Biochimica et Biophysica Acta, 356 (1974) 144–155

BBA 76683

GLYCOPROTEINS AND GLYCOLIPIDS OF OXYNTIC CELL MICROSOMES
II. GLYCOPEPTIDES AND GLYCOLIPIDS

ROBERT C. BEESLEY and JOHN G. FORTE

Department of Physiology-Anatomy, University of California, Berkeley, Calif. 94720 (U.S.A.)

(Received February 8th, 1974)

SUMMARY

Glycosylated compounds associated with the carbohydrate-rich tubular membrane system of the oxyntic cell were investigated. Two glycopeptide fractions, designated Peaks A and B, were isolated from pronase digests of bullfrog oxyntic cell microsomes. Molecular sieve chromatography and cellulose acetate electrophoresis revealed that, although somewhat heterogeneous, each peak was composed primarily of glycopeptides with similar molecular weights and net charge densities. Peak B glycopeptides had a mean molecular weight of about 6000 and contained 70 % of the recovered carbohydrate in the following molar ratios: hexose, 1.00; *N*-acetylhexosamine, 0.71; fucose, 0.61; sialic acids, < 0.03. Peak A glycopeptides were considerably larger (molecular weight approx. 100 000) and contained carbohydrates in molar ratios similar to those of Peak B. In both peaks galactose and *N*-acetylglucosamine, respectively, were the predominant hexose and amino sugar isomers.

The glycolipid content of bullfrog oxyntic cell microsomes was assessed by qualitative and quantitative thin-layer chromatography. The most abundant glycolipids were monoglucosylceramides (0.098 mole/mole phospholipid) and monogalactosylceramides (0.046 mole/mole phospholipid). Small quantities of sulfatides and gangliosides were also present.

A compilation of available data regarding the chemical composition of the microsomes revealed that these membranes resemble plasma membranes in having high molar ratios of cholesterol to phospholipid (approx. 1.0) and large quantities of carbohydrate (225 μg/mg protein). The possible significance of these compositional features in protecting the oxyntic cell is discussed.

INTRODUCTION

The oxyntic, or acid-secreting, cell of the gastric mucosa possesses an extensive smooth-surfaced tubular membrane system which is thought to play a central role in the acid secretory process [1–4]. This membrane system can be isolated in gastric microsomal fractions [5–7] and has been shown to contain large quantities of car-

bohydrate [6–9]. For instance, chemical analysis of frog oxyntic cell microsomes showed that they contained 260 μg of sugar per mg protein (primarily hexoses, hexosamines and fucose) [7]. While the majority of this carbohydrate was associated with the membrane proteins preliminary evidence suggested that significant quantities of glycolipid were also present.

In view of the apparent structural and functional importance of membrane glycoproteins and glycolipids (see reviews of Winzler [10] and Kraemer [11]), the present investigation was undertaken to further characterize these carbohydrate components in the oxyntic cell tubular membrane system. For this purpose, bullfrog gastric microsomes were digested with proteolytic enzymes and the resulting glycopeptides fractionated and characterized as to size and sugar composition. In addition, the major microsomal glycolipids were isolated and identified.

METHODS

Isolation, extraction and digestion of microsomes

Bullfrog oxyntic cell microsomes were isolated, washed and extracted of lipids as previously described [7]. The fat-free microsomes (100 mg) were suspended in 4 ml of 75 mM Tris–HCl buffer (pH 7.9), 7.5 mM $CaCl_2$. A small amount of toluene was added to prevent microbial growth. The solution was incubated at 37 °C for 120 h with additions of 1 mg of protease, (Sigma Type VI) at zero and 24 h and 0.5 mg of protease at 56 h. The course of digestion was monitored using the ninhydrin assay [12] with leucine as standard. At the end of the incubation period insoluble remnants were removed by centrifugation at 27 000 × *g* for 10 min.

Fractionation of glycopeptides

Sephadex G-75 chromatography was performed as described in the legend to Fig. 1. Molecular sieve chromatography on Sephadex G-200, G-50 and G-15 was performed on 1.5 cm × 51 cm columns equilibrated and developed with 25 mM ammonium acetate (pH 6.8). The flow rates were approx. 10 ml/h and 2-ml fractions were collected. Elution was monitored by 260- and 280-nm absorption and fractions assayed for carbohydrate with orcinol [13]. Calibration of each column was accomplished using three or more of the following known molecular weight standards: blue dextran, human γ-globulin, bovine serum albumin, ovalbumin, myoglobin, cytochrome *c*, eserine sulfate, and fucose (Sigma Chem. Co.).

