A. bilineatus* can stimulate the rate of wound healing in animals and healing of diabetic foot ulcers in humans (Al-Hassan et al., 1983,1985,1987a,b, 1991; Al-Hassan, 1990).

Our previous efforts concentrated on *A. bilineatus* because of the healing and biochemical interests associated with its skin secretion. The physical and chemical properties of its skin secretions as well as their effects on superficial wounds in humans are different from those of the skin secretions of *A. tenuispinis*. Whereas *A. bilineatus* is a migrant to the Kuwaiti territorial waters during the summer months, *A. tenuispinis* is a resident of the northern shallow mud flats of the coastal waters of Kuwait. In this study, we concentrate on the identification of the carbohydrate components of the lectins produced by the epidermal cells of *A. tenuispinis*.

Our current knowledge on the histochemical analysis of glycoconjugates in the secretory cells of the epidermis in catfish skin is very limited. In most catfish species investigated so far, the contents of the mucus have not been characterized using advanced histochemical methods. In this study, we intend to identify the carbohydrate components of the glycoproteins of the highly biologically active proteinaceous skin secretion of the catfish and in the unicellular glands that produce it. Analysis of glycoconjugates from 10 different areas of the catfish skin has been performed, using conventional methods based on periodic acid-Schiff (PAS) and alcian blue procedures as well as lectin histochemistry.

Materials and Methods

A total of 20 adult catfish (*A. tenuispinis*, *Day*) were caught with baited hook and line in the coastal waters of Kuwait. Their body lengths ranged from 25 to 32 cm and they weighed 300–450 g. Small tissue samples were obtained from 10 different areas of the skin (Fig. 1) and immediately fixed in Bouin's solution (picric acid 1500 ml, glacial acetic acid 500 ml, 37% formalin 100 ml) for 24 h. After fixation, the tissue samples were immersed in 70% ethanol (3 × 24 h) to wash out Bouin's solution. The specimens were then dehydrated in a series of graded ethanol and embedded in paraplast using an automatic tissue processor (Shandon Duplex Processor; Shandon, Frankfurt, Germany) and a Histostat Tissue Embedding Center (Reichert-Jung, Wien, Austria). Sections (5 μm thick) were cut on a Leica rotatory microtome type 1516 (Leica Ltd, Wetzlar, Germany) and collected on polylysine

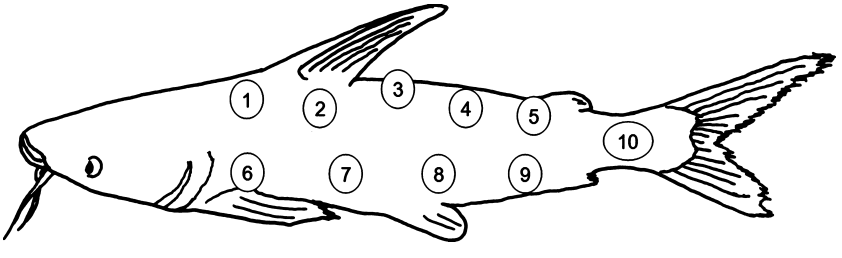

Fig. 1. Schematic representation of *Arius tenuispinis* showing the areas of sample collection.

Table 1. Survey of lectins used in this study

Lectin group	Lectin	Origin	Abbreviation	Major sugar specification	Binding inhibitor
Mannose-binding lectins	*Concanavalin ensiformis* agglutinin	Jack bean	Con A	α-D-Man>a-D-Glc	D-Mannose
	Lens culinaris agglutinin	Lentil	LCA	α-D-Man	D-Mannose
	Pisum sativum agglutinin	Garden pea	PNA	β-D-Man	D-Mannose
Galactose-binding lectins	*Arachis hypogea* agglutinin	Peanut	PNA	β-D-Gal(1–3)-D-GalNAc	D-Galactose
	Ricinus communis agglutinin	Castor bean	RCA	β-D-Galactose	β-D-Galactose
N-Acetylgalactosamine-binding lectins	*Dolichos biflorus* agglutinin	Horse gram	DBA	α-D-GalNAc (1–3)GalNAc	*N*-acetyl-D-galactosamine
	Glycine max agglutinin	Soja bean	SBA	α-D-GalNAc, α-D-Gal	*N*-acetyl-D-galactosamine
	Saphora japonica agglutinin	Japanese pagoda tree	SJA	α-D-GalNAc	*N*-acetyl-D-galactosamine
	Griffonia simplicifolia agglutinin I	Griffonia seed	GSL I	α-D-GalNAc	*N*-acetyl-D-galactosamine
N-Acetylglucoamine-binding lectins	*Triticum vulgare* agglutinin	Wheat germ	WGA	(dGlcNAc)2Neu-NAc	*N*-acetyl-D-glucosamine
Fucose-binding lectins	*Ulex europeus* agglutinin	Gorse seed	UEA	Fucose	Fucose
Lectins with complex carbohydrate configurations	*Phaseolus vulgaris* agglutinin	Garden bean	PHA E PHA L	–	–
Sialic acid-binding lectin	*Sambucus nigra*	Elderberry bark	SNA	–	–

