IDENTITY OF LYSOLECITHIN ACYLTRANSFERASE AND LECITHIN-CHOLESTEROL ACYLTRANSFERASE

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There is in normal plasma an enzyme activity which converts labeled lysolecithin to lecithin by an energy-independent low density lipoprotein-activated pathway. Studies were undertaken to compare the identity of this enzyme with lecithin-cholesterol acyltransferase. During purification of the enzyme by ultracentrifugation and by chromatography on high density lipoprotein affinity column, DEAE-Sepharose column, and hydroxylapatite column, both the lysolecithin acyltransferase activity and the lecithin-cholesterol acyltransferase activity were found in the same fractions and were enriched to the same extent at each step. The final purified preparation which had 18,000- to 24,000-fold higher specific activities than starting plasma gave a single protein band on polyacrylamide gel electrophoresis and this single band contained both the activities. Also, the effects of pH, heat, and chemical inhibitors on the enzyme activities were similar. Plasma from patients with familial lecithin-cholesterol acyltransferase deficiency also lacked lysolecithin acyltransferase activity. These results indicate that a single enzyme carries out both lecithin-cholesterol acyltransferase and lysolecithin acyltransferase activities. The purified enzyme required apolipoprotein A-I for lecithin-cholesterol acyltransferase activity, but required low density lipoprotein for lysolecithin acyltransferase activity.

Large amounts of lysolecithin are produced continuously in normal human plasma by the actions of lecithin-cholesterol acyltransferase (EC 2.3.1.43) (1) and phospholipase A (EC 3.1.1.4) (2). However, the further metabolism of this potentially cytolytic product in the plasma is not well studied. We have recently demonstrated a lysolecithin acyltransferase activity in normal human plasma which converts lysolecithin to lecithin by an energy-independent pathway (3). This enzyme is associated with the high density lipoprotein and is activated by low density lipoprotein (4). Because the enzyme associates with HDL, it transfers a fatty acid independent of acyl-CoA formation, as does lecithin-cholesterol acyltransferase, we have undertaken systematic studies to determine if this enzyme activity is due to lecithin-cholesterol acyltransferase itself. Our results indicate that the acylation of both lysolecithin and cholesterol are carried out by the same protein in human plasma; whereas the acylation of cholesterol is activated by apolipoprotein A-I, the acylation of lysolecithin is activated by LDL.

MATERIALS AND METHODS

Substrates—Egg lecithin, soy lecithin, and human serum albumin (essentially fatty acid-free) were obtained from Sigma Chemical Co. [4-14C]Cholesterol (specific activity, 54.0 mCi/mmol) was purchased from New England Nuclear Co. Lecithin-cholesterol liposomes were prepared as described before (5). [3H]Lysolecithin was prepared by the action of snake venom phospholipase A on [3H]glycerol-labeled lecithin, which in turn was prepared from germinating soybeans (6) by the following modifications: 50 g of soybeans (W. Atlee Burpee Co., Riverside, Calif.) were washed in running tap water in the presence of small amounts of bleach for 30 min. They were then allowed to absorb 5 ml of [2-3H]glycerol (ICN Pharmaceuticals, Irvine, Calif.) (specific activity, 250 mCi/mmol) dissolved in 5 ml of boiled water. The seeds were then incubated in the dark at 25°C for 3 days. The germinated seeds were homogenized in a blender with 100 ml of cold water (4°C) and the lipids were extracted by the procedure of Bligh and Dyer (7). The chloroform extract was evaporated to dryness, redissolved in 30 ml of chloroform, and loaded onto a neutral alumina column (250 g) which was prepared in chloroform. The neutral lipids were eluted with 1.5 liters of chloroform followed by the elution of lecithin with 3 liters of chloroform:methanol (1:1, v/v) mixture. Fractions of 250 ml were collected and those containing lecithin were pooled and concentrated. The lecithin was further purified on preparative thin layer chromatographic plates (0.5 mm of Silica Gel G, E. Merck, Darmstadt, West Germany) using the solvent system of chloroform:methanol:water (65:25:4 by volume). The bands corresponding to lecithin were eluted using Bligh and Dyer procedure (7). About 175 μCi of [3H]glycerol-labeled chromatographically pure lecithin with a specific activity of 1.9 μCi/μmol were obtained by this procedure. The lecithin preparation was dissolved in 20 ml of ethyl ether and incubated at room temperature for 24 h with 5 mg of crude Crotalus adamanteus venom (Sigma Chemical Co.) dissolved in 0.1 ml of 10 mM CaCl2 in 0.1 M Tris-Cl, pH 7.4. The lecithin preparation was purified on silica gel thin layer plates. Alkaline hydrolysis of the lecithin preparation prepared that over 97% of the label was in the glycerol portion. The labeled lecithin was diluted with unlabeled soy lecithin, prepared by the action of snake venom phospholipase A on unlabeled soy lecithin, to give the desired specific activity.

