Biochimica et Biophysica Acta, 688 (1982) 667–670
Elsevier Biomedical Press

BBA Report

BBA 70040

ALTERATION OF PHOSPHOLIPID COMPOSITION OF MOUSE LIVER MICROSOMES IN VIVO AND THE EFFECT ON MEMBRANE PROPERTIES

DENIS M. BOYLE and WILLIAM L. DEAN

Department of Biochemistry, University of Louisville School of Medicine, Louisville, KY 40292 (U.S.A.)

(Received November 3rd, 1981)

Key words: Phosphatidylethanolamine methylation; Membrane fluidity; Periodate-oxidized adenosine; Phospholipid composition; Membrane reconstitution; (Mouse liver microsome)

Administration of the methylation inhibitor periodate-oxidized adenosine to male Swiss-Webster mice on a choline-deficient diet produced a decrease (17%) in phosphatidylcholine to phosphatidylethanolamine ratios compared to saline-injected controls in liver, and also in kidney (11%), but not in muscle microsome preparations. Both intact liver microsomes and reconstituted membranes from lipid extracts showed a higher fluorescence anisotropy of the hydrophobic probe 1,6-diphenyl-1,3,5-hexatriene than control samples in the temperature range of 20–31°C.

Phosphatidylcholine (PC) is the major phospholipid in most animal membranes. It is important not only as a structural component but it also plays a role in the function of some membrane-bound enzymes [1]. It has been shown that many membrane-bound enzymes require phospholipid for full enzymatic activity [2], and that for some enzymes the polar head group structure may be more important for activity than the hydrophobic portion of the amphiphile [3,4]. Differences in the head group composition of phospholipid bilayers can change the phase transition temperature [5] and fluidity [6,7] of the membrane. Because these physical parameters affect membrane structure and function, and depend on the phospholipid composition, we examined the effects of altered phospholipid composition on the apparent microviscosity of biological membranes.

Two pathways have been described for the de novo biosynthesis of PC. Free choline can be incorporated into PC [8], or PE can be converted directly to PC by stepwise methylation using the methyl groups from *S*-adenosylmethionine [9,10]. Data presented here indicate that much of the phosphatidylcholine content of liver microsome membranes is supplied by an independently operating methylation pathway which is capable of modulating the physical state of the membrane.

For manipulation of the phospholipid composition of membranes in vivo, male Swiss-Webster mice weighing 25–30 g were fed a pelleted, choline-deficient diet obtained from ICN Nutritional Biochemicals. After a three week period on the diet, animals in the experimental group received 0.20 μmol freshly prepared periodate-oxidized adenosine [11] in a saline per g mouse weight administered by intraperitoneal injection and two more injections at a dose of 0.10 μmol/g each were administered at 24-h intervals. Cycloleucine (a methionine analogue) was also included in the treatment at a dose of 7.75 mmol/kg [12] in saline injected on the first day. Choline-deficient

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS+PI, total of phosphatidylserine and phosphatidylinositol.

animals in the control group received saline only. The animals were killed on the day following the last injection. Sarcoplasmic reticulum vesicles from pooled hind-leg muscles were prepared according to the method of Meissner et al. [13]. Liver and kidney membrane vesicles were prepared from the pooled tissues of each group by a modification of the method of Munro et al. [14]. Tissues were homogenized (15% w/v) in a 0.25 M sucrose solution containing 0.04 M NaCl, 0.1 M KCl, 5 mM $MgSO_4$, 0.02 M Tris-HCl (pH 7.60) and the final microsomal membranes were washed once by resuspension and centrifugation in homogenizing buffer. The washed microsomal membranes were resuspended in 50 mM potassium phosphate (pH 7.50), 10% sucrose. To prevent oxidation of lipids, all samples were aspirated under reduced pressure for at least 10 min to remove O_2 and then brought to ambient pressure with N_2. The membranes were frozen in liquid nitrogen and stored at $-11°C$.

It has been reported that administration of periodate-oxidized adenosine results in increased levels of *S*-adenosylhomocysteine, a competitive inhibitor of transmethylation reactions in mouse liver [11]. Incorporation of [^{14}C]choline into phospholipids is increased when inhibitors of methylation are administered [15]. Conversely, the methylation of phospholipids was reported to increase in liver microsomes from rats deprived of choline [16,17].

