CHANGES IN ACYL GROUP COMPOSITION OF PHOSPHOLIPIDS FROM CHICKEN EMBRYONIC FIBROBLASTS AFTER TRANSFORMATION BY ROUS SARCOMA VIRUS

Tom M. Yau* and Michael J. Weber†

*Department of Neurochemistry, Cleveland Psychiatric Institute, Cleveland, Ohio 44109 and

†Department of Microbiology, University of Illinois, Urbana, Illinois 61801

Received August 23,1972

SUMMARY

The acyl group composition of individual phospholipids isolated from both normal and Rous sarcoma virus (RSV) transformed chicken embryo fibroblasts (CEF) was analyzed by Gas-Liquid Chromatography (GLC). Each of the phospholipids analyzed displayed a characteristic pattern of acyl groups. Significant differences between normal and transformed cells in the acyl group composition of the phospholipids was detected. In the transformed cells, the content of arachidonate decreased while oleate increased. These results indicate that a reduced content of certain polyunsaturated fatty acids accompanies malignant transformation by Rous sarcoma virus.

INTRODUCTION

It is widely believed that biochemical changes in the surface of malignant cells are associated with the expression of malignant behavior. Recent work has demonstrated that the synthesis of the carbohydrate chains of glycolipids is often incomplete in transformed cells (1, 2, 3). Reduction in the content of the glycosyl extension enzymes in transformed cells was found to parallel the decrease in higher ganglioside homologs (4, 5). Similar alterations in glycolipid content were also detected in RSV-transformed CEF (6).

On the other hand, the composition and metabolism of other lipids in CEF did not change in any major way after transformation by RSV (7,8). However, since profound alterations in membrane activity accompany transformation (9, 10, 11), we decided to analyze the non-polar acyl

groups of the phospholipids, since these may be important in the control of membrane activity (12, 13).

MATERIALS AND METHODS

Virus and Cell Culture. Body walls from 10-day old chick embryos were dissociated with 0.25% Difco trypsin and plated in 100 mm plastic tissue culture dishes in Dulbecco's modified Eagle Medium containing 2% Tryptose Phosphate Broth, 1% calf and 1% heat-inactivated chicken serum. Virus (Schmidt-Ruppin strain of Rous sarcoma virus, sub-group A) was added at 6 hours, m.o.i. = 1. After 5 days as primary cultures, the cells were removed from the dishes with 0.05% trypsin and replated in 100 mm dishes at 2 x 10^6 normal cells/dish, and 3 x 10^6 transformed cells/dish in Dulbecco's medium containing 10% Tryptose Phosphate Broth, 4% calf serum and 1% chicken serum. Cells were changed to fresh medium daily and harvested for lipid analysis on day 3. Under these conditions the normal and the transformed cells multiply at the same rate (14), and so alterations in lipids associated with contact-inhibition should not occur.

Lipid Extraction and Analysis. Medium from cells was aspirated and the cells were carefully rinsed 1 or 2 times with ice-cold phosphate buffered saline. 2.5 ml of cold methanol was added to each plate, and the cells were scraped off with a rubber policeman and transferred to a glass vial. Two volumes of chloroform were then added. The mixture was homogenized with a Teflon pestle, and further lipid extraction was performed according to Sun and Horrocks (15). Major phospholipid fractions were separated by 0.01 M-$NaCO_3$ impregnated Silica Gel G TLC plates with a solvent system containing chloroform-Methanol-NH_4OH (65:25:4 v/v). Total ethanolamine phosphorylglyceride was eluted from the plate and applied to another TLC plate. Prior to chromatography with the same solvent, the plate was exposed to concentrated HCl fumes for 5 minutes to hydrolyze the alkenyl groups (16). Acyl groups were converted to methyl esters by

base-methanolysis (0.5N NaOH-MeOH) together with an internal standard for quantitative determination (17). Acyl group compositions were determined with a Hewlett Packard (5750) Gas-Liquid Chromatograph (18).

Materials. C/O, Cofal-negative embryonated eggs were from Spafas, Roanoke, Illinois. Medium and calf serum were from GIBCO, Grand Island, New York, and chicken serum was from BBL, Cockeysville, Maryland.

RESULTS

The total fatty acid composition of chicken embryonic fibroblasts (CEF) has been reported earlier (19, 20); however, no analysis was made on the individual phospholipid fractions. The acyl group composition of choline phosphorylglyceride (CPG), alkenyl acyl ethanolamine phosphorylglyceride (aGPE), diacyl ethanolamine phosphorylglyceride (dGPE), and inositol phosphorylglyceride plus serine phosphorylglyceride (IPG + SPG) from normal and RSV-transformed CEF were analyzed by GLC and expressed as percent distribution in Table I. Each of the phospholipids analyzed showed acyl group specificity. The fatty acids found in highest concentration in CPG, aGPE, dGPE and IPG + SPG were 16:0+18:1, 20:4, 18:0 and 18:0, respectively. With the exception of aGPE, which possessed a high content of polyenoic acid 20:4 and relatively high content of 22:4, 22:5 (ω3) and 22:6, the ratio of the percent of unsaturated acids to saturated acids was near unity (e.g., the low content of 20:4 in CPG was compensated by the high content of 18:1 and, to a lesser extent, 18:2).

