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LYSOLECITHIN ACYLTRANSFERASE AND LYSOLECITHIN: LYSOLECITHIN ACYLTRANSFERASE IN ADULT RAT LUNG ALVEOLAR TYPE II EPITHELIAL CELLS

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Summary

1. The specific activity of lysolecithin acyltransferase (EC 2.3.1.23) in sonicated adult rat lung alveolar type II epithelial cells, measured either alone or in combination with acyl-CoA synthetase (EC 6.2.1.3), was found to be an order of magnitude greater than that of lysolecithin:lysolecithin acyltransferase.

2. Lysolecithin acyltransferase in type II cells was found to prefer palmitoyl-CoA over oleoyl-CoA as substrate. The combination of lysolecithin acyltransferase and acyl-CoA synthetase was found to prefer palmitate over oleate for incorporation into phosphatidylcholine.

3. Compared to whole lung homogenate, sonicated adult rat type II cells are highly enriched in lysolecithin acyltransferase but not in lysolecithin:lysolecithin acyltransferase.

4. These observations indicate that in normal adult rat type II cells the deacylation-reacylation cycle is more important for the formation of dipalmitoyl phosphatidylcholine than the deacylation-transacylation process.

Introduction

The major active component of pulmonary surfactant, which is present on the alveolar surface and prevents alveolar collapse and transudation, is dipalmitoyl phosphatidylcholine [1]. Experiments with rat-lung slices [2] and studies

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in vivo [3,4] have indicated that the de novo synthesis of phosphatidylcholine in the lung yields primarily molecules with an unsaturated fatty acid at the 2-position. Remodelling mechanisms, such as deacylation-reacylation [3,5] and/or deacylation-transacylation [2,6—8] are required to convert the de novo synthesized phosphatidylcholines into dipalmitoyl phosphatidylcholine.

Because the lung is a heterogeneous organ, containing approx. 40 different cell types [9], experiments with whole lung or subcellular fractions of total lung (with the exception of lamellar bodies, which occur only in type II cells) do not yield data pertinent to any specific cell type. Studies with isolated type II alveolar epithelial cells, the most important, if not exclusive source of surfactant (for discussion see refs. 10 and 11), may yield a better understanding of the formation of surfactant. Evidence that remodelling of primarily synthesized unsaturated phosphatidylcholines occurs during formation of dipalmitoyl phosphatidylcholine in type II cells comes from studies with urethane-induced adenomas from the mouse [12] and with freshly isolated rabbit type II cells [13]. Which of the remodelling mechanisms is the most important is not yet known. Wykle et al. [12] have suggested that in urethane-induced adenomas the deacylation-reacylation cycle is involved in the transformation of unsaturated phosphatidylcholines into dipalmitoyl phosphatidylcholine. Reports on the substrate specificity of lysolecithin acyltransferase (Acyl-CoA:1-acylglycero-3-phosphocholine *O*-acyltransferase, EC 2.3.1.23), acting alone [14] or in combination with acyl-CoA synthetase (EC 6.2.1.3) [15], in long-term cultures of rabbit-lung cells presumably derived from type II cells [14] and in urethane-induced adenomas [15], respectively, are in agreement with this hypothesis. However, in these studies [14,15] the rate of lysolecithin:lysolecithin acyltransferase was not measured.

Although urethane-induced adenoma cells are morphologically similar to type II cells [16] and a large number of biochemical properties of these adenoma cells and type II cells are also similar, only 28% of the phosphatidylcholine in adenomas is disaturated [16], whereas 43—51% is disaturated in type II cells isolated from normal rabbit or rat lungs [17—19]. The adenomas also differ from normal lung tissue in their capability to synthesize alkyl glycerolipids [16]. Therefore, it is uncertain whether results obtained with adenomas can be directly extrapolated to normal type II cells. As the development of a cell line usually involves a transformation [20] a similar uncertainty applies to data obtained with cells in long-term culture [14]. A second problem encountered in the use of cells in long-term culture derived from a heterogeneous cell population is the uncertainty about the cellular origin of the cell line (for discussion see ref. 21).

