The β-Subunit of the Rabbit H,K-ATPase: A Glycoprotein with All Terminal Lactosamine Units Capped with α-Linked Galactose Residues†

Kamala Tyagarajan,‡ R. Reid Townsend,§ and John G. Forte*,‡

Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-3200, and Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143-0446

Received September 27, 1995; Revised Manuscript Received November 27, 1995⊗

ABSTRACT: The β-subunit of the gastric H,K-ATPase is the most abundant glycoprotein in the tubulovesicular compartment of the acid-secreting parietal cells. The oligosaccharides of the β-subunit have been shown to contain fucose, *N*-acetylglucosamine, mannose, galactose, and *N*-acetylgalactosamine. Previous studies have shown that the rabbit β-subunit is devoid of *N*-acetylneuraminic acid. Here we report the structural features of the *N*-linked oligosaccharides of the β-subunit from rabbit H,K-ATPase. We used glycosidase digestions and analysis by high-pH anion-exchange chromatography with pulsed amperometric detection and matrix-assisted laser desorption/ionization mass spectrometry to analyze the peptide-N^4-(*N*-acetyl-β-D-glucosaminyl)asparagine amidase (PNGase F)- and endo-β-*N*-acetylglucosaminidase H (Endo H)-released oligosaccharides. The studies showed that the oligosaccharides of the β-subunit are a mixture of both oligomannosidic and lactosamine-type structures. The high-mannose structures were identified as $Man_5Man_8GlcNAc_2$ species. A striking finding was that all the branches of the lactosamine-type structures were terminated with Galα→Galβ→GlcNAc extensions. All of the lactosamine-type structures were found to be core fucosylated and some of them contained one to three lactosamine repeats. We propose that a part of the adaptation of the gastric β-subunit to the acidic environment of the stomach is through providing acid-stable terminal residues on the oligosaccharides.

The gastric H,K-ATPase is a P-type cation-transporting ATPase which is responsible for acid secretion in the stomach. The protein is a heterodimer with a catalytic α-subunit and a glycosylated β-subunit which is essential for holoenzyme function (Chow et al., 1992; Tyagarajan et al., 1995). The β-subunit is a protein of ∼291 amino acids with seven potential sites for *N*-glycosylation (Canfield et al., 1990; Reuben et al., 1990; Shull, 1990). These sequons are conserved in all species except for pig, which lacks the Asn^{103} sequon (Toh et al., 1990). The glycoprotein migrates as a broad 60−80 kDa band in SDS−PAGE gels. On deglycosylation with PNGase F[1] a sharp 32 kDa band is seen. Treatment with Endo H results in no apparent change in migration of the 60−80 kDa band (Okamoto et al., 1990; Chow et al., 1993).

Histochemical studies using fluoresceinated lectins demonstrated an abundance of glycoconjugates in the tubulovesicular and apical membranes of the parietal cells, which are involved in HCl secretion. The H,K-ATPase enriched tubulovesicles were shown to be heavily stained by wheat germ (WGA), *Helix pomatia* (HPA), and *Ricinus communis* I (RCA) lectins (Okamoto & Forte, 1988). The β-subunit of H,K-ATPase was subsequently identified as the most abundant glycoprotein in gastric tubulovesicles (Callaghan et al., 1990). Its reactivity and purification with WGA, RCA I (Okamoto et al., 1990), and tomato lectins (Callaghan et al., 1990) suggested that the protein is glycosylated with complex and polylactosamine-type oligosaccharide structures. Because the protein did not react with concanavalin A and was insensitive to Endo H, it was previously concluded that the β-subunit did not contain oligomannosidic chains (Okamoto et al., 1990; Chow & Forte, 1993; Hall et al., 1990). Monosaccharide analysis has demonstrated the presence of Man, Gal, GlcNAc, GalNAc, and Fuc but no NeuAc or NeuOGc (Beesley & Forte, 1973; Weitzhandler et al., 1993). It has been suggested that the oligosaccharides of the β-subunit may be sialylated during biosynthesis but lose sialic acid in the acidic environment of the stomach (Goldkorn et al., 1989). Further, it has been proposed that the inhibition of acid secretion is accompanied by sialylation of parietal cell membrane glycoprotein (Usomoto et al., 1994).

In this study, we further investigated the complete absence of sialic acid on the β-subunit by structural analyses of the oligosaccharide chains on the rabbit β-subunit of the H,K-ATPase. Oligosaccharides were completely released from the purified β-subunit by PNGase F digestion. Structural information was obtained by exoglycosidase digestions with analysis using high-pH anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Molecular weights of the released oligosaccharides were determined using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). The studies demonstrated that the

† This work was supported by NIH Grant DK 38972 and the Biomedical Research Technology Program of the National Center for Research Resources, NIH NCRR BRTP 01614.

* Address correspondence to this author at 241 LSA, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200. Tel: (510) 642-1544. FAX: (510) 643-6791.

‡ University of California, Berkeley.

§ University of California, San Francisco.

⊗ Abstract published in *Advance ACS Abstracts,* February 15, 1996.

[1] Abbreviations: PNGase F, peptide-N^4-(*N*-acetyl-β-D-glucosaminyl)asparagine amidase; Endo H, endo-β-*N*-acetylglucosaminidase H; Endo F, endo-β-*N*-acetylglucosaminidase F; WGA, wheat germ agglutinin; HPA, *Helix pomatia* agglutinin; Con A, concanavalin A; RCA, *Ricinus communis* agglutinin; NeuAc, *N*-acetylneuraminic acid; NeuGc; *N*-glycolylneuraminic acid; HPAEC-PAD, high-pH anion-exchange chromatography with pulsed amperometric detection; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; 2-AB, 2-aminobenzamide; DHB, 2,5-dihydroxybenzoic acid; t_r, retention time.

0006-2960/96/0435-3238$12.00/0

oligosaccharides of rabbit gastric β-subunit are oligomannosidic structures ($Man_5GlcNAc_2$-$Man_8GlcNac_2$) and core-fucosylated bi-, tri-, and tetraantennary lactosamine-type oligosaccharides with all branches terminated by Galα→Galβ→GlcNAc extensions. Several of the oligosaccharides have one to three lactosamine repeats. We propose that a part of the adaptation of the gastric β-subunit to the acidic environment of the stomach is through providing alternative acid-stable terminal residues on the oligosaccharides.

MATERIALS AND METHODS

Materials. Glass-distilled water was used for the preparation of all buffers and eluents. Sodium hydroxide (50% solution) and acetic acid were from Fisher Scientific (Pittsburgh, PA). PNGase F (glycerol free) was provided by Dr. Tony Tarentino (New York Department of Health, Albany, NY). Chicken liver fucosidase, sialidase from *Arthrobacter ureafaciens*, jack bean α-mannosidase, and coffee bean α-galactosidase were from Oxford Glycosystems (Abingdon, U.K.). Endoglycosidase H and β-galactosidase were purchased from Boehringer Mannheim (Indianapolis, IN). Endo-β-galactosidase from *Escherichia freundii* was kindly provided by Dr. Michiko Fukuda (La Jolla Cancer Research Center, La Jolla, CA). The asialo-biantennary (C2-024300)[2] and agalacto-biantennary fucosylated (C2-004301) standard oligosaccharides were from Oxford Glycosystems (Abingdon, U.K.). DHB matrix was purchased from Hewlett Packard (Palo Alto, CA). A low-molecular weight calibration set was purchased from Bio-Rad (Richmond, CA). Adrenocorticotropic hormone fragment (18−39) was purchased from Sigma (St. Louis, MO). Bovine fetuin oligosaccharides were prepared as previously described (Hardy & Townsend, 1994). The high-mannose oligosaccharides were prepared by Dr. Peter Lipniunas.

