CHANGE OF THE UNSATURATION INDEX OF THE LYMPHOCYTE PLASMA MEMBRANE BY ETHANOL AND FATTY ACIDS

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ABSTRACT

We have shown that phospholipids fatty acid composition of lymphocyte plasma membrane(PM)alters migration"in vivo".To confirm the relationship between fatty acids composition(FA's)of phospholipids and unsaturation index(UI)of PM,we have studied BALB/c mouse spleen lymphocyte PM after incubation with FA's added directly from stock solutions in ethanol(Eth).

Cells are disrupted by sonication and PM isolated by sucrose density gradient.The purity of PM enriched fraction was tested by enzymic markers and the molar ratio cholesterol/phospholipids.Lipids were extracted by the Blight and Dyer method and phospholipids obtained by acetone precipitation.Protein was determined by the Lowry method.Fatty acid methyl esters obtained by the BF_3 method were separated and identified by GLC.The results show that Eth acts as a "fluidizing" agent,increasing the UI,and in presence of FA's the cells react metabolizing the exogenous FA's added in attempt to restore the UI.This correction seems more effective: the shorter the length of the chain,the less the unsaturation of the FA's.

INTRODUCTION

We have shown that phospholipid fatty acids composition of lymphocyte plasma membrane alters migration "in vivo",(NOVO,C.,FONSECA,E.,FREITAS,A.A.,in press)

Key words:Lymphocyte,Plasma membrane,Unsaturation index,Fatty acids,Ethanol.

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In order to confirm the influence of lenght and unsaturation of fatty acyl chain on lymphocyte plasma membrane unsaturation index(UI),we have study the phospholipid fatty acids composition of BALB/c mouse spleen lymphocytes plasma membrane after incubation "in vitro" with ethanol and stearic(18:0),oleic 18:1(n-9),linolenic 18:3 (n-3),and arachidic(20:0) acids.

MATERIAL AND METHODS

BALB/c mouse spleen cell suspensions were prepared by teasing the organ with two forceps in cold Balanced Salt Solution(BSS)and erythrocites lysed by treatment of cell suspensions with a modified Gey's solution.

3 x 10^8 cells were incubated(20 hr.)at 37ºC in RPMI 1640,0.01 M HEPES,2 mM L-Glutamine,50 µg/ml Streptomycin,50 IU/ml Penicillin,0.05 mM 2-Mercaptoethanol and supplemented with 0.2% of lipid free bovine serum albumin in a humidified 5% CO_2 atmosphere,at a concentration of 2 x 10^6 cell/ml,in presence of ethanol and stearic, oleic,linolenic and arachidic acids.

Fatty acids added directly from stock solutions of 0.04 M in 95º ethanol, were used at a final concentration of 25 µg/ml and 0.2%(V/V) ethanol (1).

Cells were washed with BSS,ressuspended in 10 mM Tris,120 mM NaCl,pH=7.4,disrupted by sonication (2) and PM enriched fraction isolated by discontinuous sucrose density gradient (3).The purity of PM enriched fraction was tested by the following markers:5'-Nucleotidase activity (4) (7),Glucose-6-phosphatase activity (5) (7) and the molar ratio cholesterol/phospholipids (6) (7).Phospholipid was calculated by assuming 25 µg of phospholipid/µg of Phosphorous and proteins were determined by Lowry method (8).

Lipids were extracted by the Bligh and Dyer method (9) and phospholipids isolated by acetone precipitation (10).

Fatty acids obtained by acid hidrolysis were converted in methyl esters by the boron trfluoride method (11) and separated by GLC in a Perkin Elmer 900 chromatograph with a dual flame ionization detector(FID).Fatty acid methyl esters were separated on a 20% DEGS on a 80-100 mesh Chromosorb AW DMCS in a stainless steel column(6 ft x 1/8 inch).The analysis was performed at programmed temperature (140 - 170ºC) with an increasing rate of 2ºC/min. in the presence of a N_2 flow of 35 ml/m followed by isothermal operation.Peaks identification was obtained by comparing their relative retention time(t'_r) in relation to an internal standard added (margaric acid) with t'_r of known standards or by more probable metabolic pathways and confirmed by overloading with known standards (Applied Sciences Lab.).The relative percentage of peaks areas was evaluated using a Hewlett Packard 3380 A integrator.

