Acyl Group Transfer from the *sn*-1 Position of Phospholipids in the Biosynthesis of *n*-Dodecyl Palmitate†

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ABSTRACT: The wax ester *n*-dodecyl palmitate is shown to be synthesized by retinal pigment epithelial membranes. The biosynthesis of this ester is phospholipid dependent and occurs via the transfer of a palmitoyl group from the *sn*-1 position of lecithin to *n*-dodecanol. When retinal pigment epithelial membranes are used as the source of enzyme, the apparent Michaelis constant for *n*-dodecanol in this process is 65.8 μM, and the maximal velocity for *n*-dodecyl palmitate synthesis is 16.2 nmol/(h·mg of protein). The enzymatic activity is membrane associated and shows a maximum velocity between pH 8 and pH 9. This transesterification process appears to be similar to the lecithin retinol acyl transferase reaction and is a further example of acyl group transfer reactions from the *sn*-1 position of phospholipids.

The visual cycle is completed by an enzyme system in the retinal pigment epithelium (RPE)[1] by a two-step process that begins with the conversion of *all-trans*-retinol (vitamin A) to its ester, followed by the direct conversion of the *all-trans*-retinyl ester into 11-*cis*-retinol by means of an isomerohydrolase enzyme (Rando, 1991, 1990). This reaction sequence uses the negative free energy of hydrolysis of an acyl ester to drive the thermodynamically uphill trans to cis isomerization (Deigner et al., 1989). The energy originates in membrane phospholipids, because retinyl esters are generated here by a transesterification reaction which is catalyzed by lecithin retinol acyl transferase (LRAT) and in which an acyl group is transferred from lecithin to vitamin A (Rando, 1991, Rando, 1990; Deigner et al., 1989; Saari & Bredberg, 1989). This type of group transfer reaction is normally associated with ATP utilization. In addition to occurring in the RPE, the LRAT reaction also occurs in the intestine and liver (Ong et al., 1991; MacDonald & Ong, 1987). The enzyme is importantly implicated in the mobilization of vitamin A in the liver and intestine. LRAT is specific for phosphatidylcholine derivatives and transfers acyl groups regiospecifically from the *sn*-1 position of the phospholipid (MacDonald & Ong, 1987; Saari & Bredberg, 1989; Cañada et al., 1990). Since the *sn*-1 position of phospholipids contains mostly saturated fatty acids, such as in palmitate, the resulting retinyl esters formed are largely saturated in nature (MacDonald & Ong, 1987; Saari & Bredberg, 1989; Cañada et al., 1990).

Acyl group transfers from the *sn*-1 position of phospholipids appear to occur infrequently, but this may simply be because these reactions have not been investigated. It was therefore of interest to determine whether further examples could be uncovered in light of the role of these transfers in the visual cycle. It is shown here that RPE membranes can also transfer palmitoyl groups from the *sn*-1 position of lecithin to small molecule acceptor species other than vitamin A. The major endogenous acceptor proved to be *n*-dodecanol. The biosynthesis of this compound occurs via an acyl transfer of a palmitoyl group from the *sn*-1 position of an endogenous lecithin. These findings indicate that acyl group transfers from the *sn*-1 position of lecithin may be of general interest.

MATERIALS AND METHODS

Materials

Palmitoyl-CoA ([1-^{14}C]palmitoyl 57.0 mCi/mmol) was prepared by the reaction of [1-^{14}C]palmitoyl chloride with CoA (Bishop & Hajra, 1980). The [9,10-^{3}H,^{3}H]palmitic acid (60.0 Ci/mmol) was from Amersham Corp. *n*-Dodecanol was from Sigma Inc. Radiolabeled *n*-dodecanol (1-^{3}H, 108 mCi/mmol) was prepared by oxidation of *n*-dodecanol to *n*-dodecanal with pyridinium dichromate (Corey & Schmidt, 1979), followed by reduction with [^{3}H]$NaBH_4$. *n*-Dodecyl palmitate and *n*-dodecyl valerate were synthesized from *n*-dodecanol and the corresponding acid chloride in the presence of pyridine (Mahadevan, 1978). Lysolecithin (L-1-palmitoyl, [1-^{14}C]palmitoyl, 57.0 mCi/mmol), palmitic acid ([1-^{14}C], 57.0 mCi/mmol), *all-trans*-retinol (11,12-$^{3}H_2$ 47.9 Ci/mmol), and [^{3}H]$NaBH_4$ (409 mCi/mmol) were purchased from New England Nuclear, Boston. Arachidonoyl-CoA and lysolecithin were products of Sigma. All other chemicals were purchased from Fluka Chemical Co.

