Biochimica et Biophysica Acta, 455 (1976) 655–664

BBA 77525

THE LIPID COMPOSITION OF PLASMA MEMBRANE SUBFRACTIONS ORIGINATING FROM THE THREE MAJOR FUNCTIONAL DOMAINS OF THE RAT HEPATOCYTE CELL SURFACE

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(Received April 27th, 1976)

SUMMARY

1. The neutral and phospholipid compositions of three rat liver plasma membrane subfractions originating predominantly from the three major functional domains of the hepatocyte viz the blood sinusoidal, contiguous and bile canalicular fractions, were determined.

2. The sinusoidal and canalicular plasma membrane subfractions, both of which were vesicular, contained a higher lipid to protein weight ratio than the contiguous plasma membrane subfraction that consisted of membrane strips, junctional complexes and some larger vesicles. The three plasma membrane subfractions contained a similar neutral lipid to phospholipid ratio. The highest unesterified cholesterol content was associated with the canalicular plasma membrane subfraction.

3. The phospholipid profiles of the three subfractions were generally similar. However, the canalicular plasma membrane subfraction contained a higher proportion of sphingomyelin than the other subfractions.

4. Correlations between the neutral and phospholipid composition of the subfractions and membrane integrity and function are discussed, especially with respect to a possible role of lipids in governing the resilience of the canalicular plasma membrane to the action of bile salts.

INTRODUCTION

Although the plasma membrane of mammalian cells is commonly isolated as a single subcellular fraction, this is clearly an oversimplification when this fraction is prepared from cells organised into tissues. Scanning electron-microscopy has most vividly emphasised the hepatocyte surface membrane to comprise of two surface areas rich in microvilli, interfacing with the blood and bile spaces, topographically separated by a smoother surface area containing intercellular junctional complexes [1]. These three morphologically and functionally distinct hepatocyte surface domains are now amenable to chemical analysis following the identification, on the basis of

biochemical and morphological studies [2, 3] of plasma membrane subfractions originating from the blood, bile and contiguous (lateral) surface areas. To account for the polar distribution on the hepatocyte surface membrane of enzyme activities, proteins and glycoproteins, knowledge of the lipid composition of membrane fractions originating mainly from each of these surface domains should prove instructive, since modern concepts of membrane structure envisage the cell surface membrane as a fluid lipid continuum in which proteins and glycoproteins may move laterally in the membrane plane [4]. The present work describes the neutral and phospholipid compositions of the appropriate liver plasma membrane subfractions now designated according to their functional origin, i.e. the sinusoidal, canalicular and contiguous plasma membrane subfractions, and shows that the overall lipid content of the fractions differs to a far lesser extent than that reported for enzymes or proteins [3]. However, some differences were noted, especially in the cholesterol and sphingomyelin composition and these are discussed especially in the context of a possible mechanism that can explain the resilience of the bile canalicular membrane towards bile salts.

EXPERIMENTAL

Preparation of plasma membrane fractions

Rat liver plasma membrane subfractions were prepared from male Sprague-Dawley rats (150–200 g) [3] fed on Charles-River diets 7RF and 4RF. The plasma membrane fractions now prefixed sinusoidal, canalicular and contiguous correspond to the microsomal-light, zonal light and combined zonal heavy A and B subfractions respectively in the nomenclature of Wisher and Evans [3]. Plasma membranes, collected at sucrose gradient interfaces, were diluted with H_2O and pelleted by centrifugation. To remove adsorbed or occluded proteins and lipoproteins the pellets were resuspended in a known volume of H_2O by homogenisation in a tight-fitting homogeniser and after approx. 5 min at 4 °C, an equal volume of 0.15 M NaCl, 10 mM Tris · HCl, pH 7.5 was added. The membranes were pelleted by high-speed centrifugation, and then resuspended in small volumes of H_2O for immediate lipid analysis or in isotonic sucrose for determination of protein content [5].

Extraction of lipids

Membrane samples suspended in 2.5 ml of H_2O, were added to 40 ml of chloroform/methanol (1 : 1, v/v) and the lipids extracted at 4 °C overnight. A further 20 ml of chloroform was added, and the extract filtered through Whatman No. 1 filter paper, which was then washed twice with a small volume of 2 : 1, (v/v), chloroform/methanol. The extract was separated into two phases by addition of 0.2 vol. of 0.04 % $CaCl_2$, 0.017 % $MgCl_2$, 0.29 % NaCl and 0.37 % KCl according to Folch et al. [6], and the lower phase collected after standing overnight at 4 °C. The extracts were evaporated to dryness, weighed and resuspended in a small volume of benzene. All organic reagents contained butylhydroxytoluene (0.05 %) to minimise oxidation of lipids.

