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Research Article

Glycoconjugates of the intestinal goblet cells of four cyprinids

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Abstract. The aim of this work was to show differences in the terminal and subterminal sugar composition of carbohydrate chains of glycoconjugates produced by the goblet cells of the intestines of four cyprinids. We analysed intestines of two herbivorous species – sneep and grass carp – and two omnivorous ones – chub and common carp. We compared four intestinal regions of every studied species. In every region, the presence of neutral and acidic glycoconjugates was confirmed. The smallest amount of acidic glycoconjugates was present in the second region of sneep intestine. Sulphated glycoconjugates were absent in the third and fourth region of chub intestine. Lectin histochemistry provided evidence for the presence of β-D-galactose, α-N-acetylgalactosamine, β-N-acetylglucosamine and sialic acids. Additionally, the occurrence of α-L-fucose in the goblet cells of chub, grass carp and sneep was confirmed. We tried to correlate the patern of glycoconjugate glycosylation with feeding habits of the studied fishes.

Key words. Intestine; goblet cell; lectin; cyprinid; glycoconjugate.

The digestive tract of fish is differentiated into four distinct sections: buccopharynx, oesophagus, stomach and intestine. However, the digestive tract of Cyprinidae lacks an acidic stomach, and an oesophagus directly joins an intestine. Some authors state that the cyprinid strong chewing and crushing devices (pharyngeal teeth) can effectively replace an acidic stomach. However, acid hydrolysis confers high assimilation efficiencies [1]. All cyprinids lack an acidic stomach, so their intestines must have different mechanisms for preparing food for digestion and absorption. One of the proposed mechanisms is associated with mucus which is produced in the intestinal epithelium by goblet cells [2].

Some published reports have found mucus to perform functions associated with feeding in lower vertebrates. Mucus-covered elements attract particles [3], playing an entrapment role in the food collection systems of anuran [4] and lamprey [5] larvae. Mucus is thought to mediate transport of particulate food in the Atlantic menhaden *Brevoortia tyrannus* [6]. Sibbing and Uribe [7] considered that highly viscous mucus, produced by the common carp (*Cyprinus carpio* L.) pharynx, traps small particles, aggregates them into boluses and lubricates the chewing plate. Murray et al. [8] suggested that mucus in the posterior oesophagus of the winter flounder (*Pleuronectes americanus* Walbaum) and yellowtail flounder (*P. ferrugineus* Storer) may have a role in pregastric digestion. Tibbetts [2] postulated that acidic mucus might play a role in the assimilation of plant nutrients by hemiramphids.

We compared the carbohydrate composition of mucus produced by intestinal goblet cells of four cyprinids. We chose two herbivorous species: sneep (*Chondrostoma nasus* L.) caught in running waters of southern Poland, which eats algal growth on stones, scraping them away with a sharp, low, slit-like mouth [9], and grass carp (*Ctenopharyngodon idella* L.), a species of Asian origin bred in aquacultures. Grass carp is primarily a ‘grazer’; it

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tends to feed on the surface and in shallow water. Grass carp prefers submerged plants and the soft tips of young tender plants. Large fish will consume hydrilla before musk grass. When the preferred food of the grass carp is not available, this fish feeds on terrestrial vegetation hanging over the surface of the water [10].

The other two species are omnivorous: chub (*Leuciscus cephalus* L.) from the river Raba and common carp from aqaculture. Chub, apart from eating plants, is an active hunter and feeds on worms, molluscs, crustaceans and various insect larvae; large chub eat considerable numbers of small fish, such as chub, eel, dace, roach, gudgeon and minnows, and they will also consume frogs, crayfish, voles and young water birds [11]. Carp is omnivorous, showing some preference for chironomids, cladocerans, oligochaetes, other invertebrates, plankton and macroalgae [12]. Juvenile common carp may feed on larval fish, when invertebrates are scarce [10, 13].

The aim of this work was to reveal differences in the pattern of glycoconjugate composition of the intestinal goblet cells between studied species, and to try to correlate them with the type of food ingested by the selected species. Second, we investigated the possibility of dividing biochemically the cyprinoid intestine, which anatomically is an almost uniform tube.

Materials and methods

Tissue preparation

Six adult sneeps, grass carps, common carps and chubs of both sexes, weighing 400–1300 g, collected from Raba River in southern Poland (sneep, chub) and aquacultures (grass carp, common carp) were used. The animals were killed by a blow to the head and the entire intestine was then quickly removed. The intestine was divided into four equal parts. From the geometrical centre of each part, 0.5-cm tissue fragments were collected for preparation. They were numbered according to their proximity to the oesophagus as 1, 2, 3 and 4. They were fixed in 10% buffered Lillie's formalin, dehydrated in a graded series of ethanol, cleared in toluene and embedded in paraffin wax. The serial sections of 5 µm were mounted on poly-L-lysine (P1274; Sigma) coated slides, deparaffinised in xylene, rehydrated in a graded ethanol series (from 100 to 50%) and rinsed in 0.05 M phosphate-buffered saline, pH 7.2, for 30 min.

