PHOSPHOLIPIDS OF OUTER AND INNER NUCLEAR MEMBRANES IN RAT LIVER AND BHK-21 CELLS

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Received March 25,1977

SUMMARY: Isolated nuclei of rat liver and BHK-21 cells were treated with citric acid and the resulting outer nuclear membrane sheets were separated from the nuclear residues surrounded by the inner nuclear membrane. Both fractions contained approximately equal amounts of phospholipid in both cell types. The phospholipid compositions of the two fractions were remarkably similar. The results are in accordance with the notion of the structural continuity of the two nuclear membranes.

The nuclear envelope consists of an outer (ONM) and inner (INM) nuclear membrane. The cisternal surfaces of both membranes may be similar (1) but their outer surfaces are different: the cytoplasmic surface of ONM is covered with ribosomes (2,3) and the nuclear surface of the INM is associated with the nuclear matrix complex (4,5).

The lipid compositions of the two nuclear membranes have been analyzed from samples treated with detergents, and differences have been reported both in the quantity and quality of their phospholipids (6-8). However, such analyses may not be reliable because detergent treatment removes phospholipids from membranes (9). In order to avoid this in the present study we used the aqueous citric acid treatment (10,11) to separate the two nuclear membrane compartments.

ISSN 0006-291X

MATERIALS AND METHODS

Rat liver cell nuclei were isolated from adult Sprague-Dawley rats by homogenizing livers in 0.32 M sucrose- 3 mM $MgCl_2$ solution and by purifying the nuclei by sucrose step-gradient ultracentrifugation (12). From cultured BHK-21 cells (13) the nuclei were isolated according to Pegorado et al. (14). The cells were first treated for 15 min at 0°C in a hypotonic solution consisting of 2 mM $CaCl_2$, 1.5 mM $MgCl_2$, 8 mM NaCl, 10 mM Tris-HCl (pH 7.5), then homogenized in a motor-driven glass-Teflon pestle homogenizer. The homogenate was made isotonic by adding 1 M sucrose, 2.5 mM $CaCl_2$, 2.5 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.5). The homogenate was centrifuged at 800 xg for 10 min and the crude nuclear fraction was purified by sucrose step-gradient ultracentrifugation as above.

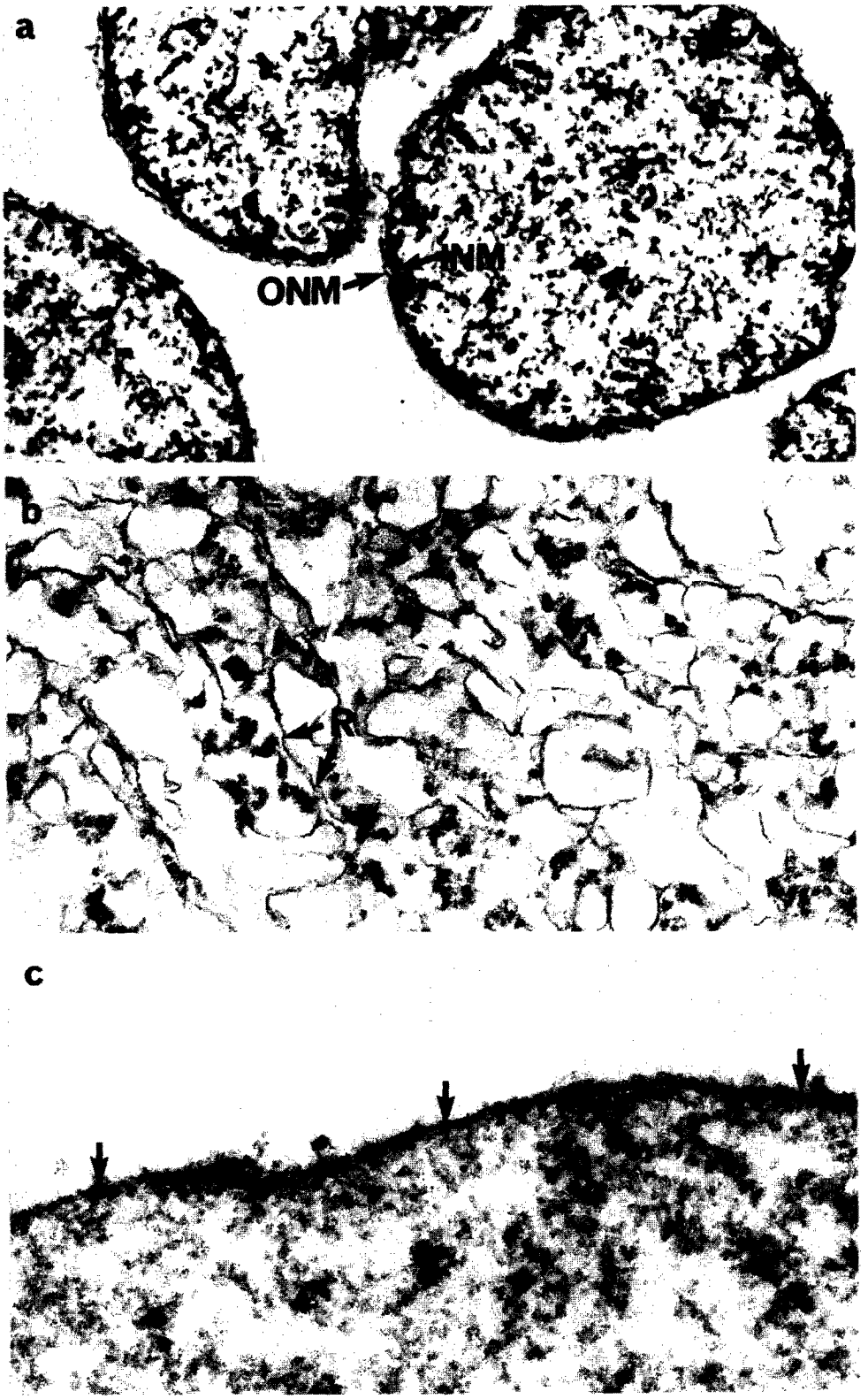
The purified nuclei were suspended in 5 vol of 2.5% citric acid by 3 strokes in a tight Dounce homogenizer and incubated 10 min at 4°C (10). After incubation the nuclear suspension was centrifuged at 600 xg for 10 min and the pellet, consisting of residual nuclei surrounded by the INM, was washed three times in 0.25 M sucrose- 3 mM $MgCl_2$ solution and pelleted as above. The supernatant of the first 600 xg centrifugation, representing the outer nuclear membrane fraction, was pelleted by centrifugation at 143,000 xg for 60 min and washed three times in the same solution as the INM fraction. The membrane pellets were stored either at -20°C or -70°C in a 0.2 M Na-cacodylate buffer (pH 7.2) prior to phospholipid analysis.