Estimation of neutral and phospholipids

The overall lipid content of the membrane fractions was determined gravimetri-

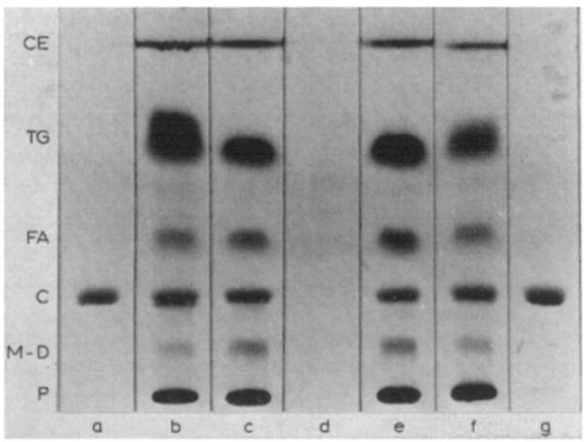

Fig. 1. One-dimensional thin-layer chromatogram of the separation of neutral lipids extracted from liver plasma membrane subfractions. (a) and (g), cholesterol standards of 200 and 400 μg respectively; (d), blank area for quantitative determination by dichromate oxidation [7]. (b) and (f), Sinusoidal plasma membrane subfraction; (c), canalicular plasma membrane subfraction; (e), contiguous plasma membrane subfraction. P, phosphatides (origin); M-D, mono- and di-glycerides; C, cholesterol; FA, fatty acids; TG, triglycerides; CE, cholesterol esters (solvent front).

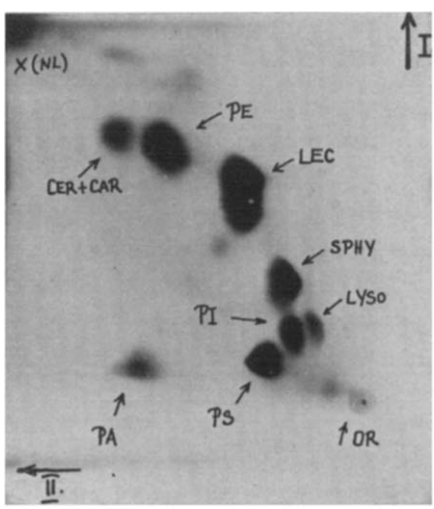

Fig. 2. Two dimensional thin-layer chromatogram of the phospholipids of rat liver plasma membrane subfractions (contiguous subfraction). PA, phosphatidic acid; PS, phosphatidylserine; PI, phosphoinositides; LYSO, lysolecithin; SPHY, sphingomyelin; LEC, phosphatidylcholine; CER and CAR, ceramide and cardiolipin; PE, phosphatidylethanolamine; NL, neutral lipids; OR, origin.

cally, and the cholesterol, neutral and phospholipid contents determined colourimetrically, [7, 8]. A typical separation of the neutral lipid fraction is shown in Fig. 1. Individual phospholipids were separated by two-dimensional chromatography on silica gel H plates (Merck, Darmstadt, G.F.R.) [9, 10]. The solvent system used in the first dimension (I) was chloroform/methanol/14 N, NH_4OH (65 : 35 : 5, : v/v) and, after air drying, in the second dimension (II) chloroform/acetone/methanol/acetic

acid/H_2O (50 : 20 : 10 : 10 : 5, v/v). Individual phospholipids were developed by using iodine vapour and the resolved components, identified by using pure phospholipid standards (Lipid Products, South Nutfield, Surrey, U.K.), scraped from the plates for determination of phospholipid phosphorus [9, 11]. Phosphorus was assumed to account for 4 % by weight of phospholipids [12]. Fig. 2 indicates the two-dimensional separation of phospholipids achieved under these conditions.

RESULTS AND DISCUSSION

Topographical origin and properties of the plasma membrane subfractions

The three plasma membrane subfractions analysed were designated according to their anatomical position on the hepatocyte surface membrane, namely sinusoidal (the plasma membrane interfacing with the blood spaces that are supplied mainly by the portal vein and to a lesser extent by the hepatic artery) canalicular (the plasma membrane interfacing with the bile spaces) and contiguous (plasma membranes in close proximity to neighbouring hepatocytes and containing the junctional specialisations). The subfractions were identified from six liver plasma membrane preparations because their enzymic and morphological properties [3] correlated with enzyme histochemical studies [13–15]. Additional corroborative evidence supporting an origin of the membrane fragments from the correct topographical positions on the hepatocyte surface was provided by enzymic iodination and ligand-binding studies using perfused livers and isolated membrane fractions (ref. 16 and Carey and Evans, unpublished work).