Preparation of β-Subunit of H,K-ATPase. H,K-ATPase-containing gastric microsomes were isolated from rabbit stomach as previously described (Reenstra & Forte, 1990). Crude microsomes were harvested from homogenized mucosa of unstimulated rabbit stomach (H_2 receptor-blocked) as the membrane pellet sedimenting between 13 000*g* for 10 min and 100 000*g* for 1 h. The pellet was resuspended in 10% sucrose, brought to 40% sucrose (9 mL), and overlaid with successive layers of 30% sucrose (11 mL) and 10% sucrose (16 mL) [all sucrose solutions with 5 mM tris(hydroxymethyl)aminomethane (Tris) and 0.2 mM EDTA, pH 7.4] in a 37 mL tube. After centrifugation at 100 000*g* for 4 h, the purified gastric microsomes were collected from the interface between 10% and 30% sucrose.

The β-subunit of gastric H,K-ATPase was isolated from sucrose density purified rabbit microsomes according to the protocol of Okamoto and co-workers (1990) by WGA affinity chromatography in the presence of dodecyl trimethyl ammonium bromide. The WGA-binding fraction was resuspended in sodium phosphate (10 mM, pH 7.0) and quantitated by Bradford assays, and the purity was checked by SDS−PAGE gels followed by Coomassie Blue staining. The staining revealed the presence of a dominant band from 60−80 kDa and a band at 120−160 kDa. Both bands immunostained with a β-subunit specific monoclonal antibody, hence the upper band was presumed to be a dimer of the β-subunit.

Release of β-Subunit Oligosaccharides. To release all the *N*-linked oligosaccharides, 8 nmol (275 μg of protein) of purified β-subunit of rabbit H,K-ATPase was incubated at 37 °C for 18 h with 100 munits of PNGase F (glycerol free) in a sodium phosphate buffer (10 mM, pH 7.5) in a total volume of 280 μL. This mixture is referred to as β-subunit PNGase F digest. The completeness of oligosaccharide release was determined by conversion of the 60−80 kDa band to a sharp 32 kDa core protein.

A milliunit of PNGase F activity is defined as the amount of enzyme required to hydrolyze one nanomole of a pentaglycopeptide from bovine fetuin per minute at 37 °C (Plummer et al., 1987).

To release *N*-linked oligomannosidic structures, β-subunit (1.5 nmol) from rabbit H,K-ATPase was incubated with 2 munits of Endo H in a sodium acetate buffer (25 mM, pH 5.5) at 37 °C in a total volume of 50 μL.

Glycosidase Treatment of Oligosaccharides. Separate aliquots (12 μL, ∼345 pmol of protein) of the β-subunit PNGase F digest were treated for 18 h at 37 °C with one of the following glycosidases: (1) β-galactosidase from bovine testes (15 munits) in a sodium acetate buffer (50 mM, pH 5.0) to a final volume of 50 μL; (2) α-galactosidase from coffee beans (50 munits) in a sodium citrate phosphate buffer (100 mM, pH 6.0) to a final volume of 50 μL; (3) α-mannosidase from jack bean (20 munits) in a sodium acetate buffer (50 mM, pH 5.0) to a final volume of 50 μL; (4) chicken liver fucosidase (50 munits) to a final volume of 70 μL; (5) endo-β-galactosidase (5 munits) from *E. freundii* in water to a final volume of 70 μL.

An aliquot of α-galactosidase-treated β-subunit oligosaccharides (12.5 μL) was treated with 6 munits of β-galactosidase to a final volume of 20 μL.

Derivitization of Oligosaccharides with 2-Aminobenzamide. The reducing oligosaccharides were coupled to 2-AB as previously described (Bigge et al., 1995). Briefly, 12 μL of β-subunit PNGase F digest was dried. The oligosaccharides were dissolved in 5 μL of a solution of 2-AB (0.35 M) in dimethylsulfoxide/glacial acetic acid (30% v/v) sodium cyanoborohydride (1 M). The glycan solution was then incubated at 65 °C for 2 h. After the conjugation with 2-AB, the reaction mixture was applied to a cellulose disk (1 cm in diameter) in a glass holder. The disk was washed 5 times with 1 mL of acetonitrile to remove unreacted dye and nonreactive oligosaccharide reactants. Labeled glycans were then eluted using two washes (0.5 mL) of water and then filtered (0.2 μm) prior to analysis. The solution was dried and resuspended to 100 μL.

Oligosaccharide Analyses. Oligosaccharides were analyzed using HPAEC-PAD on a Dionex Glycostation with a pulsed amperometric detector as previously described (Hardy & Townsend, 1994), except that the acetate-containing eluent was prepared using acetic acid and the pH was adjusted to 5.5 with 50% NaOH (Anumula & Taylor, 1991). A Carbo Pac PA-100 column (4 × 250 mm) with an eluent flow rate of 1 mL/min was used for all the analyses. Elution conditions were produced using water (eluent A), 200 mM NaOH (eluent B, prepared from a 50% NaOH solution), and 0.5 M sodium acetate, pH 5.5 (eluent C). The gradient consisted of 5 min of isocratic elution with 100 mM NaOH (50% eluent A) and 10 mM sodium acetate (2% eluent C).

[2] The nomenclature is that previously proposed for N-linked glycans (Hermentin et al., 1991).

After 5 min, a linear acetate gradient was developed over 60 min to a limit of 125 mM of sodium acetate, while the concentration of sodium hydroxide was maintained at 100 mM. The pulsed amperometric detector sensitivity was set to 300 nA (attenuation full scale), and the pulse potentials were as follows: E1 = +0.05 V, t_1 = 480 ms; E2 = +0.6 V, t_2 = 120 ms; E3 = −0.6 V, t_3 = 60 ms. The time constant was set to 3 s. Data were collected and analyzed using the Dionex Glycostation software, and the chromatographic profiles were exported as "*X, Y*" data files using the "Optimize" module in the software. The retention times were also determined using the "Optimize" module in the software.

Matrix-Assisted Laser-Desorption Mass Spectrometry. The total 2-AB labeled oligosaccharides of β-subunit were dissolved in 100 μL. A 1 μL amount was mixed with 1 μL of 2,5-dihydroxybenzoic acid matrix. The admixture (1 μL) (oligosaccharides from ~1.5 pmol of protein) was spotted onto a stainless steel target and dried in the dark at room temperature. The MALDI mass measurements were carried out on a TOFSpec SE from Fisons, equipped with a reflectron and using a nitrogen laser (337 nm). An accelerating potential of 25 kV, a reflectron voltage of 28.5 kV, and an extraction voltage of 10 kV in the reflectron ion mode were used. Typically about 50 laser shots were averaged. The instrument was calibrated with peptides from a low molecular weight calibration set. MH^+ ions of bombesin and the 18−39 clip of adrenocorticotropic hormone fragment (18−39) at *m/z* 1665.9 and 2465.2, respectively, were used as calibration standards.

RESULTS

Analysis of the Oligosaccharides of the Rabbit β-Subunit Using HPAEC-PAD. Figure 1 shows the chromatogram of the oligosaccharides released by PNGase F digest from the gastric β-subunit. The profile reveals that β-subunit oligosaccharides are a mixture of at least 16 different structures. The retention times (t_r) of the oligosaccharides are given in Table 1. Peaks that eluted at a t_r < 16 min (elution position of an agalacto-biantennary fucosylated oligosaccharide) are due to non-carbohydrate, electrochemically active analytes. The base line distortion with a $t_r \approx 19$ min was seen consistently in the HPAEC-PAD chromatograms of the β-subunit oligosaccharides. For the purpose of description the most reproducible and identifiable peaks are numbered from **1** to **16** as shown in Figure 1. These peaks eluted in the region of t_r = 16−40 min. In addition, peaks were frequently seen at a t_r = 19 min (between peaks **1** and **2**) but were not well resolved in some runs. A split peak between peaks **2** and **3** was often observed, but in some runs this peak appeared as a singlet.