Extraction and analysis of phospholipids by two dimensional chromatography was performed as described earlier (13).

For electron microscopy the pelleted nuclei and nuclear membrane fractions were fixed for 1 hour in 2.5% glutaraldehyde buffered with 0.1 M Na-cacodylate (pH 7.2) at room temperature. The residual nuclear fraction was treated with 1% tannic acid (15) for the staining of the INM. After post-fixation for 1 hour in 1.5% osmium tetroxide buffered with 0.1 M phosphate buffer (pH 7.0) the specimens were dehydrated and embedded in Epon 812. Thin sections except those from specimens treated with tannic acid were stained with uranyl acetate and lead citrate and examined in a Jeol 100B electron microscope.

RESULTS AND DISCUSSION

Isolation of liver cell nuclei yielded ultrastructurally well preserved nuclei (Fig. 1a) that were contaminated only to a limited degree by other recognizable membrane structures eg. mitochondria. The recovered ONM fraction consisted of membrane sheets covered mostly with ribosome-like structures (Fig. 1b).


a
ONM
NM
b
R
c

Treatment of the nuclei with 2.5% citric acid almost totally removed the ONM with its ribosomes whereas it left the residual nuclei with a distinct INM stained by tannic acid (Fig. 1c). The isolated BHK-cell nuclei were more damaged in their ultrastructure but the nuclear fractions obtained from them with the citric acid treatment were structurally similar to those obtained from rat liver cell nuclei.

In both cell types the ONM and INM nuclear fractions contained approximately equal amounts of phospholipid. This is conceivable as all nuclear lipids are thought to be associated with the two nuclear membranes (16) and the membranes have essentially equal surface areas. In rat liver cell nuclei the ONM fraction contained 50.4% and the residual nuclei 49.6% (SD 1.4, n=4) of the total phospholipids. In BHK-21 cells the figures were 47.5% and 52.5% (SD 1.4, n=3) respectively. These results are in contrast with the data obtained with detergent fractionation of the nuclear envelope. For example, Triton X-100 which has been claimed to extract the ONM and leave the INM intact yields only 5 to 20% of the total nuclear phospholipids in the INM fraction (8,17,18).

The distribution of phospholipids in rat liver and BHK-21 cell nuclei was similar. Phosphatidylcholine was the largest component followed by phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyelin and cardiolipin in both cell types (Table 1.). Almost identical

Figure 1. Electron micrographs of rat liver cell nuclei and of ONM and INM nuclear fractions. (a) Purified rat liver cell nuclei have an intact nuclear envelope. 14,000 x. (b) Membrane sheets in the isolated outer nuclear membrane fraction are covered with ribosomes. 50,000 x. (c) After citric acid treatment only the inner nuclear membrane (arrows) is seen around the nuclear residues distinctly visible after tannic acid treatment. 73,000 x. Outer nuclear membrane, ONM; inner nuclear membrane, INM; ribosomes, R.

Table 1.