coated slides (Menzel-Gläser, Braunschweig, Germany). Dewaxed serial sections were stained with Delafields's haematoxylin, eosin and Masson-Goldner.

Conventional histochemical glycoconjugate staining was performed using PAS (including diastase controls) (Pearse, 1985) and alcian blue staining at pH 1 and 2.5 for glycoconjugate expression (Pearse, 1985) in the skin of the catfish. The lectins used in this study are listed in Table 1 and belong to the following six groups of lectins: mannose-, galactose-, *N*-acetylgalactosamine-, *N*-acetylglucosamine and L-fucose-binding lectins, and lectins that bind to complex carbohydrate residues. The complete complement of lectins investigated and the oligosaccharide specificity associated with these probes are detailed in Table 1.

Sections (5 μm thick) of the catfish (*A. tenuispinis*) skin from 10 different areas (Fig. 1) were dewaxed with xylene (2 × 30 min), then rehydrated and thoroughly washed in TRIS buffer (3 × 5 min; pH 6.8). Incubation with the respective fluorescein isothiocyanate (FITC)-conjugated lectins (12 μg/ml TRIS buffer) was performed in a darkened humidified chamber for 1 h. The panel of 14 FITC-labelled lectins was used and their respective inhibitors (Schick et al., 2009) are shown in Table 1. After incubation, the sections were gently washed in TRIS buffer (3 × 5 min; pH 6.8) and mounted in a mixture of polyvinyl alcohol and ethylene glycol (Serva, Heidelberg, Germany) to probe the slides, and viewed with a Leitz fluorescent microscope. Microphotographs were taken using a confocal laser scanning microscope (LSM 510 Meta; Zeiss, Göttingen, Germany) equipped with a 10× Plan-Neofluar objective (numerical aperture 0.3)/a 20× Plan-Neofluar objective (numerical aperture 0.5)/ a 40× Plan-Neofluar oil immersion objective (numerical aperture 1.3). The excitation wavelengths used were: 364 nm for DAPI and 488 for FITC. The resulting fluorescence emissions were detected through emission bandpass filters at 385–470 nm (DAPI) and 505–530 nm (FITC).

Results

Depending on the special location, the epidermis of the catfish (*A. tenuispinis*) consists of three to six layers of epidermal cells, and is mucogenic in nature. The histochemical characteristics of the epidermis and its associated intra-epidermal glands (club glands and mucous goblet cells) did not show significant differences in the 10 areas of the skin studied in this investigation, but their number varied in the different regions. The mucous unicellular glands are usually located in the superficial layers of the epidermis and release their secretion to the surface, where a more or less continuous layer of mucus is formed. The club cells are also unicellular and usually possess a central ovoid nucleus, which is surrounded by a comparatively large amount of homogenous eosinophilic cytoplasm (Fig. 2a,b). Larger club cells are mostly located in the middle layer of the epidermis. Distinct staining of the mucus was not observed after H&E (Fig. 2a,b) or after Goldner staining (Fig. 2c,d).

