

ADAPTIVE ALTERATION IN PHOSPHOLIPID COMPOSITION OF PLASMA MEMBRANES FROM A SOMATIC CELL MUTANT DEFECTIVE IN THE REGULATION OF CHOLESTEROL BIOSYNTHESIS

MICHAEL SINENSKY. From the Eleanor Roosevelt Institute and Florence R. Sabin Laboratories for Genetic and Developmental Medicine, Denver, Colorado 80262

ABSTRACT

A somatic cell mutant (CR1) of a Chinese hamster ovary cell (CHO-K1) which has previously been shown to be defective in the regulation of cholesterol biosynthesis accumulates more cholesterol than the parental cell line in plasma membranes. Although such an increase in membrane cholesterol should lead to an increase in the order parameter of these membranes, as measured with an electron spin resonance spin probe, the order parameters of mutant and wild-type plasma membranes are identical—apparently because of an adaptive alteration in membrane phospholipid composition. The phospholipid compositions of mutant and wild-type cell plasma membranes are compared and the mutant is shown to have a threefold higher level of oleic acid and a twofold lower level of phosphatidyl ethanolamine than the wild type. These results are consistent with model studies which show that these compositional changes lead to lower-order parameters for phospholipid dispersions.

Although the functional significance of the diversity of membrane lipid structures has generally remained elusive, it has been possible to explain some aspects of the variation of membrane lipid composition by the concept of homeoviscous adaptation (18). This concept argues that membrane lipid composition varies in response to conditions that perturb phospholipid acyl chain conformational mobility in such a fashion as to maintain this conformational mobility constant. It is best exemplified by the decrease in membrane phospholipid acyl chain desaturation with increasing ambient temperature of bacteria (17).

Recently we have described a mutant (CR1) of the Chinese hamster ovary cell (CHO-K1) which is defective in the regulation of cholesterol biosynthesis by medium sterol (19–21). This defect, in turn, leads to a defect in the ability of this mutant to regulate plasma membrane cholesterol levels (20). In this report, evidence will be presented that shows that although the CR1 mutant accumulates more plasma membrane cholesterol than the wild-type cell when these cells are grown with whole

fetal calf serum supplements (8%) to medium F12, there is no difference in the acyl chain ordering in the plasma membranes from mutant and wild-type cells. Some of the variations in lipid composition that might account for this apparent example of homeoviscous adaptation in a mammalian cell will also be presented.

MATERIALS AND METHODS

Materials

The cells used were the Chinese hamster ovary cell line, CHO-K1, originally isolated by Kao and Puck (9), and a mutant of this cell, CR1, defective in the regulation of cholesterol biosynthesis (19–21). Cells were grown on 100-mm plastic petri plates (Lux Scientific Corp., Newbury Park, Calif.) or in suspension in medium F12 purchased from K. C. Biologicals (Lenexa, Kans.), supplemented with 8% fetal calf serum purchased from K. C. Biologicals. 5-doxyloleic acid was obtained from Syva (Palo Alto, Calif.). Radioactive sodium acetate ($1\text{-}^{14}\text{C}$, 1 mCi/mmol) was purchased from New England Nuclear (Boston, Mass.).

Plasma Membrane Preparation

Plasma membranes were prepared by the method of Brunette and Till (3). When membranes were prepared from cells growing

on petri plates, the cells were treated with 10^{-3} M $ZnCl_2$ on the plate and harvested with a rubber policeman. Protein determinations on these preparations were by the method of Biuret (10).

Lipid Analysis

Lipids were extracted from plasma membrane preparations by the method of Bligh and Dyer (2). Cholesterol levels were determined by gas-liquid chromatography with an internal coprostanol standard on a 91-cm SP-2250 column (Supelco, Inc., Bellefonte, Pa.) at $280^\circ C$. Phospholipid phosphate was measured by the method of Bartlett (1). Neutral lipids were separated from phospholipids by elution from silicic acid columns with 20-column volumes of $CHCl_3$. Phospholipids were recovered from the column in 10-column volumes of methanol. Fatty acid analysis of total plasma membrane phospholipid was performed after transesterification to methyl esters in acidic methanol (5) by gas-liquid chromatography on 10% stabilized diethylene glycol succinate (Supelco, Inc.) with a Perkin-Elmer 3920 B gas chromatograph temperature programmed from 150° to $180^\circ C$. Phospholipids were fractionated into polar head group classes by thin-layer chromatography on silicic acid plates developed with $CHCl_3$:MeOH:acetic acid: H_2O , 25:14:4:2 and visualized by radio-scanning on a Packard radio-TLC scanner (Packard Instrument Co., Inc., Downers Grove, Ill.).