Cellulose acetate electrophoresis was performed using the following conditions: 36 mM sodium citrate buffer (pH 3.5) and 36 mM sodium barbital buffer (pH 8.5) at 200 V for 30 min, or 36 mM sodium citrate buffer (pH 6.3) at 100 V for 1 h. The sample volume was 0.1 μl and the electrophorograms were stained with amido schwartz [14].

Chemical analysis of microsomes and glycopeptides

Protein, hexose, fucose, hexosamine and sialic acids were measured as previously described [7]. *N*-Acetylhexosamine was assayed using the Morgan–Elson reaction [15] after neutralization of samples previously sealed in ampules under vacuum and hydrolyzed in 1 M HCl for 30 min at 100 °C.

To determine the molar ratios of galactose/glucose/mannose and of glucos-

amine/galactosamine in the glycopeptides, samples were sealed in ampules under vacuum and hydrolyzed in 4 M HCl for 4 h at 100 °C. HCl was removed by several evaporations in vacuo over KOH and concentrated H_2SO_4. The hydrolysate was spotted in triplicate along with a mixture of known sugars on washed Whatman No. 1 paper and chromatographed in a solvent system described by Fischer and Nebel [16]. Sugar spots were identified by alkaline $AgNO_3$ staining [17]; corresponding unstained sections of test and blank strips were cut out, eluted with water overnight and assayed for reducing sugars [18].

Extraction, partitioning and trans esterification of lipids

Lipids were obtained by extraction of bullfrog microsomes with ethanol–diethyl ether (3 : 1, by vol.) and diethyl ether [19]. Total phospholipid was calculated from inorganic phosphorous analysis according to Bartlett [20] after digestion in H_2SO_4 assuming an average phospholipid molecular weight of 800. Cholesterol was assayed using *o*-phthalaldehyde [21].

The lipid extract was dried under a stream of N_2 and subjected to Folch partitioning as described by Esselman et al. [22]. The aqueous layer and washes were combined, dialyzed against double-distilled water at 4 °C for 36 h, lyophilized, redissolved in chloroform–methanol–water (65 : 35 : 4, by vol.) and used for analysis of gangliosides. An aliquot of the organic layer was saved for qualitative thin-layer chromatography of phospholipids and the remainder trans esterified in methanolic KOH as described by van Hoeven and Emmelot [23]. The trans-esterified extract was partitioned into aqueous and organic phases [23] and the latter used for qualitative and quantitative thin-layer chromatography of glycolipids.

Thin-layer chromatography of microsomal lipids

Thin-layer chromatographic plates were prepared and developed essentially as described by Skipski and Barclay [24] using 0.275-mm layers of silica gel without binder. Individual spots were detected with iodine vapor as a general lipid stain followed by resorcinol [25] or orcinol [26] sprays for specific detection of gangliosides and other glycolipids. Lipid and glycolipid spots were identified by comparison of the R_F values with those of known phospholipids, cerebrosides (Sigma Grade II), gangliosides (Sigma Type III) brain extract (Sigma Type VI) and diglycosylceramide (Miles-Yeda) run on parallel plates.

Qualitative thin-layer chromatography was performed as described in the Results (see legend to Fig. 3). For preparative chromatography of glycolipids, samples were spotted as a line on thin-layer plates and developed with chloroform–acetone–methanol–acetic acid–water (5 : 2 : 1 : 1 : 0.5, by vol.) [27]. Lipid spots were located with light iodine vapor staining and encircled. After evaporation of iodine from the plate the spot containing the mixed monoglycosylceramides was collected, eluted and a portion analyzed for neutral sugar as described by Eng and Noble [28]. The remainder of the eluate obtained in the one-step procedure described above was concentrated under a stream of N_2 and rechromatographed on borate-impregnated plates according to Kean [29]. Spots were again located with iodine vapor and encircled. Individual monoglucosyl- and monogalactosylceramide fractions were collected, eluted and assayed for neutral sugar [28].

