MODIFICATION OF THE TECHNIQUE FOR ISOLATING NUCLEAR MEMBRANES OF THE RAT LIVER AND INVESTIGATION OF THE AMINO-ACID COMPOSITION OF THE MEMBRANE PROTEINS

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The modification of the technique for isolating nuclear membranes is based on disintegration of the nuclei by incubation in 0.02 M phosphate buffer, pH 7.2, and separation of the nuclear membranes by ultracentrifuging the unpurified membrane fraction layered above a stepwise sucrose density gradient. The yield of nuclear membranes sedimented in sucrose layers with d 1.14-1.18 and d 1.18-1.19 was 0.8% of the nuclear nitrogen. Composition of the membranes: 52% protein, 39.2% lipids, 0.61% DNA, 3.4% RNA. The amino-acid composition of the membrane protein was investigated.

Pure nuclear membranes can be obtained by isolating rat liver nuclear membranes by disintegrating isolated nuclei by ultrasound or osmotic shock, as the writers have suggested previously [2]. However, the yield of membrane material is low and often insufficient for the study of the chemical composition of the nuclear membranes.

It was therefore decided to attempt to modify the method in order to increase the yield of the membrane fraction of the nuclei. Since there is no information on the protein composition of isolated nuclear membranes in the literature, their amino-acid composition also was studied.

EXPERIMENTAL METHOD

The proposed modification of the method is based on techniques used previously: disintegration of the nuclei isolated by the method of Digirolamo et al. [4] by incubating them in 0.02 M phosphate buffer, pH 7.2, and separation of the nuclear membranes by ultracentrifuging the unpurified membrane material in a stepwise sucrose density gradient [2]. However, unlike in the method proposed earlier, before the suspension of membrane material is ultracentrifuged it is layered above sucrose solutions of increasing density (d): 1.09, 1.14, 1.18, 1.19, 1.20, 1.22, made up in 0.02 M phosphate buffer, pH 7.2 (at 20°C). Centrifugation was carried out at 50,000 g for 1 h in the SW-25 bucket rotor of the Spinco L-50 ultracentrifuge. After centrifugation dense whiteish-colored layers were found between sucrose layers with d = 1.14 and 1.18 and between layers d = 1.18 and 1.19; less dense whitish layers were found between sucrose layers d = 1.19 and 1.20 and between layers d = 1.20 and 1.22 (Fig. 1).

Electron microscopy showed that the material obtained at the boundary between sucrose layers with d = 1.18 and 1.19 and with d = 1.18 and 1.14 consisted of membranes uncontaminated with nuclear and cytoplasmic impurities (Fig. 2). The membrane fraction collected from sucrose layers with d = 1.20 and 1.22 (Fig. 3) consisted of membranes with granules measuring 200-300 Å attached. Electron-microscopic investigation of preparations stained by Bernard's method [5] showed that these granules were ribosomes.

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TABLE 1. Amino-Acid Composition (in %) of Nuclear Membrane Proteins of Rat Liver (mean of 2-3 determinations)

Fractions	Amino acid																			
	alanine	arginine	aspartic acid	valine	histidine	glutamic acid	iso-leucine	leucine	lysine	methio-nine	hydroxy-proline	proline	serine	thyro-nine	tyrosine	hypo-xan-thine	phenyl-alanine	cysteine	glycine
Total proteins	9,26	4,50	10,62	8,36	1,56	12,48	6,07	9,98	6,12	0,39	—	4,14	6,61	4,87	1,32	—	5,95	0,1	11,29
HCl extract of membranes	10,20	6,52	10,59	6,06	1,51	13,73	4,98	7,80	10,35	1,03	—	4,60	5,25	4,47	1,96	—	2,57	0,0	12,28

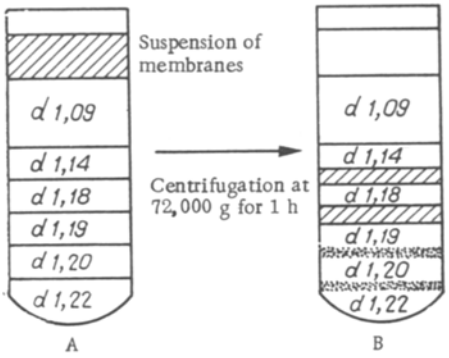

Fig. 1

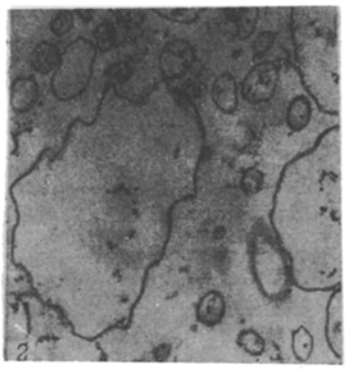

Fig. 2

Fig. 1. Scheme of sedimentation of nuclear membranes by ultracentrifuging in a sucrose density gradient: a) before; b) after centrifuging at 50,000 g for 1 h.