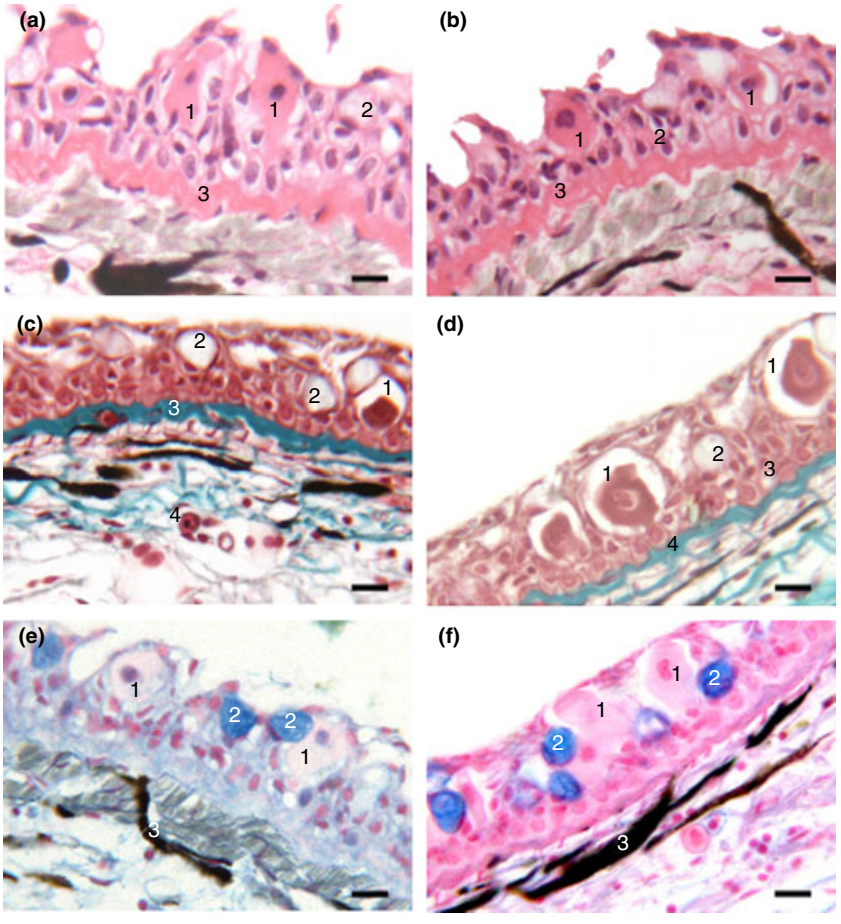

Fig. 2. Histology of the epidermis of the skin of *Arius tenuispinis*. Scale bar = 25 μm. (a) Epidermis, area 1, H&E-staining; 1 club cell; 2 mucous cell; 3 basal membrane. (b) Epidermis, area 6: H&E-staining; 1 club cell; 2 basal layer of the epidermis; 3 basal membrane. (c) Epidermis, area 1, Goldner staining; 1 club cell; 2 mucous cell; 3 basal membrane; 4 subepidermal connective tissue with numerous melanocytes. (d) Epidermis, area 1, Goldner staining; 1 club cell; 2 mucous cell; 3 basal layer of epidermis; 4 basal membrane. (e) Epidermis, area 1, alcian blue staining, pH 1.0. 1 club cell; 2 mucous cell; 3 melanocyte. (f) Epidermis, area 1, alcian blue staining, pH 1.0. 1 Club cell; 2 mucous cell; 3 melanocyte.

Using conventional glycoconjugate staining techniques (PAS staining, alcian blue at pH 1 and 2.5), it could be confirmed that the mucous cells contain a considerable amount of glycoconjugates in all locations of the skin, whereas the club cells lacked the glycoconjugates. The glycoproteins found in goblet cells stain distinctly magenta when subjected to PAS staining and strongly blue when subjected to alcian blue staining at pH 1 (Fig. 2e,f). Alcian blue at pH 2.5 resulted in a comparatively weak staining of the goblet cells. From these data, we can conclude that the mucus of the goblet cells is rich in neutral and acidic glycoproteins. The acid glycoproteins are obviously quite heavily sulphated, but contain only small amounts of carboxyl groups.