On comparison with the retention time of different *N*-linked oligosaccharides standards under similar gradient conditions, it was found that all the *N*-linked β-subunit oligosaccharides included in peaks **1**−**16** eluted earlier than a disialylated biantennary oligosaccharide from bovine fetuin (t_r = 54 min, labeled B in Figure 1) suggesting that β-subunit oligosaccharides were neutral, asialo complexes. Peak **1** had a similar t_r as that of an agalacto-biantennary fucosylated oligosaccharide standard (C2-004301) (t_r = 16 min, labeled A in Figure 1), while the retention time of the remaining β-subunit oligosaccharides (peaks numbered **2**−**16**) was greater than an asialo-biantennary complex structure (C2-024301) (t_r = 21 min) in the same gradient. The profile and elution positions of *N*-linked oligosaccharides thus suggests that β-subunit oligosaccharides are a mixture of

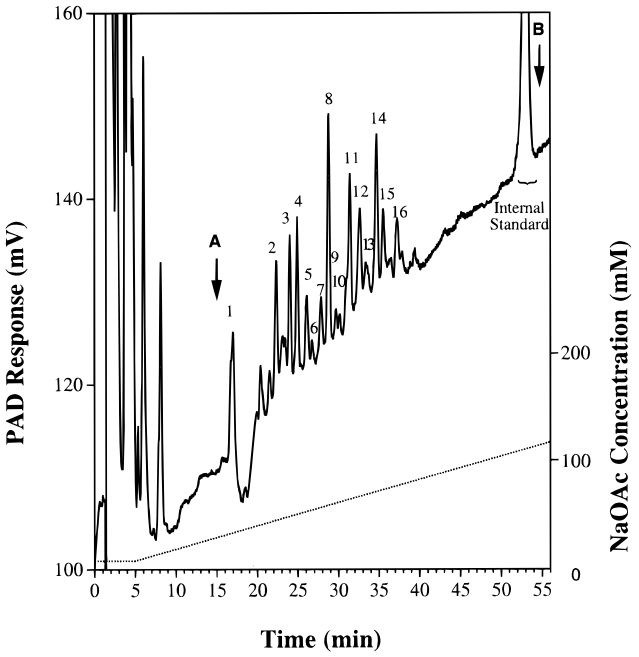

FIGURE 1: PNGase F-released oligosaccharides from the gastric β-subunit. The β-subunit PNGase F digest (6 μL) as described under Materials and Methods was diluted with water (194 μL), injected (150 μL) into the chromatograph, and analyzed using HPAEC-PAD. The linear sodium acetate gradient is shown by the dashed line. The trace represents oligosaccharides from ~130 pmol of protein. The major oligosaccharide peaks eluting after ~10 min were numbered **1**−**16**. The peak eluting at a $t_r \approx 53$ min is *N*-glycolylneuraminic acid, which was used as an internal standard. The elution positions of an agalacto-biantennary, core-fucosylated oligosaccharide standard (C2-004301) and the disialylated oligosaccharide from fetuin are indicated by arrows **A** and **B**.

Table 1: Retention Times of β-Subunit Oligosaccharides before and after Fucosidase Digestion[a]

peak no.	retention time (− fucosidase)	retention time (+ fucosidase)
1	17.0	17.2
2	22.3	22.8
3	24.0	24.5
4	24.9	25.5
5	26.1	na[b]
6	26.7	na
7	27.9	na
		28.1
		28.6
8	28.8	30.5
9	29.7	na
10	30.2	32.6
11	31.4	33.4
12	32.7	34.6
13	33.4	35.7
14	34.7	37.1
15	35.5	37.8
16	37.2	39.8

[a] The β-subunit oligosaccharides were digested with chicken liver fucosidase and analyzed by HPAEC-PAD as detailed for Figure 6, and the retention times of the individual peaks were determined as described in Materials and Methods. The peak numbers correspond to those of the peak labels shown in trace A of Figure 6. The retention times between the two chromatograpic runs were compensated for using the internal *N*-glycolylneuraminic acid standard that elutes at ~53 min. [b] Not assigned.

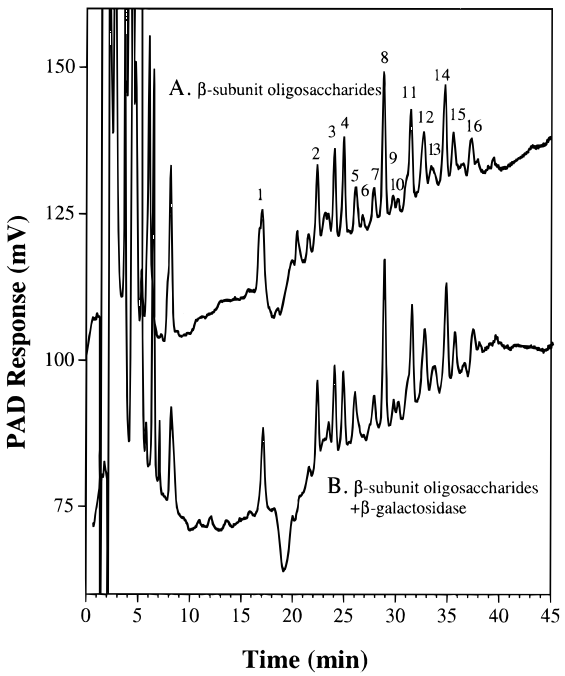

FIGURE 2: Profiles of β-subunit oligosaccharides before and after treatment with β-galactosidase. Trace A: Intact β-subunit oligosaccharides using HPAEC-PAD. Oligosaccharide peaks are labeled as in Figure 1. Trace B: The β-subunit PNGase F digest was treated with β-galactosidase as described under Materials and Methods. An aliquot of this digest (25 μL) was diluted with water (175 μL), injected (150 μL) into the chromatograph, and analyzed by HPAEC-PAD. Both traces are oligosaccharides from ~130 pmol of protein.

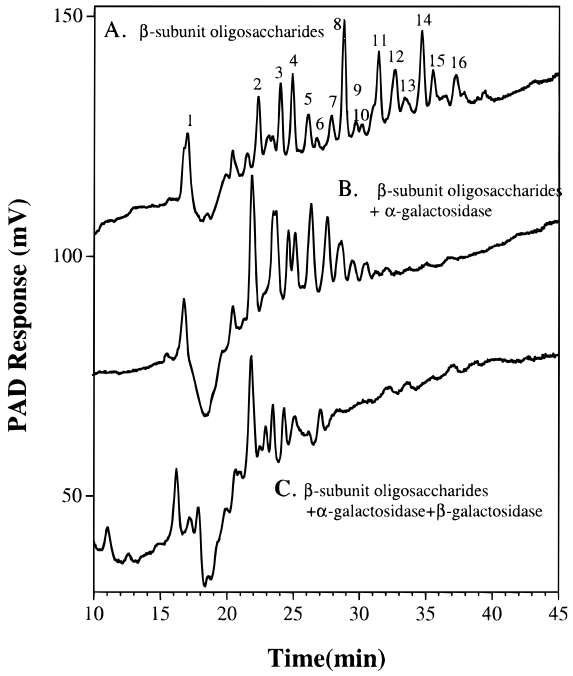

FIGURE 3: Susceptibility of β-subunit oligosaccharides to α-galactosidase. Trace A: Analysis of β-subunit oligosaccharides using HPAEC-PAD. Oligosaccharide peaks are labeled as in Figure 1. Trace B: The β-subunit PNGase F digest was treated with α-galactosidase as described under Materials and Methods. An aliquot (25 μL) of this digest was diluted with water (175 μL), injected (150 μL) into the chromatograph, and analyzed by HPAEC-PAD. Trace C: The β-subunit PNGase F digest was first treated with α-galactosidase and then with β-galactosidase as described under Materials and Methods. The entire sample (20 μL) was diluted with water (180 μL) and injected (150 μL) into the chromatograph. Traces A and B are oligosaccharides from ~130 pmol of protein, and trace C represents oligosaccharides from ~ 65 pmol of protein.

neutral, non-sialylated structures. Comparison of the HPAEC-PAD *N*-linked oligosaccharide profile from two different rabbits of the same age demonstrated that the two traces were very similar (data not shown).

