ACYLTRANSFERASES AND THE BIOSYNTHESIS OF PULMONARY SURFACTANT LIPID IN ADENOMA ALVEOLAR TYPE II CELLS

Fred Snyder and Boyd Malone

From the Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee 37830

Received August 4,1975

SUMMARY

Acyltransferases are present in microsomes from alveolar type II cell adenomas (produced by urethan injections) that transfer palmitic acid in the presence of CoA, ATP, and Mg^{++} to sn-glycerol-3-P to form phosphatidic acid, to dihydroxyacetone-P to form acyldihydroxyacetone-P, and to 1-acyl-sn-glycero-3-phosphocholine to form 3-sn-phosphatidylcholine. The data clearly demonstrate that the microsomal preparations can catalyze significant incorporation of palmitic acid into the 2-position of the disaturated species of 3-sn-phosphatidylcholine independently of phosphatidic acid formation as evidenced by the fact that sn-glycerol-3-P and calcium ions (which inhibit choline phosphotransferase) did not influence the incorporation of palmitic acid into the main surfactant lipid. Thus, a deacylation-acylation reaction involving 2-lysophosphatidylcholine appears to be an important pathway for the synthesis of surfactant lipid in alveolar type II cells; the control of acyl specificity at the 2-position is determined by the relative concentrations of the copartici-pating substrates, 1-palmitoyl-sn-glycero-3-phosphocholine and palmitoyl-CoA.

INTRODUCTION

A great difficulty in forming definitive conclusions from studies done on the biosynthesis of pulmonary surfactant(s) is that they have been carried out with whole lung tissue, which contains over forty different types of cells (1). Therefore, even though histological studies have indicated that the source of pulmonary surfactant is alveolar type II cells (2), it has been impossible for biochemists working with whole lung preparations to restrict interpretation of their results to those enzymes that only occur in type II alveolar cells. These difficulties were resolved recently when our laboratory provided direct evidence that homogenates derived from a homogeneous population of alveolar type II cells (induced as lung adenomas by urethan) were capable of synthesizing the main surfactant lipid, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (3). The adenoma is an ideal model for the investigation of pulmonary surfactant metabolism since it is comprised exclusively of alveolar type II epithelial cells that contain many lamellar bodies and are morphologically similar in all other respects to type II cells found in normal lungs (3-5).

In our current work we have used the type II cells to investigate the nature of the acyltransferases associated with the endoplasmic reticulum. Our results

show that the microsomal enzymes in alveolar type II cells can synthesize "disaturated" 3-sn-phosphatidylcholine independently of de novo synthesis from sn-glycerol-3-P or dihydroxyacetone-P. Moreover, our data demonstrate that the redistribution of acyl moieties in 3-sn-phosphatidylcholine species via an acylation-deacylation reaction can be a major route of surfactant biosynthesis in type II cells.

METHODS

Alveolar type II cell adenomas were harvested from the lungs of female BALB/c mice that had been injected intraperitoneally with urethan (ethyl carbamate, 1 mg/g body weight on 3 consecutive days) approximately 6 months earlier. The morphological ultrastructure of these adenomas, and the phospholipase A_2 and chromatographic procedures used to analyze the various molecular species of lipids have been described elsewhere (3). Microsomes were pelleted (6) from adenoma homogenates in 0.25 M sucrose by centrifugation at 100,000 g for 1 hr using an SW-40 rotor in a Beckman ultracentrifuge, Model L2-65B; the pellets were washed once.

Acyltransferase activities were determined in a system that consisted of the following components unless otherwise noted: an acyl acceptor (see table or figure), adenoma microsomes (0.8 mg protein), CoA (100 μM), ATP (10 mM), Mg^{++} (4 mM), and [1-^{14}C]palmitic acid (33.3 μM) in 3 ml Tris buffer (100 mM) at pH 7.1; in some instances $CaCl_2$ (20 mM) was included. All samples were incubated for 20 min at 37° in a Dubnoff metabolic shaker set at 150 oscillations per min. rac-Glycerol-3-P or dihydroxyacetone-P were purchased from Sigma Chemical Co., whereas 1-palmitoyl-sn-glycero-3-phosphocholine was purchased from Applied Science Laboratories, Inc. We obtained the [1-^{14}C]palmitic acid, [9,10-^{3}H]palmitic acid, and [5,6,8,9,11,12,14,15-^{3}H]-arachidonic acid from New England Nuclear.