It was, therefore, of interest to examine the effect of periodate-oxidized adenosine on membrane phospholipid methylation in mice on a choline-deficient diet. Table I shows the data from four experiments after treatment of choline-deprived mice with cycloleucine and periodate-oxidized adenosine for 3 days. The phosphatidylcholine content of liver microsomal membranes was decreased an average of 11% with a concomitant increase in the PS + PI fraction of 19%. The change in the PC/PE ratio averaged 17% which is equivalent to 54 nmol phospholipid/mg protein. This value is several orders of magnitude larger than the changes reported by Hirata and Axelrod [6]. A similar, although smaller, decrease in the PC/PE ratio was noted in microsomes from kidney (11%), but no change was observed in muscle tissue (data not shown). The lipid to protein ratios of the membranes were not consistently changed in any of the tissues by this treatment. The reduced magnitude of the response to methylation inhibition in muscle as compared to liver microsomes is in agreement with the findings of Bjørnstad and Bremer [18] who concluded that the methylation pathway for phosphatidylcholine biosynthesis is highly functional in liver but is of minor importance in extrahepatic tissues. In experiments not shown it was also observed that cycloleucine and periodate-oxidized adenosine decreased the PC/PE ratio in mice on a diet in which choline was not omitted although the magnitude of the change was much smaller.

In order to determine the effect of the observed change in phospholipid composition on membrane fluidity experiments were carried out using 1,6-di-

TABLE I

CHANGES IN MOUSE LIVER MICROSOME PHOSPHOLIPID COMPOSITION INDUCED BY CHOLINE-DEFICIENCY AND PERIODATE-OXIDIZED ADENOSINE

Phospholipids were extracted according to the method of Folch et al. [20]. Lipids were quantitatively extracted from 1.5 mg membrane protein in 0.10 ml by warming slightly and vortexing twice for 10 s in a 10-fold excess of $CHCl_3/CH_3OH$ (2:1, v/v). Membrane phospholipid compositions were analyzed by one-dimensional TLC [21]. 50 μl (about 60 nmol lipid phosphorus) of the extract was applied per lane on silica gel H plates (Supelco, Inc.). The spots were identified with appropriate standards after I_2 vapor staining and scraped from the plates for quantitation of PO_4^{3-} by the method of Bartlett [22]. The phospholipids were not eluted from the silica gel but were digested directly with 0.20 ml 10 M H_2SO_4 at 155°C for more than 3 h. Phospholipid compositions in each experiment were calculated from eight separate determinations. Values are $\bar{x} \pm$ S.E. from four separate experiments with six mice in each group per experiment.

Phospholipid	% change ($\bar{x} \pm$ S.E.)	Control phospholipid composition (100 nmol total)
PC	-11 ± 4	50.4
PE	$+13 \pm 5$	18.9
PS + PI	$+19 \pm 5$	14.0
LysoPC	-23 ± 10	10.2
Front	no change	2.8
Origin	no change	4.2
Lipid : protein (nmol/mg)	no change	730

phenyl-1,3,5-hexatriene as a fluorescent probe. It is evident from the results in Fig. 1 that the emission anisotropy of the control membranes (higher phosphatidylcholine, lower phosphatidylethanolamine content) was significantly higher from 20 to 31°C than that of the membranes from treated animals. This is due entirely to lipid-probe interactions since the difference in anisotropy was even more pronounced for reconstituted membranes (filled symbols) than for microsomes (open symbols). Thus it appears that the apparent microviscosity [7] is affected by the PC/PE ratio as proposed by Hirata and Axelrod [6] although this observation could also be a result of concomitant changes in fatty acid composition which was not determined in these experiments.

