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Characterization of plasma membrane domains of mouse EL_4 lymphoma cells obtained by affinity chromatography on concanavalin A-Sepharose

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Purified plasma membranes of mouse EL_4 lymphoma cells were fractionated by means of affinity chromatography on concanavalin A-Sepharose into two subfractions; one (MF1) eluted freely from the affinity column, the second (MF2) adhered specifically to Con A-Sepharose. Both membrane subfractions proved to be of plasma membrane origin, as evidenced by the following criteria (i) The ratio of cholesterol to phospholipid was nearly identical in plasma membrane and both subfractions. (ii) When isolated plasma membranes were labelled with tritiated $NaBH_4$, both subfractions exhibited identical specific radioactivities (iii) After enzymatic radioiodination of the cells, the total content of labelled proteins was very similar in isolated plasma membranes and in both subfractions. (iv) Some plasma membrane marker enzymes exhibited nearly identical specific activities in plasma membranes, MF1 or MF2 including γ-glutamyl transpeptidase, 5′-nucleotidase and Mg^{2+}-ATPase. Both subfractions exhibited characteristic differences. Thus the specific activities of ($Na^+ + K^+$)-ATPase, Ca^{2+}-ATPase and lysophosphatidylcholine acyltransferase were several-fold enriched in MF2 compared to MF1. SDS-polyacrylamide gel electrophoresis revealed a different polypeptide composition of the two subfractions. Polypeptides of apparent molecular mass of 116, 95, 42, 39, 30 and 28 kDa were highly enriched in MF2, whereas MF1 contained another set of proteins, of apparent molecular mass of 70, 55 and 24 kDa The phospholipid fatty acid composition of the subfractions proved to be different, as well, MF2 contained more saturated fatty acids than MF1. The data suggest the existence of plasma membrane domains in the plasma membranes of the mouse EL_4 lymphoma cells, containing a set of polypeptides, among others membrane bound enzymes, embedded in a different phospholipid milieu.

Introduction

The fluid mosaic model of cell membranes has been widely accepted to explain the basic structure of plasma membranes [1] Specialized plasma membrane areas, however, clearly exist in different cells that have an obvious morphological orientation [2,3] More recent evidence, however, suggested that also in the plasma membranes of cells, which do not exhibit an obvious orientation, domains are also present [5,6] Lymphocytes that exist physiologically as single cells were claimed to bear plasma membrane domains [7,8] Our previous work has shown that from the plasma membranes of rabbit and calf thymocytes specialized membrane areas could be isolated by affinity chromatography on Con A-Sepharose [9–11] This

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Abbreviation Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid

finding is of considerable interest in lymphoic cells because of the wide range of functions involving membrane determined events in which these cells are involved As in stimulated T lymphocytes the plasma membrane domains were claimed to be involved in the regulation of cell differentiation, growth and proliferation it was of particular interest whether similar membrane areas could be shown in proliferating tumor cells as well In this work we show that the plasma membranes of the mouse lymphoma cells, EL_4, contain plasma membrane domains, which closely resemble those described for calf and rabbit T-lymphocytes

Materials and Methods

Cells

EL_4 cells were maintained in ascites form in the peritoneal cavities of 8–10-week-old C57Bl/6 mice Immediately before use, the carrier C57Bl/6 mice were killed and their peritoneal cavities were flushed several times with phosphate-buffered saline containing 5 U/ml heparin The harvested EL_4 cells were washed twice, filtered through small nylon columns (LeukoPak Fenwall Lab) to remove cell debris and adherent cells EL_4 cells were then taken up in cell disruption buffer (0 02 mol/l Hepes, 0 14 mol/l KCl, 0 25 mmol/l $MgCl_2$ (pH 8 0)), chilled in ice and all subsequent steps carried out strictly in the cold The disruption of the cells and the isolation of plasma membranes has recently been described in detail [9] Briefly, the cells were equilibrated for 20 min under 30 atm N_2 in an Artisan pressure homogenizer with gentle stirring, and then released dropwise Immediately after the release EDTA was added to give a final concentration of 1 mmol/l Nuclei, large granules (mitochondria, lysosomes) and a microsomal fraction containing vesicles derived from the plasma membrane and the endoplasmic reticulum were sedimented by differential centrifugation After shocking the microsomal membranes hypotonically (0 02 mol/l Hepes (pH 8 0)) to remove trapped cytoplasmic protein, the membranes were suspended in plasma membrane buffer (0 02 mol/l Hepes, 0 14 mol/l KCl (pH 8 0)) and layered on top of 35% sucrose in plasma membrane buffer After centrifugation for 2 h at $250000 \times g_{max}$ the plasma membranes formed a band at the interface between sucrose and buffer, which was collected and sucrose removed by dialysis overnight against a gradient of a total of 2000 ml 20% sucrose (w/w) and plasma membrane buffer Finally, plasma membranes were dialysed against plasma membrane buffer for 4 h This slow removal of sucrose was essential to prevent the membranes from irreversible aggregation [9]