The distribution among the subfractions of these plasma membrane marker enzymes and sialic acid is shown in Table I. The distribution among the subfractions

TABLE I

PROPERTIES OF THE RAT LIVER SINUSOIDAL, CONTIGUOUS AND CANALICULAR PLASMA MEMBRANE SUBFRACTIONS

Plasma membrane subfractions corresponding to the three functional domains of the hepatocyte surface were prepared as described in Methods.

Fraction	Plasma membrane markers			
	5′-Nucleotidase (r.s.a.)*	Alkaline phosphodiesterase (r.s.a.)	Glucagon-activated adenylate cyclase (r.s.a.)	Sialic acid (nmol/mg protein)
Sinusoidal	41.7	46.0	22.9	91
Canalicular	104.5	228.1	4.2	98
Contiguous	19.3	39.1	15.0	46

* r.s.a., specific activity of isolated fraction relative to the following tissue homogenate activities: 5′-nucleotidase, 2.52 μmol/mg protein/h; alkaline phosphodiesterase, 0.77 μmol/ml protein/h; glucagon (2 μM) activated adenylate cyclase, 0.132 nmol/mg protein/h [3].

** The maximal levels of contamination were calculated by relating the specific activities of the components in the subfractions [3] to the literature values for highly-purified intracellular fractions by the stated authors. The value for plasma membrane cardiolipin in Table IV was used.

of alkaline phosphatase, leucine naphthylamidase and Mg^{2+}-activated adenosine triphosphatase was similar and with the exception of adenylate cyclase these plasma membrane marker enzymes are glycoprotein ectoenzymes [17–21]. The canalicular and sinusoidal subfractions consisted of vesicular profiles contrasted with the contiguous plasma membrane subfractions that were of a higher density on sucrose gradients and consisted of strips of membranes punctuated by cell surface specialisations intermixed with vesicular membrane profiles of large diameter. The plasma membrane subfractions derived from these three functional areas of the hepatocyte surface membrane contained similar populations of polypeptides but glycoproteins were present at higher intensities in the sinusoidal and canalicular subfractions [3].

Purity of the plasma membrane subfractions

Before analysis of the lipid compositions of the three subfractions previously shown to contain high specific activities, relative to the liver homogenate, of plasma membrane markers, their purity was assessed. Table I indicates the maximal levels of contamination of the subfractions based on the measurement of enzymic and chemical markers diagnostic for intracellular membranes and organelles. Contamination of the subfractions by nuclear membranes was also argued to be low on the basis of chemical and enzymic evidence [3]. The relatively higher content of galactosyl transferase activity in the sinusoidal subfraction was shown by morphological analysis to be due to the presence in this fraction of Golgi apparatus components but the lateral diffusion of Golgi membrane components into the sinusoidal plasma membrane during secretion may also contribute to the activity. Evidence that galactosyl transferase activity determinations may overestimate the Golgi membrane content of the blood sinusoidal subfraction is indicated by the high specific activities of plasma

Estimation of maximal levels of contamination by intracellular membranes (%)**

Endoplasmic reticulum glucose-6-phosphatase [22]	Lysosomes (acid phosphatase [23])	Mitochondria			Golgi apparatus (galactosyl transferase [26])
		Outer membrane (Monoamine oxidase [24])	Inner membrane		
			Succinate dehydrogenase [22]	Cardiolipin [25]	
10	1.5	3.5	0	10	<25
1–2	1	<1	6	10	< 1
1–2	<1	3	7	15	< 1

TABLE II

CHEMICAL COMPOSITION OF RAT LIVER PLASMA MEMBRANE FRACTIONS ORIGINATING FROM THE THREE FUNCTIONAL DOMAINS OF THE HEPATOCYTE SURFACE

The plasma membrane fractions were prepared and the lipids analysed as described in Experimental. The results represent the average of estimations made with two different membrane preparations that agreed to within 5 %. Carbohydrate content of lipid and protein fractions was not determined.

	Sinusoidal subfraction	Contiguous subfraction	Canalicular subfraction
Protein content (% by wt.)	50.6	56.8	47.4
Lipid content (% by wt.)	49.4	43.2	52.6
Phospholipid content (% by wt.)	34.6	30.5	36.5
Neutral lipid content (% by wt.)	14.8	12.7	16.1
Total cholesterol content (% neutral lipid)	9.2	8.8	12.8
Lipid/protein (w/w)	0.98	0.76	1.11
Phospholipid/protein (w/w)	0.68	0.54	0.77
Neutral lipid/phospholipid (w/w)	0.43	0.42	0.44
Cholesterol/phospholipid (molar ratio)	0.53	0.58	0.70

membrane marker enzymes in this subfraction (Table I) and the similarity of the polypeptide composition to that of the other plasma membrane subfractions [3].