The mixture of β-subunit oligosaccharides was next subjected to treatment with different exoglycosidases and then reanalyzed by HPAEC-PAD. The purity and specificity of the exoglycosidases used in these studies was established using HPAEC-PAD (Tyagarajan et al., 1996). We included *N*-glycolylneuraminic acid (NeuOGc) as an internal standard which had a unique elution (t_r = 53 min). Typically, we observed shifts of 2−3 min, and the internal standard was used to normalize chromatographic runs.

Treatment with Sialidase and β-Galactosidase. Previous composition analysis showing the absence of NeuAc (Weitzhandler et al., 1993) was confirmed by treating the β-subunit oligosaccharides with the linkage nonspecific sialidase from *A. ureafaciens*. The profile of the PNGase F released oligosaccharides when analyzed by HPAEC-PAD after treatment with sialidase was not changed (data not shown). In the absence of sialic acid, structures may terminate in Galβ(1→3)GlcNAc and Galβ(1→4)GlcNAc units (Yamashita et al., 1989; Mizuochi et al., 1988; Parekh et al., 1989). β-Galactosidase from bovine testes cleaves Galβ(1→3)GlcNAc and Galβ(1→4)GlcNAc linkages at the terminal end of glycoconjugates (Distler & Jourdian, 1973). We used this β-galactosidase to ascertain whether β-subunit oligosaccharides were terminated with β-Gal residues. Figure 2 shows the HPAEC-PAD profile of the β-subunit oligosaccharides before and after digestion with β-galactosidase. The retention times of all but one of the oligosaccharide peaks were relatively unchanged after digestion (traces A and B). Peak **6** (t_r = 26 min) disappeared upon treatment, indicating that a very minor proportion of the oligosaccharides have terminal β(1→4)- or β(1→3)-linked Gal residues. Positive controls showed the β-galactosidase preparation to be active (data not shown).

Digestion with α-Galactosidase. We next tested whether the oligosaccharide chains were terminated with α-linked Gal residues as has been found in oligosaccharide chains of other mammalian species (Eckhardt & Goldstein, 1983; Spiro & Bhoyroo, 1984; Santer et al., 1989; Anderson et al., 1985). We treated the oligosaccharides with coffee bean α-galactosidase which releases α-Gal residues linked either α(1→3), α(1→4) or α(1→6) to oligosaccharides (Courtois & Petek, 1966; Harpaz et al., 1975). Figure 3 shows that treatment with α-galactosidase produced a disappearance of peaks **11**−**16** and a dramatic change in the profile of earlier eluting peaks, indicating that peaks **11**−**16** contain α-Gal termini. (Figure 3, traces A and B). Due to the coelution of the product peaks in the region of 20−30 min, it was difficult to ascertain whether peaks **2**−**10** also contained α-Gal linkages.

Figure 3 also shows the sequential digestion of β-subunit oligosaccharides with α-galactosidase and then with β-galactosidase (trace C). The profile is further simplified after this treatment with all the oligosaccharides now eluting with t_r < 28 min (traces B and C). Disappearance of peaks **8**−**10** on sequential digestion confirms that in addition to peaks **11**−**16** they also contain Galα(1→3, 4, or 6)Galβ-(1→3, 4) extensions. Thus, removal of α-Gal termini on β-subunit oligosaccharides resulted in the exposure of

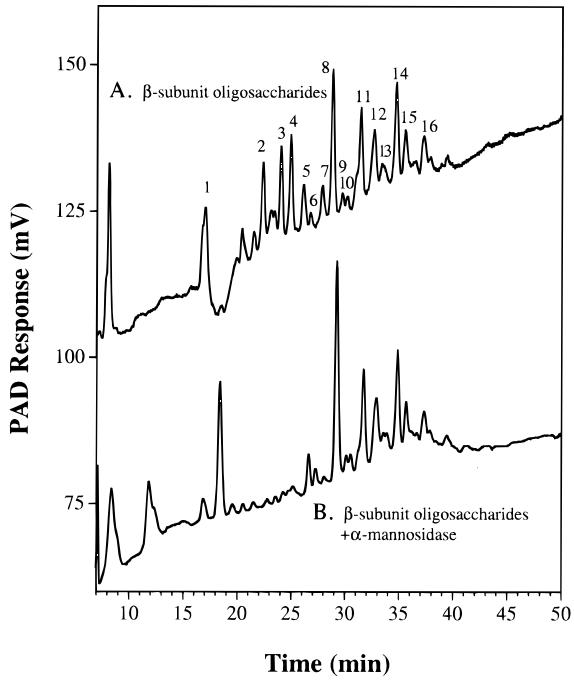

FIGURE 4: Analysis of α-mannosidase digestion of *β*-subunit oligosaccharides. Trace A: Analysis of *β*-subunit oligosaccharides using HPAEC-PAD. Oligosaccharide peaks are labeled as in Figure 1. Trace B: The *β*-subunit PNGase F digest was treated with α-mannosidase as described under Materials and Methods. An aliquot of this digest was next diluted with water (175 *μ*L) and injected (150 *μ*L) into the chromatograph. Trace A represents oligosaccharides from ∼130 pmol of protein, while B represents oligosaccharides from ∼180 pmol of protein.

β-Gal residues that are susceptible to *β*-galactosidase.

Treatment with α-Mannosidase and Endo H. Treatment of the *β*-subunit oligosaccharides with α-galactosidase and/or *β*-galactosidase did not appear to significantly alter the retention times of peaks **1**−**4**. It was also difficult to ascertain whether the peaks eluting from $t_r \approx 26-28$ min changed in shape or profile. We speculated that some of these oligosaccharides could be high-mannose or hybrid oligosaccharides. *β*-Subunit oligosaccharides were next treated with jack bean α-mannosidase, which cleaves Manα-(1→2), Manα(1→6) or Manα(1→3) linkages on oligosaccharides (Li & Li, 1972). Figure 4 shows the analysis of the α-mannosidase digest. Treatment of the *β*-subunit oligosaccharides with α-mannosidase resulted in an almost complete disappearance of peaks **2**−**4** ($t_r \approx 22.3-24.9$). Also peak **7** diminished in intensity, while peaks **5**, **6**, and **8**−**16** were unaffected by the enzyme treatment (traces A and B). From the difference in the base line at $t_r \approx 17.5$ min in traces A and B, the effect of α-mannosidase digestion on peak **1** could not be determined, and the differences in the base lines were concluded to be due to inter-run variability. These results demonstrate that at least four *β*-subunit oligosaccharides contain terminal α-Man residues and hence must be either of the oligomannosidic or the hybrid type.