Affinity chromatography of plasma membranes on Con A-Sepharose

Affinity chromatography was performed strictly at 4°C The detailed procedure of the affinity separation of plasma membrane subfractions has been published recently [9] Briefly, 50 ml of wet gel of Con A-Sepharose (Pharmacia), washed thoroughly before use, were rinsed with 3-times the gel volume with plasma membrane buffer, in a glass separation chamber as described in [9] To the wet gel about 2 mg of plasma membrane protein were added in 10 ml plasma membrane buffer Con A-Sepharose and membranes were mixed by stirring the gel for 2 min at 200 rpm with a motor driven stirrer The membranes were then allowed to bind for 20 min, within which time the gel had sedimented The plasma membrane fraction not binding to Con A-Sepharose was eluted with plasma membrane buffer at a flow rate of 2–3 ml/min, and designated MF1 After complete elution of MF1 the gel was washed with plasma membrane buffer substituted with 0 1 mol/l methyl α-mannoside To dissociate the bound membranes, Con A-Sepharose was stirred for 2 min at 200 rpm The fraction was then eluted with plasma membrane buffer containing 0 1 mol/l methyl α-mannoside and was designated MF2 Elution of membrane protein was monitored with a spectrophotometer at 280 nm (UA5 ISCO Instruments) and the fractions collected batchwise MF1 and MF2 were concentrated by ultracentrifugation for 120 min at $250000 \times g_{max}$ and resuspended in plasma membrane buffer

Analytical procedures

Protein Protein was measured by its native fluorescence as described earlier, using a MPF 44 fluorescence spectrophotometer (Perkin-Elmer, Hitachi) [12]

Cholesterol Cholesterol was measured en-

zymatically as described in Ref 13

Phospholipid Total phospholipid was measured as described in Ref 14 Separation of individual phospholipids Phospholipids were extracted as described recently [15] The phospholipids were separated by thin-layer chromatography on TLC plastic sheets Silica-gel 60, layer thickness 0 2 mm (Merck) with chloroform/methanol/acetic acid/ 0 9% NaCl (50 25 8 2 5, v/v) [15], a modification of the method described by Skipski and Barclay [16] The individual lipid fractions were visualized by exposure to iodine vapour The areas containing a lipid fraction were cut out and counted in a liquid scintillation counter (LKB, Munich)

Radioactive labelling

5×10 lymphocytes in 1 ml phosphate-buffered saline, substituted with 20 mM glucose, were incubated for 20 min at room temperature with 20 units lactoperoxidase, 80 units glucose oxidase (Boehringer), and 1 mCi $Na^{125}I$ (Amersham Buchler), a method similar to that described by Hubbard and Cohn [17] The cells were then washed four times with excess phosphate-buffered saline supplemented with 10% foetal calf serum Radioiodinated lymphocytes were then diluted with unlabelled lymphocytes

Plasma membranes (1 mg protein in 5 ml plasma membrane buffer, supplemented with 20 mM glucose) were incubated for 30 min at room temperature with 100 units lactoperoxidase, 400 units glucose oxidase and 1 mCi $Na^{125}I$ The membranes were then washed twice by ultracentrifugation (60 min at $175\,000 \times g_{max}$) and dialyzed overnight in plasma membrane buffer

Membrane glycoproteins were labelled by sodium borohydride reduction of galactose oxidase-treated membranes [18] 10 ml plasma membranes containing 2 mg membrane protein were first reacted for 5 min with unlabelled $NaBH_4$ (2 mM) at room temperature The plasma membranes were then centrifuged at $250\,000 \times g_{max}$ for 60 min and resuspended in 5 ml plasma membrane buffer The membranes were treated with neuraminidase (Sigma Type VI, 30 μg) for 20 min at room temperature, and subsequently with galactose oxidase (Boehringer, 25 units) for 15 min at 37°C The aldehydes were reduced with tritiated $NaBH_4$ at a concentration of about 1 mCi/ml for 5 min at room temperature $NaBH_4$ (Amersham Buchler, 8 Ci/mmol) was dissolved immediately before use in 0 01 M NaOH The plasma membranes were washed twice in plasma membrane buffer (centrifugation 45 min at $250\,000 \times g_{max}$) and resuspended in 3 ml plasma membrane buffer