In order to calculated unsaturation index (UI),the molar proportion (percentage) of each fatty acid in an analysis is multiplied by the number of double bonds contained in that fatty acid.The values thus obtained are summed over all the fatty acids presents.

RESULTS

The procedure of lymphocyte disruption and the fractionation method used made possible the isolation of a plasma membrane enriched fraction with a high recovery of 5'-Nucleotidase activity (42.5 ± 13.9%) as well a cholesterol/phospholipid molar ratio (0.39 ± 0.04) in agreement with others authors (12) (13),and a specific

activity enrichement in relation to homogenate of 5.6 ± 1.9.

Although the plasma membrane ehriched fraction to be partially contaminated with endoplasmic reticulum, the recovery of Glucose-6-phosphatase activity is 22.7 ± 13.5%, the percentage of pure membrane in the fraction is 77% as well the cholesterol/phospholipid ratio in pure membrane is 0.5, in agreement with Johnson and Robinson (12) (Table 1).

TABLE 1

Markers of PM enriched fraction

	5'-NUCLEOTIDASE a)		GLUCOSE-6-PHOSPHAT. a)		CHOLESTE./ PHOSPHOL. MOLAR RATIO b)	PURE MEMBRANE IN PM ENRICHED FRACTION (%) c)	CHOLESTE./ PHOSPHOL. MOLAR RATIO IN PURE MEMBRANE c)
	Specific activity enrichement in relation to homog.	Total activity recovery (%)	Specific activity enrichement in relation to homog.	Total activity recovery (%)			
PM enriched fraction	5.6 ± 1.9	42.5 ± 13.9	3.1 ± 2.2	22.7 ± 13.5	0.39 ± 0.04	77	0.5

a) The results are the mean ± SD of 8 experiments for 5'-Nucleotidase activity and 5 experiments for Glucose-6-phosphatase activity. The specific activity of enzymes are expressed in μmol(P)/h/mg Prot.

b) The results are the mean ± SD of 5 experiments for the phospholipid and cholesterol composition.

c) The results were calculated as described by Johnson and Robinson (12).

The analysis of chromatograms (Fig. 1 and 2) and the plasma membrane UI values, suggest that there is a relationship between phospholipid fatty acyl chain lenght and unsaturation with plasma membrane UI (Fig. 3).

The results obtained show that ethanol and fatty acids added promote alterations in phospholipid fatty acids composition of PM with consequences in UI of membrane. As shown in Fig. 3 the results suggest:

1-Ethanol increases the UI in relation to cells without any incubation;

2-Unsaturated fatty acids with same chain lenght show an UI increase as higher the number of double bonds;

3-Saturated fatty acids increase UI as long the chain lenght.

In conclusion the results suggest that ethanol acts as a "fluidizing" agent at expences of saturated fatty acids (16:0 + 18:0) that decrease about 86%.

In presence of exogenous fatty acids, cells reacts to this high UI, metabolizing these fatty acids in attempt to restore the UI. This correction seems more effective the shorter the lenght of the chain, the less the unsaturation of the fatty acids.

