Phospholipid Class and Fatty Acid Composition of Golgi Apparatus Isolated from Rat Liver and Comparison with Other Cell Fractions*

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ABSTRACT: Rough endoplasmic reticulum, Golgi apparatus, and plasma membrane rich cell fractions were isolated from livers of rats under similar conditions of age and diet for analysis of phospholipid classes and fatty acid composition. Phosphatidylcholine was the major phospholipid of all membrane types. Golgi apparatus was intermediate between endoplasmic reticulum and plasma membrane with respect to levels of phosphatidylcholine and sphingomyelin. Endoplasmic reticulum was highest in phosphatidylcholine and lowest in sphingomyelin. Levels of lysophosphatidylcholine, phosphatidylserine, phosphatidylinositol, and phosphatidylethanolamine were relatively constant comparing endoplasmic reticulum, Golgi apparatus, and plasma membrane. Lysophosphatidylethanolamine was detected in Golgi apparatus and plasma membrane but not in endoplasmic reticulum. Neutral lipids represented 15% of the total extractable lipids of endoplasmic reticulum, 38% of the total extractable lipids of plasma membrane, and 46% of the total extractable lipids of Golgi apparatus. Neutral lipid fractions were composed mainly of cholesterol, free fatty acids, and triglycerides with smaller amounts of cholesterol esters. Palmitate and stearate were the major saturated acids of all lipid classes except sphingomyelin, which contained palmitate and lignocerate as its major saturated acids. Sphingomyelin from each membrane contained the highest percentage of saturated fatty acids in comparison with other phospholipids from the respective membrane fraction. The major unsaturated acids encountered were oleic, linoleic, and arachidonic acids. Phosphatidylserine of endoplasmic reticulum contained the largest percentage of unsaturated acids (75%). Total phospholipid fractions of endoplasmic reticulum contained the largest percentage of unsaturated acid residues (45%), followed by Golgi apparatus (36%) and plasma membrane (29%). The fatty acid composition of phosphatidylcholine and sphingomyelin of Golgi apparatus was intermediate between that of these lipids from endoplasmic reticulum and plasma membrane. The results are compatible with partial derivation of Golgi apparatus membranes from endoplasmic reticulum and reutilization of these membranes in the production of secretory vesicle and plasma membranes.

Precise knowledge on the composition of components comprising the cytoplasmic membrane system of mammalian cells (nuclear envelope, endoplasmic reticulum, Golgi apparatus, secretory vesicles, and plasma membranes) is limited. However, during the last decade development of suitable methods for isolation have permitted detailed characterization of lipids from rat liver plasma membranes (Emmelot *et al.*, 1964; Takeuchi and Terayama, 1965; Skipski *et al.*, 1965; Ashworth and Green, 1966; Dod and Grey, 1968; Pfleger *et al.*, 1968) and rough endoplasmic reticulum membranes (Dallner *et al.*, 1966; Glauman and Dallner, 1968). More recently, methods for the reproducible isolation of a highly purified Golgi apparatus fraction of useful quantity have become available (Morré *et al.*, 1969b), and it is now possible to make detailed comparisons of the lipid composition of Golgi apparatus with those of rough endoplasmic reticulum and plasma membranes as a first step in relating morphological differences among these cell components (Sjöstrand, 1963,1968) to differences in membrane constituents. Such comparisons are of importance not only in view of the essential role played by lipids in the structure and function of membranes (Green and Tzagoloff, 1966) but also from the standpoint of clarification of the proposed functional role of the Golgi apparatus as a site of endomembrane differentiation (Grove *et al.*, 1968; Morré *et al.*, 1969a). Grove and his associates (1968) observed a progressive staining transition across stacks of Golgi apparatus cisternae of the fungus *Pythium ultimum*. At the proximal face of the stacks (the face adjacent to endoplasmic reticulum or nuclear envelope), the membranes stained like endoplasmic reticulum. At the distal face of the stacks (adjacent to secretory vesicles), the membranes stained like vesicle membrane or plasma membrane. From the transitional nature of staining, Golgi apparatus was suggested to function as sites of membrane differentiation from endoplasmic reticulum-like to plasma membrane-like. Similar staining transitions have been observed across stacks of Golgi apparatus of mammary secretory cells (Helminen and Ericsson, 1968) and in Golgi apparatus isolated from rat liver (Morré *et al.*, 1969a). This paper describes the phospholipid class distribution and fatty acid composition of a Golgi apparatus rich fraction isolated from rat liver with corresponding data for endoplasmic reticulum and plasma membrane fractions obtained from animals under similar conditions of age and diet.

