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Co-distribution of Glycoconjugates and H^+, K^+-ATPase in the Parietal Cells of the Greater Horseshoe Bat, *Rhinolophus ferrumequinum* (Schreber, 1774)

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Histochemical, lectin-histochemical, and immunohistochemical analyses were performed on parietal cells of the greater horseshoe bat, *Rhinolophus ferrumequinum*, to clarify the composition and distribution of oligosaccharide chains in the β-subunit of the protonic pump H^+,K^+-ATPase. PAS, Alcian Blue (pH 2.5) and Alcian Blue (pH 1.0) stainings detected only neutral glycoconjugates. Lectin-binding analyses included LTA, UEA-I, ConA, SBA, BSI-B4, AAA, DBA, PNA, and WGA. WGA- and PNA-bindings were also tested after β-elimination to detect O-linked glycans. Parietal cells were negative for binding to LTA and UEA-I, and to PNA and WGA after β-elimination, indicating the lack of (1,2) fucosylated residues and of N-linked glycans, respectively. Immunohistochemical tests with anti-α- and anti-β-H^+,K^+-ATPase were positive. Two alternative patterns of glycoconjugate distribution were found, i.e. a perinuclear and a diffuse one, indicating localization in the intracellular canaliculus and in the tubulovesicular system of the parietal cells, respectively. Both the subunits of the H^+,K^+-ATPase and the galactosyl/galactosaminyl residues were co-distributed in both the perinuclear and the diffuse patterns, suggesting that the residues are part of the protonic pump. Glycosyl/glycosaminyl and mannosyl groups were concentrated in the tubulovesicular system, and fucosylated residues were found almost exclusively in the intracellular canaliculi; thus they are probably not included in the oligosaccharide chains of β-H^+,K^+-ATPase. These findings indicate that the oligosaccharide chains linked to the β-H^+,K^+-ATPase subunit in *R. ferrumequinum* have distinct features compared to the other mammals studied and confirms the taxon specificity of the chains in the proton pump.

Key words: *Rhinolophus ferrumequinum*, stomach, parietal cell, lectin histochemistry, H^+,K^+-ATPase

INTRODUCTION

The parietal cells in the mammalian gastric mucosa contain a number of glycoconjugates that bind lectins (e.g., Okamoto and Forte, 1988; Ogata, 1997). One of these, *Dolichos biflorus* agglutinin (DBA), binds so intensely to parietal cells that it is considered to be a specific marker (Peschke et al., 1983; Kessimian et al., 1986), even if affinity has been demonstrated in other cells of the gastric mucosa (e.g., Ito et al., 1985; Jiang et al., 2004; Sommer et al., 2001). Most of the oligosaccharide residues responsible for lectin binding are probably linked to the β-subunit of the protonic pump H^+, K^+-ATPase, since it is the most abundant glycoprotein in the secretory membranes of parietal cells (e.g., Ogata, 1997; Tyagarajan et al., 1996). The β-subunit has six or seven N-glycosylation sites (Chow and Forte, 1995; Shin et al., 2009), and the structure of the carbohydrate chains is known in the rabbit (Tyagarajan et al., 1997). These chains are involved in a number of functions, such as assembly with the α-subunit (Asano et al., 2000), binding and transport of K^+ (Hermsen et al., 2000), maintenance of the ultrastructure of the secretory membranes (Miller et al., 2002), regulation of intracellular trafficking and sorting (Vagin et al., 2004; Vagin et al., 2007), protection against trypsinolysis (Crothers et al., 2004), and the normal development of parietal cells (Scarff et al., 1999).

The oligosaccharide chain sequences of the β-H^+,K^+-ATPase subunit are taxon specific; in particular, terminal α-galactosyl residues in the polylactosamine chains are present in rat and rabbit, but are lacking in pig, dog, and mouse (Stewart et al., 1999). Interspecific differences in β-H^+,K^+-ATPase structure can influence the functionality of the holoenzyme, as has been shown for the affinity for K^+, which differs between rat and pig (Hermsen et al., 2000). Furthermore, N-acetyl-galactosamine residues, as evidenced by DBA-binding, can sometimes be lacking in parietal cells (e.g., Suganuma et al., 1984; Danguy et al., 1986), and it is not clear whether they are present in the oligosaccharide chains of the β-H^+,K^+-ATPase subunit, since rabbit parietal cells are positive to DBA (Okamoto and Forte, 1988), but the β-H^+,K^+-ATPase subunit lacks galactosaminyl residues (Tyagarajan et al., 1997). Thus, studies on glyconjugates in