Acylation—Lyssolecithin was prepared in a similar manner from the [4-C]labeled lecithin. The latter was prepared by incubating the germinating soybeans in presence of sodium [1-14C]acetate (New England Nuclear, Boston, Mass.) (specific activity, 59 mCi/mmol). More than 95% of the label was found to be in the fatty acids after the alkaline hydrolysis of [14C]lyssolecithin.

Apo A-I and LDL—Apolipoprotein A-I was prepared as described by Cheung and Albers (8). For LDL preparation, outdated plasma...
obtained from the Puget Sound Blood Center was first centrifuged at a density of 1.019 g/ml (KBr) at 100,000 x g for 18 h and the infranatant was brought to 1.063 density with KBr and centrifuged at the same speed for 20 h. The floated LDL was removed by tube slicing and recentrifuged at the same density to remove any heavier density components. The LDL preparation was then dialyzed extensively against 0.15 M NaCl-1 mM EDTA, pH 7.4, and concentrated by ultrafiltration using Centriflo membrane filters (CF-50A, Amicon Corp., Lexington, Mass.) to give a protein concentration of approximately 4 mg/ml. The LDL preparations contained small amounts of apolipoprotein A-I (10 to 20 μg of A-I/mg of protein) as determined by the immunodiffusion method (8). However, this LDL preparation did not include the enzyme, which was then added, followed by vortexing for 15 s. LDL preparation from normal human plasma containing 0.4 mg of protein in 0.1 M mercaptoethanol in a final volume of 2.0 ml. Solutions of unlabeled cholesterol (0.257 mg), egg lecithin (2.48 mg), and [4-14C]cholesterol (80 nmol) were mixed and evaporated to dryness in a glass vial and redissolved in 1 ml of absolute ethanol. Of this substrate solution, 25 μl were used for each assay. The reactions were carried out in screw-cap culture tubes (16 x 125 mm) in which the reagents were added in the following order: 1.25 ml of Tris buffer (10 mM Tris, 1 mM EDTA, 140 mM NaCl, pH 7.4), 25 μl of apo A-I solution (0.8 mg/ml in Tris buffer), 0.5 ml of 2% human serum albumin in Tris buffer, and 25 μl of the substrate solution. The reagents were mixed immediately in a vortex mixer, kept at 4°C for 10 min, and 0.1 ml of methanol and the lipids were extracted by precipitation with petroleum ether-ethyl acetate (85:15, v/v) and the radioactivity in each lipid region was determined in a Beckman LS 7000 liquid scintillation counter. Control reactions containing no enzyme were run simultaneously to correct for any nonenzymatic reaction.