The lectin histochemical staining properties (Fig. 3a–h) of the epidermis and subepidermal structures are summarized in Table 2. The contents of the mucous cells mostly appear loosely packed, with a mass of bubble-like vesicles. With lectins, which stain the mucous cells strongly (DBA, SBA, GLS-1), usually a distinctly stronger reaction intensity is found at their periphery. Co-incubation of the FITC-labelled lectins with the corresponding inhibiting sugars (see Table 1) significantly or completely reduced binding to the sections.

Mannose-binding lectins (ConA, LCA and PSA)

Mannose-binding lectins showed a characteristic staining pattern in all 10 skin areas studied. Whereas LCA (Fig. 3 b) and PSA (Fig. 3c) staining appeared nearly identical (strong staining of mucous cells, weak staining in the epidermis, no staining of club cells), ConA-FITC (Fig. 3a) only weakly stained the mucus and the mucous cells.

Galactose-binding lectins (PNA and RCA)

Incubation with the galactose-binding lectins PNA and RCA (Fig. 3e) generally resulted only in faint and mostly discontinuous staining of the apical epithelial cells.

N-Acetylgalactosamine-binding lectins (DBA, GSL I, SJA and SBA)

These lectins which all have a nominal specificity for α-D-*N*-acetylgalactosamine showed divergent staining pattern

