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INVESTIGATION OF VINCA ALKALOID–PLASMA MEMBRANE INTERACTIONS BY DETERGENT GEL CHROMATOGRAPHY

T. KREMMER and L. HOLCZINGER
National Oncological Institute, Research Institute of Oncopathology, Budapest (Hungary)

SUMMARY

Detergent gel chromatography was successfully applied for the separation of protein subunits and lipid micelles of [^{14}C]Vincristine-treated and sodium dodecyl sulphate-solubilized liver cell plasma membranes. The elution profiles of solubilized membranes depended on the pore size of the commercial agarose and agarose–polyacrylamide gels used. It was found that most of the membrane-bound Vincristine was associated with the solubilized lipid micelles and only a very small proportion was bound to protein subunits.

INTRODUCTION

Gel chromatography of biological membranes solubilized in detergents has been reported to be a valuable method for studying membrane structure and function[1,2].

Recently, we observed that Vinca alkaloids used widely in cancer chemotherapy bound strongly to liver cell membranes[3]. In order to investigate the character of plasma membrane–drug interactions in detail, detergent gel chromatography using commercial gels of different pore sizes was performed for the separation of membrane protein and lipid constituents.

MATERIALS AND METHODS

Preparation of plasma membranes

Liver cell plasma membranes were prepared from male Wistar rats (200–250 g) by the method of Hodson and Brenchley[4] and purified according to Wisher and Evans[5] by ultracentrifugation in a surcrose density gradient. Purified plasma membranes (d = 1.14–1.16) were suspended in 10 mM piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES)–5 mM $MgCl_2$ buffer (pH 7.4) to give a protein concentration of 0.5–1 mg/ml and incubated for 1 h at 25° with N-[^{14}C-formyl]-Vincristine (Specific activity 50.13 μCi/mg; Richter, Budapest, Hungary) using $5 \cdot 10^5$ cpm activity per mg of membrane protein. [^{14}C]Vincristine-treated plasma membranes were washed and pelleted five times with PIPES–$MgCl_2$ buffer, and solubilized in 50 mM Tris–glycin buffer (pH 8.5) containing 2% sodium dodecyl sulphate (E. Merck, Darmstadt,

G.F.R.) and 1% 2-mercaptoethanol by heating for 10 min at 100°. After centrifuging, the clear plasma membrane solutions (3–5 mg protein per ml) were applied to the gel columns.

Measurements

Plasma membrane samples were analyzed by extracting their lipid content according to Folch *et al.*[6], and fractionated by quantitative thin-layer chromatography[7]. The lipid content of the eluted fractions obtained in the column chromatographic experiments was determined by the micromethod of Woodman and Price[8]. Protein measurements were made according to Hartree[9]. ^{14}C-Activity was measured in Bray-cocktail (toluene–dioxane) using an Intertechnique Type SL 30 liquid scintillation spectrometer.

Column chromatography

In an attempt to perform a perfect separation of solubilized plasma membrane protein subunits and lipid micelles in detergent gel chromatography it was necessary to:

(a) choose the appropriate amount of detergent in the solubilization procedure to attain the critical micellar concentration (CMC) which is at least 1.4–1.6 mg of sodium dodecyl sulphate per mg of membrane protein[10];

(b) maintain a low ionic strength during the chromatography to prevent aggregation of detergent micelles;

(c) choose the appropriate pore size of the gel as well as the optimal parameters for column chromatography[11–13].

In our experiments Whatman precision columns (39 × 1.5 cm, total volume (V_t) = 60 ml; or 90 × 2.54 cm, V_t = 450 ml) were filled with the gels listed in Table I and equilibrated with 50 m*M* Tris–glycin buffer (pH 8.5) containing 25 m*M* NaCl and 0.1% sodium dodecyl sulphate. The flow-rate was maintained between 6 and 10 ml/h by hydrostatic pressure as specified by the manufacturers. One (or 5) ml of solubilized membrane sample was applied on the columns and fractionated dropwise in 1 (or 5) ml portions using an LKB Ultrorac 7000 Fraction Collector and monitored by an LKB Uvicord II system at 282 nm.

TABLE I

COMMERCIAL GELS USED IN DETERGENT GEL CHROMATOGRAPHY OF SOLUBILIZED RAT LIVER CELL PLASMA MEMBRANES

Sepharoses: particle size 40–210 μm; Pharmacia, Uppsala, Sweden. Ultragels: particle size 60–140 μm; LKB Bromma, Sweden. Bio-Gel: 100–200 mesh; Bio-Rad Labs., Richmond, Calif., U.S.A.