Methods

Synthesis of L-*1-[9,10-^{3}H,^{3}H]Palmitoyl-2-pentyl-sn-glyceryl-3-phosphocholine.* To a vial was added 200 μL of [9,10-^{3}H,^{3}H]palmitic acid (60.0 Ci/mmol, 0.083 μmol/mL) and 20 μL of palmitic acid (7.41 μM). The solvent was evaporated under nitrogen; 100 μL of dry benzene was added and the solvent was evaporated again. This cycle was repeated three times. One hundred microliters of oxalyl chloride was added and the solution was incubated for 4 h at 37 °C under nitrogen. The excess oxalyl chloride was then removed by nitrogen; 100 μL of dry benzene was added, followed by the evaporation of solvent. A total of 200 μL of the lysolecithin derivative L-2-pentyl-*sn*-glyceryl-3-phosphocholine (10 mM) (Hirth et al., 1983; Chaudrakumar & Hajdu, 1983) was added in dry chloroform, along with a catalytic amount of *p*-(dimethylamino)pyridine. The mixture was heated for 12 h at 37 °C. The product L-1-[9,10-^{3}H,^{3}H]palmitoyl-2-pentyl-*sn*-glyceryl-

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[1] Abbreviations: LRAT, lecithin retinol acyl transferase; DPPC, dipalmitoylphosphatidylcholine; DTT, dithiothreitol; DP, *n*-dodecyl palmitate; DV, *n*-dodecyl valerate; HPLC, high-performance liquid chromatography; TLC, thin layer chromatography; RPE, retinal pigment epithelium; BSA, bovine serum albumin.

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3-phosphocholine was obtained by HPLC purification on a C18 column (C18-300A). The product had a retention time of 6–7 min eluting with methanol at 1 mL/min. The yield was approximately 20%. ^{1}H NMR (unlabeled compound) ($CDCl_3$) δ: 0.87 (m, 6 H); 1.27 (m, 30 H); 1.47 (m, 4 H); 2.28 (m, 2 H); 3.35 (s, 1 H); 3.49 (m, 1 H); 3.57 (m, 1 H); 3.65 (m, 1 H); 3.81 (m, 1 H); 3.87 (m, 1 H); 3.94 (m, 1 H); 4.07 (m, 1 H); 4.26 (m, 1 H); 4.34 (m, 1 H).

Acyl Transfer Reactions from Phospholipids. The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.4), 0.2 mM DTT, 100 μmol of arachidonoyl-CoA, 0.5 mg of BSA, 88 μmol (1 mCi) of lysolecithin (L-1-palmitoyl), [1-^{14}C]palmitoyl), and the enzyme preparation, in a final volume of 200 μL. Both endogenous *n*-dodecanol and endogenous *all-trans*-retinol were esterified under these conditions. After incubations for up to 4 h at 23 °C, a 40-μL aliquot of the reaction mixture was taken and added to 100 μL of methanol. Generally, the reactions were linear for minimally 1 h. For kinetic studies, aliquots were removed at between 15 min and 30 min. The reaction products were extracted with chloroform and a methanol/water system according to the published method (Bligh & Dyer, 1959) and analyzed on silica gel TLC plates using chloforom/methanol/water 65:25:4 (v/v/v) or hexane/acetone 95:5 (v/v). *n*-Dodecyl esters and retinyl esters were extracted with *n*-hexane and analyzed by HPLC using a Waters HPLC system with a silica column (0.46 × 25 cm, LiChrosorb Si60, Hibar, Merck). Elution was carried out with *n*-hexane/ether 99:1 (v/v) at a flow rate of 1.5 mL/min. The elution times of *n*-dodecyl palmitate and *all-trans*-retinyl palmitate were 4.7 and 6.1 min, respectively. The radioactivity was measured with a Berthold radioactivity monitor on-line with the HPLC system. All experiments were repeated two or three times.

Reaction of L-*1-[9,10-^{3}H,^{3}H]Palmitoyl-2-pentyl-sn-glyceryl-3-phosphocholine with 1-Dodecanol in the Presence of RPE Membrane.* *n*-Dodecanol (1.75 μL; 22.87 mM) and 10 μL of L-1-[9,10-^{3}H,^{3}H]palmitoyl-2-pentyl-*sn*-glyceryl-3-phosphocholine (4.9 μM, 6 Ci/mmol, 0.03 mCi/mL) were added to a plastic vial. After the solvent was evaporated with nitrogen, 130 μL of buffer (0.015 M Tris-HCl, pH = 8.0, 3mM DTT) and 50 μL of RPE membranes were added. The final concentrations of L-1-[9,10-^{3}H,^{3}H]palmitoyl-2-pentyl-*sn*-glyceryl-3-phosphocholine and *n*-dodecanol were 0.49 and 200 μM, respectively. This solution was incubated at 37 °C for 1 h. To quench the reaction, 500 μL of methanol and 400 μL of hexane were added. After the solution was mixed by vortexing and centrifuging, a 200-μL aliquot of the hexane layer was removed and analyzed by HPLC on a Lichrosorb Si-60 (250 mm × 4.6 mm) column eluting with *n*-hexane/ether 99:1 (v/v) at 1.5 mL/min. The HPLC was connected to an on-line Berthold radioactivity monitor for radioactivity determination.

