BBA 75587

NUCLEAR MEMBRANES FROM MAMMALIAN LIVER

III. FATTY ACIDS

J. STADLER AND H. KLEINIG

Institut für Biologie II, Lehrstuhl für Zellbiologie, Universität Freiburg i.Br. (Deutschland)

(Received October 1st, 1970)

SUMMARY

The fatty acid patterns of phosphoglycerides and neutral lipids from pig liver nuclear membranes, microsomes and "floating lipids" have been determined. There was a tendency to more saturated acids in the phosphoglycerides with the nuclear membranes. Among the neutral lipids, however, the highest amount of unsaturated fatty acids was found in the nuclear membranes. The results are discussed in terms of cytomembrane differentiation.

INTRODUCTION

Different methods have recently been reported for isolating nuclear membranes from mammalian liver in quantities sufficient for biochemical analyses[1-5]. With pig and rat liver, the phospholipid composition of nuclear membranes was shown to be similar to that of microsomes[6]. However, the cholesterol ester content of the nonpolar lipid fraction of nuclear membranes was 4 times that of the corresponding microsome fraction[6]. In the present communication these analyses have been extended to the fatty acid composition of the individual lipid classes, comparing nuclear membranes and microsomes. Such an approach seemed to be especially interesting in view of the findings of KEENAN AND MORRÉ[7] for rat liver. These authors reported a decrease in the percentage of unsaturated acid residues from the endoplasmic reticulum membranes (45 %) *via* the Golgi apparatus membranes (36 %) to the plasma membranes (29 %), which was discussed in relation to cytomembrane differentiation.

Important secretory products of the liver cell are precursors of very low density lipoproteins of plasma, and they are rich in cholesterol esters[8]. Therefore, the fatty acid analysis of a "floating lipid" fraction containing lipid droplets and contents of secretory vesicles was included in the present investigation.

MATERIALS AND METHODS

Nuclear membranes and microsomes from pig liver were isolated and characterized biochemically and electron microscopically as described previously[5]. After centrifugation of the mitochondrial supernatant of the liver homogenate at 110000 × *g* for 2 h, fractions enriched in "floating lipids" were obtained from the top of the tubes.

Phosphoglycerides and neutral lipids were separated and identified according to procedures reported elsewhere[6]. The individual lipids were scraped from thin-layer plates and eluted from the adsorbent with chloroform–methanol (2:1, v/v) or methanol. After evaporation of the solvent with nitrogen the lipids were hydrolysed with ethanolic KOH (1.3 g KOH in 10 ml 96 % ethanol). The free fatty acids then were methylated with diazomethane. Samples were applied to a column (1.8 m × 3 mm) packed with 10 % EGSSX on 100–120 mesh Gaschrom P (Serva, Heidelberg, Germany) operated isothermally at 180° in a Varian Aerograph Model 1520 gas chromatograph equipped with a flame ionization detector. When reference standards (Serva) were used for comparison the relative error of analyses for the major components (> 10 % of total fatty acids) was found to be less than 6 %. Identification of the methyl esters was based on comparison of retention times with those of the reference standards. Reference esters containing 20- and 22-carbon polyunsaturates were prepared from rat liver lipids, which are known to contain such acids (see *e.g.* refs. 9 and 10). Identification of unsaturated methyl esters was further achieved by gas chromatographic analysis of esters before and after hydrogenation on a platinum oxide catalyst. The carbon chain length composition of liver lipids as summed from the gas chromatograms was compared with the calculated data of gas chromatograms of hydrogenated samples. A reasonably good agreement was achieved.

RESULTS AND DISCUSSION

The lipid class composition of pig liver nuclear membranes, microsomes and "floating lipids" is given in Table I. The values for the microsomal fraction were similar to the values given by KEENAN AND MORRÉ[7] for rat liver microsomes.

TABLE I

LIPID COMPOSITION OF PIG LIVER NUCLEAR MEMBRANES, MICROSOMES AND "FLOATING LIPIDS"

Values are derived from 4 determinations, and are given in mole %.