Since significant losses of material might have occurred during the two-step

procedure, the amounts of glucosyl- and galactosylceramides were calculated from the absorbance measured in the assay of the mixed monoglycosylceramide fraction (one-step procedure), the ratio of galactose to glucose obtained from the subsequent two-step procedure, the extinction coefficients for galactose and glucose, and the estimated ratio of the molecular weight of the glycolipid to that of the monosaccharide (i.e. 4.6) [26].

RESULTS

The results of partial chemical analysis of bullfrog oxyntic cell microsomes used in the present study are shown in Table I. The relatively high ratio of lipid to protein (0.95) reflects the membranous nature of the microsomal fraction. In addition, as previously reported [7], considerable quantities of carbohydrate are associated with these membranes.

TABLE I

PARTIAL CHEMICAL COMPOSITION OF OXYNTIC CELL MICROSOMES

	mg
Fat free material	
protein	100
neutral sugar	11.2
Lipid	
phospholipid	64
cholesterol	31

Glycopeptides

Lipid-extracted microsomes were digested with pronase, which hydrolyzed more than 65 % of the peptide bonds as assessed by ninhydrin assay. Any remaining insoluble material was removed by centrifugation and the soluble supernatant, which contained 96 % of the neutral sugar, was chromatographed on Sephadex G-75. The elution profile (Fig. 1) demonstrated that most of the carbohydrate-containing materials had been eluted as two major peaks well separated from lower-molecular weight components. Individual fractions were pooled as depicted in Fig. 1 and subjected to orcinol and ninhydrin assays. Peak B contained the majority, 56 %, of the neutral sugar applied to the column while Peak A contained 16 %. Only 11 % of the neutral sugar was present in the combined low-molecular weight fractions, Pool C, where more than 96 % of the ninhydrin-positive substances were recovered.

Since Peak A had been eluted with the void volume it might have contained a mixture of materials of varying molecular weights greater than the exclusion limit of Sephadex G-75. Therefore, Peak A was rechromatographed on Sephadex G-200 where all materials were eluted as a single relatively symmetrical peak. In addition, Peak B from Sephadex G-75 was rechromatographed on Sephadex G-50 to determine if a separation of different-sized glycopeptides could be achieved on this more discriminating gel. Again, only a single symmetrical peak was obtained. By comparing the elution volumes for Peak A and Peak B with those of proteins of known size run

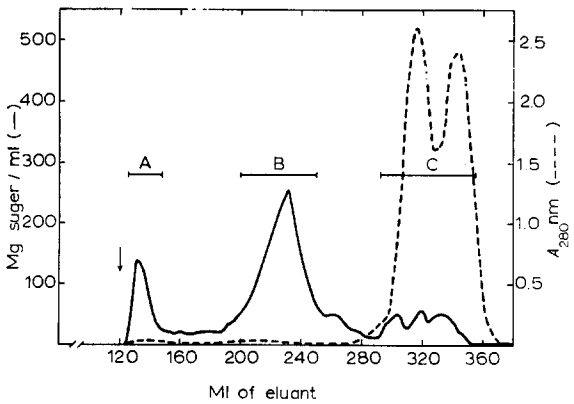

Fig. 1. Sephadex G-75 chromatography of the microsomal digest. The soluble supernatant obtained after proteolytic digestion of approx. 100 mg of microsomal protein was applied to a 2.5 cm × 53 cm Sephadex G-75 column equilibrated and eluted with 25 mM ammonium acetate (pH 6.9). The flow rate was 20 ml/h; 4-ml fractions were collected. Elution was monitored by 260- and 280-nm absorption and fractions were assayed for carbohydrate by the orcinol procedure [13]. For further analytical work fractions were pooled as indicated by the horizontal bars (A, B and C). The arrow designates the void volume.

on the identical Sephadex columns the molecular weights of Peak A and Peak B were determined to be approx. 100 000 and 6000, respectively.

Further fractionation of Pool C by chromatography on Sephadex G-15 revealed that several materials of different molecular weights were present. In addition to amino acids and small peptides, this pool contained considerable quantities of substances with $A_{260\,nm}/A_{280\,nm}$ ratios approaching 2.0. The majority of the orcinol-positive materials were associated with the 260-nm absorbing substances and were therefore probably not glycopeptides but perhaps small nucleotides. This is consistent with our previously reported finding of relatively carbohydrate-poor, 260-nm absorbing materials in low-molecular weight fractions obtained from nondigested microsomes [7].