### RESULTS

**Lecithin-Cholesterol Acyltransferase Assay**—This activity was determined from the conversion of [4-14C]cholesterol to labeled choles- terol ester in presence of egg lecithin, apo A-I, and serum albumin as described by Albers et al. (5). The reaction mixture contained 15 nmol of unlabeled cholesterol, 5 nmol of [4-14C]cholesterol, 80 nmol of lecithin, and 20 μg of apo A-I, 0.5% of human serum albumin, and 5 mM mercaptoethanol in a final volume of 2.0 ml. Solutions of unlabeled cholesterol (0.257 mg), egg lecithin (2.48 mg), and [4-14C]cholesterol (0.5% of 10 μg) were mixed and evaporated to dryness in a glass vial and redissolved in 1 ml of absolute ethanol. Of this substrate solution, 25 μl were used for each assay. The reactions were carried out in screw-cap culture tubes (16 x 125 mm) in which the reagents were added in the following order: 1.25 ml of Tris buffer (10 mM Tris, 1 mM EDTA, 140 mM NaCl, pH 7.4), 25 μl of apo A-I solution (0.8 mg/ml in Tris buffer), 0.5 ml of 2% human serum albumin in Tris buffer, and 25 μl of the substrate solution. The reagents were mixed immediately in a vortex mixer, kept at 4°C for 10 min, and 0.1 ml of methanol and the lipids were extracted by precipitation with petroleum ether-ethyl acetate (85:15, v/v) and the radioactivity in each lipid region was determined in a Beckman LS 7000 liquid scintillation counter. Control reactions containing no enzyme were run simultaneously to correct for any nonenzymatic reaction.

**Enzyme Assay**—Freshly prepared normal human plasma (in EDTA) from healthy male volunteers. The plasma was first subjected to ultracentrifugation at a KBr density of 1.25 g/ml for 26 h at 100,000 x g. The lipoproteins were separated by tube-slicing and diluted to a density of 1.21 g/ml and subjected to centrifugation again at 150,000 x g for 44 h. The middle clear layer from each tube was taken by tube slicing, dialyzed against 10 mM Tris, 1 mM EDTA, and 150 mM NaCl, pH 7.4, and then subjected to chromatography on HDL-Sepharose column (5 x 20 cm), as described by Albers et al. (5). The enzyme activities were eluted with 0.5 mM sodium taurocholate in water. Each fraction was then dialyzed against the Tris-EDTA buffer and 0.1 ml from each was tested for both activities as described in the text.

**Lysolecithin Acyltransferase Activity**—The pooled eluate from HDL affinity column chromatography. Normal human plasma was obtained from freshly drawn blood (in EDTA) from healthy male volunteers. The plasma was first subjected to ultracentrifugation at a KBr density of 1.25 g/ml for 26 h at 100,000 x g. The lipoproteins were separated by tube-slicing and diluted to a density of 1.21 g/ml and subjected to centrifugation again at 150,000 x g for 44 h. The middle clear layer from each tube was taken by tube slicing, dialyzed against 10 mM Tris, 1 mM EDTA, and 150 mM NaCl, pH 7.4, and then subjected to chromatography on HDL-Sepharose column (5 x 20 cm), as described by Albers et al. (5). The enzyme activities were eluted with 0.5 mM sodium taurocholate in water. Each fraction was then dialyzed against the Tris-EDTA buffer and 0.1 ml from each was tested for both activities as described in the text.

**Enzyme Assay**—Freshly prepared normal human plasma (in EDTA) was used as the enzyme source. The purification procedure involved ultracentrifugation to obtain a 1.21 to 1.25 fraction, and serial chromatography on HDL-Sepharose and a DEAE-Sepharose column. The elution profiles

**Other Methods**—Protein was determined by the procedure of Lowry et al. (9) using bovine serum albumin as standard. Electrophoresis on 7.5% polyacrylamide gels, pH 8.6, was performed by the method of Davis (10), in the absence of urea.
of the two activities at each chromatographic step of one isolation are shown in Figs. 1 to 3. Both activities were recovered in the same fractions in each chromatographic procedure for six different preparations. The second peak of activity shown in the DEAE-Sepharose chromatography step was not observed in all preparations (Fig. 2). There was no difference in the subsequent chromatographic behavior of enzyme activities in this peak, when compared with those in the main peak. The proteins which were not bound to the columns did not contain either activity.