Lipid class	*Fraction*		
	*Nuclear membranes**	*Microsomes**	*"Floating lipids"*
Phospholipids	75.4 ± 2.1	80.2 ± 2.6	6.3 ± 1.2
Triglycerides *plus* free fatty acids	16.1 ± 3.2	12.2 ± 3.6	89.8 ± 4.7
Cholesterol	7.8 ± 0.4	7.4 ± 0.4	1.7 ± 0.3
Cholesterol ester	0.7 ± 0.1	0.14 ± 0.05	2.2 ± 0.4

* Values derived from the data of ref. 6.

Phosphoglycerides of the nuclear membranes and the microsomes of pig liver contained the same fatty acid residues as occur commonly among mammalian tissue (Table II). The relative distribution of the acids within the two membrane systems was slightly different with a tendency towards more unsaturated acids within the microsomes. This can be derived from the ratios of saturated to unsaturated acids (Table II). These values were 0.41 (nuclear membranes) and 0.24 (microsomes) for phosphatidylcholine, and 0.21 and 0.08 for phosphatidylethanolamine, respectively. These two phospholipids account for about 85 % of the total phospholipids in these membranes[6].

TABLE II

MAJOR FATTY ACID COMPOSITION OF PHOSPHOGLYCERIDES FROM NUCLEAR MEMBRANES, MICROSOMES AND "FLOATING LIPIDS" OF PIG LIVER

Unknown acids comprise small amounts of short chain acids and traces of unidentified polyunsaturates. See text for experimental details.

Fatty acid	*Composition (weight %)*									
	Total liver	*Phosphatidylcholine*			*Phosphatidylethanolamine*			*Phosphatidylserine plus phosphatidylinositol*		
		*NM**	*MI***	*FL****	*NM**	*MI***	*FL****	*NM**	*MI***	*FL****
16:0	11.8	12.6	9.5	12.5	3.3	1.7	5.5	6.4	3.9	8.0
18:0	22.5	15.5	9.2	11.5	13.9	5.7	25.2	31.8	46.8	39.8
18:1	12.7	22.4	22.3	24.8	8.2	8.1	8.7	11.9	5.3	9.2
18:2	17.2	17.9	18.6	18.1	9.7	10.0	7.0	7.8	7.8	5.1
20:4	21.7	13.2	16.4	11.0	37.5	43.5	33.1	32.0	29.4	25.6
22:5§	4.3	5.2	9.3	5.3	6.0	6.5	4.8	2.6	3.0	3.7
22:6	9.5	10.6	12.5	10.0	18.9	22.6	15.5	3.9	3.5	4.7
Unknown	0.3	2.6	2.2	6.8	2.5	1.9	0.2	3.6	0.3	3.9
$\frac{\text{Saturates}}{\text{Unsaturates}}$	0.52	0.41	0.24	0.35	0.21	0.08	0.44	0.66	1.03	0.99

* NM, nuclear membrane.
** MI, microsomes.
*** FL, "floating lipids".
§ Tentative identification.

With the quantitatively minor phospholipids, such as phosphatidylserine and phosphatidylinositol, the content of unsaturated acids was higher in the nuclear membrane than in the microsomes (Table II). In general, the statement that each phospholipid has a rather distinctive fatty acid pattern which is more or less reproduced in all membranes[9,11,12] (but see STOFFEL AND SCHIEFER[13]) apparently holds for these membranes (Table II). In our analyses, appreciable amounts of arachidonic and docosahexaenoic acids were found, especially in phosphatidylethanolamine. The amounts varied somewhat in different experiments, seemingly depending on the specific nutritional state of the animals, but an increasing percentage of unsaturated fatty acids from the nuclear membranes to the microsomes was always noted.