Experimental Procedures

Male Holtzman[1] rats weighing 200–250 g and maintained

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[1] The Holtzman Co., Madison, Wis.

on a Purina Laboratory diet were anesthetized by intraperitoneal injection of 0.5–1 ml of pentobarbital solution (20 mg/ml). Livers (approximately 10 g each) were removed, drained of blood, minced thoroughly with scalpels, and transferred to the appropriate homogenization medium. Methods used for preparation of endoplasmic reticulum, Golgi apparatus, and plasma membrane rich cell fractions have been described in detail in previous reports (Morré *et al.*, 1969a–c; Cheetham *et al.*, 1969) except that the Golgi apparatus was washed twice with distilled water to reduce the number of secretory vesicles. Enzymatic and morphological criteria (Cheetham *et al.*, 1969), employed on a routine basis to evaluate fraction purity, revealed that less than 20% of the Golgi apparatus and plasma membrane fractions and less than 10% of the endoplasmic reticulum fraction were contributed by other cell components. Membrane fractions were held at −20° until sufficient quantities were obtained for lipid extraction.

Buffer suspensions of membranes were thawed and extracted three times with five volumes of chloroform–methanol (2:1, v/v) and then once with chloroform alone. The chloroform-rich layers were combined and washed (Folch *et al.*, 1957) and the solvents evaporated *in vacuo* or under a stream of nitrogen without heat. Lipid residues were immediately weighed, redissolved in an accurately measured volume (50 μl/mg) of chloroform, and stored at 2° in tightly sealed vials for subsequent analysis. Solvents used throughout this study were of reagent grade quality and redistilled in glass before use.

Neutral lipids were resolved by thin-layer chromatography on 500-μ layers of silica gel G[2] in a solvents ystem composed of petroleum ether (30–60°)–ethyl ether–acetic acid (90:10:1, v/v). Identity of components was verified by comparison with authentic reference compounds. The components were measured quantitatively by densitometric (Downing, 1968) and photodensitometric (Siakotos and Rouser, 1966) analysis of plates charred with chromic acid reagent. Polar lipids were separated by two-dimensional thin layer chromatography on 500-μ layers of silica gel HR[2] without added binder in the solvent systems described by Parsons and Patton (1967). Components were detected on the plates, recovered, and analyzed for phosphorus by the procedure of Rouser *et al.* (1966). Identity of phospholipids was established by spraying with the specific phospholipid spray of Dittmer and Lester (1964), ninhydrin reagent (0.2% in ethanol), and by cochromatography with authentic reference compounds (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, cardiolipin, lysophosphatidylethanolamine, and lysolecithin).[3]

For fatty acid analysis, lipids resolved on thin-layer plates were visualized by brief exposure to iodine vapors and then scraped into screw-cap vials with Teflon-lined caps. Sulfuric acid (1 ml, 1%) in methanol (v/v) was added to vials which were sealed and held for 24 hr at 40°. Sphingomyelin was methylated with 5% H_2SO_4 in methanol (v/v) for 72 hr at 40°. Visualization by exposure to iodine vapors was found to be more sensitive and to yield the same fatty acid composition as when lipids were revealed by spraying with 2′,7′-dichlorofluorescein reagent.

The vial contents were diluted with saturated aqueous sodium chloride solution and extracted three times with petroleum ether. Extracts were combined, concentrated under a stream of nitrogen, and applied to a column (10 ft × 0.25 in.) packed with 20% diethylene glycol succinate polyester on 80–90 mesh Anakrom AB[4] operated isothermally at 185° in a Varian Aerograph Model 1520 gas chromatograph equipped with flame ionization detectors. Methyl ester concentrations were determined by half-height analysis of chromatographic peaks. Analysis of reference standards (RM-6 and NIH-F[5]) revealed that the major components (greater than 5%) were being analyzed with a relative error of less than 5%. Identification of chromatographic peaks was based on comparisons of retention times with known fatty acid methyl esters on both polar (diethylene glycol succinate) and nonpolar (Apiezon L) stationary phases. Identity of unsaturated acids was further verified by gas chromatographic analysis of methyl esters before and after hydrogenation over platinum oxide catalyst.

Results

Polar lipids accounted for 62% of the total lipids extracted from plasma membranes, 85% of the lipid fraction of endoplasmic reticulum, and 54% of the total lipid fraction of Golgi apparatus. With the exception of lysophosphatidylethanolamine, which was not detected in endoplasmic reticulum fractions, all three cell fractions contained the same seven major phospholipid classes (Figure 1).