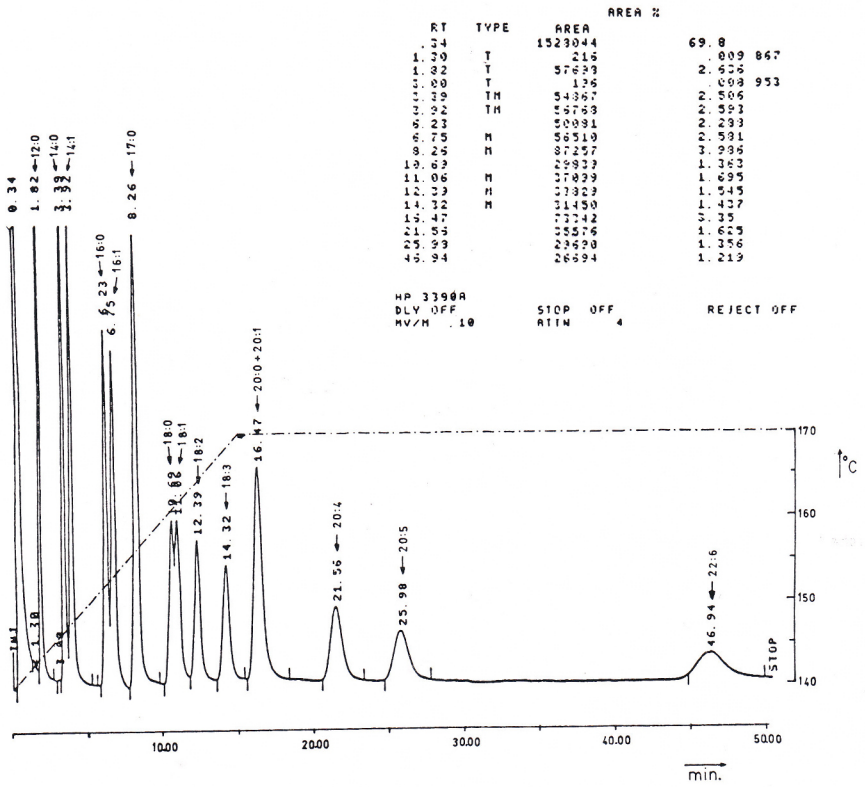

FIGURE 1

Chromatogram of Standards: Chromatogram of a mixture of standards (K-103/H-105/L-209) with internal standard (margaric acid). The temperature of injection and manifold are 240ºC and the sample size 1 µl.

DISCUSSION

The PM enriched fraction have a specific enrichement in relation to homogenate, for the 5'-Nucleotidase, lower than the obtained by others authors. Hoewever, a high recovery of activity is obtained and this is at least as important as a high specific activity, because high purification factors may represent a cell selection process, if the percentage yield is low (12) (14).