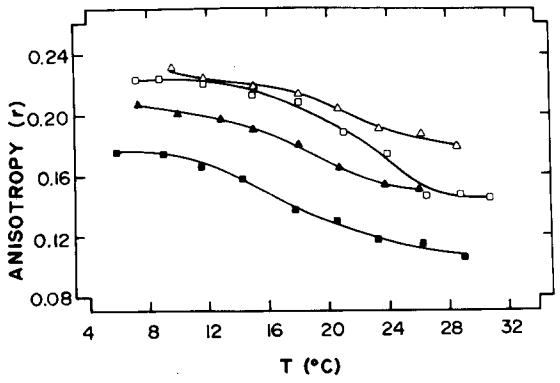

Fig. 1. Temperature dependence of anisotropy of 1,6-diphenyl-1,3,5-hexatriene fluorescence in microsomes and reconstituted phospholipid bilayers. Fluorescence measurements were made on an Aminco-Bowman Spectrophotofluorometer with ratio photometer fitted with a Forma Scientific 2006 circulating water bath and equipped with two glan prism polarizers. A YSI Telethermometer with probe was used to obtain the temperature of the sample in the cuvette after a 10 min equilibration period at each temperature and before measurement of fluorescence. The sample was excited at 368 nm and the emission at 432 nm was recorded. Emission anisotropy (r) of the probe is proportional to the microviscosity or fluidity of its surrounding medium [23] and was calculated by the procedure described by Shinitzky and Barenholz [7]. One μl of a 2 mM solution of 1,6-diphenyl-1,3,5-hexatriene (Aldrich Chemical Co., New Jersey) in tetrahydrofuran was added while stirring to one of matched quartz cuvettes containing microsomes (1000 nmol lipid phosphorus/ml), 50 mM potassium phosphate (pH 7.50), 20% sucrose, in a final volume of 1.0 ml. The mixture was stirred gently for 30 min at room temperature to allow complete incorporation of the probe into membranes. For the reconstituted vesicles, 1000 nmol of lipid phosphorus was extracted as before and the organic layer was transferred to a test tube and dried with a stream of N_2 so that all solvent had completely evaporated. Reconstitution of vesicle populations was performed by adding 1.0 ml 50 mM potassium phosphate (pH 7.50), 20% sucrose, and sonicating in a Bransonic 12 ultrasonic cleaner for 3 min. The probe was then incorporated as for microsomes. (△) Liver microsomes from choline-deprived, inhibitor-injected mice. (▲) Reconstituted membranes from lipid extracts of liver microsomes from choline-deprived, inhibitor-injected mice. (□) Liver microsomes from choline-deprived, saline-injected controls. (■) Reconstituted membranes from lipid extracts of choline-deprived, saline-injected controls. Each symbol represents the average of two determinations. The experimental variation in anisotropy was less than 5% for all temperatures.

The evidence presented in this report is in agreement with the results of others [9,16–18] showing that phosphatidylcholine is synthesized by incorporation of preformed choline and methylation reactions in the liver. The flexibility of methylation of phospholipids in liver may provide a mechanism for control of membrane physical properties and maintenance of normal body choline requirements. This control could be dependent upon the amounts of substrate, phosphatidylethanolamine [16,19], in the membrane. The possibility that the PC/PE ratio can be reduced further than we have shown in the present communication is suggested by the fact that the level of *S*-adenosylhomocysteine is highest in mouse liver 30 min to 1 h post-injection and returns to near-normal levels at 16 h [11].

The procedures described in this communication provide a method for the alteration of membrane phospholipid composition and properties that avoids extraction and reconstitution techniques. The effects of lipid composition on enzyme activity observed in vivo may lead to new insights into the effects of phospholipid composition on membrane-associated enzymes that have been obscured by the manipulations involved in extraction and reconstitution of proteins. We are currently assessing the effects of changes in membrane phospholipid composition in vivo on liver microsomal enzymes.

This work was supported by a grant from the Muscular Dystrophy Association.

References

1 Sandermann, H., Jr. (1978) Biochim. Biophys. Acta 515, 209–237

2 Jain, M.K. and Wagner, R.C. (1980) in Introduction to Biological Membranes, pp. 157–166, John Wiley and Sons, New York
3 Rice, D.M., Meadows, M.D., Scheinman, A.O., Goni, F.M., Gomez-Fernandez, D.C., Moscarello, M.A., Chapman, D. and Oldfield, E. (1979) Biochemistry 18, 5893–5903
4 Dean, W.L. and Suarez, C. (1981) Biochemistry 20, 1743–1747
5 Abramson, M.B. (1969) in Surface Chemistry of Biological Systems (Blank, M., ed.), pp. 37–53, Plenum Press, New York
6 Hirata, F. and Axelrod, J. (1978) Nature 275, 219–220
7 Shinitzky, M. and Barenholz, Y. (1974) J. Biol. Chem. 249, 2652–2657
8 Kennedy, E.P. (1961) Fed. Proc. 20, 934–940
9 Bremer, J. and Greenberg, D.M. (1961) Biochim. Biophys. Acta 46, 205–216
10 Gibson, K.D., Wilson, J.D. and Udenfriend, S. (1961) J. Biol. Chem. 236, 673–679
11 Hoffman, J. (1980) Arch. Biochem. Biophys. 205, 132–135
12 Lombardini, J.B. and Talalay, P. (1973) Mol. Pharmacol. 9, 542–560
13 Meissner, G., Connor, G.E. and Fleischer, S. (1973) Biochim. Biophys. Acta 298, 246–269
14 Munro, A.J., Jackson, R.J. and Komer, A. (1964) Biochem. J. 92, 289–299
15 Chiang, P.K., Young, S.I. and Cantoni, G.L. (1980) Biochem. Biophys. Res. Commun. 94, 174–181
16 Glenn, J.L. and Austin, W. (1971) Biochim. Biophys. Acta 231, 153–160
17 Schneider, W.J. and Vance, D.E. (1978) Eur. J. Biochem. 85, 181–187
18 Bjørnstad, P. and Bremer, J. (1966) J. Lipid Res. 7, 38–45
19 Lyman, R.L., Hopkins, S.M., Sheehan, G. and Tinoco, J. (1969) Biochim. Biophys. Acta 176, 86–94
20 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509
21 Skipski, V.P., Peterson, R.F. and Barclay, M. (1964) Biochem. J. 90, 374–378
22 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
23 Chen, L.A., Dale, R.E., Roth, S. and Brand, L. (1977) J. Biol. Chem. 252, 2163–2169