To verify the presence of high-mannose or hybrid oligosaccharides, intact *β*-subunit was digested with Endo H. Figure 5 shows the analysis of the released oligosaccharides after Endo H treatment. A minor peak (A) and three major peaks (B−D) (one with a shoulder) coeluted with oligosaccharide standards from ribonuclease B, $Man_5GlcNAc$, $Man_6GlcNAc$, $Man_7GlcNAc$, and $Man_8GlcNAc$ structures. Peak C with a shoulder coeluted with the $Man_7GlcNAc$ standard,

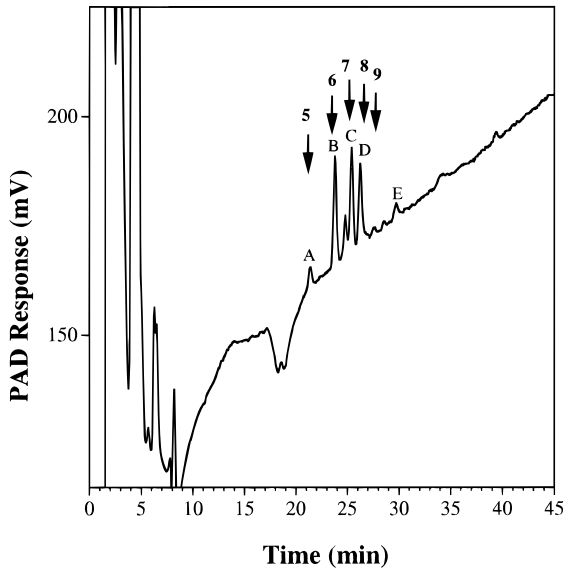

FIGURE 5: Endo H-released oligosaccharides of the *β*-subunit. An aliquot of *β*-subunit Endo H digest as described under Materials and Methods (6 *μ*L) was diluted with water (194 *μ*L) and injected (150 *μ*L) into the chromatograph and analyzed. The arrows designated **5**−**9** refer to the respective elution positions of $Man_5GlcNAc$, $Man_6GlcNAc$, $Man_7GlcNAc$, $Man_8GlcNAc$ and $Man_9GlcNAc$ oligosaccharide standards from ribonuclease B. **A**−**D** denote the *β*-subunit-derived peaks that coelute with the high-mannose standards. The profile represents oligosaccharides released from ∼130 pmol of protein.

suggesting that it is an isomer of the $Man_7GlcNAc$ species. The relative intensities of the peaks in Figure 5 suggest that $Man_6GlcNAc$, $Man_7GlcNAc$, and Man_8GlNAc are the dominant high-mannose species, while only trace amounts of $Man_5GlcNAc$ and no $Man_9GlcNAc$ species were detectable.

Digestion with Fucosidases. The presence of Fuc on the *β*-subunit has previously been shown by composition analysis (Beesley & Forte, 1973; Weitzhandler et al., 1993). We digested *β*-subunit oligosaccharides with the fucosidase from chicken liver which was found to be linkage nonspecific (Tyagarajan et al., 1996). Figure 6 shows the HPAEC-PAD analysis of *β*-subunit oligosaccharides before and after treatment with this fucosidase. Comparison of the retention time of the oligosaccharides reveals that peaks **1**−**4** were similar (with a small $\Delta \approx 0.5$ min) which we attributed to inter-run variability (Table 1). Peaks **2**−**4** were tentatively assigned to $Man_6GlcNAc$-$Man_8GlcNAc$ (see above). Peaks **5** and **6** disappeared, and this region ($t_r \approx 26-28$ min) is now occupied by two closely eluting peaks of greater intensity. The remaining of the peaks, **8**−**16**, were shifted by 1.5−2.0 min after fucosidase digestion (Table 1). It has been shown that removal of an α(1→6) Fuc residue from the chitobiose core results in the delayed elution of the product by 1−2 min (Spellman, 1990). A similar shift of ∼2 min was seen using this gradient when an asialobiantennary core-fucosylated oligosaccharide standard was treated with fucosidase (Table 1). It has been shown that removal of an α(1→2, 3, or 4)-linked Fuc residue results in a much greater increase in retention time of 10−38 min (Hardy & Townsend, 1989). From the small shift in retention time after chicken liver α-fucosidase treatment we conclude that peaks **8**−**16** contain oligosaccharides in a Fuc α(1→6) linkage.

Digestion with Endo-β-galactosidase. Endo-*β*-galactosidase is an enzyme that cleaves polylactosamine structures

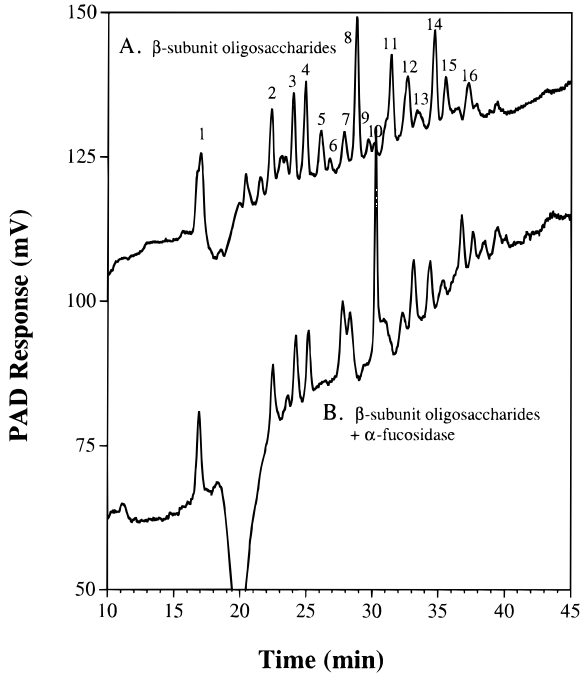

FIGURE 6: Profile after fucosidase digestion of β-subunit oligosaccharides. Trace A: Analysis of β-subunit oligosaccharides using HPAEC-PAD. Oligosaccharide peaks are labeled as in Figure 1. Trace B: β-subunit PNGase F digest was treated with α-fucosidase as described under Materials and Methods. An aliquot of this digest was diluted with water (175 μL) and injected (150 μL) into the chromatograph. Trace A represents the oligosaccharides from ~130 pmol of protein, while trace B represents oligosaccharides from ~90 pmol of protein.

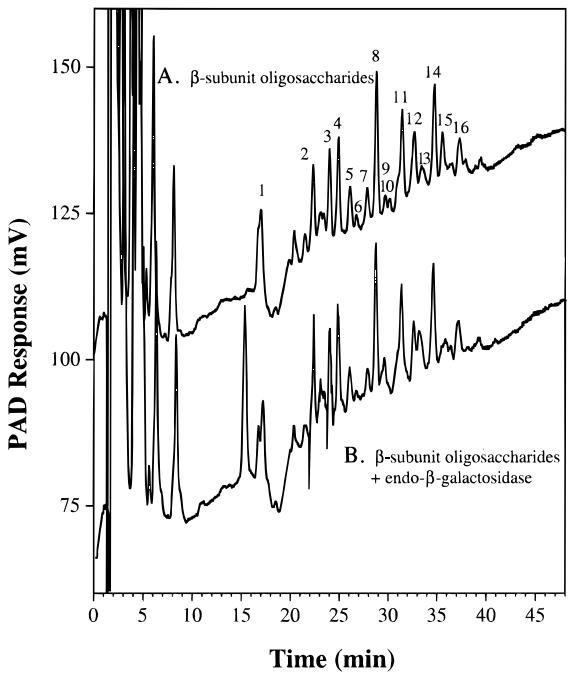

FIGURE 7: HPAEC-PAD analysis of endo-β-galactosidase digestion of β-oligosaccharides. Trace A: Analysis of β-subunit oligosaccharides using HPAEC-PAD. Oligosaccharide peaks are labeled as in Figure 1. Trace B: β-Subunit PNGase F digest was treated with endo-β-galactosidase as described under Materials and Methods. An aliquot of this digest (25 μL) was diluted with water (165 μL) and injected (150 μL) into the chromatograph. Traces A and B represent the oligosaccharides from ~130 pmol of protein.