Analysis of the distribution of phospholipids (based on percentage of total lipid phosphorus) showed phosphatidylcholine to be highest in the endoplasmic reticulum fraction and lowest in the plasma membrane fraction (Table I). The sphingomyelin content of the Golgi apparatus fraction was intermediate between endoplasmic reticulum and plasma membrane with the latter having the greater amount. Lysophosphatidylcholine increased slightly from endoplasmic reticulum to Golgi apparatus to plasma membrane and phosphatidylinositol decreased slightly across these membranes. The relative amounts of phosphatidylserine and phosphatidylethanolamine were essentially constant in the three membrane types.

Neutral lipids comprised 15% of the total lipids of endoplasmic reticulum, 38% of the total lipids of plasma membrane, and 46% of the total lipid fraction of Golgi apparatus (Table I). Thin-layer chromatographic analyses showed that cholesterol, free fatty acids, triglycerides, and cholesterol esters were the major constituents of neutral lipid fractions (Figure 2). By varying the polarity of the solvent system it was demonstrated that only trace quantities of monoglycerides and diglycerides were present. Free fatty acids accounted for the largest proportion of the neutral lipids of all membrane fractions, followed by triglycerides and cholesterol (Table I). Cholesterol esters accounted for 8 to 10% of the neutral lipid fractions.

Fatty acid analyses (through 24:1) of lipid classes revealed palmitate and stearate as the major saturated acids of all lipid classes except sphingomyelin from each membrane fraction (Table II). Palmitate and lignocerate were the major saturated acids in all sphingomyelin fractions. Sphingomyelin from

[2] Silica gel according to Stahl, Brinkmann Instruments, Inc., Westbury, Long Island, N. Y.

[3] Applied Science Laboratories, Inc., State College, Pa.

[4] Analabs, Inc., North Haven, Conn.

[5] Supelco, Inc., Bellefonte, Pa.

endoplasmic reticulum, Golgi apparatus, and plasma membrane exhibited the highest percentage of saturated acids when compared to other lipid classes from the respective membrane fraction. Overall, sphingomyelin of Golgi apparatus and plasma membrane contained the highest percentage of saturated acids. Phosphatidylserine of endoplasmic reticulum contained the largest percentage of unsaturated acids, with 75% of the total acids being unsaturated. The major unsaturated acids encountered were oleic, linoleic, and arachidonic acids. Phosphatidylinositol of endoplasmic reticulum and plasma membrane, lysophosphatidylcholine of all membrane fractions, and lysophosphatidylethanolamine of plasma membrane contained appreciable quantities of an acid tentatively identified as an 18-carbon diene isomer. This acid behaved in a manner analogous to that reported for the 6,9 isomer of linoleate on the polyester column (Ackman and Burgher, 1965) and eluted with the 18:2 fraction from the Apiezon column. On hydrogenation this acid disappeared from the chromatograms and there was a concurrent increase in the peak area for stearate. Arachidonic acid was not detected in triglycerides. With phosphatidylcholine and phosphatidylserine a decreasing percentage of unsaturated fatty acids was noted from endoplasmic reticulum to Golgi apparatus to plasma membrane. Lysophosphatidylcholine and triglycerides of Golgi apparatus contained higher quantities of unsaturated acids than these constituents from endoplasmic reticulum or plasma membrane. With total polar lipid fractions (data not shown), endoplasmic reticulum contained the largest proportion of unsaturated fatty acids (45.1%) followed by Golgi apparatus (36.1%) and plasma membrane (29.2%). Only triglycerides contained measurable quantities of fatty acids of chain lengths lower than 14 carbons. In only a few instances were the $C_{15:0}$ and $C_{17:0}$ fatty acids detected in appreciable quantities. Lysophosphatidylethanolamine contained appreciable quantities of lignoceric acid, as did sphingomyelin. These fractions did not appear to tail into one another and they were usually clearly separated on thin-layer plates. Lysophosphatidylethanolamine fractions underwent transesterification under conditions much too mild to appreciably methylate sphingomyelin. Similarly, sphingomyelin fractions did not undergo methylation under conditions used to methylate lysophosphatidylethanolamine.