Conventional histochemical staining

From each site, serial 5-µm sections were treated with the following procedures to identify the mucosubstances:

1) The periodic-acid Schiff (PAS) reaction for staining unsubstituted glycol-rich neutral mucins [14]
2) 1% Alcian blue, pH 2.5 (AB 2.5) for localisation of the carboxylated and/or sulphated type of acidic mucins [15]
3) 1% Alcian blue, pH 1.0 (AB 1.0) for selective characterisation of sulphomucins.

Alcian blue dyes and the techniques used in this study were pretested in an α-dot-blot assay for their suitability to label carboxylated and sulphated mucins, respectively [16].

Lectin histochemistry

For more specific discrimination of glycoconjugates, we applied several tetramethyl rhodamine isothiocyanate (TRITC)- and fluorescein isothiocyanate (FITC)-conjugated lectins according to the following procedure.

Deparaffinised tissue sections were incubated with lectin solutions at concentration of 0.02–0.2 mg/ml of 0.05 M phosphate-buffered saline at pH 7.2 for 45 min at room temperature. Applied lectins are presented in table 1. Before AB 2.5 and lectins were applied, some sections were incubated in 1% KOH in 70% ethanol for 20 min to cleave acetyl groups, and then at 37 °C for 16 h, in 0.86 U/mg protein of sialidase (type V, from *Clostridium perfringens*) dissolved in 0.1 M sodium acetate buffer, pH 5.5 containing 10 mM $CaCl_2$ to cleave terminal sialic acids and expose other sugar moieties for conventional staining

Table 1. Characteristics of lectins.

Lectin origin	Abbreviation	Carbohydrate-binding specificity	Inhibiting sugar
Arachis hypogea	PNA	terminal β-D-galactose(1–3)-N-acetylgalactosamine	–
Dolichos biflorus	DBA	terminal α-N- acetylgalactosamine	N-acetylgalactosamine
Ricinus communis II	RCA II	terminal α-N- acetylgalactosamine	N-acetylgalactosamine
Erythrina cristagalli	ECA	β-D-galactose (1–4)-N- acetylglucosamine	–
Triticum vulgaris	WGA	β-N-acetylglucosamine	N-acetylgalactosamine
Griffonia simplicifolia II	GSA II	β-N-acetylglucosamine	N-acetylgalactosamine
Griffonia simplicifolia IB_4	GSA IB4	terminal α-D-galactose	D-galactose
Lotus tetragonolobus	LTA	terminal α-L-fucose	α-L-fucose
Ulex europaeus	UEA-I	terminal α-L-fucose	α-L-fucose
Canavalia ensiformis	Con A	α-D-mannose	D-mannose

and lectin binding [17]. Other sections were incubated at 37 °C for 3 h in 500 U/mg protein of β-galactosidase (from *Escherichia coli*) dissolved in 0.05 M sodium citrate buffer, pH 3.8 containing 25 mM EDTA to cleave β-galactose [18]. Negative control sections for enzyme digestion were exposed to the buffer from which the enzymes had been omitted. Serial sections were incubated in lectins without previous pretreatment. Lectins and enzymes were purchased from Sigma.

The sections were mounted in glycerine containing 5% N-propyl galate (3,4,5-trihydroxybenzoidesin-N-propyl ester; Sigma) to prevent fading of fluorescence, examined, and images recorded using an Axioskope microscope (Zeiss) with epifluorescence (Filter EX 465-495; objectives: 20, NA 0.70 and 40, NA 0.85). Images were recorded on Kodak 5053 TMY and Ilford H5 Plus films with a photo camera (Zeiss: Kam MC Spot). For every lectin and every intestinal region of every species under study 24 microphotographs (6 individuals × 4 slides) were taken. From each slide, one view area was chosen at random, and photographed. Negatives were scanned at a resolution of 1200 dpi, and analysed using ACD software. The analysis was quantified as the reactive goblet cells to total goblet cells ratio (reactive cells are brighter, and non-