Differences in acyl group composition were also detected among individual phospholipids isolated from control and transformed cells. There was a definite decrease in the level of 20:4 and increase in 18:1 content accompanying cellular transformation. A slight decrease in 18:0 and increase in 18:2 was also observed.

DISCUSSION

Lipid content and metabolism in chorioallantoic membranes of chicken

ACYL GROUP COMPOSITION* OF PHOSPHOGLYCERIDES FROM NORMAL AND ROUS SARCOMA VIRUS-TRANSFORMED CHICKEN EMBRYO FIBROBLASTS

Acyl Groups	CPG		Acyl GPE		Diacyl GPE		IPG + SPG	
	Normal	Transformed	Normal	Transformed	Normal	Transformed	Normal	Transformed
16:0	28.4 ± 1.1	31.0 ± 1.0	7.2 ± 0.5	7.7 ± 0.2	6.8 ± 0.7	7.5 ± 0.3	5.2 ± 0.7	5.8 ± 0.9
18:0	17.2 ± 0.6	16.0 ± 0.6	6.9 ± 1.5	5.1 ± 0.8	41.5 ± 1.6	36.9 ± 0.5	46.0 ± 0.2	44.2 ± 0.4
18:1	28.6 ± 1.2	35.3 ± 0.9	7.6 ± 0.4	13.3 ± 1.8	14.1 ± 1.1	23.1 ± 1.7	14.6 ± 2.1	17.0 ± 1.9
18:2	10.3 ± 0.4	8.0 ± 0.3	4.9 ± 0.4	8.4 ± 0.6	8.8 ± 0.5	12.0 ± 0.4	5.8 ± 0.3	8.0 ± 0.4
20:3	2.7 ± 0.1	1.9 ± 0.2	1.8 ± 0.1	5.8 ± 0.1	2.6 ± 0.3	2.7 ± 0.4	6.1 ± 0.6	6.1 ± 1.0
20:4	6.2 ± 0.7	2.6 ± 0.3	38.4 ± 0.6	32.5 ± 0.6	18.7 ± 1.0	11.7 ± 0.6	14.7 ± 1.1	10.9 ± 1.2
22:4	1.9 ± 0.3	0.9 ± 0.2	13.2 ± 2.0	11.3 ± 0.4	2.6 ± 0.5	3.4 ± 0.8	2.7 ± 0.9	3.0 ± 0.3
22:5 (ω3)	0.9 ± 0.1	0.5 ± 0.2	10.8 ± 0.6	8.3 ± 1.5	2.4 ± 0.4	1.5 ± 0.2	2.2 ± 0.5	2.2 ± 0.6
22:6	1.1 ± 0.2	0.5 ± 0.1	9.8 ± 0.6	7.6 ± 0.3	3.0 ± 0.4	1.9 ± 0.2	1.9 ± 0.2	2.7 ± 0.1

*Each value is a mean of six determinations from two separate experiments.

eggs and in tumours induced in the chorioallantoic membrane by RSV have been analyzed previously (7). Only minor differences were revealed in the lipid composition. By using glucose and acetate as lipid precursors, enhanced lipid metabolism was found in tumours; however, the difference in incorporation between the two tissues appeared to be largely a matter of amount rather than distribution. Phospholipid compositions of control and RSV-transformed CEF whole cells and their respective plasma membranes were also reported (8). Based on data from both the flow of ^{32}P into the different phospholipid species, and endogenous phospholipid determination of control and transformed cells, Quigley et al concluded that there was no evidence to implicate changes in phospholipid composition as a possible structural factor for modifying membrane function in virally transformed cells. However, the possibility that the plasma membrane of transformed cells may contain altered microregions, whose phospholipid differences would evade detection, was not excluded.

Results from the present study indicate that although there is no apparent difference in the phospholipid distribution in normal and transformed CEF, there is a definite change in acyl group composition accompanying cellular transformation. Unpublished results (Yau and Weber) using CEF infected with RSV-SR T-5 (a temperature sensitive mutant of Rous sarcoma virus which produces virus, but does not transform cells at the non-permissive temperature [21]), indicate that the changes reported here are associated with malignant transformation, and are not simply consequences of viral infection. Furthermore, no corresponding change in acyl group composition was observed in Adenovirus Type 12 infected human fetal kidney cells (Yau and Ledinko, unpublished data), or Herpesvirus Type I and II infected human fetal lung cells (Yau and Rosenthal, unpublished data).