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parietal cells in several mammals having different dietary adaptations could help to clarify the relationship between glycosylation and function and the possible evolutionary implications. Chiroptera is an interesting group from this point of view, since chiropterans have the highest variation in feeding habits among mammals (e.g., Neuweiler and Covey, 2000). Furthermore, these studies could help clarify the relationships among carbohydrate residues, as evidenced by lectin binding and the distribution of the H^+,K^+-ATPase proton pump. Unfortunately, few data are available on the distribution of glyconjugates in the digestive tract of bats.

In previous papers (Scillitani et al., 2005, 2007), we analyzed the organization and the glycopatterns of exocrine secretion of the digestive tract of the greater horseshoe bat, *Rhinolophus ferrumequinum*. The stomach of this entomophagous species has a short cardias, a wide fundus, and a small pylorus and lacks the caeca or particular specializations found in other bats; it is thus regarded as primitive for the order (Scillitani et al., 2007). In the fundic mucosa there are several pluricellular glands, comprising many cell types, including parietal, chief, mucous (three different types), and endocrine. Chief cells disappear in the aboral fundus, whereas the number of parietal cells increases. Scillitani et al. (2005) hypothesized that this asymmetric distribution determines a gradient of pepsinogen and hydrochloric acid secretion, probably linked to requirements of fast absorption and food passage.

In this paper we present data on the histochemistry, lectin histochemistry, and immunohistochemistry of parietal cells in the gastric mucosa of *R. ferrumequinum*. We compare the results from different staining methods and the patterns of staining in the parietal cells to assess the distribution and composition of saccharide residues and their possible linking to the β-H^+,K^+-ATPase subunit, and discuss functional implications.

MATERIAL AND METHODS

Animals

Two male and one female adult greater horseshoe bats, *Rhinolophus ferrumequinum* (Schreber, 1774), were caught in September near Bari (Apulia) in a temporary roost during the diurnal resting period. Collection was authorized by the Italian Ministero dell'Ambiente, and the handling of the animals followed the current Italian laws. Each animal was sacrificed by using ethyl ether, and the stomach was quickly removed. The presence of insect remains in the stomach and/or the duodenum of the specimens suggested the fed condition, since food passes through the gastrointestinal tract rather rapidly in bats (e.g., Neuweiler and Covey, 2000). Samples of the oral and aboral portions of the fundus were fixed in 10% formalin, dehydrated through a graded alcohol series, and embedded in paraffin; details are given in Ferri et al. (1999). Serial sections 4 μm thick were cut.

Histochemistry

Histochemical methods and protocols followed Bonucci (1981), with minor modifications. Rehydrated sections were stained by using the periodic acid– Schiff (PAS)–haemallume method, and with Alcian blue (AB) at pH 2.5 or pH 1.0, to differentiate neutral and acidic glycoconjugates. Zymogen granules were identified with Bowie staining.

Lectin histochemistry

Binding of nine lectins (all from Sigma, St. Louis, USA, except for AAA from Vector Laboratories, Burlingame, California, USA) was assessed to determine the nature and distribution of glycosidic residues in the parietal cells. Lectins were labelled with horseradish peroxidase (HRP), fluoresceine isothiocyanate (FITC), or phosphatase. The lectins employed, their concentrations, and their sugar specificities are summarized in Table 1. Sections were deparaffinized and rehydrated by a routine protocol.

For binding with HRP-conjugated lectins (Con A, WGA, SBA, PNA, LTA, DBA), sections were exposed to 3% hydrogen peroxide for 10 min to inhibit endogenous peroxidase activity, and then incubated for 60 min at room temperature with HRP-lectin in 0.1 M Tris-buffered saline (TBS) pH 7.4. HRP activity was then visualized with 0.005% 3-3'-diaminobenzidine (DAB; Sigma, St. Louis, USA) and 0.01% hydrogen peroxide in 0.05 M TBS (Graham and Karnowsky, 1966) for 10 min in the dark at room temperature. Finally, the sections were dehydrated through a graded ethanol series, cleared in Histolemon (Carlo Erba, Rodano, Milan, Italy), and mounted in DPX (Fluka BioChemika, Steinheim, Germany).