The majority of the individual fatty acids of Golgi apparatus phosphatidylcholine, phosphatidylserine, and sphingomyelin are present in a relative concentration intermediate between those of endoplasmic reticulum and plasma membrane. The three membrane fractions were similar comparing the fatty acid composition of phosphatidylethanolamine. Each individual fatty acid of phosphatidylinositol of Golgi apparatus accounts for either a higher or lower per cent of the total than the corresponding acid in both endoplasmic reticulum and plasma membrane phosphatidylinositol.

TABLE I: Lipid Composition of a Golgi Apparatus Rich Cell Fraction and Comparison with Other Cell Fractions from Rat Liver.

Compound	% of Total Fraction		
	Endoplasmic Reticulum	Golgi Apparatus	Plasma Membrane
Total phospholipids[a]	84.9	53.9	61.9
Sphingomyelin[b]	3.7 ± 1.1	12.3 ± 2.5	18.9 ± 2.3
Phosphatidylcholine	60.9 ± 2.2	45.3 ± 2.7	39.9 ± 2.8
Phosphatidylserine	3.3 ± 2.2	4.2 ± 1.1	3.5 ± 1.8
Phosphatidylinositol	8.9 ± 2.3	8.7 ± 2.8	7.5 ± 1.3
Phosphatidylethanolamine	18.6 ± 1.1	17.0 ± 1.9	17.8 ± 1.5
Lysophosphatidylcholine	4.7 ± 3.4	5.9 ± 0.4	6.7 ± 0.7
Lysophosphatidylethanolamine	[c]	6.3 ± 1.0	5.7 ± 2.7
Total neutral lipids[d]	15.1	46.1	38.1
Cholesterol	24.6	16.5	34.5
Free fatty acids	40.6	38.9	35.1
Triglycerides	24.7	35.1	22.4
Cholesterol esters	10.1	9.6	8.0

[a] Mean value of three determinations, phospholipid = phosphorus value × 25. [b] Individual phospholipids expressed as percentage of total lipid phosphorus. Values are mean plus and minus standard deviation of four to six determinations. [c] Not detected. [d] Mean value of three to four determinations. Individual neutral lipids expressed as percentage of total neutral lipid fraction.

Discussion

Endoplasmic reticulum, Golgi apparatus, and plasma membranes are morphologically distinct membrane types (Sjöstrand, 1963) and studies with isolated cell fractions show them to be compositionally distinct (Morré *et al.* 1969a). The complexity of the fractions utilized in the present study precludes the possibility of directly relating these differences to differences in membrane lipid composition. Isolated Golgi apparatus fractions contain secretory vesicles and products in various stages of export (Morré *et al.*, 1969a,b). Very low density lipoprotein precursors, which are rich in neutral lipids, are major secretory products of rat liver (Mahley *et al.*, 1969) and the presence of numerous very low density lipoprotein precursor particles within tubules and forming secretory vesicles of isolated Golgi apparatus partially explains the high neutral lipid content of this fraction (Morré *et al.*, 1969a,b). Hence, phospholipids of washed Golgi apparatus preparations are of particular interest since they can reasonably be expected to be derived largely, if not entirely, from membranes.

Neutral lipid fractions from all three membrane types were rich in free fatty acids. The observed fatty acid content of plasma membrane is considerably higher than that reported by other investigators (Skipski *et al.*, 1965; Pfleger *et al.*, 1968; Dod and Grey, 1968). The possibility that these may arise by hydrolysis during isolation and handling is remote since plasma membranes isolated from other sources and handled in a similar manner do not contain appreciable levels of free fatty acids (Keenan *et al.*, 1969). The association of significant levels of free fatty acids with these membranes may indicate some lipid synthetic ability. Isolated plasma membranes

TABLE II: Fatty Acid Composition of Lipid Classes of a Golgi Apparatus Rich Cell Fraction and Comparison with Other Cell Fractions from Rat Liver.