Enzyme assays

The activities of various enzymes were measured according to the following quotations lactate dehydrogenase (EC 1 1 1 27) [19], succinate dehydrogenase (EC 1 3 99 1) [20], γ-glutamyl transpeptidase (EC 2 3 2 2) [21], alkaline nitrophenylphosphatase (EC 3 1 3 1) [22], 5′-nucleotidase (EC 3 1 3 5) [23]

ATPases (EC 3 6 1 3) were measured as described in Ref 11 The enzyme insensitive to 10^{-3} M ouabain was designated as Mg^{2+}-ATPase, the sensitive enzyme as $(Na^+ + K^+)$-ATPase Ca^{2+}-ATPase was measured according to Ref 24 Liberated phosphate was determined as described in Ref 25 Lysophosphatidylcholine acyltransferases (EC 2 3 1 23) were measured according to Ref 26 Enzyme activities were calculated from the conversion of the labelled precursor into phosphatidylcholine Arachidonoyl-coenzyme A was synthesized according to Ref 27, using arachidonoyl chloride (NuChek Prep) as substrate

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was carried out according to the method of Laemmli [28] Stacking gel was 5% and running gel 12 5% in acrylamide (Bio-Rad) In some cases, 7 5–15% polyacrylamide gradient gels were used Electrophoresis was performed at a constant current of 40 mA for 180 min 150 μg of membrane proteins were applied for Coomassie brilliant blue staining

Membranes were solubilized in sample buffer (0 025 mol/l Tris (pH 6 8), 2% SDS, 5% β-mercaptoethanol and 10% glycerol) and heated for 3 min at 100°C Gels were stained with Coomassie brilliant blue R-250 (Sigma), 0 1% in methanol/ acetic acid/water (3 6 75, v/v) for 30 min at 60°C and destained by 7% acetic acid overnight

Fatty acid determination

Lipids were extracted as described previously

[15] Phospholipids were separated from neutral lipids by TLC on silica gel plates (Schleicher & Schull) with the solvent system hexane/diethyl ether/acetic acid (80 20 2, v/v) The phospholipids remain at the starting line They were scraped off and transmethylated in the presence of silica gel Transmethylation was performed with sodium methylate as described in Ref 29 By this method acylated fatty acid residues are transmethylated Thus sphingomyelin, a minor component of lymphocytes, remains intact The fatty acid methyl esters were analyzed by capillary GLC (Fractovap a4160, Erba Strumentazione, FFAP column, carrier gas H_2) Peaks were identified by standard fatty acid methyl esters Some peaks were only partially characterized by reduction with H_2, by this method the number of carbon atoms but not the number of double bonds could be determined The peak area was calculated by a computer program from Spectra Physics

Results

Characterization of plasma membranes from EL_4 lymphoma cells

Plasma membranes were isolated from the EL_4 lymphoma cells as described in Materials and Methods For disrupting the cells the nitrogen cavitation method was used This releases small homogeneous vesicles (with a diameter between 50 and 200 nm) from the plasma membrane [30] The characteristics of the plasma membranes are summarized in Tables I–III As can be seen, the plasma membranes isolated from the EL_4 lymphoma cells proved to be highly purified, as revealed by a high cholesterol to phospholipid ratio, characteristic for plasma membranes, and the 5–10-fold enrichment of several plasma membrane bound enzymes, such as γ-glutamyl transpeptidase, 5′-nucleotidase, alkaline phosphatase, ATPases and lysophosphatidylcholine acyltransferase No activity of lactate dehydrogenase, succinate dehydrogenase and only trace amounts of β-glucuronidase were measurable in the purified plasma membrane fraction

Fractionation of plasma membranes from EL_4 lymphoma cells by affinity chromatography on Con A-Sepharose