For binding with FITC-conjugated lectins (UEA I, BSI-B4), sections were incubated for 30 min in blocking buffer (1% normal goat serum in TBS) and then incubated for 1 h at room temperature with the FITC-lectin solution in TBS. Sections were subsequently rinsed in the same buffer and mounted in 70% glycerin in TBS.

For binding with the AAA phosphate-conjugated lectin, sections were incubated for 1 h at room temperature with the lectin solution in TBS. Sections were subsequently rinsed in the same buffer and incubated in the substrate working solution (BCIP/NBT Alkaline Phosphatase Substrate Kit IV; Vector Laboratories, Burlingame, California, USA) for 15 minutes at room temperature. Endogenous alkaline phosphatase activity was inhibited by adding levamisole to the working solution. After washing in 0.1M TBS pH 9.5 for 5 min-

Table 1. Characteristics of the plant lectins utilized. Abbreviations: Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; MαM, methyl-$\tilde{\alpha}$mannopyranoside; TACT, N,N',N"-triacetylchitotriose. References: [1]Finne and Krusius, 1982; [2]Gallagher et al., 1985; [3]Bhattacharyya et al., 1988; [4]Lotan and Sharon, 1978; [5]Debray et al., 1981; [6]Baker et al., 1983; [7]Debray and Montreuil, 1989; [8]Sughii et al., 1982; [9]Wood et al., 1979.

Lectin	Source and References	Binding specificity	Lectin concentration (mg/ml)	Inhibitory sugar
Con A	*Canavalia ensiformis*[1]	D-mannose, D-Glucose	0:05	0.1 M MαM
WGA	*Triticum vulgaris*[2]	(GlcNAcβ1,4)n	0:02	0.01 M TACT
SBA	*Glycine max*[3]	GalNAc	0:02	0.2 M GalNAc
PNA	*Arachis hypogaea*[4]	Galβ1,3GalNAc	0:06	0.2 M Gal
LTA	*Tetragonolobus purpureus*[5]	L-Fucα1,6GlcNAc and L-Fucα1,2Galβ1, 4[L-Fuc1,3] GlcNAcβ1,6R	0:10	0.2 M L-fuc
DBA	*Dolichos biflorus*[6]	α-GalNAc	0:02	0.2 M GalNAc
AAA	*Aleuria aurantia*[7]	Fucα·(1,6)GlcNAc-βNAsn Fucα·(1,3), Fucα·(1,4)	0:10	0.2 M L-fuc
UEA I	*Ulex europaeus*[8]	Fucα·(1,2)	0:10	0.2 M L-fuc
BSI-B4	*Bandeiraea simplicifolia*[9]	α-Gal	0:02	0.2 M Gal

utes, the sections were counterstained with methyl green (Sigma, St. Louis, USA), dehydrated, cleared, and mounted following the HRP-lectin protocol.

PNA- and WGA-lectin binding was also tested after β-elimination, a method that removes only O-linked glycans from glycoproteins (Ono et al., 1983). Since the oligosaccharide chains of the β-H^+,K^+-ATPase glycoprotein are N-linked to the core protein (Tyagarajan et al., 1997), the suppression of lectin-binding after β-elimination would indicate that the residues were in O-linked chains, and therefore not part of the β-H^+,K^+-ATPase subunit. Sections were incubated with 0.2 M KOH in dimethylsulphoxyde-H_2O-ethanol (50:40:10) overnight at 45°C, followed by neutralization with 10 mM HCl and then labelling with the various lectins, as previously.

Two different controls for lectin labelling were used: 1) substitution of the respective lectin with TBS alone, and 2) incubation in the lectin solution with the addition of the appropriate inhibitory sugar (concentrations are in Table 1). Positive controls were included from different regions of the digestive system from two amphibians, *Pseudepidalea lineata* (formerly known as *Bufo viridis*) and *Triturus carnifex*, whose mucins were shown in previous studies to bind to the lectins tested (Liquori et al., 2002; Liquori et al., 2007).

