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LIPID COMPOSITION OF NUCLEAR MEMBRANES ISOLATED FROM BOVINE LIVER

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SUMMARY

1. Nuclear membrane fractions were prepared from rat-liver nuclei by treatment with deoxyribonuclease and $MgCl_2$.

2. The lipid distribution and fatty acid composition of total lipids and individual phospholipids was determined.

3. The ratio of protein to total lipid was 2.96. Phospholipids accounted for 62 % of the total lipid fraction. The remainder was accounted for as neutral lipid. Non-phosphorus-containing polar lipids were not detected. Based on lipid phosphorus distribution the major phospholipids were phosphatidylcholine (55 %), phosphatidylethanolamine (23 %), phosphatidylinositol (8 %), phosphatidylserine (6 %) and sphingomyelin (6 %). Cholesterol and free fatty acids were the major constituents of the neutral lipid fraction, accounting for 16 and 13 %, respectively, of the total membrane lipids.

4. Phosphatidylcholine was the least saturated and sphingomyelin the most saturated of the major phospholipids. Eighteen-carbon chain-length fatty acids accounted for 70 % of the acyl residues of the total lipid fraction. The palmitic acid to oleic acid ratio of the total lipid fraction was 0.55.

INTRODUCTION

Knowledge of the composition of membranes is crucial to an understanding of the relationships between composition, structure and function. It is beyond doubt that lipids and especially polar lipids and cholesterol are essential components of mammalian membranes. This essentiality of lipids has led many investigators to study the lipid composition of components comprising the cytoplasmic membrane system of mammalian cells (nuclear envelope, endoplasmic reticulum, Golgi apparatus, secretory vesicle and plasma membranes). Several groups have made detailed studies of the lipid composition of rat-liver plasma membranes[1–4] and endoplasmic reticulum membranes[5–8]. Detailed analyses of the lipid fraction of Golgi apparatus from rat liver have recently been conducted[7,8]. Because of a lack of suitable methods for isolation, lipids of the nuclear envelope have not been characterized. The development of a method for isolation of useful quantities of membranes from bovine liver nuclei[9] makes the present study of the lipid class distribution and fatty acid composition of the nuclear envelope possible.

METHODS AND MATERIALS

Preparation of membrane fractions

Nuclear membrane fractions were isolated from a large scale preparation of beef-liver nuclei by a newly developed procedure described in the preceding paper[9].

Lipid extraction and analysis

Membrane preparations were combined and stored at −20° until analyzed. Frozen membrane preparations were thawed at 25° and suspended in 1 mM potassium bicarbonate buffer. The suspensions were extracted twice with chloroform–methanol (2:1, by vol.) and then once with chloroform alone. The chloroform-rich layers were combined, washed by the method of FOLCH *et al.*[10] and the solvents were evaporated *in vacuo* or under a stream of N_2 without heat. Lipid residues were immediately weighed, redissolved in an accurately measured volume of chloroform and stored at −20° under N_2 in tightly sealed vials.

Neutral lipids of the total lipid extract were resolved by thin-layer chromatography on 500-μ layers of silica gel G using a solvent system of light petroleum (b.p. 30–60°)–diethyl ether–acetic acid (90:10:1, by vol.). Hydrocarbons and cholesterol esters were resolved by chromatography in a solvent system of hexane–benzene (1:1, by vol.). Identity of components was verified by comparison with authentic reference compounds. The components were measured quantitatively by densitometric analysis[11] of plates charred with 50 % aqueous H_2SO_4. The photodensitometer (Photovolt Corp., New York) was operated with a Varicord recorder and an Integraph integrator.

Polar lipids were separated by two-dimensional thin-layer chromatography[12] on 500-μ layers of silica gel HR. Lipid phosphorus was determined by the method of ROUSER *et al.*[13]. The identity of polar lipids was established by use of the specific phospholipid spray of DITTMER AND LESTER[14] and by co-chromatography with authentic reference lipids. For densitometric determination of the percentage of neutral and polar lipids and to further check for the presence of cerebrosides, plates were developed in chloroform–methanol–water (72:20:3, by vol.).

Lipid components were localized[15] on two-dimensional thin-layer plates, recovered, methylated[16] and the fatty acid composition was analyzed on a 6 ft × 1/4 in column packed with 20 % diethylene glycol succinate on Anakrom AB (Analabs, Hamden, Conn.) using a Varian–Aerograph Model 1520 gas chromatograph. The instrument was equipped with flame-ionization detectors and the oven temperature was maintained at 180°. Quantification of standard methyl ester mixtures revealed that the major components (>5 %) were being analyzed with a relative error of less than 5 %. Total lipid extracts were methylated according to METCALFE *et al.*[17]. Identification of the fatty acid methyl esters was accomplished by graphic plotting of their gas-chromatographic retention times in comparison with those of known methyl esters according to ACKMAN AND BURGHER[18]. Identity of unsaturated acids was further verified by analysis of methyl esters before and after hydrogenation over platinum oxide catalyst.

