Biochimica et Biophysica Acta, 770 (1984) 97–100
Elsevier

BBA 72022

ISOPYCNIC CENTRIFUGATION OF RAT-LIVER MICROSOMES IN ISOOSMOTIC GRADIENTS OF PERCOLL® AND RELEASE OF MICROSOMAL MATERIAL BY LOW CONCENTRATIONS OF SODIUM DEOXYCHOLATE

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(Received August 26th, 1983)

Key words: Isopycnic centrifugation; Percoll; Detergent-membrane interaction; Microsome vesicle isolation

Rat-liver microsomes were subjected to isopycnic centrifugation under isoosmotic conditions in gradients of Percoll® containing 0%, 0.05%, 0.1% and 0.2% sodium deoxycholate, respectively. The buoyant density of the microsomes was in the range 1.043 to 1.046 g/ml, independent of the detergent concentration. Absorbance measurements and polyacrylamide gel electrophoresis revealed that the detergent causes an increase in the release of material from the microsomes. Electron microscopy studies showed that membrane disassembly was avoided if the microsomes were isolated at concentrations below 0.1% sodium deoxycholate.

Introduction

There is a great need in experimental biology for pure and representative preparations of endoplasmatic reticulum when studying the transverse localisation of proteins in the membrane, their function, binding and interaction with other components and metabolic pathways [1]. This may be especially important when studying experimental and clinical pathologies, for example, chemical carcinogenesis.

A common procedure for isolating microsomal vesicles is by centrifugation in a continuous sucrose gradient. However, this procedure has some important disadvantages. The microsomes are permeable to sucrose and will initially be subjected to a great physical stress which results in loss of water and shrinkage of the vesicles. Furthermore, several hours of centrifugation is required to achieve isopycnic banding in a preformed linear gradient.

Percoll® (poly(vinyl pyrrolidone)-coated colloidal silica particles) is a density-gradient medium which overcomes the problems associated with sucrose centrifugation. With Percoll one can obtain physiological conditions throughout the gradient. Percoll does not penetrate biological membranes [2] and has been used for a number of applications involved in the isolation of biological membranes [3–8].

For functional studies of microsomal vesicles it is of great interest to achieve a selective sequential release of microsomal content and membrane-bound material without membrane disassembly. This has previously been done in sucrose gradients containing sodium deoxycholate [9].

In this work, we have studied the effect of sodium deoxycholate on microsomal vesicles subjected to isopycnic centrifugation in gradients of Percoll.

Materials and Methods

Male Sprague-Dawley rats, 150–200 g, were starved for at least 12 h. The liver was removed and homogenized in 0.25 M sucrose with a motor-driven Teflon pestle. A postmitochondrial supernatant was prepared by a two-step centrifugation

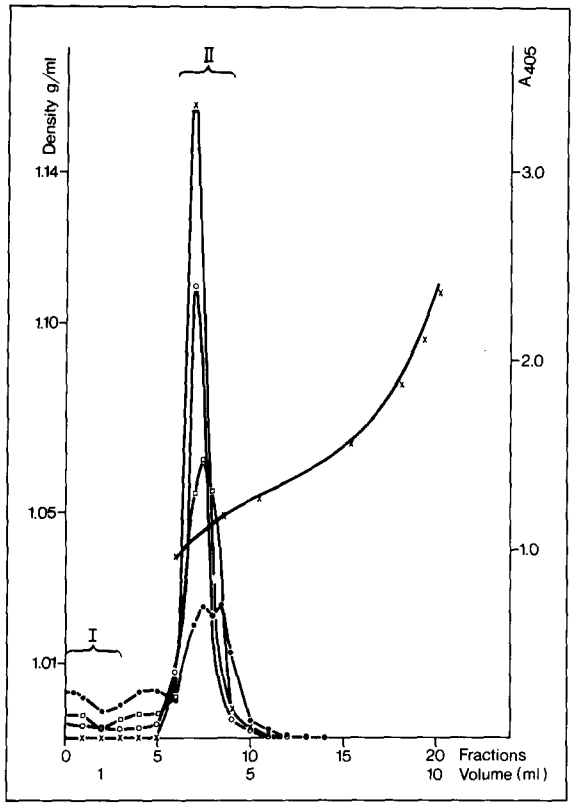

Fig. 1. Rat-liver microsomes subjected to isopycnic centrifugation in 30% Percoll containing 0.25 M sucrose and sodium deoxycholate. The gradients were fractionated from top to bottom and absorbance at 405 nm was continuously measured. Fractions indicated by bars were pooled and analyzed as described in Materials and Methods. Sodium deoxycholate: ×——×, 0%; ○——○, 0.05%; □——□, 0.1%; ●——●, 0.2%.