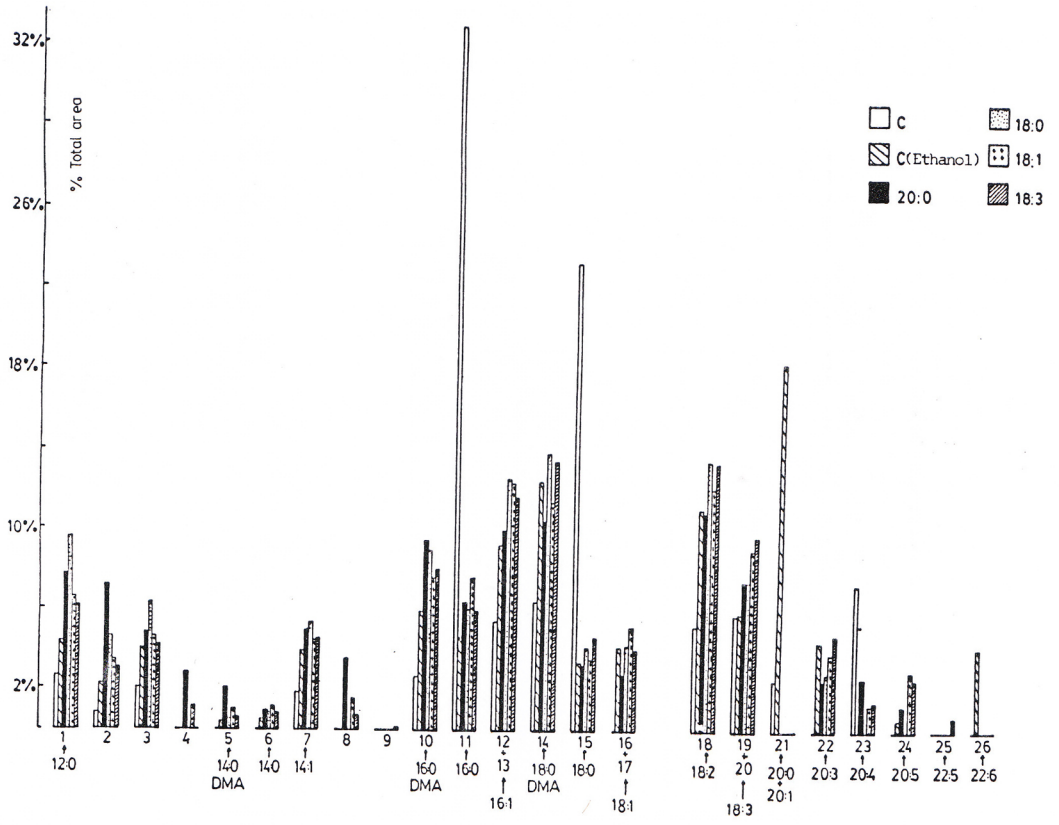

FIGURE 2

Chromatogram's analysis: Each bar represents the area covered by fatty acid individual peak expressed as a percentage of the total area of the chromatogram. Results shown represent the phospholipid fatty acids composition of plasma membrane enriched fraction of spleen cells in conditions desctibed in Material and Methods. Dymethylacetals are represented by DMA.

As the procedure of lymphocyte disruption used leaves intact nuclei (2) the major contaminating is endoplasmic reticulum. This contamination may be explained by the presence of Ca^{2+} and Mg^{2+} in the medium, that promotes aggregation of membranes during the fractionation (15).

Since the data of pure membrane percentage in fraction is about 80% and the

cholesterol/phospholipid molar ratio in the pure membrane is 0.5,this fraction is representative of plasma membrane.

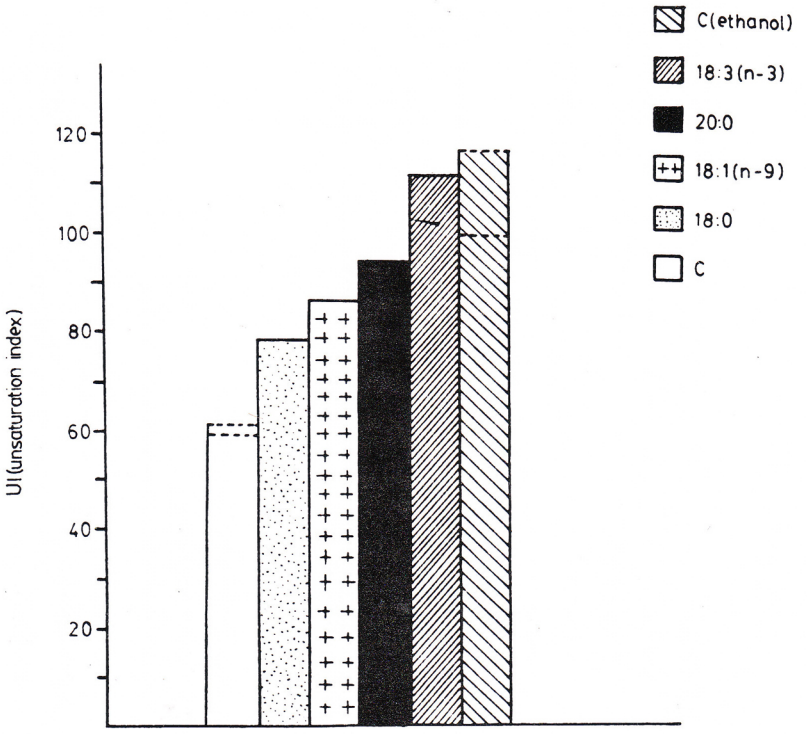

FIGURE 3

Plasma membrane UI:Each bar represents the UI calculated as described in Mate rial and Methods for PM enriched fraction.

Peak's chromatogram identification by relative retention time and overloading with standards permits to identify the majority of peaks witch agree with principal fatty acids of mammalian cells.