The presence of carbohydrate-containing components was assessed by reaction of 1 mg quantities of total lipid extracts with anthrone reagent[19].

Protein determination

Protein was determined by the method of LOWRY *et al.*[20] with bovine serum albumin as the standard.

Chemicals

Silica gel was obtained from Brinkmann Instruments (Westbury, N.Y.). Authentic phospholipids and cerebrosides were obtained from Applied Science (State College, Pa.). Neutral lipids were purchased from The Hormel Institute (Austin, Minn.). Reference methyl ester mixtures NHI-F and RM-6 were from Supelco (Bellefonte, Pa.) and individual methyl esters were from Analabs (Hamden, Conn.). NADPH, cytochrome *c* and succinic acid were from Sigma. Deoxyribonuclease was obtained from CalBiochem. All other chemicals were of reagent grade.

All solvents were glass-distilled prior to use.

RESULTS

Distribution of lipids

Nuclear membranes contained approx. 0.33 mg lipid per mg protein and gave a protein to lipid ratio of 2.96.

Phospholipids accounted for 62 % of the total membrane lipids with neutral lipids accounting for the remainder (38 %) of the lipid fraction (Table I). No non-

TABLE I

LIPID COMPOSITION OF NUCLEAR MEMBRANES OF BOVINE LIVER

Mean values ± S.D. of 4 to 6 analyses.

Compounds	*Percentage of total lipids*	*Percentage of total phospholipids*	*Percentage of total neutral lipids*
Total phospholipids	62.2 ± 3.4		
Phosphatidylcholine		54.5 ± 2.0	
Phosphatidylethanolamine		22.5 ± 3.1	
Phosphatidylserine		6.0 ± 0.8	
Phosphatidylinositol		7.6 ± 0.4	
Sphingomyelin		5.8 ± 0.4	
Lysophosphatidylcholine		1.6 ± 0.1	
Cardiolipin		2.1 ± 0.2	
Total neutral lipids	37.8 ± 3.1		
Cholesterol			42.3 ± 0.3
Free fatty acids			35.7 ± 1.6
Triglycerides			8.9 ± 0.3
Diglycerides			8.5 ± 0.3
Hydrocarbons Cholesterol esters			4.7 ± 0.1

phosphorus-containing polar lipids were observed in thin-layer chromatograms developed in either the one or two-dimensional solvent systems. Although cerebrosides are clearly separated from the other polar lipids encountered[12,16], these components were not detected in two-dimensional chromatograms when up to 1.5 mg of total

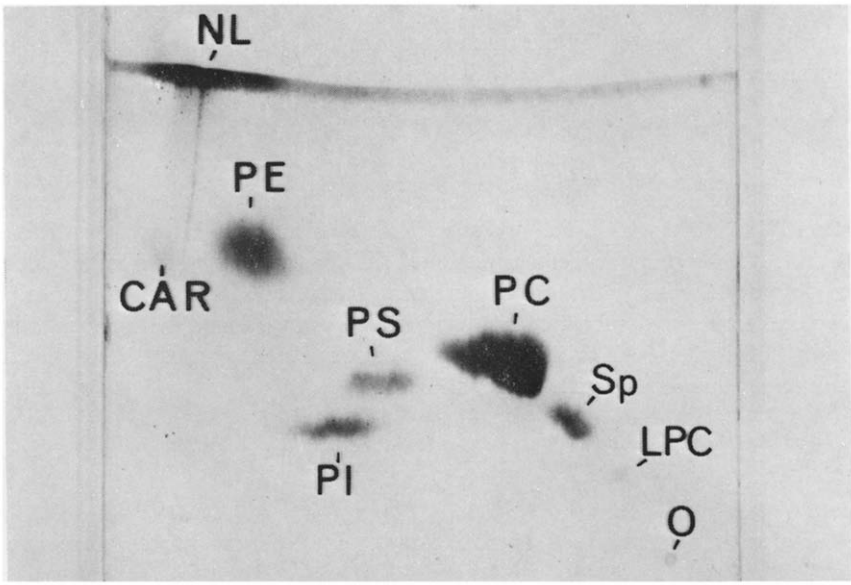

Fig. 1. Two-dimensional thin-layer chromatographic separation of polar lipids from nuclear membranes. *O*, origin; LPC, lysophosphatidylcholine; Sp, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; CAR, cardiolipin; NL, neutral lipid. Approx. 1 mg of the total lipid fraction was applied to the plate. Spots were visualized by charring with H_2SO_4.

lipid was applied to plates (Fig. 1). Total lipid fractions gave negative anthrone reactions, confirming the absence of cerebrosides in this fraction. By lipid phosphorus analysis, phospholipids were estimated to account for 62.3 ± 2.2 % (5 determinations; lipid phosphorus × 25) of the total lipid fraction. This value is in excellent agreement with the values obtained by densitometry. The latter method gave 62.2 ± 3.4 % for polar lipids and 37.8 ± 3.1 % for neutral lipids (three determinations). Thus it can be concluded that the polar lipid fraction of nuclear membrane is composed almost entirely of phospholipids.