Order Parameter Determinations

Order parameters were determined from electron spin resonance spectra of the probe molecule 5-doxylosearic acid in lipid dispersions. These were prepared by evaporating the solvent from a solution of lipid and 5-doxylosearic acid in $CHCl_3$ in the bottom of a small test tube, adding 50 μl of 0.01 M, pH 7.4 phosphate buffer, and sonicating with a Branson sonifier (power setting = 7; Branson Sonic Power Co., Danbury, Conn.) for six 10-s periods. Samples contained 1 μmol of phospholipid and 0.01 mol of 5-doxylosearic acid. Order parameters were calculated from the equation:

$$S = \frac{T_{11} - T_{11} \left(\frac{a}{a'} \right)}{T_{22} - T_{22} \left(\frac{a}{a'} \right)}$$

as described by Hubbell and McConnell (7). Measurements were taken at $37^\circ C$ on a Varian E104A spectrometer. The splittings were determined by eye.

RESULTS

The cholesterol content of CHO-K1 and CR1 plasma membranes is shown in Table I. A 50% increase in cholesterol to phospholipid ratio CR1 is structurally significant, producing a 10% change in acyl chain order parameter in model studies of cholesterol-phospholipid multilayers probed with 5-doxylosearic acid (16). However, order parameter determinations with 5-doxylosearic acid of CHO-K1 and CR1 plasma membranes (Table II) reveal that these membranes have identical order parameters. This result immediately suggests the existence of some other difference between CR1 and CHO-K1 plasma membrane compositions. When the order parameters of phospholipid dis-

TABLE I
Cholesterol and Phospholipid Content of CHO-K1 and CR1 Plasma Membranes

| | Cell type | |
|---|-----------------|-----------------|
| | CHO-K1 | CR1 |
| μmol Phospholipid/mg protein | 0.40 ± 0.01 | 0.40 ± 0.01 |
| μmol Cholesterol/mg protein | 0.18 ± 0.01 | 0.27 ± 0.02 |
| μmol Cholesterol/ μmol phospholipid | 0.45 ± 0.03 | 0.68 ± 0.04 |

Plasma membranes were prepared from 3 liters of suspension culture harvested at a titer of 3×10^5 cells/ml. Results are expressed as mean \pm SEM.

TABLE II
Order Parameters of CHO-K1 and CR1 Plasma Membrane Lipid Extracts

| Sample | Order parameter | |
|---|-------------------|-------------------|
| | CHO-K1 | CR1 |
| Plasma membranes | 0.625 ± 0.011 | 0.622 ± 0.010 |
| Plasma membrane phospholipids | 0.607 ± 0.010 | 0.533 ± 0.08 |
| Plasma membrane phospholipids + cholesterol | 0.652 ± 0.012 | 0.645 ± 0.012 |

In the samples to which cholesterol was added, 0.45 μmol of cholesterol was added to 1 μmol of CHO-K1 plasma membrane phospholipid and 0.68 μmol of cholesterol was added to 1 μmol of CR1 plasma membrane phospholipid. Results are presented as the average of three measurements \pm SD.

persions prepared from CR1 and CHO-K1 plasma membrane extracts are determined (Table II), the CR1 phospholipids are seen to be far less ordered than those from CHO-K1, indicating that an alteration in phospholipid composition has compensated for the change in cholesterol level. This notion is supported by the observation (Table II) that the addition of cholesterol to these phospholipid dispersions at levels that reconstitute the original membrane cholesterol to phospholipid mole ratio yields dispersions which have identical order parameters.

Reasoning by analogy with the adaptation of phospholipid composition to temperature in bacteria and on the basis of model physical studies, one could expect that an increase in unsaturated fatty acid content of the phospholipids is possibly responsible for these observations. When the fatty

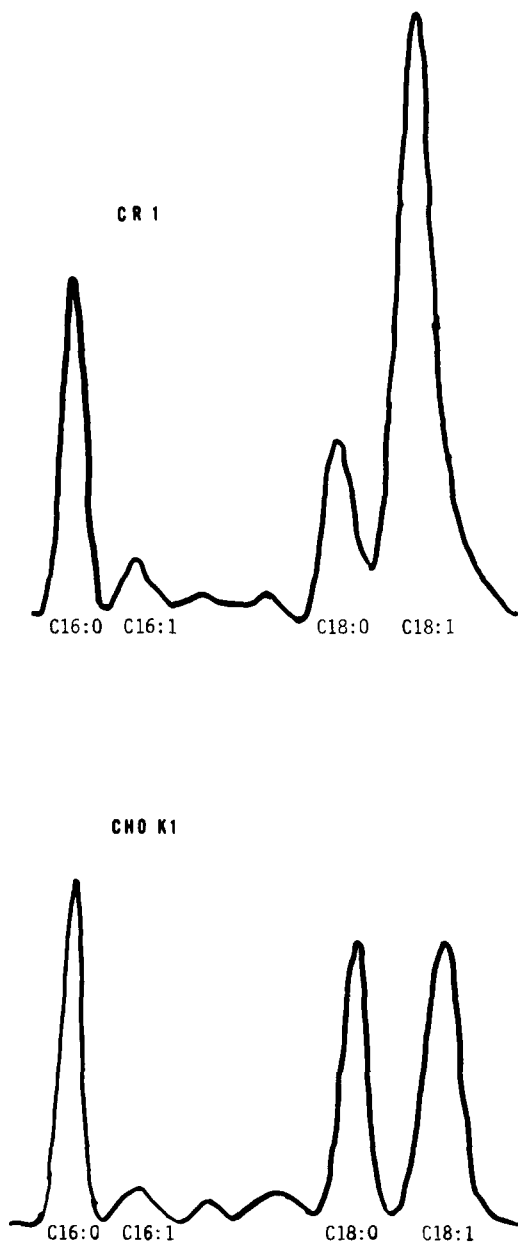


FIGURE 1 Gas-liquid chromatographic separation of the major fatty acids of the phospholipids of plasma membranes of CHO-K1 and CR1.