Table II summarizes the results of analysis of Peaks A and B. Most of the microsomal glycopeptides were present in Peak B which contained more than 70 % of the recovered carbohydrate. In general, the sugar composition of Peaks A and B was similar. Carbohydrates comprised approx. 70 % of the dry weights with the hexoses being the most abundant species. Considerable quantities of hexosamine and fucose were also present in both peaks while sialic acid was found in only negligible quantities. Galactose was the most abundant hexose isomer while the major amino sugar was glucosamine.

An aliquot of Peak B was subjected to the Morgan–Elson reaction in order to determine whether the amino sugars were N-acetylated. Without prior treatment *N*-acetyl groups were not detected. However, after acid hydrolysis 30 % of the amino sugars gave positive reactions for N-acetylation. Under identical hydrolysis conditions free *N*-acetylglucosamine and human γ-globulin gave 20 and 38 %, respectively, of the theoretical values. These results suggest that, like the amino sugars found in most glycoproteins [10, 11], the majority of the hexosamines of oxyntic cell microsomal glycopeptides are N-acetylated.

TABLE II

COMPOSITION OF GLYCOPEPTIDES

Peak A: dry wt, 4.01 mg; total carbohydrate, 2.87 mg; mol. wt 100 000. Peak B: dry wt, 13.20 mg; total carbohydrate, 9.64 mg; mol. wt 6000

	Carbohydrate analysis			
	Peak A		Peak B	
	μg/mg*	Moles per mole hexose	μg/mg*	Moles per mole hexose
Hexose	288	1.00	300	1.00
galactose		0.95		0.82
glucose		0.05		0.07
mannose		0.00		0.11
N-Acetylhexosamine	290	0.82	263	0.71
N-acetylglucosamine		0.67		0.59
N-acetylgalactosamine		0.15		0.12
Fucose	138	0.53	167	0.61
Sialic acid	< 9	0.02	< 17	< 0.03

* Values expressed as μg/mg dry wt.

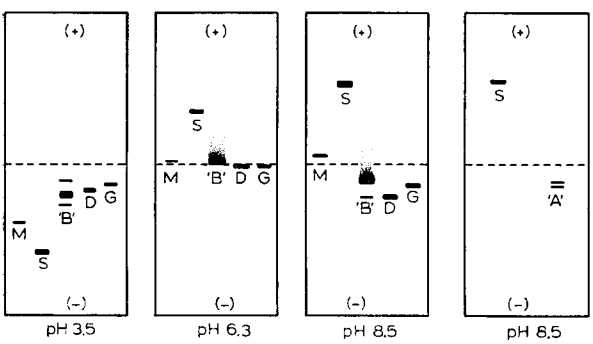

Fig. 2. Drawings of cellulose acetate electrophorograms of Peak A and Peak B glycopeptides. Details of the electrophoresis procedures are given in Methods. A, Peak A; B, Peak B; M, myoglobulin; S, bovine serum albumin. Neutral substances, glycogen (G) and dextran (D) were included to account for migration due to electro-osmosis. The strips were stained with amido schwartz [14].

Cellulose acetate electrophorograms of Peaks A and B are depicted in Fig. 2. Peak A was resolved into two bands with similar mobilities and staining intensities. Peak B, on the other hand, appeared to be composed primarily of a single major component although several minor ones were also detected. Most components exhibited relatively small electrophoretic mobilities reflecting the low charge density of these materials. This is consistent with our analytical data demonstrating that the glycopeptides are approx. 70 % carbohydrate and contain negligible quantities of acidic and/or basic sugars.

Glycolipids

Microsomal lipid extracts were subjected to Folch partitioning to obtain aqueous and organic (chloroform–methanol) phases. Examination of the aqueous phase, which would contain the gangliosides, by thin-layer chromatography revealed only one resorcinol-positive spot. Comparison of the chromatographic properties of this material with that of a mixed ganglioside standard showed that small quantities of tri- or tetrasialogangliosides were present in the microsomal extracts. Mono- and disialogangliosides were not detected.