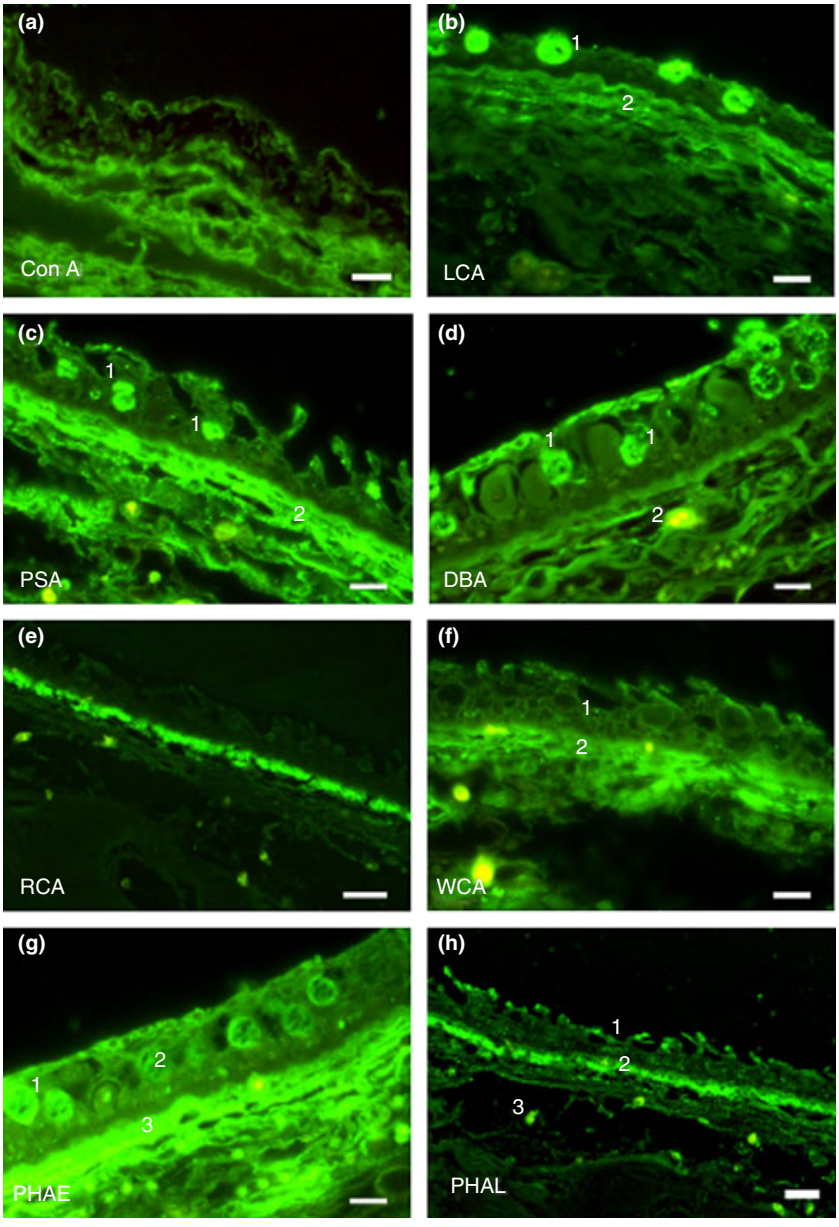

Fig. 3. Lectin-binding sites in the epidermis of the catfish *Arius tenuispinis* (area 1). (a) A weak binding of ConA-FITC is found in the mucus and the mucous cells. Scale bar = 25 μm. (b, c) Both FITC-conjugated lectins (LCA and PSA) strongly stain the mucous cells. Scale bar = 25 μm. (d) DBA-FITC strongly stains the mucous cells (1) and the mucus. Distinct staining is also found in the subepidermal connective tissue (2). Scale bar = 100 μm. (e) RCA-FITC incubation results in a faint and discontinuous staining of the apical epidermal cells. A distinct staining was observed in the dense collagen layer beneath the basal membrane. Scale bar = 25 μm. (f)-WGA-FITC shows a moderate staining of the epidermis (1), whereas the subepidermal connective tissue (2) shows a distinct to strong binding of the lectin. Scale bar = 25 μm. (g) PHA E-FITC strongly stains the mucus (1) on the surface of the epithelium and the mucous cells. In addition, the subepidermal connective tissue is distinctly stained. Scale bar = 25 μm. (h) PHA L-FITC stains the mucus on the epidermis surface (1). A distinct binding of this lectin is seen in the subepidermal collagen layer (2). Scale bar = 100 μm.

in the epidermis. Whereas DBA (Fig. 3d), SBA and GSL I distinctly stained the mucous cells of the epidermis, only a weak binding of SJA to the mucus of the goblet cells was found. The strong staining of DBA, SBA and GSL I was usually confined to the peripheral part of the goblet cells, whereas in the central part, a weaker binding of the three FITC-labelled lectins occurred. DBA and SBA also distinctly stained the apical epithelial cells, whereas the deeper cell layers showed only little binding of these two lectins.

N-Acetylglucosamine-binding lectin (WGA)

The periphery of apical cells showed a punctuate fluorescence after incubation with WGA-FITC (Fig. 3f). The mucus of the goblet cells and the mucus on the epidermal surface as well as the basal membrane were distinctly stained, whereas no effect on the club cells was noted.

Fucose-binding lectin (UEA)

Fucose-FITC did not bind significantly to any structure of the epidermis.

Lectins binding to complex carbohydrate configurations (PHA E and PHA L)

PHA E (Fig. 3g) strongly stained the mucus of the goblet cells and of the epidermal surface, whereas binding

Table 2. Lectin staining of the epidermis of the catfish *Arius tenuispinis*

Lectin	Apical epithelial cells	Epithelial cells in deeper layers	Mucous on epithelial surface	Goblet cells	Club cells	Basal membrane
Con A	+	–	+	–	–	+
LCA	+	–	+–++	+++	–	+–++
PSA	+	–	+–++	+++	–	+–++
PNA	+ (discont)	–	–	–	–	–
RCA	+–	+–	–	+–	–	–
DBA	++			+++	–	
SBA	+–++	+–	++	++–+++	–	++
SJA		+		–	–	
GSL I	+	+	+++	++	–	–
WGA	+ punc, membrane	+ punc, membrane	+	++	–	+–++
UEA	–	–	–	–	–	–
PHA E	++	+	++	++–+++	–	–
PHA L	+	+	+–++	+–++	–	+
SNA	+–++ (punctuate)	+ (punctuate)	–	–	–	–

–, negative; +, weakly positive; ++, distinctly positive; +++, strongly positive staining.

of PHA L (Fig. 3h) appeared somewhat less in both locations.

Sialic acid-binding lectin (SNA)

This lectin has an affinity for α-NeuNAc-[2 → 6]-Gal, α-NeuNAc-[2 → 6]-GalNAc, and to a lesser extent for α-NeuNAc-[2 → 3]-Gal residues. Some staining was seen in the apical and basal layers of the epidermis. The unicellular glands, goblet cells as well as club cells were always negative.