Gel type	*Lot No.*	*Operating range (mol.wt.)*
Sepharose 4B	5058	100,000–3 · 10^6
Sepharose 6B	8523	200,000–1.5 · 10^6
Ultragel AcA-44	4017	10,000–130,000
Ultragel AcA-34	3029	20,000–350,000
Ultragel AcA-22	2027	100,000–1.2 · 10^6
Bio-Gel A-5m	11961	10,000–5 · 10^6

RESULTS AND DISCUSSION

Detergent gel chromatography of the protein and lipid components as well as [^{14}C]Vincristine bound to the solubilized rat liver cell plasma membranes led to the following conclusions.

Taking into consideration the pore size and the chromatographic properties of the gels used as well as the virtual molecular weights of the sodium dodecyl sulphate-solubilized protein subunits and lipid micelles reported[1,2,10,12], the best separations were achieved on macroreticular agarose gels. The experiments also illustrated the favourable properties of mixed agarose–polyacrylamide beads (Ultragel AcA) which have a higher resistance to deformation. Using Ultragel AcA-44, -34 and -22, an increase in efficiency of separation could be observed depending on the pore size. From a practical point of view, Ultragel AcA-44, Bio-Gel A-5m and Sepharose 6B gave no useful separations. Sepharose 4B and Ultragel AcA-34, however, proved to be convenient for separating the lipid micelles from the protein subunits (Figs. 1 and 2) and there was also a tendency for subfractionation of lipids. Ultragel AcA-22 gave the best subfractionation of solubilized lipid micelles (Fig. 3). Similar observations have been reported by Helenius and Simons[13], who separated the lipids of serum low-density lipoproteins into cholesterol and mixed phospholipid–cholesterol micelles by sodium dodecyl sulphate detergent gel chromatography on Sepharose 4B.

Regarding the [^{14}C]Vincristine binding of the liver cell plasma membranes, Figs. 1–3 demonstrate that the drug is strongly associated with the solubilized lipid

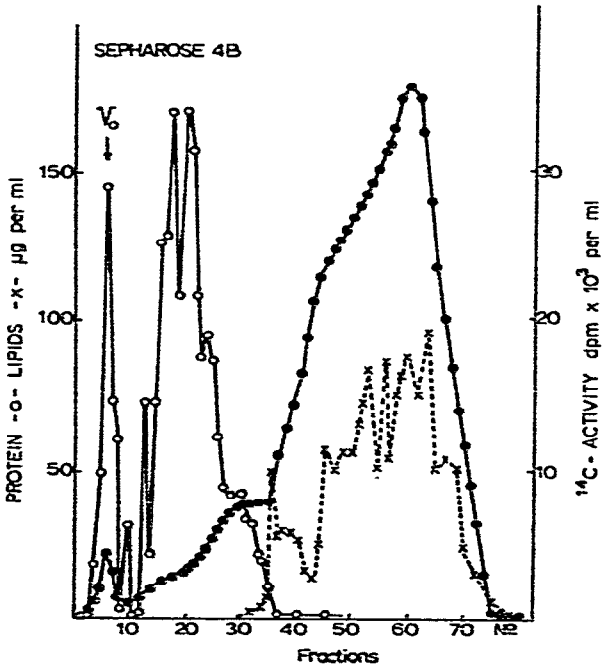

Fig. 1. Detergent gel chromatographic separation of liver cell plasma membrane proteins, lipids and [^{14}C]Vincristine on Sepharose 4B column. Experimental conditions as in Table II.

TABLE II

DISTRIBUTION OF PROTEINS, LIPIDS AND [^{14}C]VINCRISTINE IN FRACTIONS OBTAINED BY DETERGENT GEL CHROMATOGRAPHY OF LIVER CELL PLASMA MEMBRANES ON SEPHAROSE 4B

For elution pattern see Fig. 1. Column, 93 × 2.54 cm; V_t = 467 ml; flow-rate, 30 ml/h. Sample: 5 ml, 26.2 mg of membrane protein. Fractions, 5 ml. Total ^{14}C-activity, 1.029 · 10^6 cpm.