[10] and then gel-filtered on Sepharose CL-2B (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated in 10 mM Tris-HCl (pH 7.4)/0.25 M sucrose [11].

2 ml purified microsomes were carefully layered on top of 9 ml 30% Percoll (Pharmacia) containing 0.25 M sucrose and final concentrations of 0%, 0.05%, 0.1% and 0.2% sodium deoxycholate, respectively. The centrifugation was carried out in an MSE 8 × 14 ml angle head rotor at 60000 × *g* for 30 min at 4°C. Gradient density was monitored by means of colored density-marker beads (Pharmacia). The gradients were fractionated from the top to bottom and absorbance at 405 nm was continuously measured using a UV-2 monitoring system (Pharmacia). The top fractions, containing released and/or solubilized material and the fractions containing the microsomes, were each pooled.

Methods and assay

Protein was determined according to Bradford [12]. Corrections were made for the background effect of Percoll. RNA was determined according to Fleck and Munro [13]. NADPH : cytochrome *c* reductase activity was determined as described by Sottocasa et al. [14]. The detergent concentration affects the enzyme activity and was therefore adjusted to 0.2% before the activity was measured.

SDS polyacrylamide gel electrophoresis was performed in pre-cast PAA 4/30 gradient gels (Pharmacia). The electrophoresis buffer was 40 mM Tris/20 mM sodium acetate (pH 7.4)/2 mM Na_2EDTA/0.2% SDS. The sample was treated with 1.7% SDS/8.3% β-mercaptoethanol and then heated to 100°C for 5 min. The molecular weights of proteins were determined by comparing their electrophoretic mobility with those of protein standards in the low molecular weight (LMW) calibration kit (Pharmacia).

Electron microscopy was carried out with a Philips E 300 electron microscope using an acceleration voltage of 60 kV. The microsomal preparations were fixed on grids with 1.5% ammonium molybdate.

Results and Discussion

Gel filtered microsomes, subjected to density gradient centrifugation in Percoll have a buoyant density in the range of 1.043–1.046 g/ml, independent of the concentration of sodium deoxycholate (Fig. 1). The formation of the gradient or the buoyant densities of density-marker beads was not affected by the presence of sodium deoxycholate. The microsomal peak at 405 nm decreases with increasing concentration of sodium deoxycholate. The decrease starts far below the critical micellar concentration for sodium deoxycholate which is approx. 0.16%. The decrease could be due to loss of material and a change in size of the vesicles and rupture of microsomal membranes.

SDS-polyacrylamide gel electrophoresis (Fig. 2) shows that there is a clear pattern of released