Discussion

The skin of fish is continuously exposed to sea water and because of its direct contact with the environment, its structure and function have been investigated in several studies (Whitear, 1981; Burkhardt-Holm, 1997; Pinky et al., 2008). The mucus on the surface of the skin protects the animal and is an important factor in disease resistance (Shephard, 1994; Pinky et al., 2008). Mucous cells and the composition of the mucus they produce are influenced by endogenous factors (sex, developmental stage) and exogenous factors, such as stress, acid and infections (Blackstock and Pickering, 1982; Zaccone et al., 1985). After exposure to an acidic environment, modifications in the carbohydrate contents are more obvious when defined by lectin histochemistry than those detected by classical histochemical techniques. Low levels of agglutination activities have been observed in skin mucous secretions of several fishes, including gar (*Lepisosteus platyhineus*), snapper (*Lutjaneus griseus*) and bowfin (*Amia calva*) (Fletcher and Grant, 1968; Bradshow et al., 1971). It was speculated that some or all of these activities in mucus could be related to immunoglobulins, as immunoglobulins have been found in the mucus of the Atlantic salmon following immunization with erythrocytes (Harris and Hunt, 1973). Preparations from mucus from many animal sources have been shown to contain immunoglobulins (Pigman, 1977).

Mucins, the main constituents of mucus, are high molecular weight glycoproteins. Some 50% of their dry weight can consist of carbohydrate chains (Burkhardt-Holm, 1997). Although there is extensive information in the literature on this subject (Mittal et al., 1994; Kumari et al., 2009), the carbohydrate nature of the glycoproteins in the unicellular glands in fish epidermis has not been fully characterized.

The Arabian Gulf catfish *A. bilineatus*, *Val*. and *A. tenuispinis* secrete a viscous layer of proteinaceous gel when frightened or injured. The gel-like material adheres to the skin of the fish even when the fish swims at varying speeds and for a number of days. We observed that the gel on the catfish *A. bilineatus*, *Val.* peels off in an irregular manner after 3 days or more. Usually, a catfish caught 48 h after scraping the opaque proteinaceous gel did not elaborate more of the gel, but secreted a transparent, viscous, water-soluble solution, which is reminiscent of mucus. The epidermal secretion of *A. tenuispinis* is more viscous and glue-like compared with that of *A. bilineatus*. The biochemical and pharmacological properties of *A. tenuispinis* skin secretions appear to be similar to those of the secretions of *A. bilineatus*. Preparations from *A. tenuispinis* secretions induce lethal response in rabbits comparable with *A. bilineatus* secretions. However, its plasma enzyme activity is lower than that of *A. bilineatus* (Ali et al., 1989). In this study, a detailed histochemical analysis of the carbohydrate residues found in the epidermis of the catfish *A. tenuispinis* has been performed.

Glycoconjugates can be characterized using different histochemical techniques, including lectin histochemistry. In this study, we used conventional histochemical staining methods (PAS reaction, diastase-PAS, alcian blue at pH 1, and 2.5) to distinguish neutral and acidic (carboxylated and sulphated) glycoconjugates, and 14 fluorescein isothiocyanate-labelled lectins with different carbohydrate specificities to identify the presence and distribution of defined sugar residues in the oligosaccharide chains of glycoconjugates.

Using conventional glycoconjugate staining techniques (PAS staining, alcian blue at pH 1 and 2.5) showed that the mucous cells contain a considerable amount of glycoconjugates in all locations of the skin, whereas the other unicellular gland type, the club cells, was consistently lacking these glycoconjugates. The glycoproteins found in mucous cells stain magenta when subjected to PAS staining and therefore contain neutral glycoproteins. Alcian blue staining indicating acid glycoproteins was distinctly positive at pH 1, but gave only a comparable weak staining at pH 2.5. Therefore, the mucus of the goblet cells also contains acid glycoproteins rich in sulphate groups.

Using 14 different lectins, the carbohydrate compositions of the glycoproteins of mucous cells could be more fully characterized. A distinct staining of the mucus of goblet cells was found with the mannose-binding lectins LCA and PSA; the galactosamine-binding lectins DBA, SBA and GLS I; the glucosamine-binding lectin WGA; and PHA E which stains glycoproteins with complex carbohydrate configurations, whereas no reaction occurred with the fucose-binding lectin UEA and the sialic acid-specific lectin SNA. In addition, the galactose-binding lectins PNA and RCA showed only a weak or completely negative staining of the mucus in the goblet cells. The specificity of the lectin staining could be proved by inhibiting the binding of the lectins using the corresponding sugars. From these data, we can conclude that the mucus produced by the epidermal mucous cells of *A. tenuispinis* is rich in mannose, *N*-acetylgalactosamine and *N*-acetylglucosamine residues.