The fatty acid values of the phosphoglycerides from nuclear membranes of bovine liver[14], though in the same range, cannot be directly compared with our findings for pig liver. The differences regarding total unsaturates and long chain unsaturated acids, however, are noteworthy. The lower level of unsaturates in bovine liver might be attributable to the fact that large amounts of ingested fatty acids are hydrogenated in the rumen[15].

The phosphoglycerides of the "floating lipid" fraction are likely to be derived partly from the membranes and contents of secretory vesicles which contain the very low-density lipoproteins[16]. The fatty acid residues of these phosphoglycerides are less unsaturated than those from the microsomes (Table II).

The fatty acid moieties of cholesterol esters and triglycerides, and the free fatty acids of nuclear membranes, microsomes and the fractions enriched in non-membrane-bound lipids are listed in Table III. Among these lipids the proportions are reversed in

TABLE III

MAJOR FATTY ACID COMPOSITION OF NONPOLAR LIPIDS FROM NUCLEAR MEMBRANES, MICROSOMES AND "FLOATING LIPIDS" OF PIG LIVER

Unknown acids comprise small amounts of short chain acids and traces of unidentified polyunsaturates. See text for experimental details.

Fatty acids	*Composition (weight %)*								
	Cholesterol esters			*Triglycerides*			*Free fatty acids*		
	*NM**	*MI***	*FL****	*NM**	*MI***	*FL****	*NM**	*MI***	*FL****
14:0	2.0	4.0	2.4						1.0
16:0	17.9	38.4	36.6	6.8	21.8	16.0	9.3	8.9	32.0
16:1	3.2	3.0			3.3	3.6		3.8	1.4
18:0	10.0	12.5	10.6	3.2	7.9	5.1	5.1	5.0	10.0
18:1	28.6	16.5	30.9	26.4	41.5	47.5	33.2	31.8	38.0
18:2	20.9	9.5	8.0	61.2	10.2	14.6	37.5	16.5	10.0
20:4	9.9	7.0	3.8	0.5	10.4	4.0	8.2	18.8	3.9
22:5§	1.6		2.3		1.1	2.7	1.6	3.8	
22:6	2.9	4.2	3.1		1.6	1.4	2.8	6.5	
Unknown	3.0	4.9	2.3	1.9	2.2	5.1	2.3	4.9	3.7
Saturates / Unsaturates	0.45	1.36	1.03	0.11	0.44	0.29	0.17	0.17	0.81

* NM, nuclear membranes.
** MI, microsomes.
*** FL, "floating lipids".
§ Tentative identification.

that the highest amount of unsaturated acids was found in the nuclear membrane. This holds especially for the cholesterol esters in which the ratios are similar in different preparations. In contrast, the free fatty acids and the triglycerides seemed to be more variable in their content of carbon–carbon double bonds. This shows that the cholesterol esters of the nuclear membranes differ from the bulk cholesterol esters of the liver cell, which exchange rapidly with the esters of the plasma[8,17–20]. The cholesterol esters of the nuclear membrane also differed from those of the microsomes (Table III). In our nuclear membrane preparations an appreciably high amount of cholesterol esters was consistently observed[6]. These findings seem to be especially interesting, since within the liver cell two cholesterol esterifying systems were found. One is free in the cell sap resembling the system of blood plasma[21,22]. The other is particulate, presumably microsomal, and requires both ATP and coenzyme A[19,23]. To what extent the membrane-bound system synthesizes membrane-bound cholesterol esters and *vice versa* for the soluble system is not known.