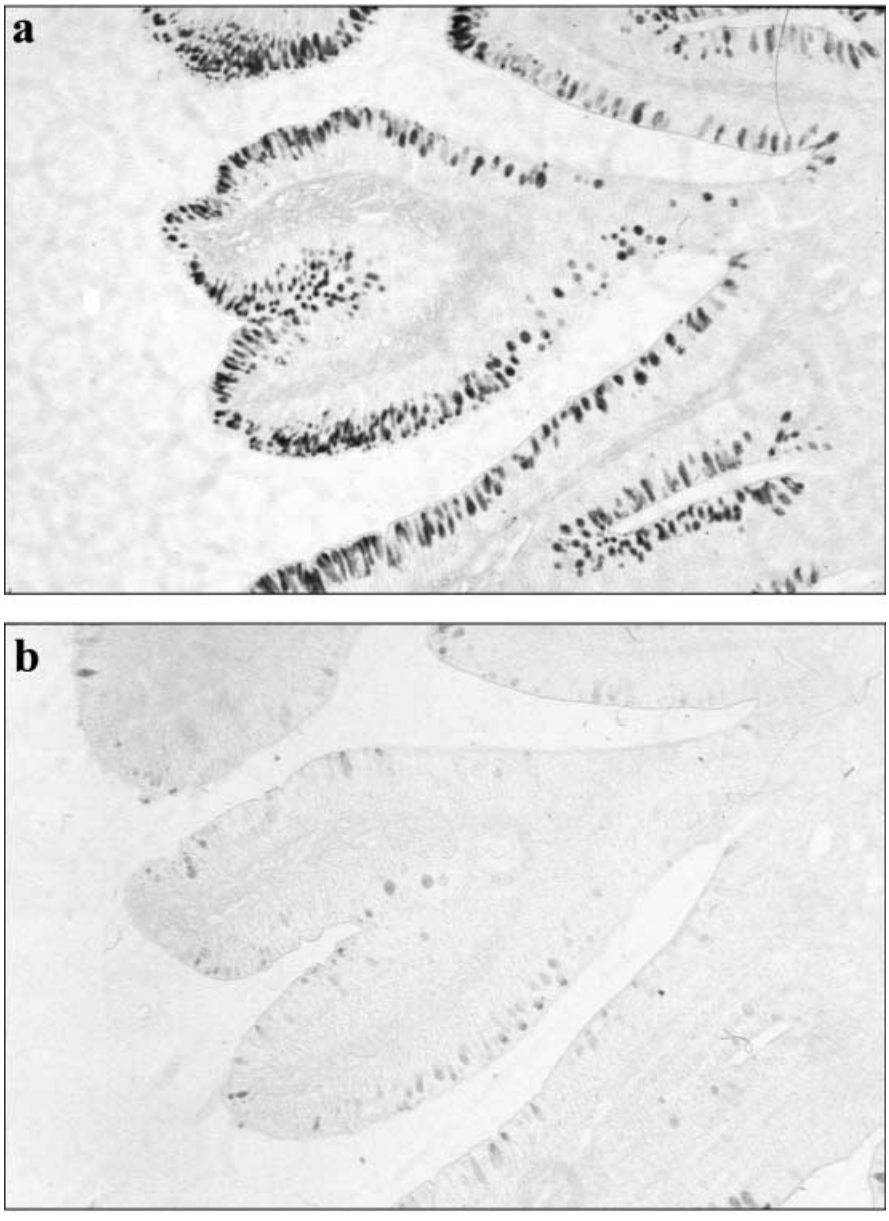

Figure 1. Cross-section of a few folds of the intestinal fourth region of chub stained with AB 2.5 without (*a*) and with (*b*) preincubation with sialidase. There is a clear difference between photographs in goblet cell staining, indicating that the majority of acidic glycoconjugates are sialylated (×240).

Table 2. Lectin binding to the goblet cells of different regions of the intestines of all studied species.