When purified plasma membranes were subjected to affinity chromatography on Con A-Sepharose under the conditions described in Materials and Methods, two fractions were recovered (Fig 1) One membrane fraction, designated MF1, was not retained on Con A-Sepharose and eluted freely Membrane vesicles, which bound to the affinity adsorbent, could be eluted after mechanical dissociation in the presence of 0 1 mol/l methyl α-mannoside From the membrane vesicles recovered after affinity chromatography 75% eluted in MF1 and 25% in MF2 In more than 15 separations the amount of MF2 varied between 19 and 26%, thus showing the high reproducibility of the separation method The total recovery of the membrane protein subjected to affinity chromatography under the conditions used was greater than 90%

Both fractions, MF1 as well as MF2, revealed

TABLE I

CHARACTERIZATION OF CELLULAR FRACTIONS OF THE EL_4 LYMPHOMA

10^{10} cells were disrupted and cellular fractions isolated as described in Materials and Methods Data are means of three separate experiments S D was always less than 5% n d not detectable

	Protein (mg)	Cholesterol (μmol/mg protein)	Phospholipid (μmol/mg protein)	Ratio (cholesterol/phospholipid)
Homogenate	70 8	0 06	0 23	0 26
Nuclei + large granules	37 9	0 07	0 28	0 25
Supernate	28 3	n d	n d	–
Microsomes	5 3	0 26	0 97	0 27
Plasma membrane	0 8	0 52	0 99	0 53
Endoplasmic reticulum	4 4	0 15	0 85	0 18

TABLE II

CHARACTERIZATION OF CELLULAR FRACTIONS OF THE EL_4 LYMPHOMA

10^{10} cells were disrupted and cellular fractions isolated as described in Materials and Methods Data are means of two separate experiments S D was always less than 5% n d , not detectable

	Marker enzyme activities (nmol (mg protein)$^{-1}$ min^{-1})		
	Lactate dehydrogenase	Succinate dehydrogenase	β-Glucuronidase
Homogenate	325	7 6	5 4
Nuclei	210	8 8	5 6
Large granules	76	46 1	25 6
Supernate	531	n d	0 2
Microsomes	n d	n d	1 4
Plasma membrane	n d	n d	0 7
Endoplasmic reticulum	n d	n d	0 8

TABLE III

CHARACTERIZATION OF CELLULAR FRACTIONS OF THE EL_4 LYMPHOMA

10^{10} cells were disrupted and cellular fractions isolated as described in Materials and Methods All data are means of 3–5 separate experiments S D less than 5% n d , not detectable γGT, γ-glutamyl transpeptidase 5′-Nucl, 5′-nucleotidase AP, alkaline phosphatase LAT, lysophosphatidylcholine acyltransferase

	Marker enzyme activities (nmol (mg protein)$^{-1}$ min^{-1})					
	γGT	5′-Nucl	AP	Mg^{2+}-ATPase	$(Na^+ + K^+)$-ATPase	LAT
Homogenate	4 7	4 2	24 5	104 6	n d	7 6
Nuclei + large granules	6 7	6 9	130 0	178 0	27 5	10 4
Supernate	0 4	–	1 0	9 9	n d	0 5
Microsomes	9 5	12 4	260 0	198 0	48 6	18 9
Plasma membrane	29 5	30 0	559 0	346 0	120 0	45 6
Endoplasmic reticulum	5 0	8 7	160 0	170 0	31 5	8 1

high homogeneity In experiments where both fractions were subjected to rechromatography on Con A-Sepharose 89% of MF1 was recovered in the non-adherent fraction (i e MF1) Similarly, 93% of MF2 was recovered in the adherent fraction (MF2)

Composition of plasma membranes and plasma membrane subfractions

In the plasma membranes and in the subfractions derived thereof, the content of cholesterol and phospholipid was very similar, resulting in a nearly identical high cholesterol to phospholipid ratio, as characteristic for plasma membranes (Table V) This strongly suggested that both subfractions were derived from the plasma membrane

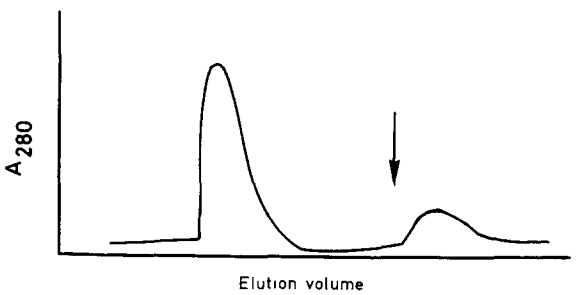

Fig 1 Fraction of plasma membranes on Con A-Sepharose Plasma membranes from the mouse EL_4 lymphoma cells were isolated as described in Materials and Methods 2 mg plasma membrane protein was applied to 50 ml Con A-Sepharose gel, and affinity chromatography carried out as described in Materials and Methods The elution profile was monitored by an ISCO UA-5 absorbance monitor The arrow indicates dissociation of bound membranes