In Table I the specific activities of the pools from each step for lysolecithin acyltransferase and lecithin-cholesterol acyltransferase activities are compared. Both the activities were

![Figure 3. Hydroxyapatite column chromatography of pooled active fractions from DEAE-Sepharose column.](image)

Fig. 3. Hydroxyapatite column chromatography of pooled active fractions from DEAE-Sepharose column. All fractions of DEAE-Sepharose column containing lecithin-cholesterol acyltransferase and lysolecithin acyltransferase activities were pooled and dialyzed against 150 mM NaCl, 4 mM sodium phosphate, pH 6.9, and loaded onto the hydroxyapatite column (2.5 × 10 cm) prepared in the same buffer. The enzyme activities were eluted with a linear gradient of 15 mM to 60 mM sodium phosphate, pH 6.9, in 150 mM NaCl. Each fraction was dialyzed against 10 mM Tris, 1 mM EDTA, and 140 mM NaCl, pH 7.4, and assayed for both the enzyme activities.  --- , lecithin-cholesterol acyltransferase activity;  -- - , lecithin-cholesterol acyltransferase activity;  - - , absorbance at 280 nm;  --- , PO4 gradient (mM).

TABLE I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Lysolecithin acyltransferase activity</th>
<th>Lecithin-Cholesterol acyltransferase activity</th>
<th>Ratio of specific activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Purification&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Specific Activity&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whole plasma</td>
<td>0.078</td>
<td>1</td>
<td>0.1017</td>
</tr>
<tr>
<td>d 1.21 to 1.25 fraction</td>
<td>4.737</td>
<td>61</td>
<td>5.691</td>
</tr>
<tr>
<td>HDL affinity column eluate</td>
<td>72.43</td>
<td>929</td>
<td>87.04</td>
</tr>
<tr>
<td>DEAE-Sepharose column eluate</td>
<td>117.5</td>
<td>1,506</td>
<td>332.4</td>
</tr>
<tr>
<td>Hydroxyapatite column eluate</td>
<td>1,541</td>
<td>24,882</td>
<td>1,708</td>
</tr>
</tbody>
</table>

<sup>a</sup> Nanomoles of lysolecithin acylated/h/mg of protein.
<sup>b</sup> Nanomoles of cholesterol acylated/h/mg of protein.

![Figure 4. Polyacrylamide gel electrophoresis of the purified enzyme preparation.](image)

Fig. 4. Polyacrylamide gel electrophoresis of the purified enzyme preparation. The active fractions from hydroxyapatite chromatography were pooled and concentrated by ultrafiltration through dialysis bags under vacuum. The concentrated sample (50 μg of protein) was then subjected to electrophoresis on 7.5% polyacrylamide gels (10) containing 0.1 mM mercaptoethanol, but no denaturing agent. The electrophoresis was done at 4°C for 2 h and the gels were immediately sliced into 3-mm sections at 4°C. Each slice was cut longitudinally and one-half used for lecithin-cholesterol acyltransferase assay, while the other half was used for lysolecithin acyltransferase assay. The slice was dropped into the complete reaction mixture and was washed with the help of a glass rod and incubated at 37°C for 2 h. Duplicate gel was stained with 0.04% Coomassie brilliant blue. Slice 1 is the top of the separating gel and the tracking dye is in slice 20.  --- , lecithin-cholesterol acyltransferase activity;  -- - , lysolecithin acyltransferase activity.

Enriched to a similar extent at each step except after the DEAE-Sepharose chromatography where the lysolecithin acyl transferase activity was found to be consistently lower than lecithin-cholesterol acyltransferase activity. This was apparently due to some inhibition of lysolecithin acyltransferase activity and not due to loss of enzyme protein because at the next step (hydroxyapatite column) the lysolecithin acyltransferase activity was comparable to that of lecithin-cholesterol acyltransferase. Similar results were obtained with five other preparations. The ratio of activities did not change appreciably from the whole plasma during the purification, except at the DEAE-Sepharose step. These results thus indicate that both activities are co-purified even up to an enrichment of 16,000- to 24,000-fold. The recovery of activities was 5 to 10% from the starting plasma in various preparations.