(Fukuda, 1981). The β-subunit oligosaccharides were digested with endo-β-galactosidase, and the digest was analyzed by HPAEC-PAD as shown in Figure 7. The overall profile changed slightly on treatment; however, a new prominent peak appeared adjacent to peak **1** ($t_r \approx 15$ min). Further, peaks **10** and **15** have diminished in intensity (Figure 7, traces A and B). The profile in the region of peaks **12** and **13** is also altered. Peaks **2**−**9**, **11**, **14**, and **16** appear not to be affected by the treatment with endo-β-galactosidase. The intensity of peaks **10**, **13**, and **15** (trace A) was much lower relative to the putative product peak at a $t_r = 15$ min. It is plausible that each branched polylactosamine structure produced multiple Galα1Galβ1GlcNAcα1β1Gal units and enhanced the electrochemical response. The appearance of a product peak and the disappearance of some oligosaccharide peaks indicate that some of the oligosaccharides contain lactosamine repeats.

Mass Spectrometric Analysis of β-Subunit Oligosaccharides. We next determined the molecular weight of the oligosaccharides of the β-subunit. An analysis using MALDI-TOF mass spectrometry of the PNGase F digest of the β-subunit PNGase F did not give interpretable ions. However, when we derivatized the unprocessed β-subunit PNGase F digest with 2-aminobenzamide, a reagent that labels the reducing termini of the oligosaccharides (Bigge et al., 1995), a heterogeneous array of signals was obtained. Figure 8 shows the analysis of the 2-AB-derivatized oligosaccharides by positive ionization mode MALDI-TOF mass spectrometry. The spectrum shows a series of strong ion signals from *m/z* 1173.5 to 4561.2. The spectra revealed mass differences between adjacent peaks characteristic of sugar residues (i.e., 162 Da for hexose, 203 Da for *N*-acetylhexosamine, and 146 Da for fucose and combinations thereof, e.g., 365 Da for hexose + *N*-acetylhexosamine), indicating oligosaccharide heterogeneity.

From the monosaccharides present in the rabbbit β-subunit we assigned a mass composition to each signal (Table 2). We found signals at *m/z* 1173.5, 1334.4, 1497.7, and 1658.9, which agreed with the theoretical masses of 2-AB-derivatized sodium adducts of $Man_5GlcNAc$, $Man_6GlcNAc$, $Man_7GlcNAc$, and $Man_8GlcNac$ species, respectively. These results are consistent with findings that oligosaccharides released by Endo H treatment coelute with $Man_5GlcNAc$ to $Man_8GlcNAc$ standards (see above). Further, no signal corresponding to the *m/z* of $Man_9GlcNAc$ species was seen in agreement with our HPAEC-PAD data. The loss of a GlcNAc was attributed to a small level of Endo F activity which may have been present in the PNGase F preparation.

The signals from *m/z* 2252−4570 were consistent with the masses of bi-, tri-, and tetraantennary α-Gal-capped oligosaccharide structures, some of them containing lactosamine repeats (Table 2). The antennarity was deduced assuming single Galα1Gal termini on each branch. The ions at *m/z* 2253.2 and 2615.9 were consistent with core-fucosylated biantennary oligosaccharides with both branches terminated in α-Gal residues, the latter having an additional lactosamine repeat. Another possibility for the ion at 2615.9 is a triantennary structure with an α-Gal terminal on two branches and one branch lacking the α-Gal residue, and this type of structure may be represented by the single peak which moves upon β-galactosidase treatment. The intense signals at *m/z* 2778.8, 3143.9, and 3508.3 agreed with the masses of core-fucosylated triantennary oligosaccharides with all branches terminated with α-Gal residues and 0, 1, and 2 lactosamine repeats, respectively (Table 2). The masses seen

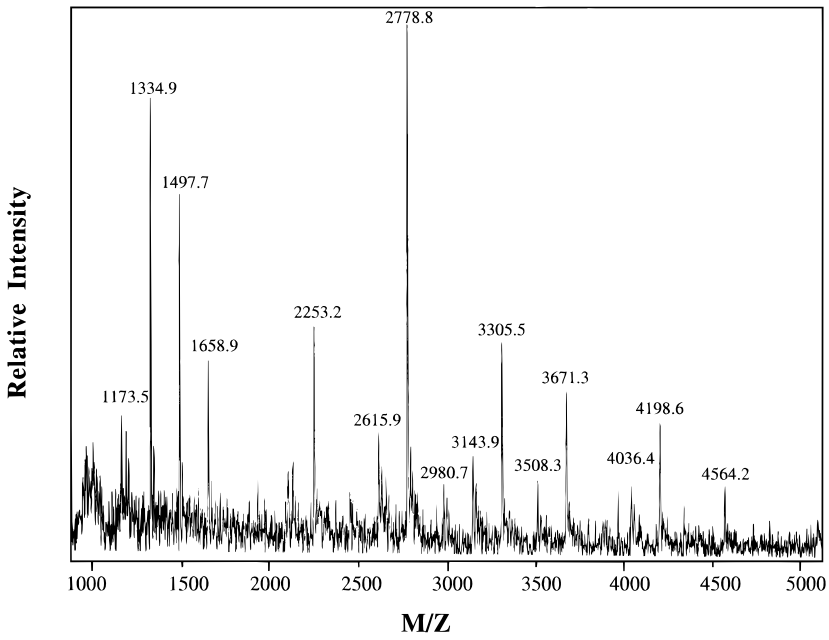

FIGURE 8: MALDI-TOF mass spectrum of N-linked oligosaccharides of β-subunit. The oligosaccharides released by PNGase F digestion of β-subunit were derivatized with 2-aminobenzamide as described under Materials and Methods. The spectra were obtained in the positive ion mode using an accelerating voltage of 25 kV as detailed under Materials and Methods. 2-5-Dihydroxybenzoic acid was used as a matrix. The spectrum represents oligosaccharides from ~1.5 pmol of protein.

Table 2: Assignment of MALDI-MS Signals of the 2-AB-Derivatized Oligosaccharides of the β-Subunit[a]

observed $[M + Na]^{+1}$	theoretical $[M + Na]^{+}$ [b]	composition	proposed structures
1173.5	1172.1	$Hex_5HexNAc$	$Man_5GlcNAc$
1334.9	1334.2	$Hex_6HexNAc$	$Man_6GlcNAc$
1497.7	1496.4	$Hex_7HexNAc$	$Man_7GlcNAc$
1658.9	1658.5	$Hex_8HexNAc$	$Man_8GlcNAc$
2253.2	2252.2	$Hex_7HexNAc_4DeoxyHex_1$	fucosylated, biantennary with α-Gal termini
2615.9	2617.9	$Hex_8HexNAc_5DeoxyHex_1$	fucosylated, biantennary with α-Gal termini + lactosamine
2778.8	2779.6	$Hex_9HexNAc_5DeoxyHex_1$	fucosylated, triantennary with α-Gal termini
2980.7	2982.8	$Hex_9HexNAc_6DeoxyHex_1$	fucosylated, triantennary with α-Gal termini + HexNAc
3143.9	3145.0	$Hex_{10}HexNAc_6DeoxyHex_1$	fucosylated, triantennary with α-Gal termini + lactosamine
3305.5	3307.1	$Hex_{11}HexNAc_6DeoxyHex_1$	fucosylated, tetraantennary with α-Gal termini
3508.3	3510.2	$Hex_{11}HexNAc_7DeoxyHex_1$	fucosylated, triantennary with α-Gal termini + 2 lactosmines
3671.3	3672.5	$Hex_{12}HexNAc_7DeoxyHex_1$	fucosylated, tetraantennary with α-Gal termini + lactosamine
4036.4	4037.9	$Hex_{13}HexNAc_8DeoxyHex_1$	fucosylated, tetraantennary with α-Gal termini + 2 lactosamines
4198.6	4200.1	$Hex_{14}HexNAc_8DeoxyHex_1$	fucosylated, tetraantennary with α-Gal termini + 2 lactosamines + Hex
4564.2	4565.4	$Hex_{15}HexNAc_9DeoxyHex_1$	fucosylated, tetraantennary with α-Gal termini + 3 lactosamines + Hex

[a] The β-subunit oligosaccharides were labeled with 2-AB and subjected to MALDI-TOF mass spectrometric analysis. The signals in Figure 8 were assigned to a mass composition using the average masses of monosaccharides found in the rabbit β-subunit. All signals corresponded to signals of the 2-AB derivatives. [b] The masses include that of the 2-AB label.

at *m/z* 3305.5, 3671.3, and 4036.4 are consistent with core-fucosylated tetraantennary structures with all branches terminated with α-Gal residues, having 0, 1, and 2 lactosamine repeats, respectively (Table 2). Interestingly, the signal seen at *m/z* 4198.6 correlates to the mass of a core-fucosylated tetraantennary structure with two lactosamine repeats, with three branches terminated with a single α-Gal residue and one branch possibly containing two α-Gal residues. The signal at *m/z* 4564.2 corresponds to the addition of a lactosamine unit to the same structure (*m/z* 4198.6). Further structural work will be needed to confirm these assignments and to determine branch locations of the lactosamine units.