TABLE IV

DISTRIBUTION OF SURFACE LABEL IN EL_4 PLASMA MEMBRANE SUBFRACTIONS

Plasma membranes were surface labelled either by the lactoperoxidase catalyzed radioiodination or by $NaB[^3H]_4$ as described in Materials and Methods The labelled plasma membranes were fractionated on Con A-Sepharose into MF_1 and MF_2

	Label (cpm/mg protein)	
	$[^{125}I]$	$[^3H]$
Plasma membrane	285093	510597
MF_1	288061	530688
MF_2	293476	556146

This was further substantiated by the identical labelling of plasma membranes and the two subfractions after radioiodination of intact cells (Table IV) In this experiment the lymphoma cells were iodinated enzymatically by the lactoperoxidase method, then plasma membranes isolated and subsequently fractionated into MF1 and MF2 by Con A-Sepharose In agreement with these findings, when membrane glycoproteins of isolated plasma membranes were labelled by reduction with tritiated $NaBH_4$ after enzymatic oxydation by galactose oxidase, plasma membranes as well as the subfractions exhibited nearly identical specific radioactivity (Table V)

Taken together these data demonstrate that both subfractions, MF1 as well as MF2, separated by affinity chromatography on Con A-Sepharose, were subfractions of the plasma membrane

Distribution of enzyme activities in plasma membrane subfractions

The plasma membrane nature of the subfractions MF1 and MF2 was further substantiated by the distribution of several plasma membrane-bound enzymes (Table V) Thus, 5′-nucleotidase, γ-glutamyl transpeptidase and Mg^{2+}-ATPase exhibited nearly identical specific activities in both membrane subfractions as well as in unseparated plasma membranes (Table V) Other membrane specific enzymes, however, showed a clearcut heterogeneous distribution Thus $(Na^+ + K^+)$-ATPase, Ca^{2+}-ATPase and lysophosphatidylcholine acyltransferase were several-fold enriched in the adherent fraction MF2

Characterization of the polypeptide composition of plasma membranes and subfractions

When plasma membranes and their subfractions separated by Con A-Sepharose were processed to SDS-polyacrylamide gel electrophoresis, the overall polypeptide pattern of the unseparated membrane and of both subfractions proved to be broadly similar (Fig 2) Among the cytoskeletal proteins the amount of actin, that was identified by comigration with a purified marker was present in equal amounts in the plasma membrane as well as in both subfractions, further substantiating the exclusive plasma membrane origin of the membrane subfractions In contrast, some polypeptides of apparent molecular mass of 116, 95, 42, 39, 30, and 28 kDa were markedly enriched in MF2 as compared to MF1 In contrast, MF1 contained significantly higher amounts of some

TABLE V

DISTRIBUTION OF ENZYME ACTIVITIES IN EL_4 PLASMA MEMBRANE SUBFRACTIONS

Plasma membranes were fractionated on Con A-Sepharose into MF1 and MF2 Data are mean values of 5–10 different plasma membrane preparations S D was less than 5% γGT, γ-glutamyl transpeptidase, LAT, lysophosphatidylcholine acyltransferase

	Specific activity (nmol (mg protein)$^{-1}$ min^{-1})						
	γGT	5′-Nucleotidase	Mg^{2+}-ATPase	$(Na^+ + K^+)$-ATPase	Ca^{2+}-ATPase	LAT	Ratio cholesterol/phospholipid
Plasma membrane	26 9	32 1	342 2	126 0	41 6	44 3	0 54
MF1	27 0	30 8	356 1	60 5	20 2	24 9	0 50
MF2	25 4	31 3	324 8	242 0	80 3	97 5	0 57