Assay of Activities in Polyacrylamide Gels—The pooled eluate from the hydroxyapatite column was subjected to electrophoresis on 7.5% polyacrylamide gels at 4°C in presence of 0.1 mM mercaptoethanol and in the absence of denaturing agent (10). As reported earlier (5) the preparation gave a single protein band when stained with 0.04% Coomassie brilliant blue (Fig. 4). A duplicate gel was sliced into 3-mm slices immediately after electrophoresis and each slice then cut into two halves longitudinally. One-half of each slice was used for assay of lecithin-cholesterol acyltransferase and the other half for lysolecithin acyltransferase activity. The slices were directly added to complete reaction mixtures containing labeled substrate and co-factors and crushed with a glass rod in the reaction mixture. Incubations were carried out at 37°C for 2 h. Both activities were found in the same slice which also corresponded to the stained protein band (Fig. 4). Similar results were obtained with three other preparations. These results thus show that both the enzyme activities are due to a single protein.

Effects of pH and Heat on the Two Activities—In order to further substantiate that lysolecithin acyltransferase and lec-
Lysolecithin Acylation by Lecithin-Cholesterol Acyltransferase

Lysolecithin and cholesterol acyl transferase activities are mediated by the same protein, we studied the effects of several physical and chemical agents on the enzyme activities of the purified preparation. The effect of pH on both the reactions was the same (Fig. 5), the optimum activities occurring at pH 8.0 in Britton and Robinson type universal buffer (11). This pH value for lecithin-cholesterol acyltransferase activity corresponds to the value reported by Aron et al. (12) for their purified preparation. With the whole plasma and partly purified preparation (d 1.21 to 1.25 plasma fraction) the pH optimum for both activities was approximately 7.4 (results not shown).

When the purified enzyme preparation was kept for 20 min at various temperatures (Fig. 6, left), there was no loss of either activity up to 30°C but both activities were rapidly lost above 37°C. No significant difference was noted in the heat lability of the two enzyme activities at any temperature. Their inactivation profiles were also similar when the purified enzyme preparation was heated at 45°C for various periods of time (Fig. 6, right).

The effect of some chemical inhibitors on both activities was also tested. p-Hydroxymercuric benzoate (0.5 mM), phenyl methyl sulfonyl fluoride (2.5 mM), sodium deoxycholate (0.1 mM), and Zn²⁺ (10 mM) inhibited both activities by more than 90% (results not shown).

Assay of Lysolecithin Acyltransferase in Lecithin-Cholesterol Acyltransferase-deficient Plasma—In further experiments to prove that the acylation of lysolecithin and cholesterol is carried out by the same enzyme, we estimated the lyssolecithin acyltransferase activity in plasma from three patients with familial lecithin-cholesterol acyltransferase deficiency. In one experiment the lyssolecithin acyltransferase activity was assayed in the whole plasma from a lecithin-cholesterol acyltransferase-deficient patient in the presence and absence of added LDL from normal human plasma. As seen in Table II, there was no labeled lecithin formation in the patient’s plasma even in presence of normal LDL. Addition of the patient’s plasma to the normal plasma did not result in any decrease of lyssolecithin acylation, indicating that the lack of activity is not due to the presence of an inhibitor in the plasma of the patient. In another experiment lyssolecithin acylation was assayed in plasma from one normal control and from two lecithin-cholesterol acyltransferase-deficient offspring of the control. These patients also lacked lyssolecithin acyltransferase activity, further supporting the fact that lyssolecithin acyltransferase activity is due to the same enzyme which carries out the esterification of cholesterol.