Because of these uncertainties it was of interest to study the properties of enzymes involved in the remodelling mechanisms in fresh type II cells from normal lung. It has been shown [22] that rat type II cells in primary monolayer culture have the capacity to synthesize phosphatidylcholine with a high percentage of the disaturated species. In the present study the activity of lysolecithin acyltransferase towards different substrates in these cells is measured and compared with the activity of lysolecithin:lysolecithin acyltransferase.

Materials and Methods

Isolation of type II cells. Type II cells were isolated from female Wistar rats by trypsinization and density gradient centrifugation exactly as described earlier [23,24] followed by differential adherence in primary monolayer culture [24,25]. Yield, purity and viability were determined as reported earlier [22] and were $8 \pm 2 \cdot 10^6$ cells per preparation of four rats, 95 ± 1% and 96 ± 3% ($N = 25$).

Preparation of type II cell sonicate. The type II cells, attached to the tissue culture dishes, were rinsed three times with 1.5 ml medium containing 125 mM NaCl, 5 mM KCl, 2.5 mM Na_2HPO_4, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 17 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and 50 μg/ml gentamycin (pH 7.4), and then scraped from the culture dishes with a rubber policeman into 1—2 ml 10 mM Tris-HCl, 1 mM EDTA (pH 7.4). Cells and medium were sonicated four times for 30 s at 0°C with an MSE ultrasonic disintegrator at 21 kcycles/s and an amplitude of 6 μm peak to peak.

Preparation of lung homogenate. The lungs from one rat were homogenized with a Potter-Elvehjem homogenizer in 7 ml 0.25 M sucrose/10 mM Tris/1 mM EDTA (pH 7.4). Nuclei and debris were removed by centrifugation at $600 \times g$ for 10 min. The resultant supernatant was used after dilution with 10 mM Tris-HCl, 1 mM EDTA (pH 7.4).

Lysolecithin acyltransferase assay. The conversion of 1-palmitoyl-*sn*-glycero-3-phosphocholine into phosphatidylcholine by acylation with palmitoyl-CoA or oleoyl-CoA was assayed in 0.5 ml of medium: 0.2 mM 1-palmitoyl-*sn*-glycero-3-phosphocholine (taken from a sonicated 1 mM emulsion in water), 65 mM Tris-HCl (pH 7.4), varying concentrations of [1-^{14}C]palmitoyl-CoA or [1-^{14}C]oleoyl-CoA (spec. act. $5.5 \cdot 10^3$ dpm/nmol) and sonicated type II cells or lung homogenate (12 μg protein). After 0 or 4 min incubation at 37°C, the reactions were stopped by addition of 2 ml methanol/chloroform (2 : 1, v/v). Following addition of 0.2 μmol egg phosphatidylcholine and 1 μmol linoleic acid as carriers, the lipids were extracted by the method of Bligh and Dyer [26] and subjected to chromatography on silica H plates with chloroform/methanol/glacial acetic acid/water (50 : 30 : 8 : 4, v/v) as eluent [27]. The phosphatidylcholine fractions were transferred into scintillation vials. Radioactivity was measured with the liquid scintillation mixture described by Pande [28]. Counting efficiency was determined by the channels-ratio method. Under the conditions used the formation of phosphatidylcholine was directly proportional to the incubation time and to the amount of protein.