RESULTS AND DISCUSSION

Table I shows that microsomal preparations from alveolar type II cells contain acyltransferases that transfer palmitate to rac-glycerol-3-P to form phosphatidic acid. The ratio of [1-^{14}C]palmitate at the 1- and 2-positions was 1:1 as determined by the analysis of the products formed after phospholipase A_2 treatment of the ^{14}C-labeled phosphatidic acid. The disaturated phosphatidic acid formed by the palmitoyl-CoA:glycerol-3-P palmitoyltransferase in lung is also the product formed by liver microsomes (7) when palmitic acid and glycerol-3-P are the substrates.

Palmitoyl-CoA:dihydroxyacetone-P palmitoyltransferase is also present in the microsomes of the type II cells as indicated by the build-up of acyldihydroxyacetone-P (Table I) and its dephosphorylated product (acyldihydroxyacetone) when dihydroxyacetone-P is substituted for rac-glycerol-3-P in the incubation system. Phosphatidic acid was not formed under these conditions because we did not include NADPH, which is required for the oxidoreductase that reduces the ketone group of acyldihydroxyacetone-P.

Data in Table I reveal that the labeling of 3-sn-phosphatidylcholine from

TABLE I. Biosynthesis of Acyldihydroxyacetone-P, Phosphatidic Acid, and Phosphatidylcholine by Microsomes of Alveolar Type II Cells

Addition*	Palmitic Acid Incorporated into Product (nanomoles)		
	Acyldihydroxyacetone-P	Phosphatidic Acid	Phosphatidylcholine
None	tr	tr	8.9; 8.9
None (boiled)	tr	tr	tr
None (CoA, ATP, and Mg^{++} deleted)	tr	tr	tr
sn-Glycerol-3-P (1.67 mM)	1.5; 1.5	24; 24	8.9; 8.7
Dihydroxyacetone-P (1.67 mM)	7.0; 6.1	tr	8.0; 8.3
Lyso-phosphatidylcholine (33.3 μM)	tr	tr	44; 45
$CaCl_2$ (20 mM)	1.5; 1.5	2.5; 1.9	6.9; 7.9

* Complete system described under "Methods;" tr = trace (< 1 nanomole)

[1-^{14}C]palmitic acid, in the presence of ATP, CoA, and Mg^{++}, procedes independently of added rac-glycerol-3-P, DHAP, or calcium ions in the system. Under any of these conditions, approximately 90% of the [1-^{14}C]palmitate that is incorporated into 3-sn-phosphatidylcholine is located at the 2-position. These results indicate that the "surfactant" type of phosphatidylcholine can be readily formed by insertion of palmitate at the 2-position of other phosphatidylcholine molecules, presumably by a deacylation-acylation enzymatic sequence.

In view of the foregoing results, we determined the acyltransferase activity in the microsomes with 1-palmitoyl-sn-glycero-3-phosphocholine as a substrate. Acylation was linear up through substrate concentrations of 33.3 μM. However, in the absence of added substrate the microsomes still incorporated 11 nanomoles of palmitic acid into phosphatidylcholine (per mg microsomal protein per 20 min). Moreover, the data in Fig. 1 demonstrate that the incorporation of fatty acid species into phosphatidylcholine formed is dependent on the concentrations of the substrates present. At a constant ratio of fatty acid to lyso-phosphatidylcholine (ratio = 4), increased concentrations of both substrates caused a preferential utilization of palmitic acid instead of arachidonic acid by the acyltransferase. Whereas,

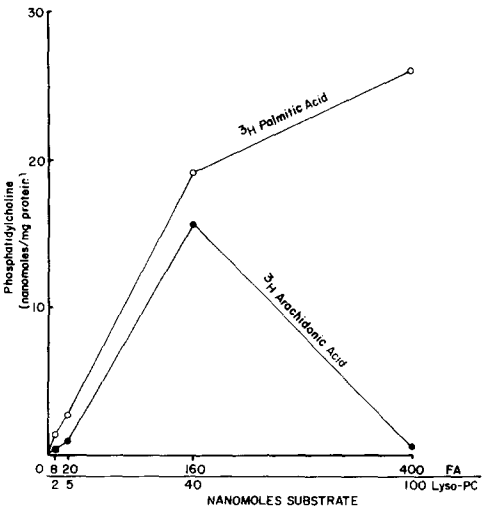

Fig. 1. Acylation of 1-palmitoyl-sn-glycero-3-phosphocholine by microsomes of alveolar type II cells (FA = fatty acid; Lyso-PC = 1-acyl-sn-glycero-3-phosphocholine). The complete incubation mixture is described under "Methods," except in this instance it contained 1 mg microsomal protein.