Preparation of Pigment Epithelium Homogenates. Pigment epithelium cells were obtained from bovine eye cups as described previously (Fulton & Rando, 1987). The cells were suspended into 0.32 M sucrose/0.1 M (sodium/potassium) phosphate buffer (pH 7.4) and disrupted with an ice-cold Micro Tissue homogenizer. The homogenate was frozen in liquid nitrogen and stored at −80 °C until use. RPE membrane and cytosolic fractions were prepared as described previously (Barry et al., 1989). The protein concentrations were measured by using the Peterson modification (Peterson, 1977) of the Lowry method.

Isolation of n-Dodecyl Palmitate from Pigment Epithelial Homogenates. A large-scale reaction (200 mL) with RPE homogenate was performed under the conditions described above, except that 500 μM cold lysolecithin (L-1-palmitoyl) was used as the acyl donor. After extraction, the chloroform extract was concentrated and subjected to preparative TLC using chloroform/methanol/water 65:25:4 (v/v/v). The product distribution was determined by developing the radioactive product on the same plate and assaying the silica gel scraped from the various regions directly for ^{14}C. The cold reaction product was eluted from the silica gel by *n*-hexane/ether 99.5:0.5 (v/v) (80 mL, 3 times) and subjected to normal phase HPLC on a Dynamax 60 A column (1.0 × 25 cm, Rainin) using *n*-hexane/ether 99.5:0.5 (v/v) as the eluant, at a flow rate of 2.0 mL/min. The peak corresponding to the radioactive peak (retention time, 28 min) was collected and further purified by reverse-phase HPLC on a C-18 column (0.46 × 25 cm, Dynamax 300 A, Rainin) using methanol/ether 9:1 (v/v) as the eluant, with a flow rate of 1.0 mL/min. The peak corresponding to the radioactive peak (retention time, 6.5 min) was collected and analyzed. Proton NMR spectra were obtained on a Varian VRX500S spectrometer operating at a proton frequency of 499.843 MHz. Chloroform-*d* was used as the NMR solvent and as the internal standard. NMR (500 MHz, $CDCl_3$) δ: 0.88 (6 H, t, J = 6.7 Hz), 1.25 (42 H, br s), 1.61 (4 H, br quint, J = 7.2 Hz), 2.28 (2 H, t, J = 7.7 Hz), 4.05 (2 H, t, J = 6.7). This NMR spectrum is identical to that of authentic *n*-dodecyl palmitate. Electron-impact mass spectra were performed on a Jeol AX-505 mass spectrometer. Mass spectrum: m/e 424 (M^+); exact mass calcd for $C_{28}H_{56}O_2$ (*n*-dodecyl palmitate) 424.4280, found 424.4300.

RESULTS

Formation of n-Dodecyl Palmitate by RPE Membranes. Initial experiments were aimed at determining if any acyl transfer reactions from the *sn*-1 position of lecithin, other than that found in retinyl ester formation, occurred in bovine retinal RPE. As long-chain phospholipids are difficult to use directly as substrates because of their low monomeric solubility, they were generated in situ using a previously published protocol (Saari & Bredberg, 1989). In this case, they were generated from L-1-palmitoyl-[1-^{14}C]lysolecithin and arachidonoyl-CoA. Lysolecithins are more soluble in water than are lecithins, and they are readily converted into phospholipids by RPE membranes in the presence of an acyl-CoA (Saari & Bredberg, 1989). Figure 1 shows the TLC analysis of the reaction mixture of L-1-palmitoyl-[1-^{14}C]lysolecithin, arachidonoyl-CoA, and the RPE homogenate. After incubation in the presence of 100 μM arachidonoyl-CoA for 4 h at 23 °C, 47% of the original radioactivity was found in lecithin and 33% was in palmitic acid (Figure 1A). Therefore, as expected (Saari & Bredberg, 1989), a substantial fraction of the lysolecithin introduced was processed to form lecithin. In the absence of arachidonoyl-CoA, only 17% of the radiolabeled lysolecithin was converted into lecithin (Figure 1B). This suggests that there are endogenous acyl esters present capable of acylating the added lysolecithin. When an experiment was carried out as in Figure 1A except that the TLC development was carried out differently, the result shown in Figure 1C was obtained. This result shows the formation of one major new radioactive spot (R_f 0.709, Figure 1C). Several other new radiolabeled compounds were also formed which were not abundant enough to pursue further at this time. The major new radiolabeled compound was more hydrophobic on silica gel TLC than long-chain retinyl esters.