Assay of the combined action of acyl-CoA synthetase and lysolecithin acyltransferase. The formation of phosphatidylcholine from 1-palmitoyl-*sn*-glycero-3-phosphocholine and palmitate or oleate by the combined action of acyl-CoA synthetase and lysolecithin acyltransferase was measured at 37°C in 0.25 ml of a medium containing 100 mM KCl, 80 mM Tris-HCl (pH 7.4), 0.2 mM 1-palmitoyl-*sn*-glycero-3-phosphocholine (taken from a sonicated 2 mM emulsion in 125 mM KCl/100 mM Tris-HCl (pH 7.4)), 10 mM $MgCl_2$, 10 mM ATP, 0.2 mM CoASH, 0.2 mM [1-^{14}C]palmitate or [1-^{14}C]oleate (spec. act. $4 \cdot 10^3$ dpm/nmol, taken from a sonicated 2.0 mM emulsion in 125 mM KCl/100 mM Tris-HCl (pH 7.4)) and sonicated type II cells (19 μg protein). After the appropriate

incubation times (0 and 2 min) the reactions were stopped by addition of 2 ml methanol/chloroform (2 : 1, v/v). After addition of 0.2 μmol egg phosphatidylcholine, lipids were extracted by the method of Bligh and Dyer [26] and analysed as described above, except that the thin-layer plates were pre-run in chloroform/light petroleum (b.p. 40—60° C)/glacial acetic acid (65 : 33 : 2, v/v).

Lysolecithin:lysolecithin acyltransferase assay. The formation of phosphatidylcholine by transesterification between 2 molecules of 1-palmitoyl-*sn*-glycero-3-phosphocholine was assayed in 0.05 ml of a medium containing 160 mM phosphate buffer pH 6.0, varying concentrations of 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine (spec. act. $5 \cdot 10^3$ dpm/nmol, taken from a sonicated 0.8 mM emulsion in 320 mM phosphate buffer, pH 6.0) and sonicated type II cells (21.5 μg protein) or whole lung homogenate (44.2 μg protein). After 0 or 5 min incubation at 37°C the reactions were stopped by addition of 2 ml methanol/chloroform (2 : 1, v/v). Following addition of lung lipid extract (0.2 μmol phosphorus) as a carrier, the lipids were extracted and analysed as described for lysolecithin acyltransferase. Under the conditions used, the formation of phosphatidylcholine was directly proportional to the incubation time and the amount of protein.

Protein assay. Protein was determined according to Lowry et al. [29].

Materials. Trypsin (twice crystallized) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.); 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine was prepared by hydrolysis of 1,2-di[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine with phospholipase A_2 from snake venom [30]. 1,2-Di-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine (spec. act. 50 Ci/mol) was obtained from Applied Science (State College, PA, U.S.A.); [1-^{14}C]palmitoyl-CoA (spec. act. 58 Ci/mol), [1-^{14}C]palmitic acid (spec. act. 58 Ci/mol) and [1-^{14}C]oleic acid (spec. act. 58 Ci/mol) were purchased from The Radiochemical Centre (Amersham, U.K.) and [1-^{14}C]oleoyl-CoA (spec. act. 50 Ci/mol) from NEN Chemicals (Dreieichenhain, F.R.G.). Phospholipase A_2 from snake venom was supplied by Boehringer (Mannheim, F.R.G.) and unlabelled 1-dipalmitoyl-*sn*-glycero-3-phosphocholine by Serdary (London, Ontario, Canada).

Results

Lysolecithin acyltransferase

The activity of lysolecithin acyltransferase in sonicated type II cells and in whole lung homogenate with palmitoyl-CoA and oleoyl-CoA as acyl donor is shown in Fig. 1. As Frosolono et al. [5] observed that in total lung microsomes the enzyme is optimally active at pH 7.4, the activity was assayed at this near-physiological pH. It can be seen that the specific activity of the enzyme in sonicated type II cells is far greater than that in whole lung homogenate with both acyl-CoA species tested. It can also be seen that the enzyme has a preference for palmitoyl-CoA over oleoyl-CoA over a wide range of substrate concentrations. Although the activity with palmitoyl-CoA appears to be slightly lower than that with oleoyl-CoA at a concentration of 25 μM when measured in the absence of Mg^{2+} (Fig. 1A), palmitoyl-CoA is the preferred substrate at all concentrations up to 50 μM in the presence of 10 mM Mg^{2+} (Fig. 1B). The increased utilization of palmitoyl-CoA compared to oleoyl-CoA at higher acyl-