DISCUSSION

We have used a combination of digestions with glycosidases and analysis by high-resolution HPLC and MALDI-TOF mass spectrometry to determine the structural features of the oligosaccharides on the β-subunit of the rabbit gastric H,K-ATPase. Our results demonstrate that the oligosaccharides are a mixture of oligomannosidic- and lactosamine-type structures. The oligomannosidic structures include the $Man_5GlcNAc_2$, $Man_6GlcNAc_2$, $Man_7GlcNAc_2$, and $Man_8GlcNAc_2$ species. The complex oligosaccharides on the β-subunit are a mixture of bi-, tri-, and tetraantennary structures with Gal$\alpha\rightarrow$Gal$\beta\rightarrow$GlcNAc termini on all of the branches. Some of the branched structures have either 1, 2, or 3 lactosamine repeats. All the lactosamine type oligosaccharides contained a single Fuc residue which is likely linked to the chitobiose core.

One of the novel findings of our studies was the presence of oligomannosidic structures on the β-subunit of H,K-ATPase. From the earlier observations on the non-reactivity of the gastric β-subunit to Con A lectin and the lack of a distinct shift of the 60−80 kDa band on gels after treatment with Endo H, it was concluded that the β-subunit did not

contain hybrid or oligomannosidic structures (Okamoto et al., 1990; Chow & Forte, 1993; Hall et al., 1990). Our analysis of the oligosaccharides demonstrated unequivocally that the β-subunit possesses oligomannosidic structures. The polypeptide distribution of different oligosaccharides is currently under investigation. Recent studies of the biosynthesis of the H,K-ATPase β-subunit in the presence of glycosylation inhibitors indicated that non-glycosylated β-subunit (tunicamycin) does not leave the endoplasmic reticulum whereas β-subunit modified by oligomannosidic-type chains (deoxynojirimycin) is targeted to the apical membrane (Chow et al., 1994). The role of oligomannnosidic structures in the biosynthesis of a functional proton pump will require further investigation. It will be of interest to determine if oligomannosidic structures on the gastric β-subunit are conserved across different species and found at the same peptide loci.

The most striking finding of these studies was that all branches of the lactosamine-type structures were capped with Galα→Galβ→GlcNAc units. Each branch of this type of glycoprotein glycans is often capped with either sialic acid or α-linked Gal residues within the same structure (Dorland et al., 1984; Geyer et al., 1984; Goulut-Chassaing et al., 1995; Santer et al., 1989; Waard et al., 1991; Pfeiffer et al., 1989; Anderson et al., 1985). Since the sialyltransferase and α-1,3-galactosyltransferase are likely in the same subcellular compartment (Smith et al., 1990), our results suggest that parietal cells lack the former enzyme activity. Kittagawa and Paulson (1994) have shown that sialyltransferases are expressed in all human tissues that they examined, including the small intestine and colon; however, their studies did not include the stomach. Sialylated glycolipids have been isolated from the fundus of the stomach, but their cellular location has not been ascertained (Dohi et al., 1991). In fact, it has been reported that H,K-ATPase-rich microsomes from frog had no sialic acid in either glycolipids or glycoconjugates (Beesley & Forte, 1974). Thus, it appears that gastric parietal cells may be an example of normal differentiated cell type which lacks glycoprotein sialyltransferase activity.

Structural studies on individual oligosaccharides suggest that α-galactosyltransferase preferentially adds Gal to the α(1→6) extension from the trimannose core. This branch specificity apparently complements the activity of CMP-sialic acid: Galβ(1→4)GlcNAc-Rα(2→6) sialyltransferase which quantitatively sialylates the Manα(1→6) extension before adding Neu5Ac to the other branches (Joziasse et al., 1990). Using synthetic oligosaccharides, Elices and Goldstein (1989) showed that the α-galactosyl transferase from Ehrlich cell carcinoma preferentially galactosylated the Man α(1→6) branch. A tetraantennary structure with all branches terminated in GalαGal was isolated from NIH3T3 fibroblasts (Santer et al., 1989). Our results show that the α-galactosyl transferase in rabbit parietal cells efficiently transfers Gal to all branches of bi-, tri-, and tetraantennary oligosaccharides. Thus, the branch specificity demonstrated by in vitro experiments is not absolute for bi-, tri-, or tetraantennary structures.

Some of the structural features of the rabbit β-subunit oligosaccharides are present in other species. The complete absence of sialylation on the gastric β-subunit in rabbit, frog, and pig have previously been demonstrated (Beesley & Forte, 1973; Weitzhandler et al., 1993; Goldkorn et al., 1989). More recently, we have found that the *Griffonia simplicifolia* (GSI) lectins bind not only to the β-subunit from rabbit but also to that from frog and cow (Tyagarajan et al., 1995). The absence of sialic acid and the exclusive presence of α-Gal termini in more than one species suggests that an acid-stable terminal group is important to prevent hydrolysis in the stomach. Whether the fidelity of glycosylation within the parietal cell is necessary for the proton pump to function in the harsh acidic environment of the secretory canaliculus requires further studies. Interestingly, the oligosaccharides of the closely related Na,K-ATPase β-subunit are sialylated (Treuheit et al., 1993). Thus, the lack of sialylation is not a characteristic of heterodimeric P-type ATPases.

The gastric β-subunit is involved specifically in recognition by parietal cell autoantibodies during autoimmune gastritis. It has been demonstrated that PNGase F treatment of the pig β-subunit abrogates binding (Goldkorn et al., 1989), and the β-subunit expressed in COS cells, which produce only oligomannosidic-type structures, fails to bind the autoantibody (Callaghan et al., 1993). Additionally, it has been shown that the β-subunit from rabbit, pig, mouse, and dog are recognized by the autoantibodies present in the serum of patients with autoimmune gastritis but not by control sera (Goldkorn et al., 1989). The reactivity suggests that there may be a common glycosylation motif on the β-subunit from different species that is involved in the recognition by parietal cell autoantibodies. Human sera contain a high titer of antibodies (1% of total IgG) against the Galα1→3Galβ1→4GlcNAc-R epitope (Galili, 1993). Since the rabbit β-subunit is glycosylated with α-Gal-terminated oligosaccharides containing this epitope, it is unclear why the normal human sera did not react with the rabbit β-subunit. In summary, the determination of the novel structural features for the oligosaccharides on the β-subunit of rabbit H,K-ATPase has raised several intriguing questions regarding the biosynthesis of oligosaccharides, the conservation of relatively acid-resistant terminal groups, and the autoimmune processes.

ACKNOWLEDGMENT

The authors acknowledge Dr. Peter Lipniunas for helpful discussions. MALDI-MS was performed at the UCSF Mass Spectrometry Facility (A. L. Burlingame, Director).