	PC			PE			PI			PS			Sp			LPC			LPE		TG		
Fatty Acid[a]	ER	GA	PM	ER	GA	PM	ER	GA	PM	ER	GA	PM	ER	GA	PM	ER	GA	PM	GA	PM	ER	GA	PM
<14:0	Tr[b]	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	1.5	2.6	Tr
14:0	0.7	0.9	0.9	0.3	0.7	1.9	Tr	3.9	Tr	9.0	7.1	1.5	Tr	Tr	Tr	3.4	3.7	2.1	5.2	1.1	7.2	10.3	5.5
14:1	—[b]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.1	2.1	Tr
15:0	0.6	0.5	0.5	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	7.6	Tr	Tr	Tr	1.2	—	—	—
16:0	24.8	34.7	36.9	31.4	33.5	25.8	12.8	36.3	14.0	11.1	29.6	38.7	26.0	24.8	24.3	24.4	31.7	37.6	22.3	18.2	34.5	32.8	35.7
16:1	—	—	—	Tr	0.4	2.0	—	—	—	1.0	11.8	Tr	—	—	—	Tr	5.3	Tr	5.4	Tr	2.0	1.4	2.2
17:0	Tr	Tr	Tr	Tr	Tr	Tr	2.7	6.2	1.7	—	—	—	Tr	3.8	5.5	8.1	Tr	Tr	Tr	1.1	—	—	—
18:0	23.7	22.5	31.2	34.4	31.8	33.2	41.9	19.9	49.0	4.7	8.2	46.1	7.2	10.0	11.6	23.7	17.4	27.1	12.4	16.9	17.2	13.1	17.4
18:1	11.0	8.7	6.4	8.0	5.1	10.4	1.8	21.9	2.3	21.8	40.3	8.4	12.5	1.1	Tr	1.7	17.2	21.2	32.8	3.0	30.0	31.7	30.2
(18:2 i)[c]	—	—	—	—	—	—	10.9	Tr	12.5	Tr	Tr	Tr	Tr	Tr	Tr	21.3	3.2	6.1	Tr	6.4	—	—	—
18:2	18.9	18.1	12.9	13.6	10.0	8.7	10.9	1.6	1.3	52.3	2.9	1.0	13.9	Tr	Tr	8.1	12.0	3.2	2.8	36.8	2.8	4.1	1.3
18:3	Tr	Tr	Tr	0.3	Tr	Tr	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.5	0.4	Tr
20:0	1.0	Tr	Tr	—	—	—	—	—	—	—	—	—	Tr	1.7	2.1	—	—	—	Tr	Tr	—	—	—
20:4	19.2	14.5	11.1	12.1	18.3	17.9	18.9	10.2	19.1	Tr	Tr	4.2	3.7	9.4	11.4	1.7	9.6	2.6	1.3	0.9	—	—	—
22:0	—	—	—	—	—	—	—	—	—	—	—	—	7.3	13.6	7.1	—	—	—	1.6	1.6	1.1	0.4	1.4
24:0	—	—	—	—	—	—	—	—	—	—	—	—	29.3	35.5	37.9	—	—	—	16.3	12.7	1.0	1.1	6.3
% Unsaturated	49.1	41.3	30.4	34.0	33.9	39.0	42.5	33.7	35.2	75.1	55.0	13.6	30.1	10.5	11.4	32.8	47.3	33.1	42.3	47.1	37.4	39.7	33.7

[a] The number before the colon gives the number of carbon atoms and the number after the colon gives the number of double bonds. All values are expressed as weight percentage of total fatty acids in each fraction. ER, endoplasmic reticulum; GA, Golgi apparatus; PM, plasma membrane; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; Sp, sphingomyelin; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; TG, triglycerides. [b] Tr, trace quantity (<0.3%); —, not detected. [c] Tentatively identified as an isomer of linoleic acid.

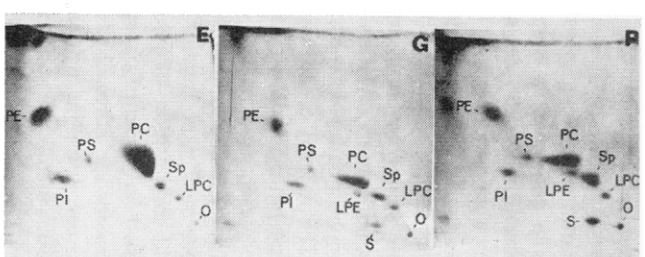

FIGURE 1: Two-dimensional thin-layer chromatographic separation of polar lipid fractions of endoplasmic reticulum (E), Golgi apparatus (G), and plasma membranes (P) from rat liver. Silica gel HR plates developed in the solvent systems described by Parsons and Patton (1967). O, origin; LPC, lysophosphatidylcholine; Sp, sphingomyelin; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; S, carbohydrate and/or protein contaminant. Material at upper left corner of plates is neutral lipid. Approximately 1 mg of each lipid fraction was applied to plates.

(Stein *et al.*, 1968), endoplasmic reticulum (Stein and Stein, 1967; S. Nyquist, 1969, unpublished data), and Golgi apparatus (S. Nyquist, 1969, unpublished data) are all capable of incorporating fatty acids into ester lipid or phospholipid classes. Neutral lipid fractions from Golgi apparatus contained higher levels of triglycerides and cholesterol esters, distinctive components of very low density lipoprotein particles (Mahley *et al.*, 1969), than did these fractions from plasma membrane and endoplasmic reticulum.