	Region	WGA	Neu WGA	GSA II	Neu GSA II	PNA	Neu PNA	β Neu PNA	RCA	Neu RCA	DBA	Neu DBA	LTA	Neu BSA IB_4
Sneep	1	60.0 ± 9.3 (2)	69.1 ± 9.1* (2,3,4)	51.3 ± 6.4 (2)	61.3 ± 5.3* (2,3,4)	52.8 ± 8.9 (2,3,4)	52.1 ± 9.4 (2,3,4)	25.2 ± 9.8 * (2,3,4)	35.4 ± 5.5 (2)	38.1 ± 8.0 (2)	58.3 ± 10.3 (2,3,4)	59.5 ± 9.5 (4)	0	0
	2	88.3 ± 3.0 (1)	88.8 ± 2.6 (1,3,4)	79.2 ± 3.8 (1)	79.4 ± 4.3 (1,3,4)	66.9 ± 5.0 (1,3,4)	67.0 ± 5.1 (1,3,4)	20.9 ± 6.5 * (1,3,4)	58.4 ± 4.9 (1)	58.2 ± 5.1 (1)	72.1 ± 8.8 (1,3,4)	66.5 ± 13.8 (3,4)	0	0
	3	0	55.0 ± 4.8* (1,2)	0	49.6 ± 3.8* (1,2)	5.9 ± 12.2 (1,2)	30.9 ± 10.5* (1,2)	34.4 ± 7.9* (1,2)	0	0	16.0 ± 3.8 (1,2)	50.7 ± 7.2* (2,4)	87.4 ± 4.1	0
	4	0	57.3 ± 2.7* (1,2)	0	52.4 ± 2.7* (1,2)	6.2 ± 8.8 (1,2)	31.8 ± 11.4* (1,2)	33.1 ± 7.6* (1,2)	0	0	9.5 ± 6.0 (1,2)	17.4 ± 5.0* (1,2,3)	85.6 ± 8.2	0
Chub	1	21.5 ± 8.2 (3,4)	45.3 ± 5.5* (3,4)	19.4 ± 8.2 (3,4)	43.5 ± 5.5* (3,4)	0	0	0	0	0	0	0	52.8 ± 6.8	0
	2	17.7 ± 8.8 (3,4)	30.4 ± 18.9* (3,4)	18.2 ± 8.8 (3,4)	26.2 ± 18.9* (3,4)	0	0	0	0	0	0	0	54.3 ± 6.9	0
	3	71.2 ± 10.2 (1,2)	83.6 ± 5.5* (1,2)	65.7 ± 8.7 (1,2)	75.4 ± 4.8* (1,2)	0	78.0 ± 6.6*	75.2 ± 6.8*	19.3 ± 5.9	51.3 ± 8.1*	21.8 ± 3.8	76.3 ± 8.8*	0	35.5 ± 5.5
	4	73.0 ± 13.3 (1,2)	82.7 ± 5.9* (1,2)	69.4 ± 14.3 (1,2)	79.4 ± 6.7* (1,2)	0	77.4 ± 6.2*	74.3 ± 6.3*	18.3 ± 4.7	45.5 ± 10.2*	20.4 ± 3.3	73.9 ± 7.0*	0	37.1 ± 5.8
Grass carp	1	26.9 ± 11.5 (3,4)	24.3 ± 12.5 (4)	21.6 ± 18.5 (3,4)	23.1 ± 8.5 (4)	0	84.9 ± 11.1*	78.7 ± 11.5*	16.5 ± 3.3 (2,3,4)	91.7 ± 5.8* (2)	21.6 ± 6.4 (2,3,4)	88.3 ± 2.9* (3,4)	58.72 ± 7.5 (2,3,4)	0
	2	21.6 ± 8.0	20.8 ± 7.9	19.8 ± 8.8	19.5 ± 6.4	0	77.4 ± 12.4*	80.3 ± 9.5*	35.9 ± 10.0 (1,3,4)	83.2 ± 6.0* (1)	36.6 ± 7.7 (1,3,4)	88.8 ± 2.6* (3,4)	87.9 ± 11.4 (1,3,4)	0
	3	16.4 ± 8.7 (1)	17.4 ± 6.5	13.7 ± 5.2 (1)	14.5 ± 4.5	0	76.6 ± 10.8*	76.9 ± 9.8*	51.8 ± 11.6 (1,2)	86.4 ± 9.0*	50.9 ± 7.2 (1,2)	80.9 ± 7.2* (1,2)	42.5 ± 5.9 (1,2)	0
	4	15.8 ± 7.9 (1)	15.3 ± 6.1 (1)	11.8 ± 6.8 (1)	13.9 ± 7.2 (1)	0	81.3 ± 8.6*	74.8 ± 10.5*	52.0 ± 13.1 (1,2)	88.0 ± 7.9*	47.9 ± 9.5 (1,2)	78.9 ± 7.9* (1,2)	46.2 ± 4.8 (1,2)	0
Common carp	1	33.2 ± 6.0	30.8 ± 11.2	28.5 ± 6.9	25.5 ± 10.4	71.2 ± 7.2	71.0 ± 7.2	38.6 ± 11.7*	71.5 ± 8.8	74.3 ± 7.9	80.6 ± 11.3	82.1 ± 11.1	0	0
	2	34.4 ± 6.7	35.4 ± 8.4	31.4 ± 5.7	32.4 ± 8.4	77.0 ± 6.2	77.7 ± 5.1	37.9 ± 6.7*	73.5 ± 8.5	73.5 ± 7.7	81.7 ± 9.4	83.6 ± 8.6	0	0
	3	35.3 ± 6.3	30.8 ± 10.5	29.4 ± 9.2	28.6 ± 9.6	72.3 ± 6.3	75.7 ± 5.1	35.2 ± 5.3*	74.6 ± 8.6	74.2 ± 8.9	81.4 ± 12.2	81.8 ± 8.8	0	0
	4	34.0 ± 7.1	30.8 ± 15.6	28.5 ± 5.1	27.9 ± 13.8	64.9 ± 16.5	62.2 ± 13.9	34.7 ± 6.2*	73.6 ± 7.6	73.7 ± 8.2	82.7 ± 11.8	83.0 ± 10.2	0	0

The regions are consecutively numbered from 1 to 4. Statistical differences between intestinal regions are indicated by different numbers in parentheses. Differences between reactions with lectins with and without pretreatment for every region are indicated by asterisks (pretreatments: β, β-galactosidase; Neu, sialidase; $p < 0.05$).

reactive cells are darker than background). Results were evaluated using the STATISTICA analysis package (StatSoft Inc.) by one-way analysis of variance (ANOVA) followed by Tukey's tests. Probabilities less than or equal to 0.05 were considered significant.

Results

The intestine walls of all species are composed of serose, muscularis externa, submucosa and mucosa. The muscularis externa of the intestine consists of a longitudinal and circular smooth muscle layer. The mucosa of the anterior intestine in chub, sneep and grass carp is organised in highly Z-shaped folds. In common carp, the mucosal folds join one another to form small pits. The epithelium, which covers the free surface of the mucous membrane, is of a simple columnar type.

Goblet cells, which were the subject of our study, were situated between cylindrical epithelial cells. In the intestine of sneep and chub they were very numerous. The content of goblet cells was generally PAS and AB positive at pH 2.5 and 1.0, although at pH 1.0 the reaction was much weaker. There were a few exceptions. Goblet cells of sneep in the second region of the intestine were barely stained after AB application at pH 2.5 and 1.0. After sialidase digestion, AB 2.5 failed to stain the content of goblet cells of chub in the third and fourth region (fig. 1).

Lectin reactivity is summarised in table 2. All other lectins failed to bind to the sections.

Sneep

A positive PAS reaction with all the intestinal goblet cells indicated that mucus produced by these cells contains neutral glycoproteins. In regions 1, 3 and 4, all the goblet cells stained with AB 2.5 and AB 1.0, indicating the common production of acidic glycoconjugates, sialylated as well as sulphated. The second region was an exception because the goblet cells produced only minor quantities of acidic glycoconjugates.