Non identified peaks with t'_r lower than 16:0 are probably degradation products since don't exist in control or individual area are less than 2% and are localized in region of unfisiologic fatty acids.

Assignment of three peaks to DMA are justified by its elution precede the cor - responding methyl esters and by the existence of plasmalogens in lymphocytes with chains of 16:0 and 18:0 (16),just the peaks that we have found in control without any incubation.

REFERENCES

(1) HORWITZ,A.F.,HATTEN,M.E.,BURGER,M.M. Membrane fatty acid replacements and their effect on growth and lectin-induced agglutinability.Proc.Nat.Acad.Sci., USA 71:3115-3119,1974

(2) KLAUSNER,R.D.,KLEINFELD,A.M.,HOOVER,R.L.,KARNOVSKY,M.J. Lipid Domains in Membrane:evidence derived from structural perturbations induced by free fatty acids and lifetime heterogeneity analysis. J.Biol.Chem. 255:1286-1295,1980

(3) KOIZUMI,K.,SHIMIZU,S.,KOIZUMI,K.T.,NISHIDA,K.,SATO,C.,OTA,K.,YAMANAKA,N. Rapid characterization of plasma membrane from normal and malignant lymphoid cells of mouse.Biochem. Biophys.Acta 649:393-403,1981

(4) WEAVER,R.C. and BOYLE,W. Purification of plasma membrane of rat liver.Application of zonal centrifugation to isolation of cell membranes.Biochem.Biophys. Acta 173:377-388,1969

(5) NORDLIE,R.C. and ARION,W.J. Glucose-6-phosphatase,in Methods in Enzymology, Vol 9,Wood,W.A.(ed.),Academic Press,New York,London,1966,pp 619-625

(6) STADTMAN,T.C. Preparation and assay of Cholesterol and Ergosterol,in Methods in Enzymology,Vol. 3,Colowick,S,P.,Kaplan,N.O.(eds.),Academic Press,New York, 1957,pp 392-394

(7) AMES,B.N. Assay of Inorganic Phosphate,total Phosphate and Phosphatase,in Methods in Enzymology,Vol. 8,Neufeld,E.F.,Ginsburg,V.(eds.),Academic Press,New York,1966,pp 115-116

(8) LOWRY,O.H.,ROSEBROUGH,N.J.,FARR,A.L.,RANDALL Protein Measurement with the Folin Phenol reagent.J.Biol.Chem. 193:265-275,1951

(9) BLIGH,E.G. and DYER,W.J. A rapid method of total lipid extraction and purification.Can.J.Biochem.Physiol. 37:911-917,1959

(10) KATES,M. Techniques of lipidology:isolation,analysis and identification of lipids.Pocked edition of Vol. 3,Part II of Laboratory Techniques in Biochemistry and Molecular Biology,North-Holland Publishing Company,Amsterdam,1972

(11) MORRISON,W.R. and SMITH,L.M. Preparation of fatty acid methyl esters and dymethylacetals from lipids with boron fluoride-method.J.Lipid Res. 5:600-608, 1964

(12) JOHNSON,S.M. and ROBINSON,R. The composition and fluidity of normal and leukaemic or lymphomatous lymphocyte plasma membrane in mouse and man.Biochem. Biophys.Acta 558:282-295,1979

(13) FERBER,E.,RESCH,K.,WALLACH,D.F.H.,IMM,W. Isolation and characterization of lymphocyte plasma membrane.Biochem.Biophys.Acta 266:494-504,1974

(14) De PIERRE,J.W. and KARNOVSKY,M.L. Plasma membrane of mammalian cells.J.Cell Biology 56:275-303,1973

(15) ALLAN,D. and CRUMPTON,M.J. Preparation and characterization of the plasma membrane of pig lymphocytes.Biochem. J.120:133-143,1970

(16) RUGGIERI,S.,ROBLIN,R.,BLACK,P.H. Lipids of whole cells and plasma membrane fractions from Balb/c 3T3,SV 3T3,and concanavalin A-selected revertant cells. J.Lipid Res. 20:772-783,1979