Although the percentage of the neutral lipid fraction of the Golgi apparatus accounted for as cholesterol is less than that of the endoplasmic reticulum (16.5% *vs.* 24.6%), this comparison is misleading. When compared on the basis of mg of cholesterol/100 mg of total lipid, the cholesterol content of the Golgi apparatus fraction (7.6 mg) is greater than that of endoplasmic reticulum (3.7 mg) but still less than that of plasma membrane (13.1 mg). The relatively high total cholesterol content of the Golgi apparatus fraction cannot be readily ascribed to the presence of lipoproteins of the secretory product. The secretory lipoprotein particles, when isolated from the Golgi apparatus fraction, are rich in neutral lipids, of which about 80% is triglyceride and 4.9% is cholesterol (Mahley *et al.*, 1969) *vs.* 35% triglyceride and 16.5% cholesterol for the total fraction. Even if all of the triglyceride of the Golgi apparatus fraction was presumed to be derived from lipoprotein particles of the secretory product, the cholesterol content of the remainder of the Golgi apparatus would not be greatly affected (calculated to be approximately 9 mg/100 mg of total lipid) and still intermediate between that of endoplasmic reticulum and plasma membrane. A similar argument can be extended to cholesterol esters (11% of the neutral lipid fraction of the secretory lipoproteins).

The decreasing phosphatidylcholine content and increasing content of sphingomyelin comparing endoplasmic reticulum, Golgi apparatus, and plasma membrane showed the Golgi apparatus to be intermediate between endoplasmic reticulum and plasma membrane (Table I). The fatty acid composition of sphingomyelin and phosphatidylcholine from Golgi apparatus was also intermediate between that of endoplasmic reticulum and plasma membranes (Table II). Assuming that Golgi apparatus membranes are derived from endoplasmic reticulum and then reutilized in the production of secretory vesicle membranes capable of fusing with plasma membrane (Grove *et al.*, 1968), then the resultant transformations at the Golgi apparatus must involve synthesis and/or selective incorporation of sphingomyelin. Another possibility is that the Golgi apparatus may be a site of selective exchange of certain phospholipids. Such selective exchanges are known to take place between mitochondria and microsomes *in vitro* (Wirtz and Zilversmit, 1968).

A high sphingomyelin content appears to be a distinctive feature of plasma membrane fractions. Other cells fractions from rat liver do not contain sphingomyelin in concentrations approaching that of the plasma membrane (Getz *et al.*, 1962; Dallner *et al.*, 1966; Dod and Grey, 1968; Yunghans *et al.*, 1970). Plasma membranes from bovine mammary gland also contain high levels of sphingomyelin and other cell fractions from this source do not contain sphingomyelin in concentrations approaching this level (Keenan *et al.*, 1969).

The relative amounts of lysophosphatidylcholine, phosphatidylserine, phosphatidylinositol, and phosphatidylethanolamine were essentially constant comparing endoplasmic reticulum, Golgi apparatus, and plasma membrane (Table I). The fatty acid composition of phosphatidylethanolamine was also similar comparing the three membrane fractions. However, distinct differences in fatty acid composition of phosphatidylserine, phosphatidylinositol, and lysophosphatidylcholine were evident comparing these membranes. This is suggestive of selective incorporation and/or exchange of these phospholipids or their acyl moieties within the Golgi apparatus. Each fatty acid in phosphatidylinositol of the Golgi apparatus was present in either higher or lower relative concentrations than the corresponding acid in phosphatidylinositol of both endoplasmic reticulum and plasma membrane. Interestingly, phosphoinositides appear to be selectively involved in secretion of proteins from pancreatic acinar cells (Hokin and Hokin, 1958) and in secretion of epinephrine by the adrenal medulla (Hokin *et al.*, 1958).

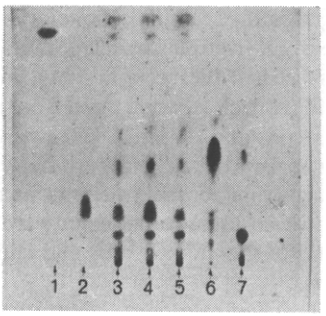

FIGURE 2: Thin-layer chromatogram of total lipid fractions extracted from endoplasmic reticulum (3), Golgi apparatus (4), and plasma membranes (5) of rat liver. Silica gel G plate with a solvent system of petroleum ether (30–60°)–diethyl ether–acetic acid (90:10:1, v/v). Approximately 600 μg of each lipid fraction was applied to the plate; (1) cholesterol palmitate, (2) palmitic acid; (6) triolein, (7) cholesterol. Material at origin is polar lipid.