In order to identify the new compound, a large-scale reaction was conducted with nonradioactive lysolecithin and arachi-

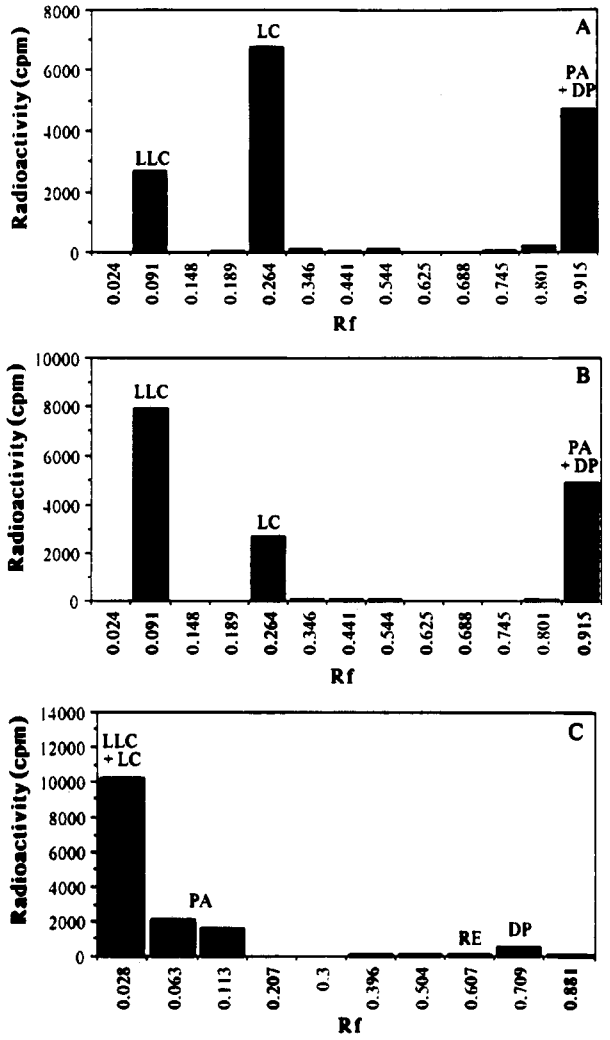


FIGURE 1: TLC analysis of acyl transfer reaction. RPE homogenate (containing 1.03 mg of protein) was incubated for 4 h at 23 °C in the presence of 0.1 M potassium phosphate buffer (pH 7.4), 2 mM DTT, 100 μM arachidonoyl-CoA (0 μM for B), 2.5 mg/mL BSA, and 88 μM (5 μCi/mL) lysolecithin (L-1-palmitoyl, [1-^{14}C]palmitoyl) in a final volume of 200 μL. The reaction was terminated by the addition of 500 μL of methanol. Reaction products were extracted with chloroform and spotted on silica gel TLC plates, which were developed in panels A and B (chloroform/methanol/water 65:25:4 (v/v/v)) and C (*n*-hexane/acetone 95:5 (v/v)). Relevant spots were scraped off the silica gel plates and counted for radioactivity. Abbreviations: LLC, lysolecithin; LC, lecithin; PA, palmitic acid; RE, retinyl ester, and DP, *n*-dodecyl palmitate.

donoyl-CoA, as described in the Materials and Method section. A product eluting at the same R_f as that of the previous radioactive spot was collected, and its structure was determined by ^{1}H-NMR and mass spectroscopy. As described in the Methods section, the NMR and mass spectra of the extracted material are identical to the spectra of authentic *n*-dodecyl palmitate. The formation of this ester in RPE was surprising in that similar wax esters are generally found to be a product of sebaceous cells (Doran et al., 1991).

Effect of Arachidonoyl-CoA on n-Dodecyl Palmitate Formation. The formation of *n*-dodecyl palmitate was further explored by demonstrating that its formation was strongly stimulated by the addition of 1 mM *n*-dodecanol to the RPE reaction mixture (Figure 2). The *n*-dodecyl palmitate ester formed enzymatically, because product was not formed when a boiled enzyme preparation was used (Figure 2). The formation of *n*-dodecyl palmitate was also stimulated by the addition of arachidonoyl-CoA (Figure 2), indicating that lecithin was required before the acyl transfer reaction occurred.

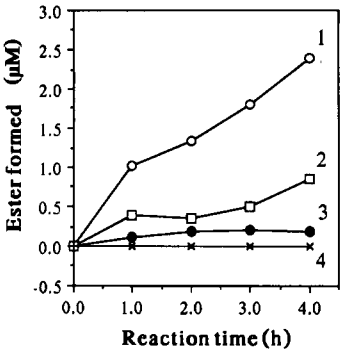
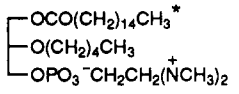


FIGURE 2: Effects of *n*-dodecanol and arachidonoyl-CoA on *n*-dodecyl palmitate formation. RPE homogenate (containing 1.03 mg of protein) was incubated at 23 °C in the presence of 0.1 M potassium phosphate buffer (pH 7.4), 2 mM DTT, 100 μM arachidonoyl-CoA, 1 mM dodecanol, 2.5 mg/mL BSA, and 88 μM (5 mCi/mL) lysolecithin (L-1-palmitoyl, [1-^{14}C]palmitoyl) in a final volume of 200 μL. After incubation for indicated times, a 40-μL aliquot of reaction mixture was added to 200 μL of methanol. *n*-Dodecyl palmitate was extracted with 200 μL of hexane and determined with HPLC. Curve 1 (○), control experiment. Curve 2, (□) arachidonoyl-CoA was removed from reaction mixture. Curve 3, (●) dodecanol was removed from reaction mixture. Curve 4, (×) RPE homogenate was boiled for 1 min prior to reaction.