at lower substrate concentrations the relative incorporation rates at the 2-position were more nearly the same for both fatty acids. These data indicate that one must be extremely cautious in interpreting results obtained on the incorporation of specific fatty acids into surfactant phosphatidylcholine, since the specificity varies with the substrate concentrations used. The influence of 1-acyl-sn-glycero-3-phosphocholine concentrations on the specificity of liver acyltransferases has also been noted (8).

Hasegawa-Sasaki and Ohno (9) have suggested that there are two separate acyl-CoA:1-acyl-sn-glycero-3-phosphocholine acyltransferases in microsomes from rat lungs that utilize palmitoyl-CoA (low K_m) and arachidonyl-CoA (high K_m) as substrates; the acyltransferase that used palmitoyl-CoA as a substrate is markedly inhibited by other acyl-CoAs (including palmitoyl-CoA), whereas other acyl-CoAs have little effect on the acyltransferase that uses arachidonyl-CoA. Although the authors (9) conclude that the transfer of palmitoyl-CoA to 1-acyl-sn-glycero-3-phosphocholine is not the main pathway for the biosynthesis 1,2-dipalmitoyl-sn-glycero-3-phosphocholine in lung, interpretation of their results is complicated by the fact that their data was obtained with microsomal preparations from the many diverse cell types found in whole rat lung. Earlier studies (10-12) with microsomal enzymes, also from mixed populations of cells from whole lungs, have indicated that palmitoyl-CoA:1-acyl-sn-glycero-3-phosphocholine palmitoyltransferase is more active in lung than in liver.

Using preparations containing 95% type II epithelial cells isolated from rabbit lungs, Kikkawa et al. (13) reported that disaturated phosphatidylcholine was synthesized by the cytidine diphosphocholine pathway. However, their interpretation of the data (based solely on the incorporation of precursors at the sn-3-position of phosphatidylcholine) did not take into account the possibility of base exchange or deacylation-reacylation reactions (see ref. 7 for review). In contrast, our enzymatic results show that the biosynthesis of the major surfactant lipid, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, in type II cells can be formed from pre-existing phosphatidylcholine molecules by an acyl replacement. The difficult assessment of the quantitative significance of this pathway for the production of surfactant lipid remains to be explored in vivo.

ACKNOWLEDGEMENT

This work was supported in part by the Energy Research and Development Administration, the National Heart and Lung Institute (HL-18181-01), and the American Cancer Society (BC-70F).

REFERENCES

1. Sorokin, S. P. (1970) Morphology of Experimental Respiratory Carcinogenesis, (P. Nettesheim, M. G. Hanna, Jr., and S. W. Deatherage, Jr., Eds.), USAEC, Symposium Series 21, Conf. 70051, pp. 3-43.
2. Tierney, D. F. (1974) Adv. Intern. Med. 14, 209-231.
3. Snyder, C., Malone, B., Nettesheim, P., and Snyder, F. (1973) Cancer Res. 33, 2437-2443.
4. Svoboda, D. J. (1962) Cancer Res. 22, 1197-1201.
5. Brooks, R. E. (1968) J. Natl. Cancer Inst. 41, 719-742.
6. Soodsma, J. F., Piantadosi, C. and Snyder, F. (1972) J. Biol. Chem. 247, 3923-3929.
7. van den Bosch, H. (1974) Annu. Rev. Biochem. 43, 243-277.
8. Okuyama, H., Yamada, K., and Ikezawa, H. (1975) J. Biol. Chem. 250, 1710-1713.
9. Hasegawa-Sasaki, H. and Ohno, K. (1975) Biochem. Biophys. Acta 380, 486-495.
10. Frosolono, M. F., Slivka, S. and Charms, B. L. (1971) J. Lipid Res. 12, 96-103.
11. Vereyken, J. M., Montfoort, A., and van Golde, L. M. G. (1972) Biochim. Biophys. Acta 260, 70-81.
12. Hasegawa, H. (1973) Sapporo Med. J. 42, 344-349.
13. Kikkawa, Y., Yoneda, K., Smith, F., Packard, B., and Suzuki, K. (1975) Lab. Invest. 32, 295-302.