Reactivity of Human Lecithin Cholesterol Acyltransferase with Synthetic Micellar Complexes of Apolipoprotein A-I, Phosphatidylcholine, and Cholesterol*

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Micellar, discoidal complexes of human apolipoprotein A-I (apo A-I) with phosphatidylcholines and cholesterol, prepared by the method described in the preceding paper (Matz, C. M., and Jonas, A. (1982) J. Biol. Chem. 257, 4535-4540), were used as substrates for human lecithin cholesterol acyltransferase, purified 10,000-fold.

The micellar complexes of apo A-I + egg yolk-phosphatidylcholine-cholesterol were compared to commonly used substrates of lecithin cholesterol acyltransferase, consisting of small unilamellar vesicles of egg yolk-phosphatidylcholine and cholesterol in the presence of apo A-I. Under identical reaction conditions, the micellar complexes had 4- to 5-fold higher initial velocities and 3-fold greater capacities for cholesterol ester formation than did the corresponding vesicular substrates.

Micellar complexes, labeled with 5-dimethylamino-naphthalene-1-sulfonyl fluorescent groups in the apo A-I, as substrates for lecithin cholesterol acyltransferase, were isolated by density gradient centrifugation. After reaction with lecithin cholesterol acyltransferase, they had a shorter rotational relaxation time (290 ns) and smaller Stokes radius (47 Å) than the unreacted complexes (530 ns and 57 Å, respectively). The characteristic stacked, discoidal particles observed on electron micrographs of negatively stained micellar, unreacted complexes disappeared after enzymatic reaction and were replaced by structures with spheroidal shapes.

Lecithin cholesterol acyltransferase (EC 2.3.1.43) is the plasma enzyme responsible for the formation of most of the cholesterol esters found in circulation (1). The normal substrates for this enzyme are nascent and mature high density lipoproteins which provide the lecithin and cholesterol reagents and contain the activator apolipoprotein A-I (2).

Studies on the plasma of patients with familial lecithin cholesterol acyltransferase deficiency (3, 4), or with alcoholic hepatitis (5, 6) have shown the presence of discoidal particles of HDL density, rich in phospholipids and apo E but low in cholesterol esters. Replacement of lecithin cholesterol acyltransferase in the plasma of patients with the familial deficiency and normalization of liver function (with restored enzyme activity) in the alcoholic hepatitis patients, lead to marked changes in the composition and morphology of these particles with HDL density, formation of spherical particles rich in apo A-I and in cholesterol esters and depletion of apo E. The similarity of the discoidal particles to nascent HDL from perfused rat liver (7) and their transformation into spherical particles by the action of lecithin cholesterol acyltransferase support the hypothesis that the activity of this enzyme is essential for the biogenesis of circulating HDL, not only from nascent HDL secreted by liver but also from surface components of triglyceride-rich lipoproteins released during lipolysis (8).

The study of the properties of this very important enzyme has been limited by the nature of the available substrates. Unfractionated plasma or isolated HDL are physiological substrates but with variable and uncontrollable lipid compositions (9). Small vesicles of PC and cholesterol with added apo A-I, as substrates for lecithin cholesterol acyltransferase (10), offer the advantage of a chemically defined system but present the problems of stability and reproducibility of vesicle preparations, a limited capacity for cholesterol ester storage, and a major morphological difference from the natural substrates.

In this paper, we describe the properties of synthetic, discoidal complexes of apo A-I-PC-cholesterol, chemically defined, stable analogues of nascent HDL particles, as substrates for lecithin cholesterol acyltransferase.

EXPERIMENTAL PROCEDURES

Preparation of Micellar Complexes and Vesicles of Aps A-I-PC-Cholesterol—Human apo A-I or apo A-I-Dns were prepared, characterized, and reacted with PC-cholesterol-sodium cholate mixtures as described in the preceding paper (11). Different PCs were used in the complex formation: DMPC, DPDC, and egg-PC. Cholesterol was mixed with ["C"]cholesterol in redistilled chloroform; its specific activity (300-400 cpm/nmol) was determined by liquid scintillation counting and dry weight of cholesterol. All the lipid mixtures contained 20 mol % cholesterol. The aqueous dispersions of the lipids and the complexes were prepared in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM NaNO3, 150 mM NaCl buffer.

After dialysis to remove sodium cholate, the reaction mixtures were fractionated by gel chromatography on a Bio-Gel A-5m column (1.8 × 65 cm). Protein was determined by a modified Lowry procedure (12) using BSA or human apo A-I as the standards. The fractions with relatively constant cholesterol/apo A-I ratios were pooled, and

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‡ The abbreviations used are: HDL, high density plasma lipoprotein; apo A-I, major protein component of HDL; apo E, apolipoprotein found in very low density lipoproteins, nascent HDL, and in certain abnormal HDLs; Dns, fluorescent dansyl (5-dimethylamino-naphth-

len-1-sulfonyl) label; PC, phosphatidylcholine; egg-PC, egg yolk phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPDC, dipalmitoylphosphatidylcholine; BSA, bovine serum albumin.
the PC content was determined by the organic phosphorus assay of Chen et al. (13).

The pooled fractions were concentrated by dialysis in the presence of polyethylene glycol and were changed to a 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM NaCl buffer prior to use as substrates for lecithin cholesterol acyltransferase. These complexes were stored at 4 °C for 7 days. The vesicles were stable for at least 6 weeks at 4 °C, maintaining their density and integrity during density gradient ultracentrifugation.