This kind of stimulation has also been observed in the formation of retinyl ester by LRAT (Saari & Bredberg, 1989). In addition, added radioactive palmitic acid was also not processed to form *n*-dodecyl palmitate, which is consistent with the idea that the ester arises by means of a transesterification reaction.

Acyl Group Transfer Is from the sn-1 Position of Phospholipids. Dipalmitoyl-lecithin proved not to be efficient as an acyl donor for *n*-dodecanol, and variable results were obtained using this phospholipid. This is not unexpected, and the result is ascribed to solubility problems which have been observed before in published studies on LRAT (Saari & Bredberg, 1989). To demonstrate acyl group transfer from phospholipids directly and unequivocally, the more soluble divaleroyl-lecithin was utilized as the potential acyl donor. It could readily be shown that divaleroyl-lecithin, a "soluble" lecithin, was an acyl donor with respect to added *n*-dodecanol (Figure 3). The maximal rate of *n*-dodecyl valerate synthesis is 0.324 nmol/(h·mg).

The result described above with divaleroyl-lecithin demonstrates that acyl transfer can occur from a "soluble" lecithin derivative to *n*-dodecanol. The result is not informative with respect to whether the transfer occurs from the *sn*-1 or the *sn*-2 position of the phospholipid. Moreover, it is still possible, although unlikely, that hydrolysis at the *sn*-2 position occurs prior to acyl transfer so that acyl group transfer occurs via a 1-acyl-lysophospholipid. In order to rule these possibilities out, L-1-[9,10-^{3}H,^{3}H]palmitoyl-2-pentyl-*sn*-glyceryl-3-phosphocholine (shown below) was prepared; it contains a



nonhydrolyzable ether linkage at the *sn*-2 position. When the RPE enzymatic activity was incubated with this labeled analog and *n*-dodecanol, the formation of *n*-dodecyl palmitate could readily be demonstrated, as shown in Figure 4. It is noteworthy that no evidence for diglyceride formation was observed so that phospholipase C mediated hydrolysis did not occur. Thus, the observed acyl group transfer must occur from the *sn*-1 position of the phospholipid.

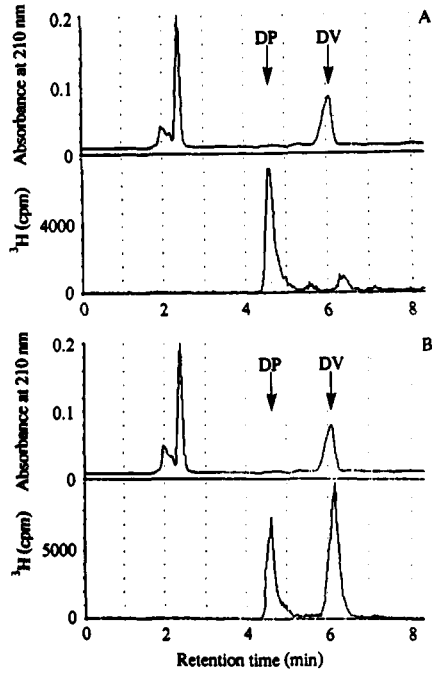


FIGURE 3: HPLC elution profiles of acyl transfer product from divaleroyl lecithin. RPE homogenate (containing 1.03 mg of protein) was incubated at 23 °C in the presence of 0.1 M potassium phosphate buffer (pH 7.4), 2 mM DTT, 2.5 mg/mL BSA, and 90 μM (9.7 mCi/mL) dodecanol (1-^{3}H) without (A) or with (B) 5 mM divaleroyl lecithin, in a final volume of 200 μL. After incubation for an hour, a 40-μL aliquot of reaction mixture was added to 200 μL of methanol, and *n*-dodecyl esters were extracted with 200 μL of *n*-hexane. Nonradioactive *n*-dodecyl valerate (10 mM) was added to each extract as a standard, prior to the analysis. Samples were applied on a normal-phase HPLC column (0.46 × 25 cm, LiChrosorb Si60) and eluted with *n*-hexane/ether 99:1 (v/v) at a flow rate of 1.5 mL/min. Arrows indicate the positions of *n*-dodecyl palmitate (DP) and divaleroyl lecithin (DV).