Distribution of phospholipids in isolated nuclei and nuclear fractions of rat liver and BHK-21 cells (% of phosphorus of total phospholipids)[x]

	Rat liver cells			BHK-21 cells		
	Whole nuclei	Outer nuclear membrane	Residual nuclei	Whole nuclei	Outer nuclear membrane	Residual nuclei
Phosphatidylcholine	58.3	54.6	65.3	67.9	63.1	66.4
Phosphatidylethanolamine (includes plasmalogens)	19.1	18.7	14.5	19.6	21.9	22.6
Phosphatidylinositol	9.9	8.5	10.2	4.9	3.3	3.9
Phosphatidylserine	4.2	2.5	1.5	2.2	3.4	2.6
Sphingomyelin	2.9	4.2	3.4	2.1	3.0	2.6
Cardiolipin	2.4	4.3	0.3	1.6	3.8	0.7
Lysophosphatidylcholine	2.4	3.0	3.3	0.8	0.6	0.6
Phosphatidic acid	0.4	1.4	0.6	0.5	0.8	0.5
Lysobisphosphatidic acid	-	0.7	0.4	0.5	0.8	0.6

[x]means of two determinations

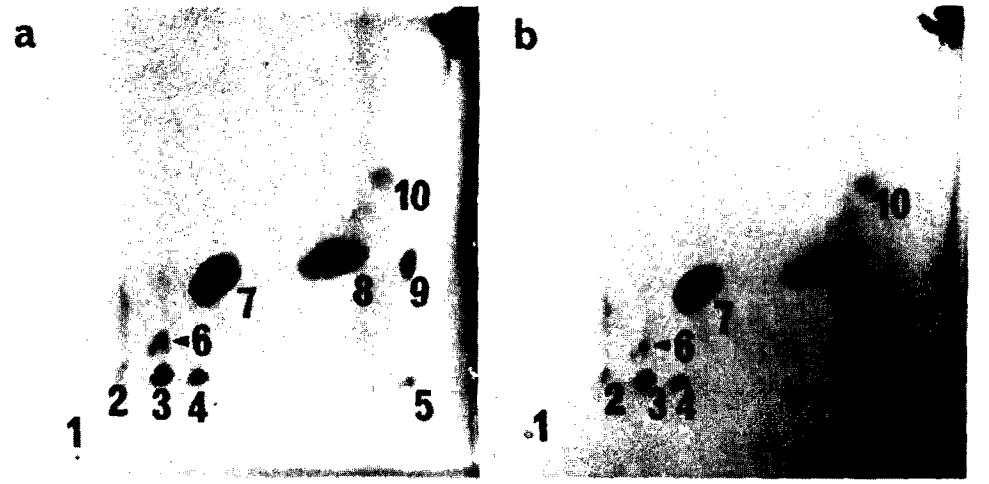


Figure 2. Two dimensional thin layer chromatographs of phospholipids of rat liver cell (a) outer nuclear membrane and (b) residual nuclei surrounded by the inner nuclear membrane. The extracted lipids were applied to the lower left corner of the precoated silicagel plates (F254, Merck AG, Darmstadt). The chromatograms were run in the first dimension (upwards) twice with chloroform-methanol-25% aqueous ammonia-water (65-20-2-2, v/v) and in the second dimension (from left to right) with chloroform-acetone-methanol-acetic acid-water (50-20-10-10, v/v) and the plates were stained with iodine vapor. 1, start; 2, lysophosphatidylcholine; 3, phosphatidylinositol; 4, phosphatidylserine; 5, phosphatidic acid; 6, sphingomyelin; 7, phosphatidylcholine; 8, phosphatidylethanolamine; 9, cardiolipin; 10, lysobisphosphatidic acid.

profiles have previously been described in the nuclei of a number of cells (eg. 19,20).

The phospholipids of the ONM and the INM nuclear fractions were similar in both cell types (Table 1, Fig. 2). Of particular interest is the even distribution of sphingomyelin in the two fractions. The discrepancy of this finding with previous reports in which detergent fractionation of nuclei was employed, and an enrichment of sphingomyelin in the residual nuclear fraction was found (6-8), might be explained by the observed poor extractability of sphingomyelin with Triton X-100 (21).

The only phospholipid displaying an asymmetric distribution between the two nuclear fractions was cardiolipin,

a minor component amounting to ca. 2% of the total phospholipid. It was enriched in ONM of both cell types to such an extent that only minimal amounts remained in the residual nuclei (Table 1.). This enrichment in the ONM fraction supports previous observations which have suggested that the small amounts of cardiolipin usually found in whole nuclei (22) are due to mitochondrial contamination (23,24) rather than to endogenous nuclear origin (16).

In summary, our results indicate a similarity in the amounts and the composition of the phospholipids in the two nuclear fractions. This is in agreement with the notion of the structural continuity of the two nuclear membranes (3).

ACKNOWLEDGEMENTS

Ms. Anneli Asikainen, Ms. Pirkko Leikas and Ms. Elina Waris are kindly acknowledged for their technical assistance. The study was supported by grants from the Finnish Medical Research Council, The Sigrid Jusélius Foundation and the Finnish Culture Foundation.

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