The organic chloroform–methanol phase, which would contain the other lipids, was also examined by thin-layer chromatography (Fig. 3) revealing the normal complement of neutral lipids and phospholipids [30]. In addition, orcinol-positive materials with R_F values corresponding to monoglycosylceramides, containing "normal" and hydroxy fatty acids, and sulfatides were present.

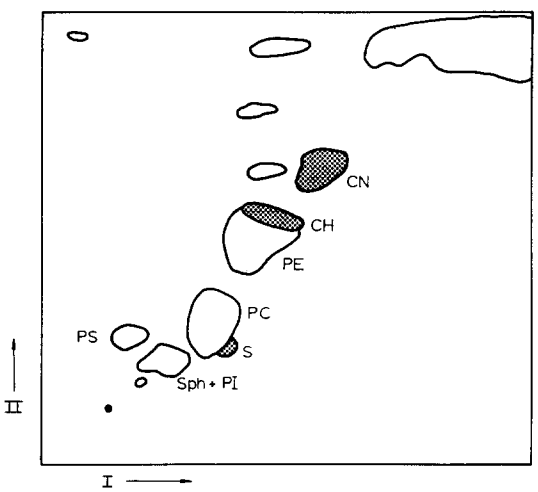

Fig. 3. Tracing of a thin-layer chromatogram of the organic layer obtained from Folch partitioning of bullfrog oxyntic cell microsomal lipids. The chromatogram was developed with a solvent system described by Rouser et al. [27]: Solvent I, chloroform–methanol–14 M ammonia (65 : 35 : 5, by vol.) and Solvent II, chloroform–acetone–methanol–acetic acid–water (5 : 2 : 1 : 1 : 0.5, by vol.). Spots staining with iodine vapor are outlined and those staining with orcinol are represented by the shaded areas. Those lipids identified were phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (Sph), sulfatides (S), monoglycosylceramides with "normal" (CN) and hydroxy (CH) fatty acids.

Since in the above system dihexosylceramides might not have been detected due to overlapping by nonglycolipid components, particularly phosphatidylethanolamine, the chloroform–methanol-soluble lipids were trans esterified in methanolic KOH and the water-soluble products removed. Subsequent chromatograms showed that the majority of the phospholipids had been destroyed by the methanolic KOH treatment. Of the glycolipids only monoglycosylceramides and sulfatides were seen. Although sphingomyelin, which is not altered by trans esterification, might have masked the presence of ceramide polyhexosides, dihexosylceramides would have been detected if present in significant quantities.

In order to determine the quantities of different glycolipid species present in the microsomes, trans esterified lipids were subjected to two-step preparative chromatography to isolate separate monoglucosyl- and monogalactosylceramide fractions. These fractions were assayed for carbohydrate and the ratio of galactose to glucose in the monoglycosylceramides determined to be 0.46.

The above ratio was used to calculate the amounts of glucosyl- and galactosylceramides in a mixed monoglycosylceramide fraction obtained by one-step preparative chromatography. Thus it was determined that the microsomal lipid extracts contained 0.098 moles of monoglucosylceramide and 0.046 moles of monogalactosylceramide per mole of phospholipid. A mixed fraction that would contain sulfatides and ceramide polyhexosides, if present, was also obtained. Although this fraction contained considerable quantities of nonglycolipid components which appeared to interfere in the orcinol assay, it was apparent that sulfatides and ceramide polyhexosides could account for less than 2 % of the glycolipid neutral sugar.

DISCUSSION

Glycopeptides

Glycopeptides present in oxyntic cell microsomal digests were separated into two major fractions each of which was composed primarily of glycopeptides with similar molecular weights and net charge densities. The carbohydrate composition of both fractions resembles that reported for several other glycoproteins found in the stomach. For instance, the "principle" human gastric glycoprotein [31], hog gastric blood group substances [32], and human gastroferrin [33], all have hexose, hexosamine, fucose and sialic acids in molar ratios approximating those reported here (i.e. 1.0 : 0.7 : 0.6 : 0.03). In addition, as in the frog microsomal glycopeptides, galactose and glucosamine are the most abundant hexose and amino sugar isomers. While this might be interpreted as reflecting similar and restricted biosynthetic capabilities of gastric cells from several species, it should be noted that not all gastric glycoproteins share these compositional features. For example, hog intrinsic factor [34] has mannose as the predominant hexose isomer and contains significant quantities of sialic acid while glycopeptides from dog mucosa [35] have equal amounts of glucosamine and galactosamine and only small quantities of fucose. Thus, additional information about the carbohydrate content of gastric glycoproteins of a number of species will be required in order to determine whether these compounds can be grouped together as a single class based on similarities in chemical composition.