Comparison of the lipid composition of the subfractions

The overall lipid analysis of the three plasma membrane subfractions is shown in Table II. The sinusoidal and canalicular subfractions contained higher lipid or phospholipid to protein ratios than the contiguous subfraction, thus agreeing with the lower densities recorded in sucrose gradients of the two vesicular fractions [3]. However, the neutral lipid to phospholipid weight ratios in all subfractions were similar. Differences in the cholesterol to phospholipid molar ratios were noted, the canalicular subfraction containing the highest and the sinusoidal subfraction the lowest ratio. The molar ratios of total cholesterol to phospholipid, typically high for mammalian plasma membranes, fell within the range of values recorded by others [27–31]. However, it should be noted that these literature values apply to plasma membranes prepared from nuclear pellets by variations of the Neville method [32] and thus correspond more to the values obtained for the contiguous and canalicular plasma membrane subfractions.

A more detailed analysis of the neutral lipid composition of the three fractions is shown in Table III. The higher triglyceride and cholesterol ester content of the sinusoidal subfraction indicates that despite washing and dispersing the membranes in hypotonic and high salt media, the possibility must be considered that very low density lipoprotein particles still persist in this fraction. These very low density lipoproteins that contain cholesterol mostly in the esterified form may be occluded inside Golgi vesicles present in this fraction [3] or may be strongly associated with the sinusoidal plasma membrane during their discharge into the blood. Calculation of the unesterified cholesterol to phospholipid molar ratios indicated a more marked difference between the fractions (Table III).

TABLE III

NEUTRAL LIPID COMPOSITION OF RAT LIVER PLASMA MEMBRANE FRACTIONS

The plasma membrane fractions were prepared and the lipid components extracted, separated and estimated as described in Experimental. The results represent the average of estimations made with two different preparations of membrane that agreed within 5 %.

	Sinusoidal subfraction	Contiguous subfraction	Canalicular subfraction
Triglycerides (% by wt.)	25.6	18.4	8.2
Free fatty acids (% by wt.)	12.0	11.9	11.2
Cholesterol (% by wt.)	51.0	60.7	73.4
Cholesterol esters (% by wt.)	11.0	8.8	5.9
Cholesterol ester/total cholesterol (wt./wt.)	17.7	12.7	7.4
Unesterified cholesterol/phospholipid (molar ratio)	0.42	0.49	0.65

TABLE IV

PHOSPHOLIPID COMPOSITION OF RAT LIVER PLASMA MEMBRANE FRACTIONS

Plasma membrane subfractions corresponding to the three surface areas were prepared, and the phospholipid separated as described in Experimental and shown in Fig. 2. Results are from 3 separate membrane preparations. Values are expressed as mol%.

Phospholipids	Sinusoidal subfraction	Contiguous subfraction	Canalicular subfraction	Range* of values reported for unfractionated rat liver plasma membranes
Origin	3.3±2.9	3.5±2.5	0.62±0.3	–
Lysolecithin	1.1±1.0	2.6±0.8	3.6 ±0.1	0.5– 6.7
Phosphatidylserine	4.1±0.8	7.8±0.8	7.8 ±1.6	3.5– 9.0
Phosphatidylinositol	10.3±0.9	8.9±1.8	7.0 ±2.3	4.2– 8.8
Sphingomyelin	12.8±0.9	15.6±1.1	24.4 ±0.5	10.7–23.2
Phosphatidylcholine	46.7±3.8	32.1±3.1	32.5 ±4.5	30.0–46.2
Phosphatidylethanolamine	14.8±0.2	20.3±2.8	14.0 ±0.9	18.5–24.7
Cerebroside	0.6±0.5	1.6±0.5	2.6 ±0.5	–
Phosphatidic Acid	1.9±0.7	2.3±0.4	3.2 ±0.6	2.0– 2.3
Cardiolipin	1.8±0.51	2.7±0.2	1.8 ±0.7	1 – 5.7

* Results reported by Zambrano et al. [25], Ray et al. [29], Colbeau et al. [27], Dorling and Lepage [33], Keenan and Morre [34], Van Hoeven and Emmelot [35], Bergelson et al. [36], Wood [37].