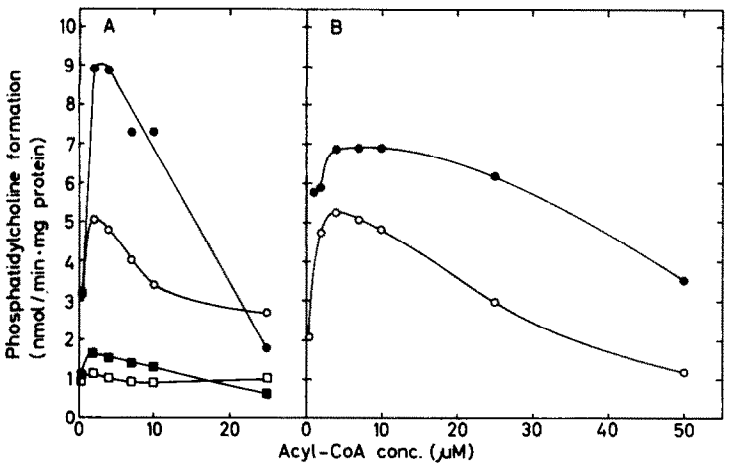

Fig. 1. The activity of lysolecithin acyltransferase in type II cell sonicate and in whole lung homogenate as a function of the acyl-CoA concentration. Beside the standard components the medium in B contained 10 mM $MgCl_2$. ●———●, type II cell sonicate, 16 : 0-CoA as substrate; ○———○, type II cell sonicate, 18 : 1-CoA as substrate; ■———■, whole lung homogenate, 16 : 0-CoA as substrate; □———□, whole lung homogenate, 18 : 1-CoA as substrate. The figure is representative for results obtained in three experiments (each carried out in duplicate; variation 6%).

CoA concentrations under the influence of Mg^{2+} was also observed with total lung microsomes, both in the absence and in the presence of 0.1 mM EDTA.

The amounts of palmitoyl- and oleoyl-CoA remaining at the end of the incubation of sonicated type II cells at a concentration of 4 μM acyl-CoA (Fig. 1A) were 77 and 87%, respectively, of the added amounts. The hydrolysis rates of palmitoyl- and oleoyl-CoA did not differ significantly and were low compared to the utilization of these CoA-esters in the formation of phosphatidylcholine.

The activity of lysolecithin acyltransferase as a function of 1-palmitoyl-*sn*-glycero-3-phosphocholine concentration exhibited saturation kinetics, the apparent K_m being approx. 0.016 mM (not shown).

Combined action of acyl-CoA synthetase and lysolecithin acyltransferase

When palmitate or oleate were added to sonicated type II cells as the free fatty acid at a concentration in the physiological range for blood (0.2 mM) [31], the formation of phosphatidylcholine from palmitate and oleate was 5.00 and 2.98 nmol/min per mg protein, respectively. This means that also when the fatty acids are added unesterified to CoASH and have to be activated by the type II cell sonicate itself, type II cell sonicate has a preference for incorporation of palmitate into phosphatidylcholine. The rate per min and per mg protein of this combined reaction is of the same order of magnitude as that of lysolecithin acyltransferase alone (Fig. 1).

Lysolecithin:lysolecithin acyltransferase

Fig. 2 shows the specific activities of lysolecithin:lysolecithin acyltransferase in sonicated type II cells and total lung homogenate as a function of the sub-