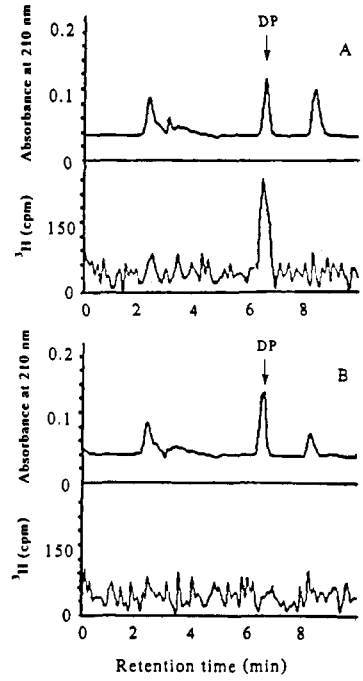


FIGURE 4: Formation of *n*-dodecylpalmitate by incubating L-1-[9,-10-^{3}H,^{3}H]palmitoyl-2-pentyl-*sn*-glyceryl-3-phosphocholine with *n*-dodecanol in the presence of RPE membranes. The incubations and analyses were performed as indicated in the *Methods* section. (**A**) The reaction with RPE membrane in the presence of *n*-dodecanol and L-1-[9,10-^{3}H,^{3}H]palmitoyl-2-pentyl-*sn*-glyceryl-3-phosphocholine. (**B**) The control experiment without RPE membrane. The upper part of each graph shows UV detection at 210 nm. The lower part shows radioactivity detected by an on-line radioactivity monitor. The counting efficiency was approximately 1%. The retention time of *n*-dodecanol palmitate (DP) is 6.4 min, and nonradioactive DP was added as standard sample before HPLC analysis. No hydrolysis of the added phospholipid to the corresponding diglyceride was observed.

Biosynthesis of n-Dodecyl Palmitate from Endogenous Phospholipids. Figure 5 shows the kinetic plots for *n*-dodecyl palmitate synthesis with RPE homogenates. In these experiments, [^{3}H]*n*-dodecanol at the indicated concentrations was added to RPE membranes and the formation of [^{3}H]*n*-dodecyl palmitate was monitored. Under the conditions described in the legend of Figure 5, the apparent Michaelis constant for *n*-dodecanol was 65.8 μM, and the maximal velocity for *n*-dodecyl palmitate synthesis was 16.2 nmol/(h·mg of protein). The pH profile of *n*-dodecyl palmitate synthetase activity is shown in Figure 6. The apparent pH maximum is approximately 9.

It was of some interest to determine if the *n*-dodecyl palmitate synthetase resided in the cytosolic or membrane fractions of the RPE. A 5-mL solution of RPE membrane (containing 3.7 mg/mL of protein) and a cytosolic fraction (containing 2.8 mg/mL of protein) were prepared from 5-mL of RPE homogenate (containing 20 mg/mL of protein) as described previously (Barry et al., 1989). As shown in Table I, the *n*-dodecyl palmitate synthetase activity is largely, if not entirely, located in the membrane fraction (Table I).

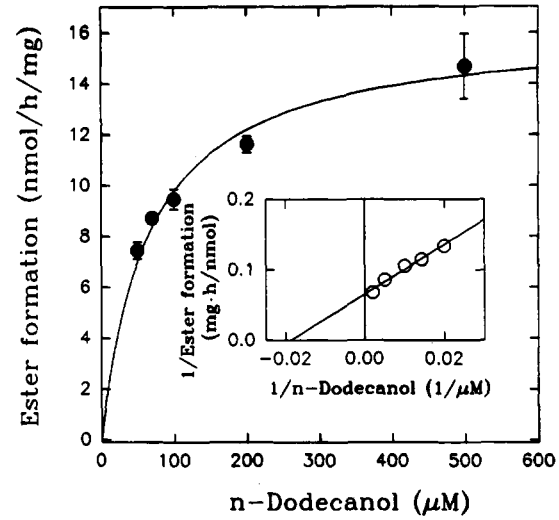


FIGURE 5: Kinetic plots for *n*-dodecyl palmitate synthesis with RPE membranes. The reaction mixture containing RPE membrane (containing 0.185 mg of protein) was incubated at 23 °C in the presence of 0.1 M Tris-HCl (pH 8.0), 2 mM DTT, 2.5 mg/mL BSA, and the indicated concentration of [1-^{3}H]*n*-dodecanol in a final volume of 200 μL. Symbols represent average values of triplicate experiments, and error bars give the standard deviation from the mean.