Analysis of the phospholipid compositions of the subfractions indicated that these fell within the range of values reported by others who used mainly plasma membranes prepared by variations of the Neville procedure (Table (V). The three subfractions contained similar overall phospholipid profiles in contrast, for example, to the radically different values of phosphatidylcholine and ethanolamine reported between the baso-lateral and microvillar intestinal epithelial plasma membranes [38]. Although the small differences observed may arise in part from the differential contamination of the plasma membrane subfractions by intracellular membranes of

different lipid composition e.g. Golgi membranes in the sinusoidal fraction [25], the larger differences observed in sphingomyelin and unesterified cholesterol are considered indicative of these performing a functional role in the canalicular plasma membranes.

The differences recorded between the subfractions indicate that care should be exercised when comparing the neutral and phospholipid composition of plasma membranes from normal liver and hepatoma. The plasma membrane fragments of hepatoma are of a different density and it is conceivable that differences in composition [36, 41] may be related not only to changes occuring in neoplasia but also to differential recovery of plasma membrane fragments from different areas of the cell surface.

The present study did not examine differences in the glycolipid composition of the three fractions. However, previous work [44] has indicated that the fraction now identified as originating from the canalicular plasma membrane contained a higher glycolipid content than the contiguous fraction. Also, the distribution of sialic acid (Table I) that may be bound to either protein or lipid suggests that the sinusoidal fraction also contains a higher glycolipid composition than the contiguous fraction.

Membrane lipid composition in relation to the functional differentiation of hepatocyte plasma membrane

The question of how far differences in the overall composition of the membrane lipids and proteins underlie the grossly different metabolic functions associated with the sinusoidal, contiguous and canalicular surface domains of the hepatocyte constitutes an important aspect of explaining the polarity of the hepatocyte surface membrane in biochemical terms. The present study shows that the overall neutral and phospholipid composition of the three functional plasma membrane domains is less variable than the differences recorded in the polypeptide, glycoprotein and enzymic composition. Modern concepts of surface membrane structure [4] emphasize that the plasma membrane of animal cells is sufficiently fluid to allow rotational and rapid translational movements of lipids [43, 44]. The present results indicate that the functionally complex surface membrane of the hepatocyte is constructed of fairly uniform populations of lipids. Thus, lipids synthesized on cytoplasmic membranes and inserted into one or more of the functional surface domains achieve a relatively uniform final distribution on the hepatocyte surface membrane. Indeed, the major differences so far determined in the composition of the functional domains of the hepatocyte surface membrane appear to be in the distribution of polypeptide hormone receptors, glycoenzymes and glycolipids [3, 42, 45] that probably result from the topographical confinement of the interaction of Golgi components with the sinusoidal plasma membrane. Plasma membrane glycoproteins that are externally orientated in the membrane plane may then move laterally between membrane domains possibly by diffusion towards the canalicular face before being gradually lost by detergent dissolution into the bile spaces [46]. The association of 5′-nucleotidase, a glycosylated hepatocyte ectoenzyme [20], with sphingomyelin [39], a phospholipid shown to be present mainly in the outer lipid layer in erythrocyte membranes [47], and now indicated to be present in the canalicular plasma membrane, at significantly higher amounts, provides an example of a possible membrane lipo-protein complex with a preferred location on the hepatocyte surface. Current views on the arragement of lipids in the inner and

outer bilayers of the erythrocyte membrane postulate that in addition to choline containing lipids, cholesterol is also predominantly located in the outer half of the bilayer [47, 48].

Analysis of phospholipid fatty acids of liver plasma membranes showed that sphingomyelin contained the highest proportion of saturated fatty acids [41]. Cholesterol and saturated phospholipids stabilise membranes, limiting membrane fluidity and imposing rigidity [49]. For example, phosphatidylcholine liposomes are less soluble in deoxycholate when cholesterol is incorporated into the bilayers (Green, N. M., personal communication). The asymmetrical distribution of lipids in the bilayer parallels the asymmetrical location of membrane glycoproteins and enzymes and is probably a general feature of mammalian plasma membranes, being predetermined by similar biosynthetic mechanisms. Hence, a higher amount of cholesterol and sphingomyelin in the outer leaflet of the hepatocyte canalicular plasma membrane may constitute a molecular mechanism for limiting the breakdown and solubilisation of this membrane by bile salts present in the bile canalicular spaces. The increased rigidity, especially of the outer lipid layer of the membrane may facilitate the anchoring of the membrane-bound glycoprotein enzymes, thereby limiting their elution into bile. Thus, the lipid composition of the hepatocyte canalicular plasma membrane, in turn, may control the lipid and protein composition of bile.

ACKNOWLEDGEMENTS

The authors thank the Royal Society and Hungarian Academy of Sciences for financial support. M. H. Wisher thanks the M.R.C. for a postgraduate studentship.

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