Lectin staining revealed the presence of β-N-acetylglucosamine in a terminal and internal location along the glucidic chains in regions 1 and 2, although expression of this carbohydrate was statistically higher in the second region (fig. 2) than in the first. Application of sialidase revealed β-N-acetylglucosamine as a sugar subterminal to sialic acid in the third and fourth region, and also in the first region. LTA reactivity suggested the presence of reactive sites containing α-L-fucose, which binds via $\alpha(1 \rightarrow 6)$ linkage to the penultimate glucosaminyl residues and/or difucosylated oligosaccharides. Lack of UEA-I (*Ulex europaeus* agglutinin) reactivity suggested the absence of α-L-fucose bound via $\beta 1 \rightarrow 2$ linkage to penultimate D-galactose ($\beta 1 \rightarrow 4$) N-acetyl-D-galactosamine [19].

A considerable fraction of the goblet cells in the first and second region produce glycoconjugates with β-D-galactose, and α-N-acetylgalactosamine as terminal sugar (fig. 3), while there is hardly any N-acetylgalactosamine in the terminal position in the third and fourth region. Preincubation with sialidase caused significant augmentation in α-N-acetylgalactosamine reactivity in the third and fourth region.

Figure 2. Fluorescence micrograph showing a cross-section of two intestinal folds of sneep stained with FITC-conjugated WGA lectin. A very intense fluorescence in goblet cells marks the presence of many N-acetyloglucosamine residues (×180).

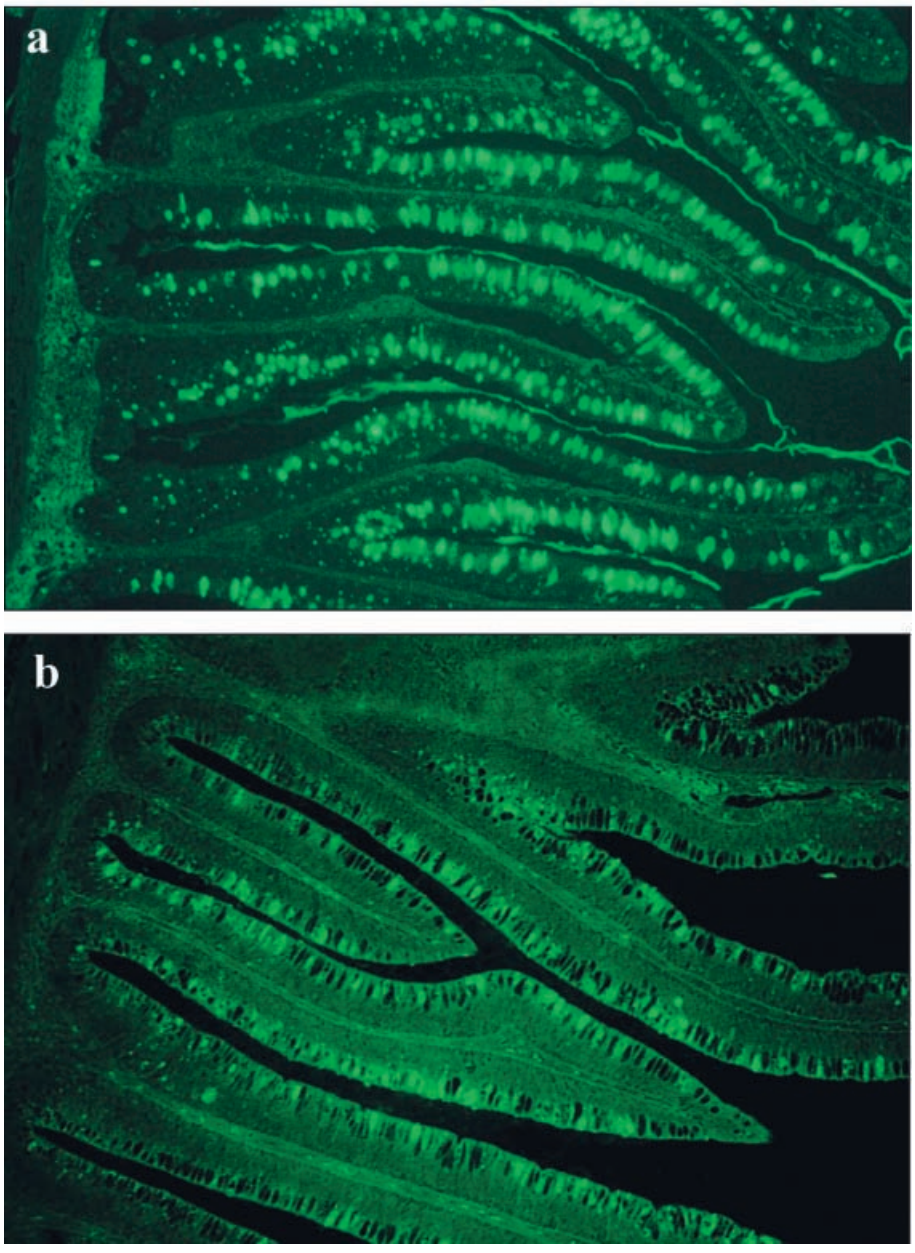

Figure 3. Cross-section of several intestinal folds of the second region of sneep stained with FITC-conjugated PNA after preincubation with sialidase (*a*) and after preincubation with β-D-galactosidase and then with sialidase (*b*). The pattern of staining shows that β-D-galactose(1–3)-N-acetylgalactosamine is present in a terminal location, and is not an acceptor sequence to sialic acids (×180).