Immunohistochemistry

Rehydrated sections were microwave pre-treated in pH 6.0 citrate buffer for three five-minute cycles for antigen retrieval (Gown et al., 1993). For the α-subunit of H^+/K^+-ATPase, the primary antibody was a rabbit antiserum against the C-terminus of the pig H^+/K^+-ATPase α-subunit (Chemicon International, Temekula, California, USA). The secondary antibody was anti-Alexa 488-labelled goat anti-rabbit IgG (Molecular Probes, Eugene, Oregon, USA). For the β-subunit of H^+/K^+-ATPase, the primary antibody was a mouse antiserum against a deglycosylated core peptide of pig H^+/K^+-ATPase β-subunit (Novus Biologicals, Littleton, Connecticut, USA). The secondary antibody was anti-Alexa 568-labelled goat anti-mouse IgG (Molecular Probes, Eugene, Oregon, USA). Rehydrated sections were incubated in blocking buffer (1% normal goat serum in TBS) for 30 min at room temperature, and then incubated overnight at 4°C with the primary antibody diluted 1:500. After washing in TBS, the sections were incubated with the secondary antibody (1:500) in the dark for 5 hours at room temperature, washed in PBS, and mounted in 70% glycerin in TBS. Controls were performed by omitting the primary antibody.

Each experiment was repeated twice on tissues taken from the three different animals giving, a total of six replicates. Staining/labelling in each experiment was assessed by at least two independent

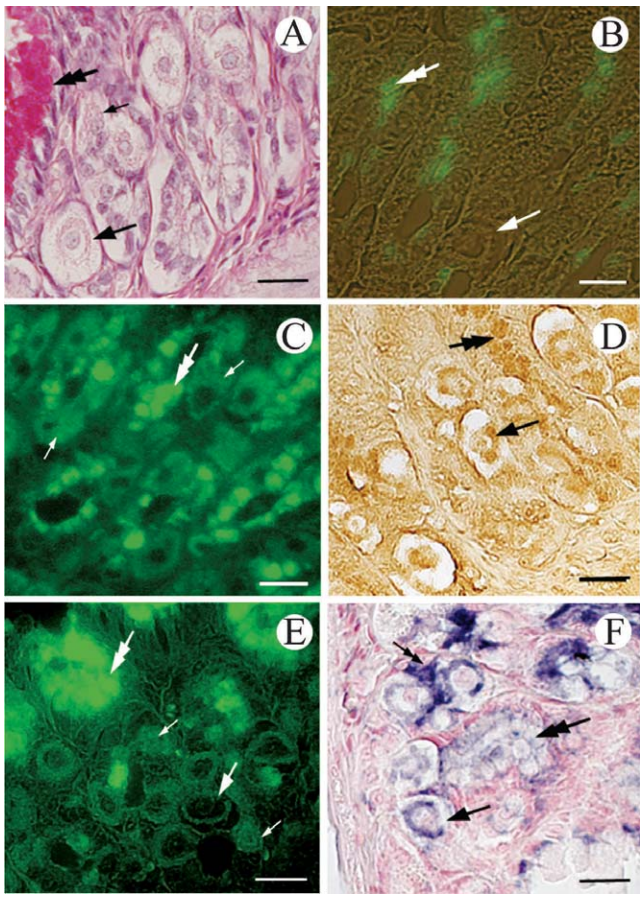

Fig 1. Gastric glands of *Rhinolophus ferrumequinum* stained by different histochemical and lectin-binding methods. **(A)** PAS-hemallume. **(B)** UEA-FITC. **(C)** ConA-FITC. **(D)** SBA-peroxidase. **(E)** BSI-B4-FITC. **(F)** AAA-phosphatase. Small arrows, parietal cells with the diffuse pattern of staining/binding; large arrows, parietal cells with the perinuclear-ring pattern of staining; large, double arrows, muciparous gland cells; small, double arrow, muciparous neck cell. Scale bars: 20 μm.