The carbohydrate composition of the glycopeptides in one of the gastric microsomal glycopeptide fractions, Peak B, can be estimated from the analytical data presented here (cf. Table II). The values for the weight of each sugar per g dry wt of glycopeptide, the estimated molecular weight of the glycopeptide, and the molar ratios of the various sugar isomers suggest that the average glycopeptide in this peak contains 8 galactose, 1 mannose, 1 glucose, 6 *N*-acetylglucosamine, 1 *N*-acetylgalactosamine and 6 fucose residues. Using these values and accounting for the water lost in formation of glycosidic bonds gives a formula weight of 3917 daltons for the oligosaccharide unit(s).

The exact relationship between the oligosaccharide and polypeptide components of Peak B glycopeptides cannot be specified at this time. Since approx.

1/3 (2000 daltons) of the molecular weight of the glycopeptide is not accounted for by carbohydrate it appears that the polypeptide portion could consist of as many as 17–20 amino acids. Therefore, each glycopeptide might be composed of (a) one large highly branched oligosaccharide attached to a single amino acid in the polypeptide or (b) several smaller heterogeneous oligosaccharides covalently linked at a number of sites along the polypeptide backbone. In the former case the glycopeptides might resemble those found in blood group substances [36] while the latter model would be similar to that proposed for some of the glycopeptides derived from the major glycoprotein of erythrocyte membranes [37].

Glycolipids

Although the present study clearly demonstrated that gastric microsomes contain glycolipids, the amounts measured were considerably less than had been predicted from the difference in carbohydrate content of native and lipid-extracted microsomes [7]. This discrepancy was probably not due to loss of glycolipids during the preparative procedures since 95–100 % of known glycolipid standards were recovered after similar treatments. It seems likely, therefore, that the elevated levels of orcinol-positive substances apparent in direct analysis of native microsomes were due in part to nonspecific interference by lipid-soluble materials. Nevertheless, the oxyntic cell microsomes do contain glycolipids in significant quantities which, while lower than those of glycolipid-rich brain myelin [38] and intestinal epithelial cells [39], are comparable to the levels reported for other membranes such as red blood cell [40] and rat liver plasma membranes [41].

Chemical composition of bullfrog oxyntic cell microsomes

The cellular and subcellular origin of gastric microsomal fractions has been previously assessed in a number of investigations [5–7]. Morphological, chemical, histochemical and developmental evidence has been used to suggest that this fraction contains the smooth-surfaced tubular membrane system found in the apical portion of oxyntic cells. Although the presence of membranes derived from other subcellular organelles such as the Golgi apparatus and plasma membrane cannot be excluded, the relative abundance of these membranes in the intact cell indicates that the tubular membranes are the predominant source of microsomal materials.

The results obtained from a number of studies involving chemical analysis of bullfrog oxyntic cell microsomal preparations are summarized in Table III. It is of interest that these tubular membranes have compositional features characteristic of plasma membranes from several sources rather than those associated with microsomal fractions derived from more typical intracellular membranes. For instance, the tubules, like plasma membranes [41, 45], have a high molar ratio of cholesterol/phospholipid (1.0) and contain large quantities of carbohydrate (225 μg/mg protein). Intracellular membranes, on the other hand, normally have smaller cholesterol/phospholipid ratios [42, 43] and low levels of carbohydrate [46, 47].

The compositional similarity of plasma membranes and oxyntic cell tubular membranes is perhaps not surprising when one considers the probable role of the latter membrane system in oxyntic cell function. When oxyntic cells are resting and not secreting acid the tubular membranes are sequestered within the apical cytoplasm of the cell [3, 48]. It has been proposed that stimulation of these cells to secrete results

TABLE III

CHEMICAL COMPOSITION OF BULLFROG OXYNTIC CELL MICROSOMES

Data compiled from this publication and [7].