Terminal rests of β-D-galactose(1$\rightarrow$3)-N-acetylgalactosamine were the components of glycoconjugates in the first and second region of the intestine, while in the goblet cells of the third and fourth region, this sugar in the terminal position was very rare. Intensification of staining with PNA (peanut agglutinin) after sialidase digestion in the third and fourth region showed terminal sialic acid linked to β-D-galactose(1$\rightarrow$3)-N-acetylgalactosamine.

One can conclude that terminal sugar residues of the first and second region – N-acetylgalactosamine and N-acetylglucosamine – are replaced by α-L-fucose in the third and fourth region. Glycoconjugate sialylation is much higher in the third and fourth region than in the first, and in the second region, sialic acids are almost absent.

Chub

PAS- and AB 2.5-positive reactions in the goblet cells of all intestinal regions confirmed the presence of neutral and acidic glycoconjugates.

After preincubation with sialidase, goblet cells of the third and fourth region became almost totally unstained, indi-

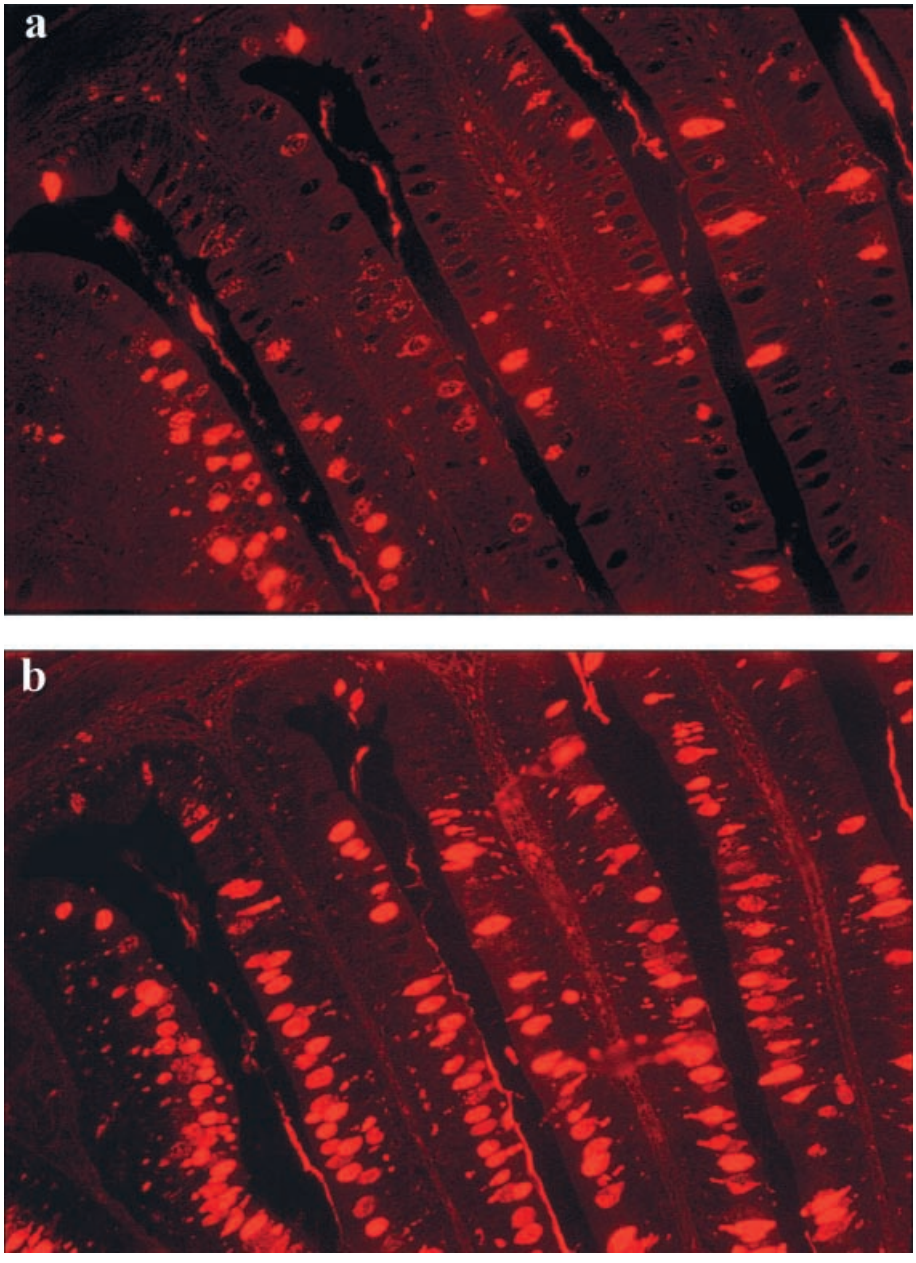

Figure 4. The fluorescence micrograph shows a cross-section of a few intestinal folds of the fourth region of chub stained with TRITC-conjugated DBA lectin without (*a*) and with (*b*) preincubation with sialidase. Intense fluorescence in goblet cells after sialidase pretreatment marks the presence of many N-acetylgalactosaminyl residues in subterminal positions to sialic acids (×360).

cating that the goblet cells secrete only sialylated and not sulphated glycoconjugates. This supposition was confirmed by the lack of AB staining at pH 1.0 in the last two regions.