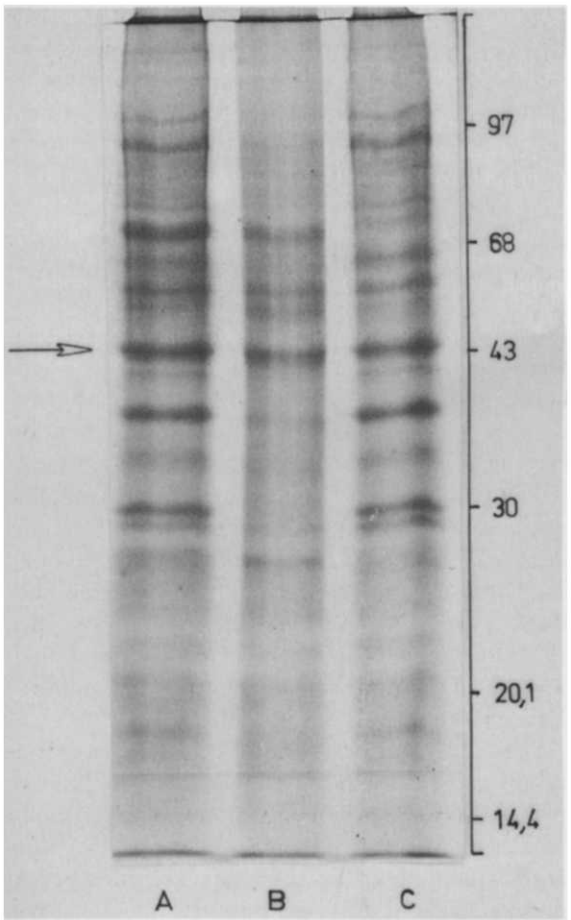

Fig 2 Polypeptide pattern of plasma membranes and subfractions of EL_4 lymphoma cells Purified plasma membranes from EL_4 lymphoma cells were fractionated into MF1 and MF2 by affinity chromatography on Con A-Sepharose as described in Materials and Methods Plasma membranes and subfractions, MF1 and MF2, were concentrated by ultracentrifugation and taken up in sample buffer, containing 0 1% Bromophenol blue Proteins were solubilized at 100°C for 3 min 150 μg protein per gel was run at 40 mA constant current for 3 h in 12 5% acrylamide gels Gel slabs were stained by Coomassie brilliant blue and destained by acetic acid as described in Materials and Methods Molecular mass of single proteins were determined by the comigration of purified molecular weight markers (Pharmacia), consisting of phosphorylase *b* (97 kDa), albumin (68 kDa), ovalbumin (43 kDa) carbonic anhydrase (30 kDa) trypsin inhibitor (20 1 kDa) and lactalbumin (14 4 kDa) The amounts of the single proteins were evaluated by the means of a laser densitometer, 2202 Ultroscan, LKB A Plasma membrane, B MF1, C MF2 The arrow indicates actin

other polypeptides of apparent molecular mass of 70, 55, and 24 kDa (Fig 2)

Characterization of the phospholipid fatty acid composition of plasma membranes and subfractions

Besides the differences observed in the polypeptide and enzyme composition of the plasma membrane subfractions, a distinct phospholipid fatty acid composition of the subfractions was found Table VI shows a typical example of a fatty acid analysis The subfraction MF1 is very similar to the plasma membrane, while MF1 and MF2 showed marked differences The amounts of saturated fatty acids, palmitic and stearic acid, were enhanced in the phospholipids of MF2 with a concomitant decrease in the amounts of polyunsaturated fatty acids The ratio polyunsaturated fatty acid to saturated fatty acid was decreased by 40 to 60% in three independent experiments

Discussion

Using affinity chromatography with Con A-Sepharose plasma membranes from the mouse EL_4

TABLE VI

FATTY ACID COMPOSITION OF THE PHOSPHOLIPIDS FROM EL_4 PLASMA MEMBRANE SUBFRACTIONS

Plasma membranes were isolated and fractionated on Con A-Sepharose The phospholipids were isolated and their fatty acid content determined For experimental details see Materials and Methods Data are expressed as weight per cent 20 *n* and 22 *n* are two and three fatty acids, respectively, with a known carbon number and an unknown number of double bonds ($n > 1$)