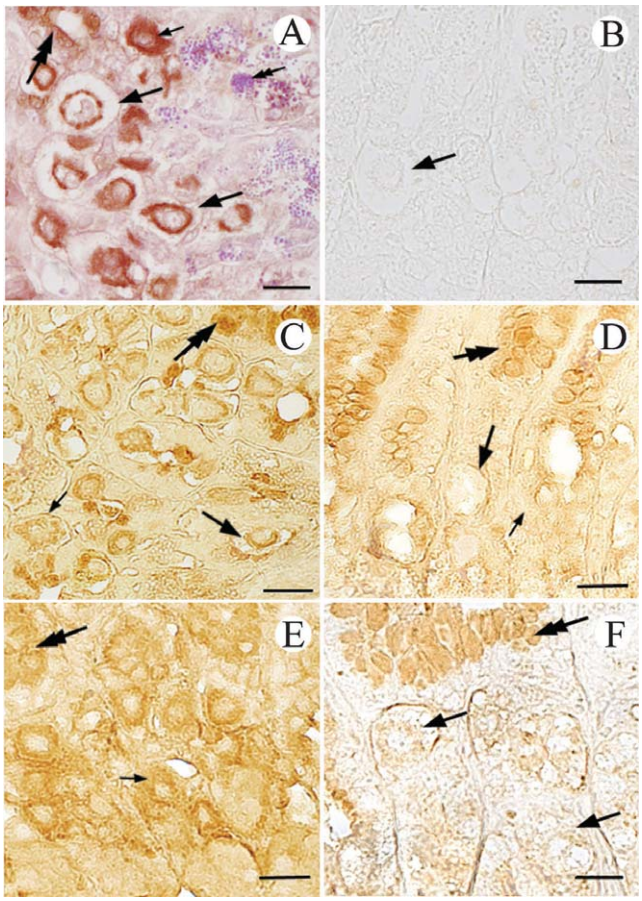

Fig 2. Gastric glands of *Rhinolophus ferrumequinum* stained by different lectin-binding methods. **(A)** DBA-peroxydase-Bowie. **(B)** Negative control for DBA. **(C)** PNA-peroxydase. **(D)** β-elimination PNA-peroxydase. **(E)** WGA-peroxydase. **(F)** β-elimination WGA-peroxydase. Small arrows, parietal cells with the diffuse pattern of staining/binding; large arrows, parietal cells with the perinuclear-ring pattern of staining; large, double arrows, muciparous gland cells; small, double arrow, chief cell. Scale bars: 20 μm.

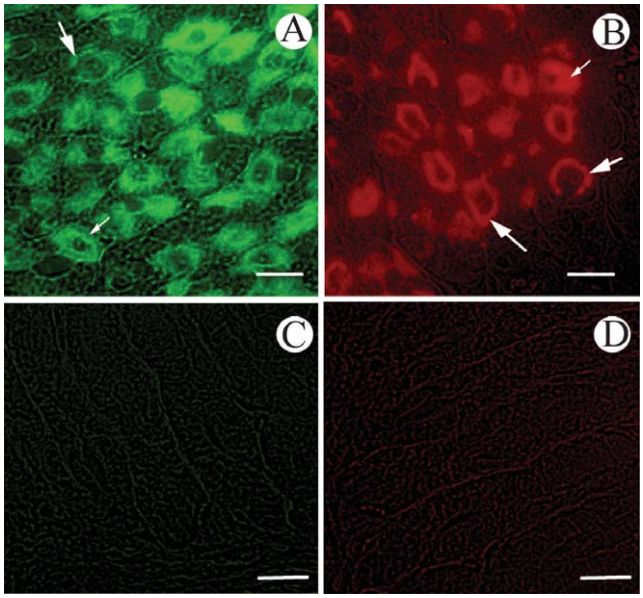

Fig. 3. Gastric glands of *Rhinolophus ferrumequinum* immunostained with anti-H^+,K^+-ATPase. **(A)** Anti-α-H^+,K^+-ATPase. **(B)** Anti-β-H^+,K^+-ATPase. **(C)** Negative control for anti-α- H^+,K^+-ATPase. **(D)** Negative control for anti-β- H^+,K^+-ATPase. Small arrow, parietal cell with the diffuse pattern of staining/binding; large arrow, parietal cell with the perinuclear-ring pattern of staining. Scale bars: 20 μm.

observers and scored as positive (+), moderately positive (–/+), or negative (–), according to the staining intensity. Images were captured with a Nikon Eclipse E600 photomicroscope equipped with a DMX 1200 digital camera (Nikon, Kawasaki, Japan).