Lysophosphatides are known to be precursors of certain cellular phospholipids and intermediates in the interconversion of diacyl and monoacyl phosphoglycerides (Van Deenen and DeHass, 1966). Our finding of lysophosphatidylcholine and lysophosphatidylethanolamine in these membrane fractions suggests that these membranes may be capable of acylation of lysophosphatides. Plasma membrane fractions from rat liver catalyze acylation of exogenously supplied lysolecithin and lysophosphatidylethanolamine *in vitro* and the resultant phospholipids are bound to the membrane fraction (Stein *et al.*, 1968). Lysophosphatidylethanolamine has not heretofore been reported to occur in plasma membrane lipids. Comparing the fatty acid compositions of lysophosphatidylcholine with phosphatidylcholine from each membrane fraction, it appears that the lysophosphatide could yield the phosphatide by predominant incorporation of linoleic and arachidonic acids. Further work on fatty acid positioning within the molecule will be necessary to evaluate this possibility. The fatty acid compositions of lysophosphatidylethanolamines were so different from those of the corresponding phosphatidylethanolamines that a precursor–product relationship between more than a small fraction of these two lipid classes is precluded.

Our results do not take into account the quantities (7–11% of the total membrane lipids) of phosphorus-free polar lipids which have been detected in plasma membrane lipids by other investigators (Pfleger *et al.*, 1968; Dod and Grey, 1968). Dod

and Grey (1968) identified ceramide monohexoside, dihexoside, and trihexoside in rat liver plasma membrane lipids. With the solvent systems used in the present study, monohexose and dihexose ceramides are clearly separated from the components encountered (Parsons and Patton, 1967; Keenan *et al.*, 1969) and under appropriate conditions were detected and identified in the plasma membrane fractions analyzed (Keenan *et al.*, 1969). However, these components were not detected in the lipid fractions of Golgi apparatus and endoplasmic reticulum preparations.

Current knowledge of membrane structure does not permit a detailed assessment of the significance of fatty acid composition of lipids in membrane structure. Current concepts of membrane structure propose that the hydrocarbon chain of fatty esters specifically complements certain hydrophobic amino acid residues in the membrane protein (for a discussion see Benson, 1966, and Korn, 1969). As discussed by Benedetti and Emmelot (1968), phospholipids containing high proportions of unsaturated fatty acids form expanded films, whereas hydrogenated phospholipid films are tightly packed, resembling the situation with saturated fatty acids. Insertion of cholesterol into the unsaturated phospholipid film has a condensing effect (Dervichian, 1964; VanDeenen, 1965). The combined presence of phospholipids containing relatively large amounts of saturated phospholipids and cholesterol (important for the tight packing of the remaining unsaturated phospholipids) in a membrane would provide a relatively stable membranous sheet as is encountered in plasma membrane. The converse seems to apply to endoplasmic reticulum which is characterized by phospholipid fractions relatively high in unsaturated fatty acids, low cholesterol content, and a marked tendency to vesiculate. The Golgi apparatus is both chemically and morphologically intermediate between these two extremes. Although considerable variation between individual fatty acids of each phospholipid was evident comparing the three membrane types, the most striking difference was the decreasing percentage of unsaturated fatty acids of total phospholipid fractions comparing endoplasmic reticulum to Golgi apparatus to plasma membrane.

Endoplasmic reticulum, Golgi apparatus, and plasma membrane are morphologically distinct, and with regard to lipids they are compositionally distinct. Golgi apparatus membranes are intermediate in content and fatty acid composition of sphingomyelin and phosphatidylcholine. The observations are particularly critical to the functional role in endomembrane differentiation proposed for the Golgi apparatus by Grove *et al.* (1968). The occurrence of membrane transformations within the Golgi apparatus is inherent in the concept of a maturing and a secreting face of the apparatus (Mollenhauer and Whaley, 1963). Since cisternae from the maturing face ultimately separate from the stack, maintenance of a constant number of cisternae depends upon the formation of new cisternae on the opposite or forming face. Thus, membrane material derived from the endoplasmic reticulum may be transformed in the Golgi apparatus into vesicle membranes which are plasma membrane-like and capable of fusing with plasma membrane. Most important, these results amplify the morphological uniqueness of Golgi apparatus and provide a basis for biochemical studies of endomembrane differentiation.