Five major components were present in the phospholipid fraction (Fig. 1); phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and sphingomyelin accounted for approx. 55, 23, 8, 6, and 6 %, respectively, of the total lipid phosphorus (Table I). Small amounts of lysophosphatidylcholine (1.6 %) and cardiolipin (2.1 %) were also present. Other phospholipids were not encountered.

The neutral lipids comprised 38 % of the total lipids present in bovine liver nuclear membranes. Thin-layer densitometric analysis of this fraction showed that it contained 42 % unesterified cholesterol and 36 % free fatty acids. Diglycerides (9 %), triglycerides (9 %) and hydrocarbons *plus* cholesterol esters (5 %) accounted for lesser amounts of the neutral lipid fraction. By chromatography in a system which resolves hydrocarbons and cholesterol esters (hexane-benzene, 1:1, by vol.), cholesterol esters were found to account for less than 2 % of the neutral lipid fraction.

TABLE II

MAJOR FATTY ACID COMPOSITION OF LIPIDS FROM NUCLEAR MEMBRANES OF BOVINE LIVER

Wt. % of major fatty acids (>1 % of total) calculated from peak areas of gas chromatograms.

Fatty acid	*Composition* (%)					
	Total lipids	*Phosphatidyl-choline*	*Phosphatidyl-ethanolamine*	*Phosphatidyl-serine*	*Phosphatidyl-inositol*	*Sphingo-myelin*
16:0	12.3	16.0	6.6	2.2	6.0	30.1
18:0	21.3	9.4	38.0	54.5	49.5	13.4
18:1	21.8	34.1	14.0	28.2	13.0	2.4
18:2	31.1	39.1	11.3	2.7	6.8	
20:0						10.2
20:4	7.0	1.4	19.5	2.9	12.8	
22:0	3.0		2.6	6.5	5.0	20.6
22:4*	2.1		5.3	3.1	6.9	
22:6	1.4		2.8			
24:0						23.3

* Tentative identification.

Fatty acid composition

The major fatty acid (>1 % of the total) composition of the total lipid fraction and individual phospholipid classes is given in Table II. Palmitate (12 %), stearate (21 %), oleate (22 %), linoleate (31 %) and arachidonate (7 %) were the principal acyl groupings present in the total lipid fraction. Smaller amounts of behenate, docosahexaenoate and an acid tentatively identified as a docosatetraenoate were also detected in this fraction. Only trace quantities of short chain acids (<16 carbons) were detected in any of the lipid fractions examined. Palmitate and stearate were the only major saturated acids in phosphatidylcholine. The saturated fatty acid fraction of phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol contained behenate in addition to palmitate and stearate. Sphingomyelin, the most highly saturated of the phospholipids, contained appreciable quantities of palmitate (30 %), arachidate (10 %), behenate (21 %), and lignocerate (23 %).

TABLE III

CARBON CHAIN-LENGTH COMPOSITION OF FATTY ACIDS FROM TOTAL LIPIDS OF NUCLEAR MEMBRANES FROM BOVINE LIVER

Wt. % calculated from peak areas of gas chromatograms. Computed values obtained by summation of data in Table III. The second method involved gas chromatographic analysis of the completely hydrogenated methyl esters.

Method	*Carbon chain length* (%)			
	16	*18*	*20*	*22*
Computed	12.3	74.2	7.0	6.5
Hydrogenation	13.9	68.9	11.8	5.3

Sphingomyelin was the only phospholipid which contained appreciable levels of arachidate and lignocerate. All phospholipids except sphingomyelin contained oleate, linoleate and arachidonate. The only unsaturated acid detected in sphingomyelin was oleate. Phosphatidylcholine contained the highest percentage of unsaturated acids (75 %), followed by phosphatidylethanolamine (53 %).

Comparisons of carbon chain-length composition of the total lipid fraction as summed from the compositional data in Table II and as calculated from the peak areas of the hydrogenated sample are presented in Table III. These analyses show reasonably good agreement and tend to confirm the identities assigned to the unsaturated acids. Fatty acids with eighteen carbon atoms accounted for approx. 70 % of the total fatty acid residues in the lipid fraction of the nuclear membrane.