RESULTS

Histology

The gastric glands of *R. ferrumequinum* were tubular, simple, or branched and opened at the bottom of gastric pits. They comprised six cell types: 1) surface mucous cells; 2) foveolar mucous cells; 3) mucous neck cells; 4) chief cells; 5) parietal cells, and 6) endocrine cells. Parietal cells were present in both the oral and aboral portions of the fundus, whereas chief cells were lacking in the aboral fundus.

Histochemistry

The parietal cells were moderately positive to PAS and negative to AB pH 2.5, AB pH 1.0, and Bowie's staining. In several parietal cells, PAS-positive glycoconjugates were concentrated in a ring surrounding the nucleus, whereas in others a rather uniform intracellular distribution was observed (Fig. 1A). No differences were observed in the parietal cells between the oral and the aboral fundus.

Lectin histochemistry

Parietal cells were negative to LTA- and UEA-I-binding (Fig. 1B); moderately positive to ConA (Fig. 1C), SBA (Fig. 1D), and BSI-B4 (Fig. 1E); and strongly positive to AAA (Fig. 1F), DBA (Fig. 2A), PNA (Fig. 2C), and WGA (Fig. 2E). The β-elimination pre-treatment suppressed affinity for both PNA (Fig. 2D) and WGA (Fig. 2F). Similarly to the PAS staining, the lectins bound to the parietal cells in two alternative patterns, either a rather uniform or a perinuclear pattern. A mainly uniform, diffuse staining pattern was observed for ConA and WGA, whereas SBA and AAA labelled cells mostly in the perinuclear-ring pattern. In the binding of DBA, PNA, and BSI-B4, about half the parietal cells exhibited the diffuse pattern, and the other half the perinuclear pattern. No labelling differences were observed between parietal cells of the oral and the aboral fundus.

Table 2. Intensity and patterns of binding of the tested lectins to parietal cells. Intensity: ––, negative; –+, moderate; ++, strong. Pattern: *, observed.

Lectin	Intensity	Pattern	
		Perinuclear	Diffused
Con A	–+		*
WGA	++		*
β elimination-WGA	––	–	–
SBA	–+	*	
PNA	++	*	*
β elimination-PNA	––	–	–
LTA	––	–	–
DBA	++	*	*
AAA	++	*	
UEA I	––	–	–
BSI-B4	–+	*	*

Immunohistochemistry

Only the parietal cells were positive to both α- and β-subunit H^+/K^+-ATPase immunostaining (Fig. 3A, B). Similarly to the PAS and lectin binding, two main alternative patterns were observed, uniform and perinuclear. No differences were observed between the oral and the aboral fundus.

Controls

Substitution of the respective lectin with TBS alone and incubation in the lectin with the addition of the appropriate inhibitory sugar resulted in no staining or non-specific staining. No binding with either anti-α or anti-β H^+,K^+-ATPase resulted when the primary antibody was omitted.

DISCUSSION

The organization of the gastric glands of *R. ferrumequinum* and the composition of the secretion of the muciparous and chief cells have been reported elsewhere (Scillitani et al, 2005, 2007), so they will be not discussed here.

In the parietal cells of *R. ferrumequinum*, PAS-staining, lectin binding, and immunostaining of both the α- and β-H^+,K^+ATPase subunits were distributed in two alternative patterns, perinuclear and diffuse. Similar patterns have been observed in several mammals (e.g., Kessimian et al., 1986; Fischer, 1987; Okamoto and Forte, 1988; Baintner et al., 2000) and can be explained by the compartmentalization of the membranes. The apical membrane invaginates into secreting canaliculi that run around the nucleus, lined by long microvilli, whereas the smooth endoplasmic reticulum forms a tubulovesicular system extending into the cytoplasm (Ito and Winchester, 1963). Thus, perinuclear staining indicates that the glycosidic residues and the protonic pump are located near the apical membrane of the canaliculi, whereas the diffuse pattern of staining suggests a cytoplasmic distri-

bution associated with the tubulovesicular system (Okamoto and Forte, 1988; Ogata, 1997). The presence of both patterns for a given staining indicates that the molecules stained can move between the tubulovesicular system and the intracellular canaliculi according to the functional status of the cell.