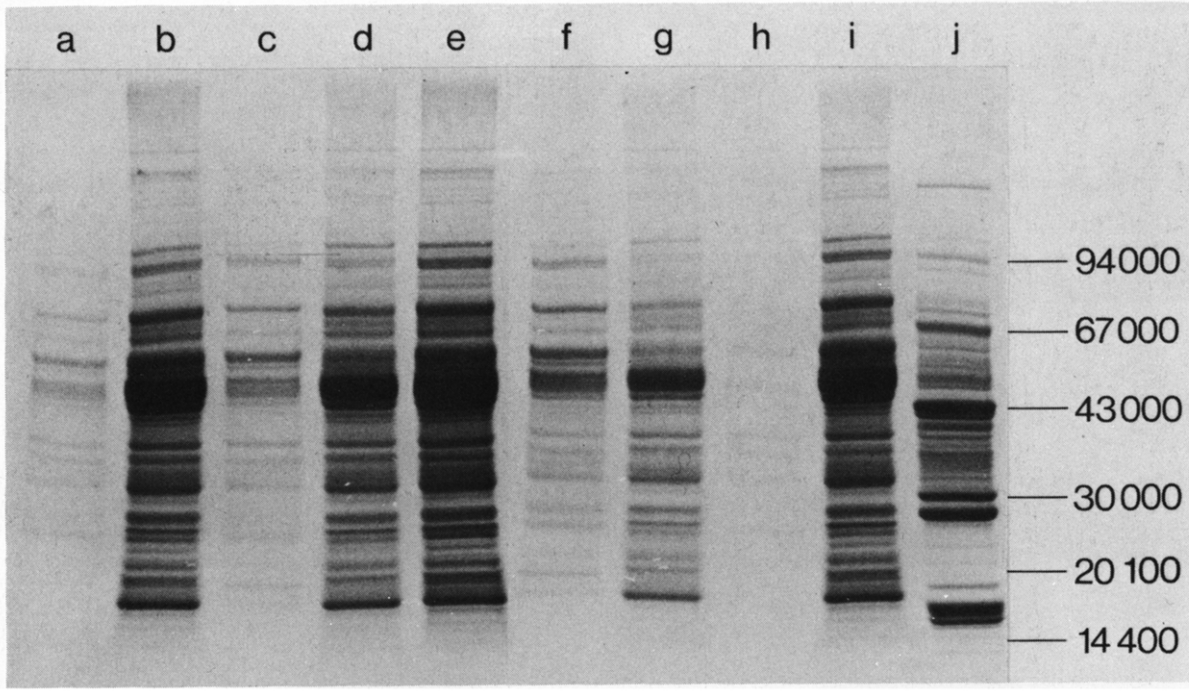

Fig. 2. SDS-polyacrylamide gradient gel electrophoretic analysis of microsomal proteins and released microsomal material. Released proteins from top fractions of gradients containing 0.05%, 0.1%, 0.2% and 0% sodium deoxycholate, respectively (a, c, f and h). Microsomal proteins from the microsomal peak of the gradients containing 0.05%, 0.1%, 0.2% and 0% sodium deoxycholate, respectively (b, d, g and i). Total microsomes loaded on the gradients (e). Cytoplasmic proteins removed in the gel-filtration step during the microsomal preparation (j).

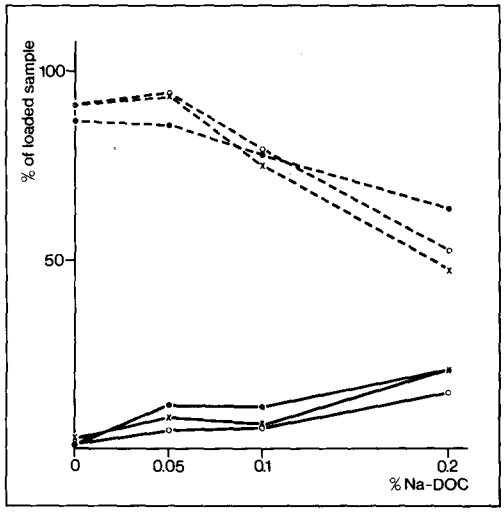

Fig. 3. Release of material from rat-liver microsomal vesicles subjected to isopycnic centrifugation in gradients of Percoll containing different amounts of sodium deoxycholate (Na-DOC). Dotted lines represent the fractions containing microsomal vesicles and full lines represent the top fractions of the gradients containing the released materials. ○, NADPH : cytochrome *c* reductase activity; ×, protein concentration; ●, RNA concentration.

proteins in small concentrations in the top fractions of the gradient even without sodium deoxycholate. These proteins are probably of microsomal origin, since all of them increase in concentration with the addition of sodium deoxycholate and no unique protein band appears. The use of gel filtered microsomes eliminates the contaminating cytoplasmatic proteins which would make the interpretation of the results difficult.

Fig. 3 demonstrates the loss of material from microsomes subjected to centrifugation in gradients of Percoll containing sodium deoxycholate. Between 0.05% and 0.2% sodium deoxycholate there is a dramatic decrease in the amount of protein, RNA and NADPH-dependent cyto-