Fig. 2. Isolated nuclear membranes from sucrose layers with d = 1.14 and 1.18 and d = 1.18 and 1.19. Epon 812, 48,000×.

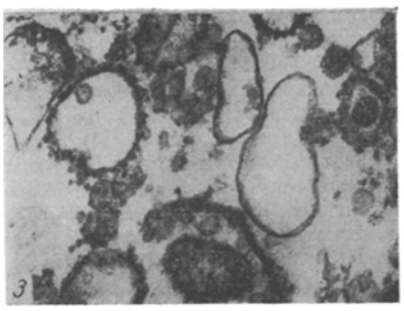

Fig. 3

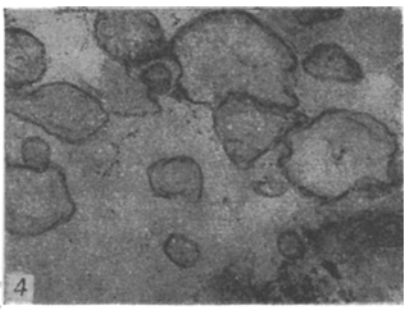

Fig. 4

Fig. 3. Membrane fraction from sucrose layers with d = 1.20 and 1.22: numerous granules measuring 200-300 Å can be seen. Epon 812, 48,000×.

Fig. 4. Materials of membrane fraction from sucrose layers with d = 1.20-1.22, treated with ribonuclease (1 mg/ml, incubation for 1 h at 37°C). Epon 812, 60,000×.

Electron-microscopic examination of ultrathin sections of the material after treatment with ribonuclease showed absence of these granular structures on the membranes, further confirmation of their ribosomal origin (Fig. 4). Membranes with ribosomes attached to them, possibly from the outer layer of the nuclear membranes, were perhaps concentrated between sucrose layers with d = 1.20 and 1.22.

The yield of membrane material from sucrose layers with d = 1.14 and 1.18 and d = 1.18 and 1.19, expressed as nitrogen, amounted to 0.8% of the nuclear nitrogen (mean of 5 determinations).

EXPERIMENTAL RESULTS AND DISCUSSION

To study the composition of the nuclear membranes a mixture of membrane fractions isolated from layers with d = 1.14 and 1.18 and with d= 1.18 and 1.19, washed to remove sucrose, was dried to constant weight at a temperature not exceeding 40°C. Lipids were determined by the use of organic solvents: methanol–chloroform (1:1) and ethanol–ether (3:1). Protein was determined by Lowry's method [6].

DNA and RNA were determined spectrophotometrically by Spirin's method [3] after hydrolysis of the membrane material by the Schmidt–Thannhauser

method in 0.5 N KOH solution for 18 h at 37°C. The nuclear membranes contained (as dry weight) 52.0% protein, 39.2% lipids, 0.61% DNA, and 3.4% RNA (mean of 4 determinations). In their composition the total nuclear membranes were close to the heavy fraction of nuclear membranes [2].

To study the properties of the proteins of the nuclear membranes their amino-acid composition was determined. A lipid-free specimen of nuclear membranes (2-3 mg) was hydrolyzed in 6 N HCl solution at 105-120°C for 24 h. The digest was treated in the usual way. Quantitative analysis of the amino acids was carried out with the KLA-3B (Hitachi) automatic amino-acid analyzer by the method of Moore and Speckman [7].

The amino-acid content was determined in the total protein of the nuclear membranes and in the membrane fraction obtained by extraction with 0.1 N HCl solution. The results are given in Table 1. They indicate certain characteristic differences between the membrane proteins. The proteins of the nuclear membranes contained relatively more aspartic and glutamic acids, glycine, leucine, and valine. Small quantities of methionine, tyrosine, and cysteine were found. The proline content was higher than in other cell proteins. Comparison of the amino-acid composition of the HCl extract of the nuclear membranes with that of total proteins showed that the lysine content was almost twice as high but the content of valine and phenylalanine somewhat lower in the former.

Comparison of the amino-acid composition of total protein of the nuclear membranes obtained in these experiments with the amino-acid composition of the residual protein derived from nuclear membranes obtained by other workers [1, 8] shows that in the present experiments a higher content of aspartic and glutamic acids, isoleucine, leucine, and phenylalanine but a lower content of proline and glycine were obtained. These differences, however, were slight and could be the result of differences in the methods used to isolate the nuclear membranes and in the method used to determine the amino-acid composition.

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