REFERENCES

Anderson, D. R., Atkinson, P. H., & Grimes, W. J. (1985) *Arch. Biochem. Biophys. 243*, 605−618.

Anumula, K. R., & Taylor, P. B. (1991) *Eur. J. Biochem. 195*, 269−280.

Beesley, R. C., & Forte, J. G. (1973) *Biochim. Biophys. Acta. 307*, 372−385.

Beesley, R. C., & Forte, J. G. (1974) *Biochim. Biophys. Acta. 356*, 144−155.

Bigge C., Patel, T. P., Bruce, J. A., Goulding, P. N., Charles, S. N., & Parekh, R. B. (1995) *Anal. Biochem. 230*, 229−238.

Callaghan, J. M., Khan, M. A., Alderuccio, F., Van-Driel, I. R., Gleeson, P. A., & Toh, B. H. (1993) *Autoimmunity 16*, 289−295.

Callaghan, J. M., Toh, B. H., Pettitt, J. M., Humphris, D. C., & Gleeson, P. A. (1990) *J. Cell Sci. 95*, 563−576.

Canfield, V. A., Okamato, C. T., Chow, D. C., Dorfman, J., Gros, P., Forte, J. G., & Levenson, R. (1990) *J. Biol.Chem. 265*, 19878−19884.

Chow, D. C., & Forte J. G. (1993) *Am. J. Physiol. 265*, C1562−1570.

Chow, D. C., Browning, C. M., & Forte, J. G. (1992) *Am. J. Physiol. 263*, C39−46.
Chow, D. C, Boyd, K. L., Scalley, M. L., & Forte, J. G. (1994) *FASEB J. 8*, 2240, A387.
Courtois, J. E., & Petek, F. (1966) *Methods Enzymol. 8*, 565−571
Distler, J. J., & Jourdian, G. W. (1973) *J. Biol. Chem. 248*, 6772−6780.
Dohi, T., Hanai, N., Yamaguchi, K., & Oshima, M. (1991) *J. Biol. Chem. 266*, 24038−24043.
Dorland, L., van Halbeek, H., & Vilegenthart, J. F. G. (1984) *Biochim. Biophys. Res. Commun. 122*, 859−866.
Eckhardt, A. E., & Goldstein, I. J. (1983) *Biochemistry 22*, 5290−5297.
Elices, M. J., & Goldstein, I. J. (1989) *J. Biol. Chem. 264*, 1375−1380.
Fukuda, M. (1981) *J. Biol. Chem. 256*, 3900−3905.
Galili, U. (1993) *Springer Semin. Immunopathol. 15*, 155−171.
Geyer, R., Geyer, H., Egge, H., Schott, H. H., & Stirm, S. (1984) *Eur. J. Biochem. 143*, 531−539.
Goldkorn, I., Gleeson, P. A., & Toh, B. H. (1989) *J. Biol. Chem. 264*, 18768−18774.
Goulut-Chassaing, C., & Bourrillon, R. (1995) *Biochim. Biophys. Acta, 1244*, 30−39.
Hall, K., Perez G, Anderson, D., Gutierrez, C., Munson, K., Hersey, S. J., Kaplan, J. H., & Sachs, G. (1990) *Biochemistry 29*, 701−706.
Hardy, M. R., & Townsend, R. R. (1989) *Carbohydr. Res. 188*, 1−7.
Hardy, M. R., & Townsend, R. R. (1994) *Methods Enzymol. 230*, 208−225.
Harpaz, N., Flowers, H. M., & Sharon, N. (1975) *Arch. Biochem. Biophys 170*, 676−683.
Hermentin, P., Witzel, R., Vliegenthart, J. F. G., Kamerling, J. P., Nimtz, M., & Conradt, H. S. (1992) *Anal. Biochem. 203*, 281−289.
Joziasse, D. H., Shaper, N. L., Salyer, L. S., Van den Eijnden, D. H., van der Spoel, A. C., & Shaper, J. H. (1990) *Eur. J. Biochem. 191*, 75−83.
Kitagawa, H., & Paulson, J. C. (1994) *J. Biol. Chem. 269*, 17872−17878.
Li, Y.-T., & Li, S.-C. (1972) *Methods Enzymol. 28*, 702−713.
Mizuochi, T., Spellman, M. W., Larkin, M., Solomon, J., Basa, L. J., & Feizi, T. (1988) *Biomed. Chromatogr. 2*, 260−70.
Okamato, C. T., & Forte, J. G. (1988) *Gastroenterology 95*, 334−342.
Okamoto, C. T., Karpilow, J. M., Smolka, A., & Forte J. G. (1990) *Biochim. Biophys. Acta. 1037*, 360−372.
Parekh, R. B., Dwek, R. A., Thomas, J. R., Opdenakker, G., Rademacher, T. W., Wittwer, A. J., Howard, S. C., Nelson, R., Siegel, N. R., Jennings, M. G., et al. (1989) *Biochemistry 28*, 7644−7662.
Pfeiffer, G., Schmidt, M., Strube, K.-H., & Geyer, R. (1989) *Eur. J. Biochem. 186*, 273−286.
Plummer, T. H., Jr., Phelan, A. W., & Tarentino, A. L. (1987) *Eur. J. Biochem. 163*, 167−173.
Reenstra, W. W., & Forte, J. G. (1990) *Methods Enzymol. 192*, 151−165.
Reuben, M. A., Lasater, L. S., & Sachs, G. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 6767−6781.
Santer, U. V., DeSantis, R., Hard, K. J., van Kuik, J. A., Vliegenthart, J. F., Won, B., & Glick, M. C. (1989) *Eur. J. Biochem. 181*, 249−260.
Shull, G. E. (1990) *J. Biol. Chem. 265*, 12123−12126.
Smith, D. F., Larsen, R. D., Mattox, S., Lowe, J. B., & Cummings, R. D. (1990) *J. Biol. Chem. 265*, 6225−6234.
Spellman, M. W. (1990) *Anal. Chem. 62*, 1714.
Spiro, R. G., & Bhoyroo, V. D. (1984) *J. Biol. Chem. 259*, 9858−9866.
Toh, B. H., Gleeson, P. A., Simpson, R. J., Moritz, R. L., Callaghan, J. M., Goldkorn, I., Jones, C. M., Martinelli, T. M., Mu, F. T., Humphris, D. C., Pettitt, J. M., Mori, Y., Masuda, T., Sobieszczuk, P., Weinstock, J., Mantamadiotis, T., & Baldwin, G. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 6418−6422.
Treuheit, M. J., Costello, C. E., & Kirley, T. L. (1993) *J. Biol. Chem. 268*, 13914−13919.
Tyagarajan, K., Chow, D. C., & Forte, J. G. (1995) *Biochim. Biophys. Acta. 1236*, 105−113.
Tyagarajan, K., Crothers, J. M., Jr., Mulvihill, S. J., Lipniunas, P., Townsend, R. R., & Forte, J. G. (1995) *Gastroenterology 108*, A247.
Tyagarajan, K., Forte, J. G., & Townsend, R. R. (1996) *Glycobiology 6*, 83−93.
Usumoto, R., Okada, Y., & Tsuji, T. (1994) *Digestive Dis. Sci. 39*, 169−176.
Waard, P. de, Koorevaar, A., Kamerling, J. P., & Vliegenthart, J. F. G. (1991) *J. Biol. Chem. 266*, 4237−4243.
Weitzhandler, M., Kadlecek, D., Avdalovic, N., Forte, J. G., Chow, D. C., & Townsend, R. R. (1993) *J. Biol.Chem. 268*, 5121−5130.
Yamashita, K., Totani, K., Iwaki,Y., Kuroki, M., Matsuoka, Y., Endo, T., & Kobata, A. (1989) *J. Biol. Chem. 264*, 17873−17881.

BI952303P