Co-factor Requirements for Lysolecithin Acylation—Our previous results showed that lyssolecithin acylation in normal human plasma required LDL for maximal activity (4). In further experiments to corroborate this observation, we found that the purified enzyme preparation also requires LDL for lyssolecithin acylation (Table III). Apo A-I, which is obligatory

<table>
<thead>
<tr>
<th>Table II</th>
<th>Assay of lecithin synthesis in lecithin-cholesterol acyltransferase-deficient plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Addition</td>
<td>Lecithin synthesis (nmol/h/ml plasma)</td>
</tr>
<tr>
<td>Experiment I: Plasma (0.2 ml)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.85</td>
</tr>
<tr>
<td>LDL</td>
<td>10.50</td>
</tr>
<tr>
<td>Patient (M. R)</td>
<td>0.05</td>
</tr>
<tr>
<td>Patient (M. R) LDL</td>
<td>0.36</td>
</tr>
<tr>
<td>Control</td>
<td>7.85</td>
</tr>
<tr>
<td>Experiment II: Plasma donor</td>
<td></td>
</tr>
<tr>
<td>D. F. (control)</td>
<td>8.89</td>
</tr>
<tr>
<td>D. H. (patient)</td>
<td>0.35</td>
</tr>
<tr>
<td>S. F. (patient)</td>
<td>0.25</td>
</tr>
</tbody>
</table>

For Experiment I a sample of plasma prepared in acid-citrate dextrose from a patient with familial lecithin-cholesterol acyltransferase deficiency was obtained from Drs. John Glomset and Kaare Norum. The sample was kept at 4°C until the assay which was performed within 4 days of plasma preparation. Control plasma from a normal female donor which was prepared in a similar manner was assayed simultaneously for lyssolecithin acyltransferase activity using 0.2-ml aliquots. In Experiment II control and lecithin-cholesterol acyltransferase-deficient plasma samples were obtained from Dr. J. Frohlich and assays were carried out using 0.1-ml aliquots of plasma. The reaction mixtures contained plasma from the patient or control, 0.17 μmol of [³H]lyssolecithin, 10 μmol of Tris-Cl, pH 7.4, and, where indicated, LDL (1.019 to 1.063 density) from pooled normal plasma at a concentration of 0.4 mg of protein/reaction mixture. Incubations (in duplicate) were carried out for 2 h at 37°C.

Fig. 5. Effect of pH on lecithin-cholesterol-acyltransferase and lysolecithin acyltransferase. The pooled fractions from the HDL affinity column were dialyzed against 10 mM Tris, 1 mM EDTA, and 140 mM NaCl, pH 7.4, and 0.1 ml of the dialyzed sample was used for an enzyme assay. The assay of both enzyme activities was as described in the text, except that the buffer in the reaction mixture was replaced by the universal buffer (Britton and Robinson type) (11) at the indicated pH. ●-●, lyssolecithin acyltransferase; ○—○, lecithin-cholesterol acyltransferase.

Fig. 6. Effect of heat on acylation of lyssolecithin and cholesterol by purified enzyme preparation. Left, effect of heating the purified enzyme preparation at various temperatures. Aliquots of the enzyme preparation were preincubated at 45°C for various periods of time. A sample of enzyme preparation preincubated at 4°C was taken as 100% enzyme activity and all other values were expressed as percentages of this value. ●-●, lyssolecithin acyltransferase; ○—○, lecithin-cholesterol acyltransferase.

Experiment | Plasma donor |
--- | --- |
Control | 6.85 |
LDL | 10.50 |
Patient (M. R) | 0.05 |
Patient (M. R) LDL | 0.36 |
Control | 7.85 |

Addition | Lecithin synthesis (nmol/h/ml plasma) |
--- | --- |
Control | 6.85 |
LDL | 10.50 |
Patient (M. R) | 0.05 |
Patient (M. R) LDL | 0.36 |
Control | 7.85 |
D. F. (control) | 8.89 |
D. H. (patient) | 0.35 |
S. F. (patient) | 0.25 |
for the esterification of cholesterol and for phospholipase activity, could not stimulate lyssolecithin acylation. Addition of LDL alone was sufficient for the lecithin formation, and addition of apo A-I with LDL did not stimulate the activity further. Treatment of LDL with 0.1 mg of IgG from rabbit anti-A-I sera did not have a significant effect on the LDL-activated lyssolecithin acylation although it inhibited 75% of the A-I-activated cholesterol esterification (Table III).