DISCUSSION

Our work is the first to have examined the lipid distribution and fatty acid composition of the nuclear membrane. Such analyses are an important step toward an understanding of the compositional–structural interrelationships of membranes. In view of their essential role in the structure and function of membranes[21], lipids would be expected to contribute to morphological differences observed with cellular cytomembranes[22]. Our nuclear envelope preparations were composed of both inner and outer membranes and, consequently, the figures presented herein represent average values for the two membranes[9]. Moreover, we assume that the low recovery of nuclear phospholipids in the nuclear membrane fraction (approx. 47 % (ref. 9)) is due to loss of membrane material. Such an assumption seems warranted in view of the close similarity between the phospholipid distribution of our nuclear membrane preparations and that of liver nuclei fractions[23].

The bovine liver nuclear membrane fraction had a phospholipid to protein ratio of 0.21. This value is lower than the values of 0.26 and 0.27 reported for rough endoplasmic reticulum[5] and plasma membrane[4], respectively, from rat liver and may reflect some contamination of the preparation by unextracted nucleoplasm. Our values for the distribution of the major phospholipids of nuclear membrane agree closely with the values reported for whole nuclei of rat liver[23]. Distribution of phospholipids in the nuclear membrane fraction was similar to the distribution reported for rough endoplasmic reticulum of rat liver[5,8], but is considerably different than the distribution of phospholipids in Golgi apparatus[7,8] and plasma membrane[1,3,4,7,8] from rat liver. The low levels of cardiolipin (2 % of the total lipid phosphorus) in our nuclear membrane preparation emphasizes the low level of contamination of this fraction by mitochondria. Cardiolipin of bovine liver mitochondria accounts for approx. 17 % of the total lipid phosphorus of this fraction[24].

Free cholesterol accounted for 16 % of the nuclear membrane total lipid fraction, a value which is somewhat lower than the 17 % and 21 % obtained with plasma membranes by DOD AND GRAY[3] and PFLEGER *et al.*[4] but which is higher than the values obtained with rough endoplasmic reticulum[5,7,8]. The neutral lipid fraction of the nuclear membrane was high in comparison with rough endoplasmic reticulum[5,7,8], as was the amount of free fatty acid. This elevated level of free fatty acids may have been caused, in part, by some hydrolysis during freezing and thawing of the membrane fractions. Diglycerides, triglycerides and hydrocarbons *plus* choles-

terol esters accounted for only a small proportion (8 %) of the total nuclear membrane lipids, in agreement with results for other endomembrane components[1,3–8,25].

Although current knowledge of membrane structure does not permit a detailed assessment of the significance of fatty acyl groupings of lipids in membrane structure, it is assumed that the hydrocarbon chain of fatty esters specifically complements certain hydrophobic amino acid residues in membrane proteins[26,27]. Thus differences in fatty acid composition would be expected to result in differences in membrane structure. In general, individual phospholipids of the nuclear membrane had lower levels of palmitic acid and higher levels of the eighteen-carbon acids than we have observed with these phospholipids from rough endoplasmic reticulum, Golgi apparatus and plasma membrane fractions isolated from rat liver[8]. Phosphatidylcholine and phosphatidylethanolamine of the nuclear membrane contained a higher percentage of unsaturated fatty acids than these lipids from the above mentioned endomembrane components[8]. Sphingomyelin of the nuclear membrane contained a larger percentage of saturated fatty acids than did the sphingomyelin fraction of the rat-liver endomembranes. We obtained a ratio of palmitic to oleic acid in the nuclear membrane (0.55 in total lipids) that was considerably lower than the ratio (2.2) observed by PFLEGER *et al.*[4] in rat-liver plasma membranes.

Since the work reported herein was done with bovine liver nuclear membranes, comparison with endomembrane lipids from rat liver may not be valid, especially with regard to fatty acid composition. However, it is of interest to note that the nuclear membrane phospholipid distribution is similar to that of rough endoplasmic reticulum and that these two membranes are morphologically similar[22]. The phospholipid distribution of both of these membranes is different than that of plasma membranes; these membranes are morphologically different[22]. GROVE *et al.*[28] 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 plasma membrane. Similar staining transitions have been observed across stacks of Golgi apparatus of mammary secretory cells[29] and in Golgi apparatus isolated from rat liver[25]. From the transitional nature of staining, Golgi apparatus have been suggested to function as sites of membrane differentiation from endoplasmic reticulum-like to plasma membrane-like[25,28]. The distribution of phospholipids in Golgi apparatus membranes from rat liver has been found to be intermediate between that observed with endoplasmic reticulum and plasma membranes from the same source[8]. In view of these observations and the data presented in this paper, it seems reasonable to conclude that at least part of the morphological difference among endomembranes is correlated with differences in phospholipid distribution within the membranes.

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