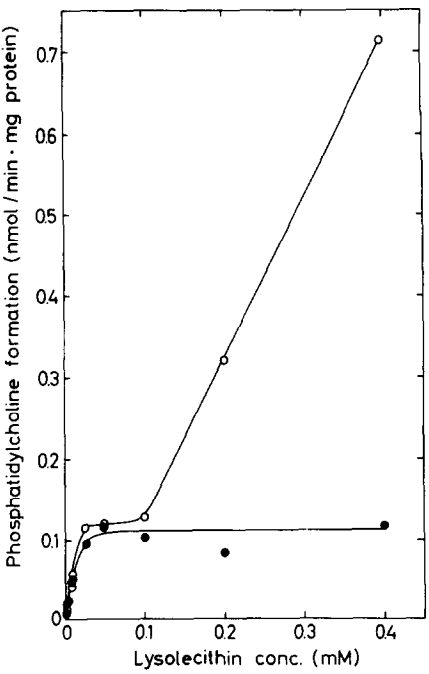

Fig. 2. The activity of lysolecithin:lysolecithin acyltransferase in type II cell sonicate and in whole lung homogenate as a function of the 1-palmitoyl-*sn*-glycero-3-phosphocholine concentration. ●———●, type II cell sonicate; ○———○, whole lung homogenate. The figure is representative for results obtained in two experiments (each carried out in duplicate; variation 13%).

strate concentration. The activity was measured at pH 6.0, at which the activity of whole rat and rabbit lung lysolecithin:lysolecithin acyltransferase has been reported to be near the optimum [32,33]. It can be seen that the enzyme in sonicated type II cells shows saturation kinetics, the apparent K_m being approx. 0.013 mM. However, the enzyme in total lung shows a different pattern. At substrate concentrations of 0.1 mM and lower the specific activity of the enzyme in whole lung homogenate is about the same as in type II cell sonicate, but at substrate concentrations above 0.1 mM the specific activity in whole lung homogenate is much higher than that in type II cell sonicate.

Comparison of the data for lysolecithin acyltransferase with those for lysolecithin:lysolecithin acyltransferase shows that in type II cell sonicate the specific activity of lysolecithin:lysolecithin acyltransferase is at least one order of magnitude lower than that of lysolecithin acyltransferase, acting alone or in combination with acyl-CoA synthetase.

Discussion

As mentioned in the preceding paragraph, lysolecithin acyltransferase and lysolecithin:lysolecithin acyltransferase in type II cells have nearly equal apparent K_m values for 1-palmitoyl-*sn*-glycero-3-phosphocholine (0.016 and 0.013 mM, respectively), which means that the relative rates of the reactions catalysed by these two enzymes will probably be determined by the V values.

In view of the large difference in activities observed with lysolecithin acyltransferase on the one hand (Fig. 1) and lysolecithin:lysolecithin acyltransferase on the other hand (Fig. 2) this suggests that in type II cells the velocity of the lysolecithin acyltransferase reaction is an order of magnitude greater than that of lysolecithin:lysolecithin acyltransferase at all lysophosphatidylcholine concentrations.

A net formation of dipalmitoyl phosphatidylcholine from phosphatidylcholine molecules containing an unsaturated fatty acid at the 2-position by removal of the unsaturated fatty acyl group and reacylation with palmitoyl-CoA can only take place if palmitoyl-CoA is the predominantly available acyl-CoA species or if the lysolecithin acyltransferase has a preference for palmitoyl-CoA as the acyl donor. In the preceding paragraph it was shown that lysolecithin acyltransferase in type II cells indeed has a preference for palmitoyl-CoA over oleoyl-CoA as the acyl donor at all substrate concentrations from 0.5 up to 22 μM, and in the presence of Mg^{2+} even up to 50 μM. The physiological acyl-CoA concentration in type II cells might well be in this range. Although the long-chain acyl-CoA content of type II cells or in total lung is unknown, isolated hepatocytes have been reported [34] to contain 250—400 nmol long-chain acyl-CoA/g dry weight which, according to the conversion data of Geelen and Gibson [35], amounts to 60—90 nmol/ml. As a large part of the acyl-CoA esters is probably bound to protein the average concentration of the free acyl-CoA ester is probably much lower and somewhere in the range of concentrations employed in the experiments shown in Fig. 1. Nothing is known, however, about the subcellular distribution of the acyl-CoA esters. The Mg^{2+} content of type II cells or whole lung tissue is also unknown. In human liver the Mg^{2+} concentration is approx. 15 mequiv./kg [36], but this Mg^{2+} is probably partly complexed to cellular constituents.