	16 0	16 1	18 0	18 1	18 2	20 *n*	20 4	22 *n*	polyunsaturated/ saturated
Plasma membrane	9 2	0 2	26 8	14 8	8 0	4 6	16 5	19 8	1 36
MF1	9 5	0 3	26 3	16 5	5 6	4 9	17 6	19 2	1 32
MF2	14 4	0 9	39 4	19 5	5 0	3 4	9 3	8 1	0 48

lymphoma cell line were fractionated By the method used two plasma membrane subfractions were obtained, MF1 and MF2, which were homogeneous by rechromatography under identical conditions Several findings indicated that both subfractions were of plasma membrane origin (i) The plasma membrane preparations used were highly purified, as revealed by a high cholesterol to phospholipid ratio, characteristic for plasma membranes and the several-fold enrichment of some membrane bound enzymes, such as γ-glutamyl transpeptidase, 5′-nucleotidase, alkaline phosphatase and $(Na^+ + K^+)$-ATPase (see Table I and III) Furthermore, no mitochondrial or cytoplasmic contamination was detectable, as shown by the lack of succinate dehydrogenase and lactate dehydrogenase in the plasma membrane fraction (Table II) (ii) The cholesterol to phospholipid ratio was identical in the plasma membrane subfractions, MF1 and MF2 (iii) Enzymatic surface radioiodination or labelling of surface carbohydrates by $NaBH_4$ reduction was identical (Table IV) (iv) In both fractions a number of plasma membrane marker enzymes showed identical specific activities, including γ-glutamyl transpeptidase, 5′-nucleotidase and Mg^{2+}-ATPase (Table V) (v) The amount of the cytoskeletal protein actin was essentially the same in the unseparated plasma membranes and in both subfractions (Fig 2)

Our previous results have shown that by the nitrogen cavitation method plasma membrane vesicles were obtained that have preserved their original orientation, e g were right-side-out [9,10], excluding the possibility that the fraction not binding to Con A-Sepharose does not expose carbohydrate to the outer surface

Although both plasma membrane subfractions share some properties – indicating their exclusive plasma membrane nature as discussed above – there also exist marked differences considering their chemical and functional properties (i) The adherent fraction MF2 consisted in a set of polypeptides of apparent molecular mass of 116, 95, 42, 30, 30, and 28 kDa, that were reduced in their amounts in MF1 (Fig 2) (ii) The phospholipid fatty acid composition of MF2 proved to be quite different as compared to MF1, showing a marked increase in the amounts of saturated fatty acids in the phospholipids extracted from MF2 (Table VI) (iii) The specific activities of several membrane bound enzymes, i e $(Na^+ + K^+)$-ATPase, Ca^{2+}-ATPase and lysophosphatidylcholine acyltransferase were several-fold higher in the adherent fraction MF2 as compared to MF1 (Table V)

Our recent work on the functional ultrastructure of lymphocyte plasma membranes has shown that plasma membrane domains from calf and rabbit thymocytes, with strikingly similar characteristics to those presented here, can be separated by affinity chromatography on Con A-Sepharose [9–11,30] Although EL_4 cells were reported to represent a homogeneous cell population [31], it cannot be ruled out that actually cells at different stages of the cell cycle were present The fact, however, that plasma membrane subfractions with nearly identical characteristics were obtained by affinity chromatography of plasma membranes from resting T-lymphocytes strengthens the idea that by affinity chromatography on Con A-Sepharose different domains of the plasma membrane of an individual cell were isolated

Thus, our data suggest that the plasma membranes of transformed lymphocytes, similarly to normal ones are not entirely homogeneous, but contain domains consisting of a set of polypeptides, among others of some membrane bound enzymes, embedded in a phospholipid milieu different from that of the bulk membrane Although their precise nature has to be established, recent experiments suggested that these domains are kept together by strong forces involving protein-protein, as well as protein-lipid interactions (Refs 10, 32, and Szamel, M , Kaever, V and Resch, K , manuscript in preparation)

The demonstration that in the plasma membranes of normal and transformed lymphocytes there exist specialized domains bears implications for the regulation of cell activation and proliferation In fact, we have shown recently that at least two enzymes specifically enriched in these plasma membrane areas, $(Na^+ + K^+)$-ATPase and lysolecithin acyltransferase, are also functionally coupled in the plasma membrane domains of lymphocytes [11,33] Blocking the activation of $(Na^+ + K^+)$-ATPase by ouabain prevented the activation of lysophosphatidylcholine acyltransferase and also the subsequent induction of macro-

molecular synthesis, supporting the role of the plasma membrane domains in the regulation of lymphocyte differentiation and proliferation The fact that these enzymes are represented with very high specific activities, also in the plasma membrane domains of transformed lymphocytes, suggests a similar regulatory function also in transformed cells

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