In parietal cells of the rat, Jiang et al (2001) found two alternative patterns of distribution of H^+,K^+-ATPase and defined them "reticulated" and "diffused", corresponding to our perinuclear and diffuse patterns, respectively. Jiang et al. (2001) associated the reticulated pattern with the condition of active secretion, in which H^+,K^+-ATPase is localized mainly in the intracellular canaliculi, while the diffuse pattern indicates the resting condition, in which H^+,K^+-ATPase concentrates mostly in the tubulovesicular system. Studies on the functional status of gastric secreting cells usually compare laboratory animals in the resting condition induced by starving with the active condition induced by food and/or secretagogues (e.g., Jiang et al., 2001; Mastrodonato et al., 2009). This is difficult to achieve in *R. ferrumequinum* due to problems in keeping captive animals and in being sure of appropriate food consumption (which can be affected by handling stress, acclimation, season of collecting, sudden death of apparently healthy individuals due to metabolic disorders, etc.) (e.g., Barnard, 1995; Gozalo et al., 2005). However, in a previous study (Scillitani et al., 2005), we observed that in the fed condition, as indicated by the presence of food remains in the stomach and duodenum, anti-H^+-K^+ ATPase immunostaining detected parietal cells with both the perinuclear (supposed to indicate an active condition) and the diffuse condition (assumed to indicate the resting condition). We compared the results from lectin histochemistry and immunohistochemistry to learn whether the carbohydrate residues evidenced by lectin binding are part of the β-H^+,K^+-ATPase subunit.

The parietal cells of *R. ferrumequinum* were moderately PAS-positive and not alcianophilic. This can be explained by the presence of neutral carbohydrate residues in the glycoproteins, as observed in other mammals (e.g., Spicer et al., 1978; Goldkorn et al., 1989). Similarly, Kessimian et al. (1986) found in human parietal cells a faint PAS staining in a perinuclear pattern and attributed it to an unidentified glycoprotein related to the secretion of HCl, probably the β-H^+,K^+-ATPase subunit.

Galactosyl/galactosaminyl residues, as demonstrated by the BSI-B4, PNA, and DBA lectins, were distributed in both the diffuse and the perinuclear patterns, similarly to the β-H^+,K^+-ATPase subunit. The BSI-B4 lectin binds α-galactose residues (Wood et al., 1979). These residues are present in the oligosaccharide chains of the β-H^+,K^+-ATPase subunit in rabbit and in rat (Tyagarajan et al., 1997; Stewart et al., 1999), and we hypothesize that the same condition holds for *R. ferrumequinum.* The PNA-lectin binds to galactose β1,3-N-acetylgalactosamine (Lotan and Sharon, 1978). Even if in *R. ferrumequinum* the PNA-bound residues are co-distributed with the β-H^+,K^+-ATPase subunit, they are probably not included in the oligosaccharide chains of the proton pump, because suppression of PNA positivity after β-elimination indicated that most residues are in O-linked glycans, and not in N-linked glycans, which characterize the β-H^+,K^+-ATPase subunit in other mammals (Asano et al., 2000). The lectin DBA binds to N-acetylgalactosamine (GalNAc) residues (e.g., Baker et al., 1983). DBA binding to the parietal cells in the diffuse and the perinuclear patterns in *R. ferrumequinum* indicates that these residues are in the oligosaccharide chains of the β-H^+,K^+-ATPase subunit. Similar DBA-binding residues have also been observed in rat (Fischer, 1987) and in rabbit (Okamoto and Forte, 1988). However, in the latter, Tyagarajan et al. (1997) demonstrated that the β-H^+,K^+-ATPase subunit lacks GalNAc residues, so that it is probably linked to other glycoproteins. Ito et al. (1997) did not observe changes in DBA staining patterns in the canaliculi of human parietal cells between the resting and stimulated conditions, which suggests that there is no relationship between the GalNAc residues and the proton pump. Differing from those bound by the lectin DBA, GalNAc residues bound by the lectin SBA in *R. ferrumequinum* were distributed only in the perinuclear pattern, indicating that they are not linked to H^+,K^+-ATPase. ConA and WGA binding indicated the presence of mannosylic and/or glicosylic and glicosamminylic residues, respectively, distributed only in the diffuse pattern, which suggests that these residues are only in the tubulovesicular system, but not in the proton pump. Similarly to the binding of PNA lectin, WGA positivity disappeared after β-elimination, indicating that the residues are in O-linked rather than N-linked chains. It should be noted that the lectin WGA also binds to Neu5Ac sialic acid residues (Monsigny et al., 1980), but the presence of the latter in the parietal cells of *R. ferrumequinum* can be excluded because of the lack of alcianophily. ConA and WGA positivity only in the diffuse pattern is seen also in human (Kessimian et al., 1986), cow (Sommer et al., 2001), and rabbit (Okamoto and Forte, 1988) parietal cells. In the last, the oligosaccharide chains of the β-H^+,K^+-ATPase subunit contain several mannosylic and glycosamminylic residues (Tyagarajan et al., 1997), so it is unusual that the perinuclear binding pattern was not observed. A possible explanation was given by Ogata (1997), who hypothesized that β-H^+/K^+-ATPase undergoes a masking and/or activating process, moving from one compartment to another, and that this could affect lectin binding to some residues.