DISCUSSION

The current studies were initiated to determine if novel acyl group transfer reactions occurred from the *sn*-1 position of phospholipids in retinal pigment epithelial (RPE) tissues. It was already clear that the RPE could produce retinyl esters by this kind of mechanism, using LRAT as the enzyme and lecithin as the acyl donor (Rando, 1990, 1991). Rat intestinal microsomes can also generate retinyl esters utilizing lecithin as the acyl donor (Barry et al., 1989). The retinyl esters serve a dual purpose in vision: they are a physically inert storage form of vitamin A, and they are a depot of energy to drive the thermodynamically unfavorable trans to cis isomerization reaction critical for vision (Rando, 1990, 1991). In the study

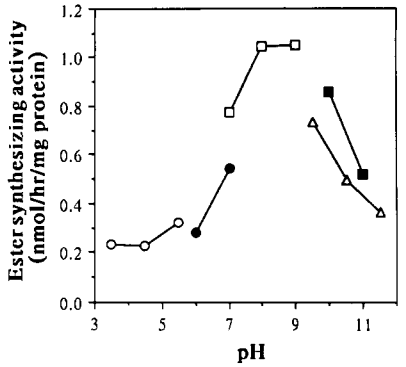


FIGURE 6: Effect of pH on *n*-dodecyl palmitate synthetase activity. The reaction mixture containing RPE homogenate (containing 1.03 mg of protein) was incubated at 23 °C in the presence of 0.1 M buffer, 2 mM DTT, 2.5 mg/mL BSA, and 90 μM (9.7 mCi/mL) *n*-dodecanol in a final volume of 200 μL. Buffers: sodium acetate buffer (○), potassium phosphate buffer (●), Tris-HCl buffer (□), glycine–KOH buffer (containing 0.1 M KCl) (■), and sodium carbonate buffer (△).

Table I: Location of *n*-Dodecyl Palmitate Synthetase Activity[a]

fraction	protein concn (mg/mL)	activity (nmol/(h·mg of protein))	total activity (nmol/h)
RPE homogenate	20.0	0.508	10.2
RPE membrane	3.7	4.02	14.8
cytosol fraction	2.8	0.587	1.64

[a] The RPE enzyme preparations were incubated for 1 h at 23 °C in the presence of 0.1 M potassium phosphate buffer (pH 7.4), 2 mM DTT, 2.5 mg/mL BSA, 200 μM dipalmitoyl-lecithin, and 90 μM (9.7 mCi/mL) *n*-dodecanol (1-^{3}H) in a final volume of 200 μL.

reported here, it was found that RPE membranes can also generate *n*-dodecyl palmitate by a route reminiscent of the LRAT route for the formation of retinyl esters.

RPE homogenates incubated with added *n*-dodecanol generated substantial quantities of *n*-dodecyl palmitate. Most if not all of the wax ester synthetase activity resided in the membrane fraction rather than in the cytosol. The K_M for *n*-dodecanol was measured to be 65.8 μM, and the maximal velocity for *n*-dodecyl palmitate synthesis was 16.2 nmol/(h·mg of protein). These membranes were also competent to synthesize *n*-hexadecyl palmitate from *n*-hexadecanol. The nature of the acyl donor is of some interest; the studies reported here strongly suggest that it is lecitin. *n*-Dodecyl[^{14}C]-palmitate was formed from added L-1-palmitoyl-[1-^{14}C]-lysolecithin and arachidonoyl-CoA. The formation of radioactive *n*-dodecyl palmitate was greatly stimulated by the addition of arachidonoyl-CoA. This suggests that lecithin is required as the acyl donor. Indeed, RPE membranes can readily form lecithins from L-1-palmitoyl-[1-^{14}C]lysolecithin and arachidonoyl-CoA, as shown in Figure 1A. Moreover, it had previously been shown that retinyl esters could be synthesized in vitro via LRAT from added lysolecithins and fatty acyl-CoA derivatives (Saari & Bredberg, 1989). Due to presumed solubility problems, added long-chain fatty acid lecithin derivatives were not generally useful acyl donors in this study, nor were they in the present work (Saari & Bredberg, 1989).

Direct acyl transfer from a phospholipid to *n*-dodecanol could be conveniently demonstrated using "soluble" divaleroyl-lecithin as the acyl donor. Furthermore, direct transfer from the *sn*-1 position could be demonstrated using the *sn*-2 ether-based phospholipid as the acyl donor. Here it could be shown unambiguously that acyl group transfer occurs from the *sn*-1 position of the phospholipid. In this case no hydrolysis at the phosphoryl moiety of the lipid was observed, so that acyl transfer could also not have occurred from the putative ether based diglyceride. It was also determined that RPE membranes are incompetent to synthesize palmitate esters from added free palmitic acid.

The experiments described above demonstrate that lecithin is the major acyl donor in *n*-dodecyl palmitate synthesis. As mentioned above, LRAT catalyzes a similar acyl transfer reaction between lecithin and retinol. A question that arises is whether there was any relationship between LRAT and the acyl transferase described here. Structure–activity studies on LRAT showed a relatively narrow spectrum of action with respect to acyl donor and alcohol (Cañada et al., 1990). Primary aliphatic alcohols were essentially inert as substrates, making it unlikely that *n*-dodecanol would be a substrate (Cañada et al., 1990). Two further lines of evidence are relevant here. First, retinyl ester formation by partially purified LRAT is not affected by the addition of 0.1 mM *n*-dodecanol (Furuyoshi and Rando, unpublished experiments). Moreover, purified LRAT preparations are virtually without activity with respect to producing *n*-dodecyl palmitate esters (Furuyoshi and Rando, unpublished experiments). Thus, it is highly unlikely that LRAT is responsible for *n*-dodecyl palmitate biosynthesis. However, it is likely that LRAT and the enzymatic activity described here will be mechanistically similar.