In the first two regions of the intestine, the vast majority of neutral glycoconjugates are fucosylated, while in the last ones, β-D-galactose and β-N-acetylgalactosamine prevail. In regions 3 and 4, sialylation of glycoconjugates was intense (fig. 4), while the level of sulphation is low, whereas in the first and second region, glycoconjugates are mainly sulphated.

Grass carp

In the first two regions of the intestine, glycoconjugate with α-L-fucose and β-N-acetylglucosamine in the terminal positions of the glucidic chain were in the majority. In the other two regions, there was much more β-D-galactose and α-N-acetylgalactosamine. Acidic glycoconjugates were sialylated in almost every goblet cell (fig. 5). Along with them, sulphated glycoconjugates are present in every region.

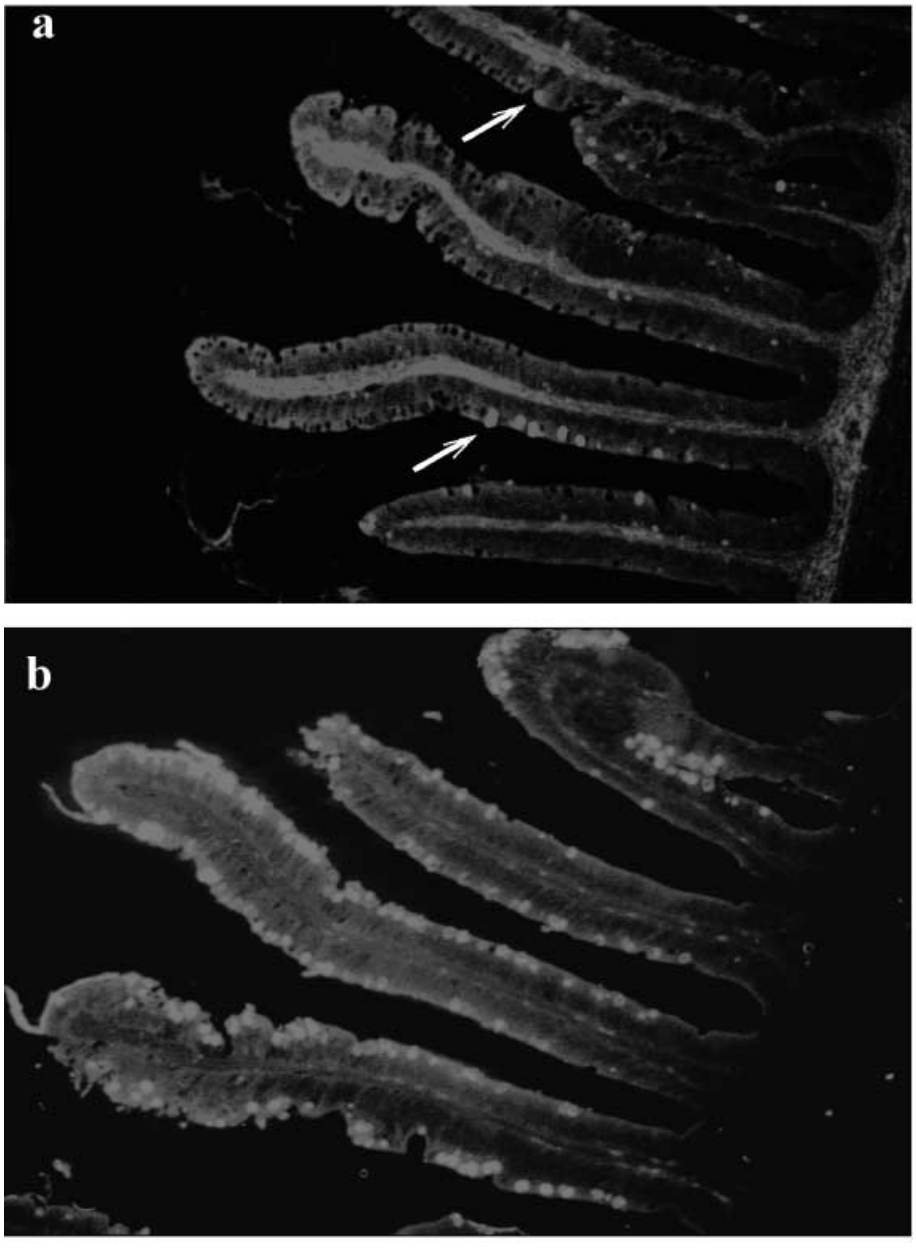

Figure 5. Cross-section of several intestinal folds of the first intestinal region of grass carp stained with FITC-conjugated RCA without (*a*) and with (*b*) preincubation with sialidase. β-D-galactose(1–3)-N-acetylgalactosamine is almost exclusively located subterminally to sialic acids (×360).

Common carp

The intestine of common carp had the most uniform pattern of terminal and subterminal glucidic residues of all the species studied. In every region acidic glycoconjugates, mainly sulphated, were abundant. Sialic acids appeared in a scanty number of goblet cells. The main terminal residue of the glucidic chains was β-N-acetylgalactosamine. β-N-acetylglucosamine was also present. There was no α-L-fucose.