acid compositions of CR1 and CHO-K1 plasma membranes are compared, the results shown in Fig. 1 are obtained, showing a threefold increase in the ratio of oleic acid to stearic acid in the mutant cells. It is interesting to note that the acyl chain composition of CHO-K1 plasma membrane phospholipid shown in Fig. 1 differs considerably

from that reported for whole cell phospholipid (14).

The phospholipids were also fractionated by thin-layer chromatography after acetate labeling

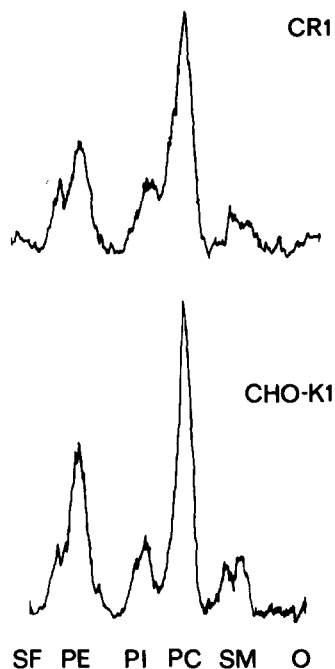


FIGURE 2 Incorporation of ^{14}C -labeled acetate into plasma membrane phospholipids of CHO-K1 and CR1. O; origin, SM; sphingomyelin, PC; phosphatidylcholine, PI; phosphatidyl inositol, PE; phosphatidylethanolamine, SF; solvent front. Phospholipids were separated by thin-layer chromatography and visualized by radio-scanner.

TABLE III
Relative Incorporation of Labeled Acetate into Plasma Membrane Phospholipids of CHO-K1 and CR1

| Phospholipid | % of total | |
|--------------------------|----------------|----------------|
| | CHO-K1 | CR1 |
| Phosphatidylethanolamine | 13.3 \pm 2.8 | 6.8 \pm 0.2 |
| Phosphatidylcholine | 69.0 \pm 3.0 | 74.6 \pm 3.5 |
| Phosphatidylinositol | 4.0 \pm 0.1 | 4.0 \pm 0.1 |
| Sphingomyelin | 13.8 \pm 0.2 | 14.2 \pm 2.8 |

Cells were grown from an initial inoculum of 10^5 to a final titer of $\sim 10^6$ cells on 100-mm petri plates in medium F12 supplemented with 8% fetal calf serum and $10 \mu\text{Ci}$ of sodium acetate. Additional cells were added as carrier, and plasma membranes were prepared and fractionated as described in Methods. The results are presented \pm the standard deviation of the mean for three independent determinations.

to examine whether alterations in biosynthesis of phospholipid polar head group classes also occur in response to altered membrane cholesterol levels. The results of a typical experiment (Fig. 2) suggest that the ratio of phosphatidylcholine to phosphatidylethanolamine is increased in the CR mutant. This experiment was repeated several times, and the various phospholipid bands were scraped and counted to quantitate this effect (Table III).

DISCUSSION

It is generally accepted that cholesterol increases the order parameter of phospholipid bilayers in the liquid crystalline state and decreases the order parameter of phospholipid bilayers in the gel state (8). As can be seen from Table II, the effect of cholesterol on the cell plasma membrane phospholipids of the Chinese hamster ovary cell is to increase their order parameter at physiological temperature (37°C). Thus, adaptive alterations in phospholipid composition in response to an increased cholesterol to phospholipid ratio would be expected to be those that produced a decrease in phospholipid acyl chain order parameter. That decreases in order parameter of phospholipid acyl chains can be produced by increasing phospholipid unsaturation is well documented (6). Other model studies have suggested that phosphatidylethanolamine can decrease the order parameter of phosphatidylcholine vesicles (11) consistent with the compositional studies in this report.

The compositional changes that were observed in Chinese hamster ovary cells are also consistent with those observed in other biological systems. In mycoplasma, an increase in the cholesterol phospholipid ratio results in a decrease in the ratio of saturated to unsaturated fatty acids (14). In the protozoan, *Tetrahymena*, conditions which result in more fluid phospholipids do so by causing an increase in unsaturation and in the phosphatidylcholine to phosphatidylethanolamine ratio (12, 13)—changes which are comparable to those that we have observed. These compositional changes are apparently not, however, consistent with those reported by Freter et al. for L-cell cholesterol auxotrophs depleted of cholesterol by starvation (4). Perhaps this difference in observations may be ascribed to the difference in our studying an in-

crease rather than a decrease in cholesterol levels compared to the normal.

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