Fatty acid composition of tissue culture cells was thought to be regulated in a passive manner by uptake of available fatty acids from

the serum (22). However, this could not account for the changes seen in our experiment since medium of the same composition was used for growth of both normal and transformed cultures, and cultures were changed to fresh medium daily during the course of the experiment.

Of the changes in fatty acid composition reported here, the drop in the content of 20:4 in transformed cells is especially noteworthy. Arachidonate is the immediate precursor to the prostaglandins PGE_2 and PGF_2a and in turn is formed from dihomo-γ-linolenic acid, which is the precursor to PGE_1 and PGF_1a (23). Thus, a decrease in the content of 20:4 may be symptomatic of major alterations in cellular control mechanisms. Moreover, because of its chain length and number of double bonds, 20:4 might be expected to help maintain the "fluidity" of membranes. Maintenance of the proper "fluidity" may be important in determining the permeability, enzymatic activity and transport characteristics of the membrane (12, 13, 24, 25).

Since 18:2 is a precursor for the synthesis of 20:4 (26, 27), it is tempting to postulate that the elaboration of chain elongation and desaturation enzymes is impaired in RSV-transformed CEF. Alternatively, an altered permeability of transformed cells to either 18:2 or 20:4 in the serum could also explain the results observed. Whether these changes in acyl group composition, along with alterations in glycolipid (6) and hexose transport (9), are the result of transformation, or whether these changes cause transformation, cannot be answered at present. Experiments are now in progress to elucidate the mechanism of changes in acyl group composition.

ACKNOWLEDGMENTS

This work was supported by USPHS grants CA 12467. Miss Shirley Yau provided excellent technical assistance. We thank Dr. G. Y. Sun for encouragement and interest in this project.

REFERENCES

1. Hakomori, S., Murakami, W. T., Proc. Nat. Acad. Sci., U.S.A., 59, 254 (1968).

2. Hakomori, S., Teather, C. and Andrews, H. D., Biochem. Biophys. Res. Commun., 33, 563 (1968).
3. Mora, P. T., Brady, R. O., Bradley, R. M. and McFarland, V. W., Proc. Nat. Acad. Sci., U.S.A., 63, 1290 (1969).
4. Mora, P. T., Cumar, F. A. and Brady, R. O., Virol., 46, 60 (1971).
5. Kijimoto, S. and Hakomori, S., Biochem. Biophys. Res. Commun., 44, 557 (1971).
6. Hakomori, S., Saito, T. and Vogt, P. K., Virol., 44, 609 (1971).
7. Figard, P. H. and Levine, A. S. Biochim. Biophys. Acta, 125, 428 (1966).
8. Quigley, J. P., Rifkin, D. B. and Reich, E., Virol., 46, 106 (1971).
9. Hatanaka, M. and Hanafusa, H., Virol., 41, 647 (1970).
10. Foster, D. O. and Pardee, A. B., J. Biol. Chem., 244, 2675 (1969).
11. Isselbacher, K. J., Proc. Nat. Acad. Sci., U.S.A., 69, 585 (1972).
12. McConnell, H. M., In, The Neurosciences Second Study Program, Schmitt, F. O., ed. Rockefeller University Press, New York, (1970)pp. 697-706.
13. Singer, S. J. and Nicholson, G. L., Science, 175, 720 (1972).
14. Martin, G. S., Venuta, S., Weber, M. J. and Rubin, H., Proc. Nat. Acad. Sci., U.S.A., 68, 2739 (1971).
15. Sun, G. Y. and Horrocks, L. A., Lipids, 5, 1006 (1970).
16. Horrocks, L. A., J. Lipid Res., 9, 469 (1968).
17. Sun, G. Y. and Horrocks, L. A., Lipids, 3, 79 (1968).
18. Sun, G. Y. and Horrocks, L. A., J. Lipid Res., 10, 153 (1969).
19. McSharry, J. J. and Wagner, R. R., J. Virol., 7, 59 (1971).
20. David, A. E., Virol., 46, 711 (1971).
21. Martin, G. S., Nature, 227, 1021 (1970).
22. Bailey, J. M., Howard, B. V., Bunbar, L. M. and Tillman, S. F., Lipids, 7, 125 (1972).
23. Horton, E. W., Prostaglandins, Springer-Verlag, New York-Heidelberg-Berlin, 1971, pp. 53-66.
24. Chapman, D., Owen, N. F. and Walker, D. A., Biochim. Biophys. Acta, 120, 148 (1966).
25. Esfahani, M., Limbrick, A. R., Knutton, S., Oka, T. and Wakil, S. J., Proc. Nat. Acad. Sci., U.S.A., 68, 3180 (1971).
26. Mead, J. F., In, Drugs Affecting Lipid Metabolism, Garatti, S. and Paoletti, R., Eds., Elsevier, Amsterdam (1961) p. 16.
27. Klenk, E., In, Drugs Affecting Lipid Metabolism, Garatti, S. and Paoletti, R., Eds., Elsevier, Amsterdam (1961) p. 21.