Discussion

Information about the function and diagnostic value of glycoconjugate secretion in fish is still limited. However, the function of gut glycoconjugates is known to include: lubrication; protection of the tunica mucosa against chemicals, parasites, hypertonic media and acidity in the stomach and intestine; formation of a diffusion barrier for various ions, and perhaps a role in transepithelial ion transport [20–25]. Neutral and sialylated glycoconjugates are generally accepted as components of fluid secretions,

whereas sulphated glycoconjugates are denser. A shift towards acid glycoproteins coincides with an increase in viscosity of the mucus in mammals, birds and corals. Sulphated glycoconjugates are usually abundant in goblet cells of distal intestinal tracts, where protein digestion and trapping of bacteria (or other pathogens) occur, and may have a role in the stimulation of immunity there. Goblet cells in all parts commonly contain mixtures of acidic and neutral forms [26, 27]. Mucus containing sulphoglycoconjugates is insoluble in water, but its feathery structure traps water molecules [28], which are responsible for over 90% of the mass of mucous gel [29]. Tibbetts [2] suggests that while highly viscous sulphomucins aid in trapping small particles, the less viscous intestinal sialomucins and neutral glycoproteins may lubricate the sheath of viscous acidic glycoprotein coat and merely provide localised protection for tissues against attack by digestive enzymes. He also thinks that thanks to sialoglycoconjugates, presumed physical barriers to diffusion and absorption of plant 'juice' are overcome. Due to their negative charge, sialic acids are involved in binding and transport of positively charged molecules as well as in attraction and repulsion phenomena between cells and molecules. Their exposed terminal position in the carbohydrate chain enables them to function as a protective shield for the subterminal part of the molecule (preventing glycoproteins from being degraded by proteases) or cell. In infectious processes, the colonisation of bacteria can be limited by the sialic acid coat covering the host cell surface. Another interesting phenomenon is the spreading effect that is exerted on sialic-acid-containing molecules due to repulsive forces acting between their negative charges. This stabilises the correct conformation of enzymes or cell surface glycoproteins, and is important for the slimy character and the resulting gliding and protective function of mucous substances, as on mucous epithelia [30].

Buddington et al. [31] observed that herbivorous species of fish have higher rates of intestinal sugar transport and lower rates of amino acid transport than carnivorous fish, if each is studied while eating their respective natural diets. The ratio of proline to glucose uptake decreased in the sequence: carnivores > omnivores > herbivores. The uptake capacity of the intestine for the nutrient glucose was much higher in herbivores than in carnivores and correlated with species differences in carbohydrate content of the natural diet. Proline uptake varied much less among species, since species with different natural diets still have similar protein requirements.

Draw a demarcation line between the herbivorous and omnivorous animals studied is very difficult. Glycoconjugate composition seems to be characteristic for each studied species. Interestingly, the two herbivorous species – grass carp and sneep – handle food differently. A high degree of sialylation of intestinal glycoconjugates in grass carp, along with a short intestine, is surely the cause of rapid gut passage rates. This fact has far-reaching consequences. Eaten food moves in the digestive tract of a grass carp rapidly, and is not completely digested and absorbed. The energetic value of absorbed food is not high, so the animal has to consume large amounts of foodstuff. This feeding strategy is difficult to explain. One possibility is that sialic acids in terminal positions of carbohydrate chains first of all protect intestinal mucosa from pathogens, and the rapid transport of ingested food through the intestine is a sort of side effect. The second possible explanation assumes improvement in speed of movement due to fast emptying of the gut. This mechanism has been observed in some geese [32].

In the case of common carp, lack of terminal sialic acids and uniformity of intestinal regions can be ascribed to the diet applied in aquacultures. Food based on grains can possibly change the composition and cause homogeneity of gut glycoconjugates.

Neutral glycoconjugates produced by goblet cells in chub and sneep are distinctive along the intestine, although the pattern of diversity is different in each studied species. This reverse pattern is difficult to explain, but may suggest localisation of different bacterial strains along the alimentary canal. Mucus is thought to be an ecological niche for bacteria. Many reports confirm the presence of bacteria producing enzymes in digestive tracts of different fish species. In the intestine of carp, bacteria responsible for the production of vitamin B12 [33], chitin-decomposing microflora [34, 35] and amylase-producing [36] bacteria have been described.

A change in the glycoconjugate composition caused by a modified diet can decrease the amount of *Campylobacter jejuni* in chicks [36]. Lower mucin viscosity caused by the loss of terminal sialic acids residues prevents *C. jejuni* attaching to the intestinal mucosa. Conversly, high quantities of this bacteria induce the production of highly sialylated glycoconjugates. A wheat diet supplemented with xylanase can alter intestinal glycoconjugate composition in chicks [37]. Whether these observations also apply to fish remains to be elucidated.

We conclude that glycoconjugate composition of the cyprinid digestive tract depends on the species, and on the intestinal region. There is no convincing correlation between the type of ingested food and the type of terminal and subterminal sugar moieties, although there are certain differences between sneep and grass carp, which can be ascribed to different food preparation mechanisms. The use of lectins conjugated with fluorochromes allows data to be analysed quantitatively.

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