Taken together, the lipids of the nuclear membrane show a fatty acid composition different from the pattern of the microsomes as well as from that of fractions enriched in "floating lipids". As discussed by KEENAN AND MORRÉ[7] in comparing plasma membranes, Golgi membranes, and endoplasmic reticulum membranes (see also ref. 7 for further literature), the decrease of free cholesterol content and the increase of the relative amount of unsaturated fatty acids could induce a decrease of membrane sheet stability. Although the cholesterol content of nuclear membranes and microsomes is nearly the same[6], there is a tendency towards more saturated acids in the phospho-

glycerides with the nuclear membranes. This then would imply an increased stability of the nuclear membranes compared with the endoplasmic reticulum. Our nuclear membrane fraction, however, is "heterogeneous" in that it consists of both inner and outer membranes of the envelope. Thus, it is not unlikely that the differences observed might reflect the inner nuclear membrane which, interestingly, can be distinct in certain cells from the outer nuclear membrane also as to its ultrastructure.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft. We wish to thank Dr. D. J. Morré, Purdue University, Lafayette, Ind., for reading the manuscript, and Dr. W. W. Franke from this laboratory for helpful discussion.

REFERENCES

1 M. M. BORNENS, *Compt. Rend. Acad. Sci. Paris*, 266 (1968) 596.
2 I. B. ZBARSKY, K. A. PEREVOSHCHIKOVA, L. N. DELEKTORSKAYA AND V. V. DELEKTORSKY, *Nature*, 221 (1969) 257.
3 D. M. KASHNIG AND C. B. KASPER, *J. Biol. Chem.*, 244 (1969) 3786.
4 R. BEREZNEY, L. K. FUNK AND F. L. CRANE, *Biochim. Biophys. Acta*, 203 (1970) 531.
5 W. W. FRANKE, B. DEUMLING, B. ERMEN, E. JARASCH AND H. KLEINIG, *J. Cell Biol.*, 46 (1970) 379.
6 H. KLEINIG, *J. Cell Biol.*, 46 (1970) 396.
7 T. W. KEENAN AND D. J. MORRÉ, *Biochemistry*, 9 (1970) 19.
8 R. W. MAHLEY, R. L. HAMILTON AND V. S. LEQUIRE, *J. Lipid Res.*, 10 (1969) 433.
9 M. G. MACFARLANE, G. M. GRAY AND L. W. WHEELDON, *Biochem. J.*, 77 (1960) 626.
10 G. S. GETZ AND W. BARTLEY, *Biochem. J.*, 78 (1961) 307.
11 W. BARTLEY, in R. M. C. DAWSON AND D. N. RHODES, *Metabolism and Physiological Significance of Lipids*, Wiley, London, 1964, p. 369.
12 J. G. PARKES AND W. THOMPSON, *Biochim. Biophys. Acta*, 196 (1970) 162.
13 W. STOFFEL AND H. G. SCHIEFER, *Z. Physiol. Chem.*, 349 (1968) 1017.
14 T. W. KEENAN, R. BEREZNEY, L. K. FUNK AND F. L. CRANE, *Biochim. Biophys. Acta*, 203 (1970) 547.
15 S. PATTON AND E. M. KESLER, *Science*, 156 (1967) 1365.
16 D. J. MORRÉ, T. W. KEENAN AND H. H. MOLLENHAUER, *Proc. 1st Intern. Symp. Cell Biol. Cytopharmacol., Raven, New York*, 1970, in the press.
17 D. S. GOODMAN, *J. Clin. Invest.*, 41 (1962) 1886.
18 P. S. ROHEIM, D. E. HAFT, L. I. GIDEZ, A. WHITE AND H. A. EDER, *J. Clin. Invest.*, 42 (1963) 1277.
19 D. S. GOODMAN, D. DEYKIN AND T. SHIRATORI, *J. Biol. Chem.*, 239 (1964) 1335.
20 O. STEIN, Y. STEIN, D. S. GOODMAN AND N. H. FIDGE, *J. Cell Biol.*, 43 (1969) 410.
21 J. A. GLOMSET AND D. M. KAPLAN, *Biochim. Biophys. Acta*, 98 (1965) 41.
22 M. AKIYAMA, O. MINARI AND T. SAKAGAMI, *Biochim. Biophys. Acta*, 137 (1967) 525.
23 K. T. STOKKE AND K. R. NORUM, *Biochim. Biophys. Acta*, 210 (1970) 202.