Our results are in close agreement with those reported by others for phospholipid distribution (Skipski *et al.*, 1965; Dod and Grey, 1968; Pfleger *et al.*, 1968) and fatty acid composition (Pfleger *et al.*, 1968) of rat liver plasma membranes. We have obtained a ratio of palmitate to oleate in plasma membrane phospholipids (1.8) which is very similar to the ratio (2.2) observed by Pfleger *et al.*, (1968) for plasma membrane total lipids. Since rats utilized in these studies represented different breeds and ages, the constancy observed indicates a distinct functional role for phospholipids in plasma membrane structure and function.

Acknowledgment

The excellent technical assistance of Diane E. Olson and Dorothy Werderitsch is gratefully acknowledged. The authors are grateful to W. Sweet, the Schoefel Instrument Co., P. T. Chandler, Virginia Polytechnic Institute, and G. A. Porter, The Pennsylvania State University, for performing the densitometric analyses.

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The Composition and Structure of Carbohydrate Moiety of Stem Bromelain*

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ABSTRACT: The composition and structure of the carbohydrate prosthetic group of stem bromelain have been investigated by using the glycopeptide preparation obtained from the Pronase digest of the parent protein. The composition of the carbohydrate unit, as expressed in nearest integers, is 3 moles of mannose, 1 of fucose, 1 of xylose, and 2 of *N*-acetylglucosamine per mole; none of the other sugars are present. There is a possibility of partial deletion or addition of some terminal residues since the actual data of analysis do not give strictly integral numbers, particularly for mannose and fucose. Two of the three mannoses, one fucose, and one xylose are specifically liberated by the action of α-D-mannosidase from the liver of *Turbo cornutus* and pig kidney, α-L-fucosidase from *T. cornutus*, and β-D-xylosidase from the liver of *Charonia lampas*, respectively. β-D-*N*-Acetylglucosaminidase from *T. cornutus* releases one *N*-acetylglucosamine from the neutral sugar-free glycopeptide. Partial acid hydrolysis of the glycopeptide yields, in addition to free monosaccharides, mannobiose, mannotriose, and mannosyl-*N*-acetylglucosamine. The glycopeptide consumes approximately 8 moles of periodate to produce more than 2 moles of formic acid, and, upon reduction and hydrolysis, close to 2 moles of glycerol, 1 mole of propylene glycol, and none of erythritol. In sequential Smith degradation, neutral sugar residues are first oxidized and removed, and two *N*-acetylglucosamine residues are then oxidized in stepwise fashion. From these and other pieces of evidence, the following structure is proposed for the carbohydrate moiety of stem bromelain:

α-D-Man(1→2)-α-D-Man-(1→2 or 6)-[α-L-Fuc-(1→6 or 2)]-α-D-Man-(β-D-Xyl)-β-D-GlcNAc-(1→3 or 4)-β-D-GlcNAc-(1→β-NH_2-*N* of Asn)-peptide

Stem bromelain is a proteolytic enzyme found in tissues of pineapple stem. It is a thiol protease and in this respect it resembles papain and ficin. However, unlike papain and ficin, stem bromelain was found to be a glycoprotein (Murachi *et al.*, 1964) which contains carbohydrate prosthetic group as a distinct single moiety (Murachi *et al.*, 1967). From the digest of stem bromelain by Pronase, glycopeptides were isolated and analyzed for their amino acid sequences around the carbohydrate moiety. The one with the longest sequence was shown to be Asn-Asn(sugar)-Glu-Ser-Ser (Takahashi *et al.*, 1969). The sugar moiety contained mannose, xylose, fucose, and *N*-acetylglucosamine.

Using the glycopeptide obtained from the Pronase digest, we have studied the composition and structure of the carbohydrate moiety. Various methods were employed for quantitative determination of component sugars in order to minimize fluctuation of data that would have arisen from different degrees of destruction of sugars depending upon the methods of assay. The sequence of the heterooligosaccharide moiety was studied by using several different kinds of glycosidases with different specificities, partial acid hydrolysis, and periodate oxidation. The latter two methods were rather conventional, while most of the specific glycosidases used in the present experiments were the enzymes only recently

* From the Department of Biochemistry, Nagoya City University School of Medicine, Nagoya, Japan. *Received September 3, 1969.* This work was supported in part by a grant from the Ministry of Education, Japan, and by a U. S. Public Health Service research grant (GM08714) from the National Institute of General Medical Sciences.