**Mechanism of Lyssolecithin Acylation**

There are two known pathways in the biological systems for the acylation of lyssolecithin to lecithin (13). One pathway involves the formation of an acyl-CoA and the esterification of lyssolecithin with the activated fatty acid, while the other pathway is energy-independent and involves a transesterification between two lyssolecithin molecules, producing one molecule of lecithin and one molecule of glyceryl phosphorylcholine. The acylation of lecithin in human plasma does not require ATP and CoA (3). On the basis of this observation and the preliminary results from snake venom treatment of the lecithin synthesized from [acyl-14C]lyssolecithin, we postulated that the lecithin is formed by transesterification between two lyssolecithin molecules. However, a more direct method of determining the reaction mechanism is now used, utilizing a doubly labeled lyssolecithin as substrate. The lyssolecithin obtained 14C in the fatty acid side chain and 3H in the glycerol portion. A transesterification between two such lyssolecithin molecules should result in a doubling of 3H/14C ratio in the synthesized lecithin when compared to the ratio in lyssolecithin. However, the results presented in Table IV show that the ratio of 3H/14C in lecithin is the same as in the substrate lyssolecithin, indicating that the acyl group for lyssolecithin acylation is coming from endogenous sources. Although it is not possible to identify the endogenous source of acyl group from the results, it is likely that one of the LDL lipids is acting as acyl donor in the reaction, because the amount of lipid present in the enzyme preparations used here is negligible and because there is little synthesis of lecithin in the absence of LDL.

**TABLE III**

Co-factor requirement for lecithin synthesis by purified enzyme preparation

<table>
<thead>
<tr>
<th>Addition</th>
<th>Lecithin synthesis nmol/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme only (6 μg protein)</td>
<td>0.097</td>
</tr>
<tr>
<td>+ Apo A-I (20 μg)</td>
<td>0.102</td>
</tr>
<tr>
<td>+ LDL (0.5 mg)</td>
<td>9.54</td>
</tr>
<tr>
<td>+ LDL + apo A-I</td>
<td>9.12</td>
</tr>
<tr>
<td>+ LDL + IgG from rabbit A-I</td>
<td>8.22*</td>
</tr>
<tr>
<td>+ LDL + IgG from nonimmune A-I</td>
<td>7.30</td>
</tr>
<tr>
<td>LDL only</td>
<td>0.0</td>
</tr>
<tr>
<td>+ Apo A-I</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*This amount of IgG from anti-A-I sera inhibited 75% of the lecithin-cholesterol acyltransferase activity.

**DISCUSSION**

The enzymatic acylation of lyssolecithin has been shown in several tissues (13) and has been implicated in membrane phospholipid turnover (13, 14), intestinal absorption of phospholipids (15), and in increased synthesis of membrane phospholipids in response to external stimuli (16, 17). We have recently demonstrated acylation of lyssolecithin in normal human plasma (3, 4). Because of the possible importance of the reaction in the metabolism of lyssolecithin which is continuously produced in the plasma by the actions of lecithin-cholesterol acyl transferase and phospholipase A, the characterization of the enzyme catalyzing this reaction is of great interest. The results presented here indicate that the same enzyme which is responsible for the production of most of the lyssolecithin in the plasma can carry out the acylation of lyssolecithin to lecithin. The identical profiles of lyssolecithin acyltransferase and lecithin-cholesterol acyl transferase activities during purification by different chromatographic procedures, as well as the presence of both activities in the same protein band on the polyacrylamide electrophoretic gels, indicates that a single protein carries out both the reactions. Further evidence that both activities are carried out by the lecithin-cholesterol acyl transferase protein is obtained by the results on the effects of pH, heat, and inhibitors on the purified enzyme preparation, and from the absence of lyssolecithin acyltransferase activity in patients with familial lecithin-cholesterol acyltransferase deficiency.