	%
Fat-free material	
Dry wt	100
Protein	74
Carbohydrate	14.8
Hexose	6.9
galactose	(5.9)
glucose	(0.5)
mannose	(0.6)
N-Acetylhexosamine	5.3
N-acetylglucosamine	(4.3)
N-acetylgalactosamine	(1.0)
Fucose	2.7
Unindentified	11
Lipid	
Dry wt	107
Phospholipid	53
Cholesterol	26
Monoglycosylceramides	7.9
Glucosylceramide	5.4
Galactosylceramide	2.5
Unidentified	20

in fusion of the tubular membranes with the apical plasma membrane thereby increasing the surface area of the cell and establishing continuity between the lumen of the gastric glands and the space within the tubular membrane system [3, 48, 49]. Thus during the process of acid secretion the tubular membranes are functioning as limiting membranes of the oxyntic cell and therefore can be anatomically defined as plasma membranes.

One important function of the plasma membrane is to provide a permeability barrier, separating the cell interior from the external environment, and to this end it is well established that lipid constituents are of fundamental importance [50]. Chemical analyses shown here suggest that oxyntic cell tubular membranes are well suited for this function in as much as they have relatively high lipid to protein ratios and contain large quantities of cholesterol, which is known to reduce the permeability of lipid films [51]. Thus the tubular membranes should provide an excellent barrier, preventing possible damage to the cell which would result from the back flux of HCl that is present in high concentration in the gastric lumen.

Various functions have been proposed for the carbohydrates in plasma membranes, and available evidence suggests that these functions may vary with the type of cell (see reviews of Winzler [10] and Kraemer [11]). Our studies of oxyntic cell glycopeptides provide suggestive evidence regarding the role of the sugars found in the tubular membranes. Incubation of the microsomes with nonspecific proteolytic enzymes resulted in hydrolysis of the vast majority of the proteins into their constituent

amino acids and small peptides. However, the glycopeptides which we isolated appeared to contains relatively large polypeptide segments. These results suggest that the glycopeptides and perhaps the native glycoproteins may possess an intrinsic resistance to digestion conferred upon them by the large quantities of covalently bound sugar. Therefore, as previously proposed [7], the carbohydrate of the tubular membranes may serve to stabilize the conformation of surface membrane components and prevent hydrolysis of membrane proteins by the proteolytic enzymes found in the stomach.

ACKNOWLEDGEMENTS

This study was supported in part by U.S. Public Health Service Grant AM 10141. R. C. B. is a trainee on U.S. Public Health Service Grant 5T01-GM-1021.

The authors gratefully acknowledge the excellent technical assistance of Liisa Van Duyts.