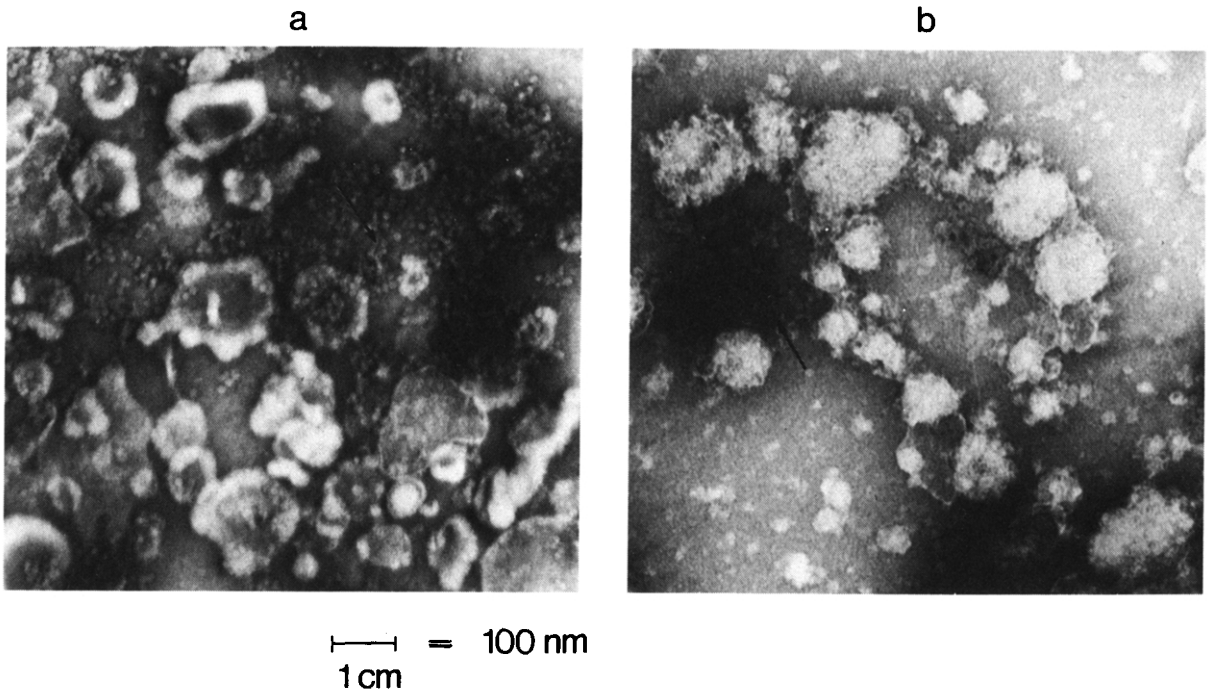

Fig. 4. Electron micrographs of microsomal vesicles. (a) Microsomes isolated in a gradient of Percoll without sodium deoxycholate. (b) Microsomes isolated in a gradient of Percoll containing 0.2% sodium deoxycholate. The small arrow indicates Percoll particles.

chrome *c* reductase in the microsomal fractions. At the same time, there is an increase in microsomal marker proteins in the top fraction with increasing concentration of sodium deoxycholate. The decrease of material in the microsomal peak should correspond with a similar increase of material in the top fractions. However, since only the first three fractions from the top of the gradient were pooled, some of the material was not detected.

Electron microscopy show that the microsomes have a smooth surface and are nonbroken in the absence of sodium deoxycholate (Fig. 4a). Above 0.05% sodium deoxycholate there is an increase in broken membrane fragments and the surface of the microsomes becomes rough and irregular. Fig. 4b shows the rough surface of microsomes isolated in the presence of 0.2% sodium deoxycholate.

Rat-liver microsomes can be isolated on a Percoll gradient without subjection to osmotic stress and nonbroken vesicles can thereby be obtained. This procedure is fast and simple. With a low concentration of sodium deoxycholate, one can obtain a release of microsomal content without membrane disassembly. These results illustrate the usefulness of Percoll® in functional and topological studies of membrane vesicles.

References

1 DePierre, J.W. and Dallner, G. (1975) Biochim. Biophys. Acta 415, 411–472
2 Pertoft, H., Laurent, T.C., Låås, T. and Kågedal, L. (1978) Anal. Biochem. 88, 271–282
3 Inui, K.-I., Okano, T., Takano, M., Kitazawa, S. and Hori, R. (1981) Biochim. Biophys. Acta 647, 150–154
4 Gratzl, M., Krieger-Brauer, H. and Ekerdt, R. (1981) Biochim. Biophys. Acta 649, 355–366
5 Bernhardt, J., Moss, K.M., Luckinger, R.M. and Neumann, E. (1981) FEBS Lett. 134, 245–248
6 Scalera, V., Huang, Y.-K. and Hildmann, B. (1981) Membrane Biochem. 4, 49–61
7 Mamelok, R.D., Groth, D.F. and Prusiner, S.B. (1980) Biochem. 19, 2367–2373
8 Belsham, G.J., Denton, R.M. and Tanner, M.J.A. (1980) Biochem. J. 192, 457–467
9 Kreibich, G., Debey, P. and Sabatini, D.D. (1973) J. Cell Biol. 58, 436–462
10 Kaderbhai, M.A. and Freedman, R.B. (1980) Biochim. Biophys. Acta 601, 11–21
11 Tangen, O., Jonsson, J. and Orrenius, S. (1973) Anal. Biochem. 54, 597–603
12 Bradford, M. (1976) Anal. Biochem. 72, 248–254
13 Fleck, A. and Munro, H.N. (1962) Biochim. Biophys. Acta 55, 571
14 Sottocasa, G.L., Kuylenstierna, B., Ernester, L. and Bergstrand, A. (1967) J. Cell Biol. 32, 415