On the contrary, the second type of unicellular glands in the skin of *A. tenuispinis*, the club cells, stained neither with conventional glycoprotein stains such as PAS and alcian blue, nor with any of the carbohydrate-specific lectins used in this study. Therefore, we can safely assume that their secretions contain no or only little carbohydrate material. These findings confirm earlier observations of Al-Hassan et al. (1982), who clearly showed by using biochemical techniques that the Arabian Gulf catfish secretes copious amounts of viscous proteinaceous gel from epidermal cells when threatened or injured. The term mucus applies to substances in which carbohydrates constitute a predominant percentage (mostly 60% or more). The epidermal gel secretion of *A. bilineatus* is unlike what can be generally termed mucus. Over 85% of the dry weight of the gel secretion is protein, with lipids (13.4% of the dry weight) and only small amounts of carbohydrates and nucleic acids. The lipid fraction contains a relatively high level of arachidonic acid, a mixture of eicosanoids, cholesterol and cholesteryl esters, as well as polar lipids (Al-Hassan et al., 1986a). The proteins are composed of two major fractions, the water-insoluble α-helical structures and the phosphate buffer-soluble fraction (Al-Hassan et al., 1987b). The soluble fraction contains amongst other biologically active proteins and peptides a mixture of enzymes whose properties resemble those of some components of animal venom. However, other proteins that have been studied include a lectin-like haemagglutination factor (Al-Hassan et al., 1982, 1986c), a haemolytic factor (Al-Hassan et al., 1982; Al-Lahham et al., 1987) and vasoactive components (Al-Hassan et al., 1986b; Al-Bow et al., 1997). The catfish gel haemagglutination factor (CHF) has a specific activity expressed as titre per mg protein, is extremely high, $>10 \times 10^6$, making it one of the most active soluble animal lectins isolated to date. Our previous studies showed that CHF recognizes red blood types (Al-Hassan et al., 1986c). Human blood types A and B had similarly high titre levels of CHF in all cases tested, but type O was variable. One-half of the type O donors tested gave a positive agglutination response, whereas the other half was negative (20 subjects in total), indicating that additional determinants may be involved. Sheep red blood cells also showed variability similar to that noted for human type O cells. Several studies have convincingly established that the repeated application of preparations involving catfish epidermal secretions to wounds and persistent diabetic foot ulcers in human subjects had a positive effect on wound healing (Al-Hassan, 1990; Al-Hassan et al., 1991). The complex mixture of components contained in the epidermal secretion obviously induces a balanced stimulation of the early stages of wound healing. Despite intensive research, the exact nature and role of the wound healing components secreted by club cells are not known, although synergistic effects by the different biologically active components are expected. Our observations during treatment of diabetic and gangrenous foot ulcers using preparations from the skin of the Arabian Gulf catfish *A. bilineatus* showed that healing progressed smoothly in most cases without the use of antibiotics, although the lesions were highly infested with a variety of micro-organisms. The appearance of large numbers of macrophages during the early stages of treatment of incised wounds in test animals only points to the enhancement of the immune system of the treated animals by the catfish preparations (Al-Hassan et al., 1991). Recent studies of the Japanese conger eel

Conger myriaster have shown that their club cells secrete a specific galectin called congerin into the epidermal mucus (Nakamura et al., 2001). The authors hypothesize that congerin participates in innate immunity on the intra- and the extra-body surface of the conger. In future studies, we intend to establish whether galectins or galectin-like proteins are components of the proteinaceous secretions of the club cells in the epidermis of the catfish *A. tenuispinis*, and the other three Ariid catfish species found in the Arabian Gulf, namely *A. bilineatus*, *A. thalassinus* and *Arius dussumieri*, and whether they play a role in the positive wound healing effects of the epidermal secretions in these species.

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