REFERENCES

1 Vial, J. D. and Orrego, H. (1960) J. Biophys. Biochem. Cytol. 7, 367–372
2 Sedar, A. W. (1961) J. Biophys. Biochem. Cytol. 10, 47–57
3 Sedar, A. W. (1965) Fed. Proc. 24, 1360–1367
4 Forte, G. M., Limlomwongse, L. and Forte, J. G. (1969) J. Cell Sci. 4, 709–727
5 Forte, J. G., Forte, G. M. and Saltman, P. (1967) J. Cell. Physiol. 69, 293–304
6 Forte, T. M. and Forte, J. G. (1970) J. Cell Biol. 47, 437–452
7 Beesley, R. C. and Forte, J. G. (1973) Biochim. Biophys. Acta 307, 372–385
8 Sedar, A. W. (1969) J. Ultrastruct. Res. 28, 112–124
9 Sedar, A. W. (1971) Anat. Rec. 169, 423
10 Winzler, R. J. (1970) Int. Rev. Cytol. 29, 77–125
11 Kraemer, P. M. (1971) in Biomembranes (Manson, L. A., ed.), Vol. 1, pp. 57–190, Plenum Press, New York
12 Moore, S. and Stein, W. H. (1954) J. Biol. Chem. 211, 907–913
13 Francois, C., Marshall, R. D. and Neuberger, A. (1962) Biochem. J. 83, 335–341
14 Weiss, J. B. and McGale, E. H. F. (1960) in Chromatographic and Electrophoretic Techniques (Smith, I., ed.), Vol. 2, p. 66, William Heinemann Medical Books, London and Interscience, New York
15 Reissig, J. L., Strominger, J. L. and Leloir, L. F. (1955) J. Biol. Chem. 217, 959–966
16 Fischer, F. G. and Nebel, H. J. (1955) Z. Physiol. Chem. 302, 10–19
17 Smith, I. (1958) in Chromatographic and Electrophoretic Techniques (Smith, I., ed.), p. 169, William Heinemann Medical Books, London and Interscience, New York
18 Park, J. T. and Johnson, M. J. (1949) J. Biol. Chem. 181, 149–151
19 Rosenberg, S. A. and Guidotti, G. (1968) J. Biol Chem. 243, 1985–1992
20 Bartlett, G. R. (1959) J. Biol. Chem. 234, 466–468
21 Zlatkis, A. and Zak, B. (1969) Anal. Biochem. 29, 143–148
22 Esselman, W. J., Laine, R. A. and Sweeley, C. C. (1972) Methods in Enzymology 28, 141–156
23 van Hoeven, R. P. and Emmelot, P. (1972) J. Membrane Biol. 9, 105–126
24 Skipski, V. P. and Barclay, M. (1969) Methods in Enzymology 14, 530–598
25 Svennerholm, L. (1957) Biochim. Biophys. Acta 24, 604–611
26 Svennerholm, L. (1956) J. Neurochem. 1, 42–53
27 Rouser, G., Galli, C. and Krotchevsky, G. (1965) J. Am. Oil Chem. Soc. 42, 404–410
28 Eng, L. F. and Noble, E. P. (1968) Lipids 3, 157–162
29 Kean, E. L. (1966) J. Lipid Res. 7, 449–452
30 Kasbekar, D. K., Forte, G. M. and Forte, J. G. (1968) Biochim. Biophys. Acta 163, 1–13
31 Schrager, J. and Oates, M. D. G. (1970) Digestion 3, 229–240

32 Slomiany, B. L. and Meyer, K. (1972) J. Biol Chem. 247, 5062–5070
33 Multani, J. S., Cepurneek, C. P., Davis, P. S. and Saltman, P. (1970) Biochemistry 9, 3970–3976
34 Allen, R. H. and Mehlman, C. S. (1973) J. Biol. Chem. 248, 3670–3680
35 Pamer, T., Glass, G. B. J. and Horowitz, M. I. (1968) Biochemistry 7, 3821–3829
36 Lloyd, K. O. and Kabat, E. A. (1968) Proc. Natl. Acad. Sci. U.S. 61, 1470–1477
37 Lisowska, E. and Jeanloz, R. W. (1973) Carbohydr. Res. 29, 181–191
38 Dalal, K. B. and Einstein, E. R. (1969) Brain Res. 16, 441–451
39 Forstner, G. G. and Wherrett, J. R. (1973) Biochim. Biophys. Acta 306, 446–459
40 Sweeley, C. C. and Dawson, G. (1969) in Red Cell Membrane (Jamieson, G. A. and Greenwalt, T. J., eds), pp. 172–227, Lippincott, Philadelphia
41 Stahl, W. L. and Trams, E. G. (1968) Biochim. Biophys. Acta 163, 459–471
42 Dewey, M. M. and Barr, L. (1970) in Current Topics in Membranes and Transport (Bronner, F. and Kleinzeller, A., eds), pp. 1–33, Academic Press, New York
43 Chandrasekhara, N. and Narayan, K. A. (1970) Life Sci. Part II, 9, 1327–1334
44 Rosenberg, S. A. and Guidotti, G. (1968) J. Biol. Chem. 243, 1985–1992
45 Quirk, S. J. and Robinson, G. B. (1972) Biochem. J. 128, 1319–1328
46 Funakoshi, L. and Yamashina, I. (1972) J. Biochem. Tokyo 72, 459–467
47 Helgeland, L., Christensen, T. B. and Janson, T. L. (1972) Biochim. Biophys. Acta 286, 62–71
48 Forte, T. M. and Forte, J. G. (1970) J. Cell Biol. 47, 782–786
49 Sedar, A. W. (1969) J. Cell Biol. 43, 179–184
50 O'Brien, J. S. (1967) J. Theoret. Biol. 15, 307–324
51 Papahadjopolos, D., Nir, S. and Ohki, S. (1971) Biochim. Biophys. Acta 266, 561–583