**TABLE IV**

Mechanism of lyssolecithin acylation

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>LDL affinity column fraction</th>
<th>Lysolecithin acylated (nmol/2 h)</th>
<th>Lysolecithin (A)</th>
<th>Lecithin (B)</th>
<th>3H/14C ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 1.21 to 1.25 fraction</td>
<td>No</td>
<td>1.143</td>
<td>0.1939</td>
<td>0.2331</td>
<td>1.1505</td>
</tr>
<tr>
<td>d 1.25 to 1.25 fraction</td>
<td>Yes</td>
<td>7.419</td>
<td>0.1783</td>
<td>0.1644</td>
<td>0.9266</td>
</tr>
<tr>
<td>HDL affinity column eluate</td>
<td>No</td>
<td>0.139</td>
<td>0.1945</td>
<td>0.2290</td>
<td>1.1773</td>
</tr>
<tr>
<td>HDL affinity column eluate</td>
<td>Yes</td>
<td>2.954</td>
<td>0.1752</td>
<td>0.1683</td>
<td>0.9090</td>
</tr>
</tbody>
</table>

Further evidence that both activities are carried out by the lecithin-cholesterol acyl transferase protein is obtained by the results on the effects of pH, heat, and inhibitors on the purified enzyme preparation, and from the absence of lyssolecithin acyltransferase activity in patients with familial lecithin-cholesterol acyltransferase deficiency.

Purified lecithin-cholesterol acyltransferase has been shown to have phospholipase A activity, especially in the absence of unesterified cholesterol (12, 18). The present research shows for the first time that the purified lecithin-cholesterol acyltransferase also has lyssolecithin acyltransferase activity. The requirement of apoprotein A-I for the lecithin-cholesterol acyltransferase and phospholipase activities of the purified enzyme preparation is well established (5, 12, 18, 19) although the mechanism of the apoprotein-dependent activity or its physiological importance is not clear. Unlike this reaction, however, the acylation of lecithin requires the presence of LDL. LDL alone stimulates lyssolecithin acylation by the purified enzyme, and addition of apo A-I to the LDL-containing reaction mixtures did not have any further effect on lyssolecithin acylation. Pretreatment of the LDL with IgG from rabbit anti-human A-I serum did not have any inhibitory effect on this reaction. Thus it appears that the enzyme preferentially esterifies cholesterol in presence of HDL, but esterifies lyssolecithin in presence of LDL.

The precise role of LDL in lyssolecithin acylation is not
clear, but its affinity to lysolecithin has been shown to be higher than that of serum albumin (20) and therefore it may be providing a binding surface for the substrate. It also appears likely that the LDL acts as an acyl donor for the lysolecithin acylation reaction, because in a reaction mixture with purified enzyme, the LDL is the only source of acyl groups except for lysolecithin itself. Although our preliminary studies suggested that a transesterification between two lysolecithin molecules may be taking place (3), the results presented here using doubly labeled lysolecithin show that the acyl group may be coming from LDL. There are several potential acyl donors in LDL: phospholipids, cholesteryl esters, triglycerides, and monoo- and diglycerides. However, since lecithin is the substrate for lecithin-cholesterol acyltransferase and phospholipase reactions, it may provide the acyl group for lysolecithin acylation. Thus it is tempting to speculate that the formation of an acyl-enzyme intermediate with the 2-acyl group of lecithin in lecithin-cholesterol acyltransferase reaction. Such a mechanism is also supported by the fact that the acyl acceptor specificity of lecithin-cholesterol acyltransferase is not strictly toward cholesterol, but other sterols and even fatty alcohols can accept the acyl group (21). The formation of an acyl intermediate is also supported by the inhibition of enzyme activity by serine esterase inhibitors like diisopropyl fluorophosphate and diethyl p-nitrophenyl phosphate. It should be pointed out that the concentration of lysolecithin used for the lysolecithin acyltransferase assay (0.5 mM) is inhibitory for the esterification of cholesterol. Thus, in accordance with the results of Fielding et al. (22) we found that lysolecithin at concentrations as low as 3 μM inhibited the lecithin-cholesterol acyltransferase activity by over 50% in the absence of serum albumin. This inhibitory action of lysolecithin can at least partly be explained by the postulate that lysolecithin competes with cholesterol for the acyl group during the transfer reaction.

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