It is of interest to compare the experiments reported here on *n*-dodecyl palmitate biosynthesis to those reported in the literature on related wax esters in animals. Palmitate esters are well-known components of skin lipids and are secreted by some glands (Doran et al., 1991; Wheatley et al., 1971). Palmitate ester synthesis from free palmitic acid and alcohol is a thermodynamically uphill reaction in water, and there have been several investigations into the nature of the acyl donor. There are several published reports regarding stimulation of palmitate ester synthesis by exogenous cofactors such as ATP and CoA. An enzyme system present in dogfish (*Squalus acanthias*) liver was described which incorporated hexadecanol into hexadecyl palmitate esters (Friedberg & Greene, 1967). Neither the rate nor the extent of incorporation of *n*-hexadecanol into *n*-hexadecyl palmitate esters was influenced by ATP, CoA, Mg^{2+}, or CTP. Thus, no requirement for activation or cofactors could be shown. It was reported that the incorporation of radioactivity from [^{14}C]-palmitic acid into hexadecyl palmitate ester catalyzed by a microsomal protein from rat liver is independent of the presence of ATP, glutathione, acyldihydroxyacetone phosphate, or Mg^{2+} (Hardeman & van den Bosch, 1991). However, no synthesis of *n*-dodecyl palmitate from *n*-dodecanol and palmitic acid occurred in the retinal pigment epithelial system. Oleyl oleate ester formation from [^{14}C]oleic acid and [^{3}H]oleyl alcohol was studied using supernatant fractions obtained by centrifuging dogfish (*S. acanthias*) liver homogenates at 1000*g*, 10000*g*, and 105000*g* (Sargent et al., 1971). In contrast with earlier results (Friedberg & Greene, 1967), with all three supernatants, the formation of the wax esters was greatly stimulated by added ATP. Of the radioactivity incorporated into oleyl oleate, the carbon-14 was found solely in the acid moiety, and the bulk of the tritium was found in the alcohol moiety. The esterification of *n*-hexadecanol to *n*-hexadecyl palmitate esters catalyzed by the bovine meibomian gland microsomal preparation was investigated, and it was shown that the reaction required exogenous acyl-CoA or ATP and CoA (Kolattukudy & Rogers, 1986). In none of the above preparations were phospholipids excluded, nor were phos-

pholipids or their precursors tested as acyl donors. It is possible that some or all of the biosynthesis of the waxy esters is phospholipid dependent, as reported here.

A complicated situation is also found with plant extracts. For example, it has been reported that multiple routes of wax ester synthesis are found in broccoli (Kolattukudy, 1967). It has been suggested that enzyme-catalyzed acylation of stearyl alcohol proceeds in three ways simultaneously: (1) directly with fatty acids, (2) by acylation with CoA derivatives, or (3) by acylation with phospholipids (Kolattukudy, 1967). However, no compelling evidence for the existence of any of these routes has been presented.

Much attention has been given in the literature to the processing of phospholipid at the *sn*-2 position (Hamberg et al., 1975; Samuelson, 1975). In general, hydrolysis of phospholipids at the *sn*-2 position generates precursors to effectors involved in signal transduction, such as the prostaglandins and leukotrienes (Hamberg et al., 1975). The noteworthy exception here is the lecithin cholesterol acyl transfer (LCAT) reaction, which transfers acyl groups from the *sn*-2 position of lecithin to cholesterol (Jauhianinen & Dolphin, 1986). Here we are concerned with acyl transfers from the *sn*-1 position of phospholipids. There are now at least two acyl transfer reactions of this type: the LRAT reaction and the *n*-dodecyl palmitate ester synthetic system described here. It should be noted that our finding of *n*-dodecyl palmitate synthesis by RPE homogenates was quite fortuitous. It depended on the fact that there was sufficient *n*-dodecanol present in RPE membranes to engage in a reaction with the radioactive lecithin. It would not be surprising if many possible reactions were missed because the alcohol was not present in sufficient amounts to form detectable product. Moreover, rat brain homogenates showed many different products (not including *n*-dodecyl palmitate synthesis) from 1-acyl group transfers, all of whose structures remain to be determined (Furuyoshi and Rando, unpublished experiments). Thus, it appears that there may be a substantial number of new biochemical reactions which are based on acyl group transfer from the *sn*-1 position of phospholipids.

The observation of the biosynthesis of a wax ester by RPE homogenates raises the issue of the possible function of wax esters in this tissue. First, it must be remembered that the RPE is an epithelial tissue and this type of tissue is known to produce wax esters (Doran et al., 1991; Wheatley et al., 1971)). The general role of wax esters is probably that of a membrane sealant or antiwetting agent.

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