Fraction No.	*Distribution (%)*		
	Proteins	*Lipids*	*[^{14}C]Vincristine*
1–22	71.0	0	4.7
23–34	29.0	7.4	15.4
35–75	0	92.6	79.9

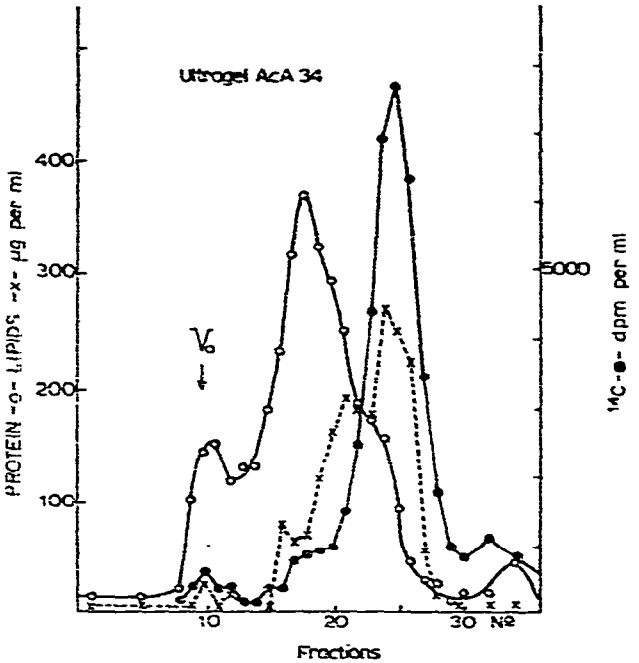

Fig. 2. Detergent gel chromatographic separation of liver cell plasma membrane proteins, lipids and [^{14}C]Vincristine on Ultrogel AcA-34 column. Experimental conditions as in Table III.

micelles, while protein subunits bind considerably less [^{14}C]Vincristine. For example, Table III shows that in the case of Ultragel AcA-34, 85.1% of the [^{14}C]Vincristine is associated with 71.5% of the membrane lipids and only 28.2% of the protein. With Sepharose 4B (Table II), 79.9% of the [^{14}C]Vincristine eluted with 92.6% of the lipids and was not associated with any proteins. These results are in good agreement with our earlier observations based on the consecutive washing procedures of [^{14}C]-Vincristine-treated plasma membranes (with buffers, 2 *M* NaCl, 10% $HClO_4$, etc.), and also on the solvent extraction of membrane lipids[3]. Therefore, it is suggested that

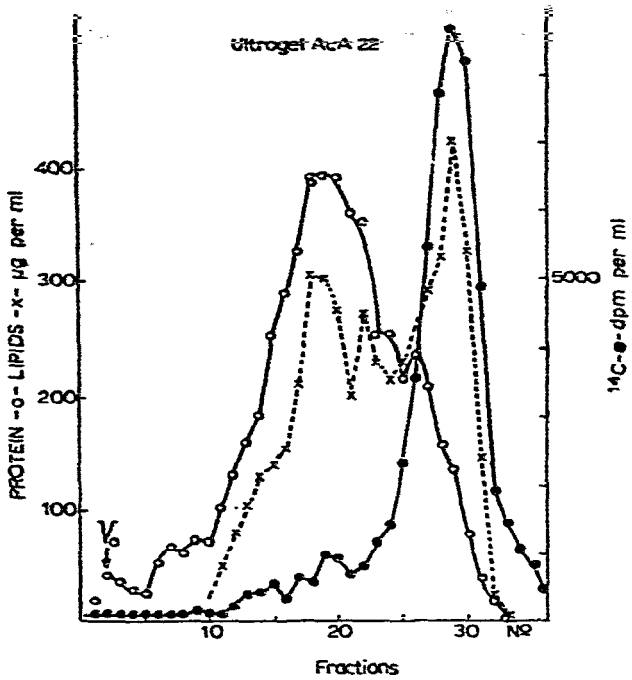

Fig. 3. Detergent gel chromatographic separation of liver cell plasma membrane proteins, lipids and [^{14}C]Vincristine on Ultrogel AcA-22 column. Experimental conditions as in Fig. 2.

TABLE III

DISTRIBUTION OF PROTEINS, LIPIDS AND [^{14}C]VINCRISTINE IN FRACTIONS OBTAINED BY DETERGENT GEL CHROMATOGRAPHY OF LIVER CELL PLASMA MEMBRANES ON ULTROGEL AcA-34

For elution pattern see Fig. 2. Column, 39 × 1.5 cm. V_t = 60 ml, flow-rate, 6 ml/h. Sample: 1 ml, 3.47 mg of membrane protein. Fractions, 1 ml. Total ^{14}C-activity: 42,790 cpm.

Fraction No.	*Distribution (%)*		
	Proteins	*Lipids*	*[^{14}C] Vincristine*
8–14	22.9	2.2	4.8
15–20	48.9	26.3	10.1
21–25	24.5	56.1	53.8
26–30	3.7	15.4	31.3

there are hydrophobic–apolar interactions (Van der Waals–London forces) between the drug molecules and the membrane lipids and this may be the most characteristic feature of the binding of Vinca alkaloids to the hepatocyte membranes.

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