It might well be that palmitoyl-CoA is indeed the acyl-CoA species predominantly available in type II cells. Studies with whole lung [37—39] have shown that palmitate is the major product of de novo fatty acid synthesis from acetyl-CoA. In addition Voelker et al. [40], who used urethane-induced adenomas from the mouse as a model for type II cells, observed that fatty acid synthesis from acetate yields predominantly palmitate. The results of Batenburg et al. [22] and Smith and Kikkawa [13] indicate that this is also the case in type II cells from normal rat and rabbit lung. These authors [13,22] observed that the degree of saturation of phosphatidylcholine synthesized by type II cells reflects the availability of saturated fatty acid and that with [1-^{14}C]acetate as the substrate about the same percentage label is found in the disaturated species of phosphatidylcholine as with [1-^{14}C]palmitate.

As mentioned in Results the combination of acyl-CoA synthetase and lysolecithin acyltransferase incorporates palmitate more rapidly than oleate into phosphatidylcholine even if palmitate is added in the same concentration as oleate. Whether this is due only to the substrate specificity of the lysolecithin acyltransferase or whether the substrate specificity of the acyl-CoA synthetase also plays a role, cannot be concluded from these data.

The observations reported in this study that the specific activity of lysolecithin:lysolecithin acyltransferase in these normal adult rat type II cells is much lower than that of lysolecithin acyltransferase (acting alone or in combi-

nation with acyl-CoA synthetase), that lysolecithin acyltransferase in the type II cells has a preference for palmitoyl-CoA over oleoyl-CoA and that the combination of acyl-CoA synthetase and lysolecithin acyltransferase has a preference for palmitate over oleate, together with previous observations (see above) that palmitate is the most important product of fatty acid synthesis in type II cells, indicate that in type II cells from adult rat lung the deacylation-reacylation cycle is more important for the formation of dipalmitoyl phosphatidylcholine than the deacylation-transacylation process. The observations that, as compared to whole lung homogenate, the type II cells are highly enriched in lysolecithin acyltransferase but not enriched in lysolecithin:lysolecithin acyltransferase point in the same direction.

Some caution, however, is appropriate. Recent work of Finkelstein and Mavis [41] suggests that in lung cells isolated after trypsin treatment some enzymes involved in phospholipid metabolism are significantly damaged. Damaging effects on lysolecithin acyltransferase or lysolecithin:lysolecithin acyltransferase were not reported, but different sensitivities of these two enzymes to trypsin treatment would modify the interpretation of the results described in this paper. On the other hand the results of Finkelstein and Mavis [41] were obtained in cells isolated directly after trypsin treatment, whereas the experiments reported here were performed with cells which had been cultured for 20 h after trypsinization. Kasten [42] has observed that the intracellular damage to cardiac cells caused by trypsin was repaired within 15 h after removal of trypsin.

The curve for the relationship between substrate concentration and enzyme activity observed for lysolecithin:lysolecithin acyltransferase in whole lung homogenate (Fig. 2) is similar to that found by Brumley and van den Bosch [43] for lysolecithin:lysolecithin acyltransferase purified from rat lung. The observation of a different pattern for lysolecithin:lysolecithin acyltransferase in sonicated type II cells raises the question whether type II cells have a lysolecithin:lysolecithin acyltransferase with different properties. The answer to this question has to await purification of the enzyme from isolated type II cells.

It is possible that in fetal type II cells the deacylation-transacylation process is relatively more important than in adult cells. This is suggested by observations with whole lung that the activity of lysolecithin:lysolecithin acyltransferase increases considerably 1 day before term in the fetal mouse [7] and rat [44]. This possibility will have to be studied with isolated type II cells from fetal lung.

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