The intense AAA staining indicated the presence of fucosylated residues, even though no binding was observed with lectins UEA-I or LTA. All three of these lectins recognize fucosylated residues, but their binding varies in relation to the links between fucose (Fuc) and its neighbors (e.g., Spicer e Schulte, 1992). The major binding affinity of AAA is to Fuc (1,6)-linked to the proximal GlcNAc of the N-linked glycans, as well as to the terminal Fuc with a (1,3) or (1,4) linkage, whereas LTA and UEA I have higher affinity for Fuc with a (1,2) linkage (e.g., Alonso et al., 2003). Thus, we assume that the fucosylated residues in the parietal cells of *R. ferrumequinum* are linked to the adjacent residues mainly in the (1,6), (1,3) and (1,4) patterns, and not in the (1,2). In contrast to the glicosyl/glicosamminyl and mannosyl residues, the fucosylated residues are localized only in the intracellular canaliculi, as indicated by the perinuclear binding pattern of AAA. Differing from parietal cells in *R. ferrumequinum,* those in other mammals are usually positive for UEA-binding (e.g., Kessimian et al., 1986; Ito et al., 1985; Baintner et al., 2000; Sommer et al., 2001), except for the Guinea pig (Lueth et al., 2005).

In conclusion, our results indicate that in the parietal cells of *R. ferrumequinum*, galactosyl/galactosaminyl groups are present in both the intracellular canaliculi and the tubulovesicular system, and thus they are probably part of the β-H^+,K^+-ATPase subunit. Glycosyl/glycosaminyl and mannosyl groups concentrate in the tubulovesicular system, and fucosylated residues are found in the intracellular canaliculi and are probably not present in the β-H^+,K^+-ATPase subunit but in other glycoproteins.

The oligosaccharide chains of the β-H^+,K^+-ATPase subunit of *R. ferrumequinum* show some features unusual in mammals, such as the presence of GalNAc residues and the lack of (1,2) fucosylated residues, and this confirms the taxon specificity of their sequences. At the state of present (scarce) knowledge, it is rather difficult to hypothesize functional implications for the H^+,K^+-ATPase interspecific heterogeneity, and to relate them to dietary adaptations, rates of food passage, metabolic rates, etc. Hermsen et al. (2000) found that the sensitivity of rat gastric H^+,K^+-ATPase for K^+ is higher than that of pig, and this is primarily due to differences in the β-subunit. Rat β-H^+,K^+-ATPase has terminal α-galactosyl residues in polylactosamine chains; these residues are lacking in pig (Stewart et al., 1999), but it is not clear whether this affects the affinity for K^+. Our data suggest that *R. ferrumequinum* β-H^+,K^+-ATPase has terminal α-galactosyl residues like those in rat, but at present no inferences can be made due to the paucity of comparative data from other bats and details about the endocrine regulation of secretion, which is known to vary among animals (e.g., Machado-Santos et al., 2009). This also obscures whether differences in the gastric proton pump are responsible for variation in food absorption and metabolic rates, which are generally higher in bats than in other mammals (e.g., Altringham, 1996). Further studies on the molecular sequences of the oligosaccharide chains will support our histochemical findings and clarify their functional implications, possibly confirming that sequences vary among animals, and with adaptation in feeding habits and/or metabolic rates.

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