Small unilamellar vesicles of egg-PC and cholesterol were prepared by sonication (14) using [14C]cholesterol (300-400 cpm/nmol) and a 4,1 PC/cholesterol molar ratio. The dispersions of the lipids contained 0.5 mM cholesterol in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM NaCl. Sonication was performed with an Heat Systems-Ultrasonics Inc., sonifier, Model W185, at a power of 70 watts, at temperatures not exceeding 25 °C, under a nitrogen atmosphere, using short 2- min bursts during 30 min. The vesicles were centrifuged at 15,000 rpm for 30 min, and the supernatant, after passage through a Millipore filter (0.20 μm), was fractionated on a Sepharose CL-4B column (1.8 × 50 cm). Only the fractions corresponding to small unilamellar vesicles (14, 15) were pooled and used as substrates for lecithin cholesterol acyltransferase. The isolated vesicles were stored at 4 °C and were used within 24 h. The purity and composition of the lipids extracted from small unilamellar vesicles before or after enzymatic reaction was determined by TLC in a chloroform/methanol/H2O (60:25:5, v/v/v) solvent.

Human apo A-I in a stock solution, whose concentration was determined from the extinction coefficient of the protein (11.5 μM × 107 g·cm2 at 280 nm), was added to the egg-PC-cholesterol vesicles to give a final PC/a-I molar ratio of 80:1, i.e. equivalent to the PC/apo A-I molar ratio of the isolated micellar complexes (11). The vesicle-apo A-I mixture was incubated at 37 °C for 1 h prior to reaction with the enzyme. Under these experimental conditions, the lipid vesicle structure is retained as judged by the elution position of the egg-PC from the Sepharose CL-4B column and from the detailed investigation of the system by other authors (16, 17).

Lecithin Cholesterol Acyltransferase Assay—Initial velocity determinations of enzymatic activity were performed in capped glass tubes. The incubation mixtures contained 25 nmol of cholesterol in complexes with apo A-I and PC, 2.0 mg of defatted BSA, 4 μM mercaptoethanol, 3 units of enzyme activity (1 unit = 1 nmol of cholesterol ester formed/h) in a final volume of 0.5 to 1.0 ml of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM NaCl buffer. The reactions were initiated by adding enzyme at 37 °C, and were carried out for 1, 30, 60, 120, and 240 min, in a shaker water bath. The reactions were stopped and the lipids were extracted with chloroform/methanol (2:1, v/v) according to the procedure of Folch et al. (18). Carrier cholesterol and cholesterol ester (>100 μg each) were added to the organic solution of the lipids prior to drying. The lipids were dissolved in n-heptane and were spotted on TLC silica gel plates which were developed either in ether/diethyl ether/acetone (90:15:5, v/v/v) solvent system. The lipid spots were visualized by short exposure to I2 vapors; the spots were scraped into vials, and [14C]cholesterol and [3H]labeled cholesterol esters were determined from counts/min in a dioxyne/naphthalene/2.5-diphenyloxazole scintillation fluid. All reactions were performed in duplicate. Initial velocities were linear for about 1 to 2 h, and were proportional to enzyme concentrations.

The studies of enzyme kinetics as a function of substrate concentrations were performed over a range of complex and vesicle concentrations containing from 5 to 60 μM cholesterol. The final incubation volume of 0.5 ml contained 7.2 units of purified enzyme. The final incubation volume of 0.5 ml contained 7.2 units of purified enzyme.

Purification of Lecithin Cholesterol Acyltransferase—The purification scheme is a modification of the procedures of Albers et al. (19) and Chung et al. (16). Approximately 1 liter of pooled human serum was obtained from local hospital pathology labs. The density of the serum was adjusted to 1.21 g/ml and centrifuged at 55,000 rpm for 30 h in a Ti-55.2 rotor at 10 °C. The top lipoprotein layer was removed and reisolated in a linear NaBr gradient by centrifugation in a SW 41 rotor at 41,000 rpm, 20 °C, for 84 h.

Rotational relaxation times and electron micrographs of the cholesterol ester containing complexes were obtained as described in the preceding paper (11).

RESULTS

Table I summarizes the purification scheme for human lecithin cholesterol acyltransferase. Typically, a 15 to 20% yield and over 10,000-fold purification were obtained after the hydroxylapatite column step. The final enzyme fraction gave a single protein band on polyacrylamide gel electrophoresis in 8 M urea or in 0.1% sodium dodecyl sulfate in the presence of 1% β-mercaptoethanol, when 40 μg of protein were applied to the gels. By reference to transferrin, BSA, ovalbumin, and chymotrypsinogen (Sigma) migration on the sodium dodecyl sulfate gels, the purified enzyme had Mr = 60,000 ± 4,000, which is in reasonable agreement with reported molecular weights for human lecithin cholesterol acyltransferase (20).

The kinetics of the reaction of the purified enzyme with three micellar complexes of apo A-I-PC cholesterol (containing egg-PC, DPPC, or DMPC) and with small vesicles of egg-PC-cholesterol plus apo A-I is shown in Fig. 1 and is summarized in Table II. Under the experimental conditions used, the velocity of the enzymatic reactions was only linear during the initial transformation of about 10% of the cholesterol into cholesterol esters. The subsequent decrease in rates was due to product inhibition, since the enzyme activity remained stable (within 90%) over the entire period of the experiment. After 24 h, the cholesterol ester yields were 90, 87, and 42% of the total, for the egg-PC, DPPC, and DMPC micellar complexes, respectively. Also for these particles, the reactions...
TABLE I

Purification of human lecithin cholesterol acyltransferase

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Yield</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>nmol CE/h</td>
<td>nmol CE/h/mg</td>
<td>%</td>
<td>fold</td>
</tr>
<tr>
<td>Human serum</td>
<td>85,400</td>
<td>6,234</td>
<td>0.073</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>Density, 1.21-1.25 g/ml</td>
<td>858</td>
<td>6,991</td>
<td>0.072</td>
<td>97.7</td>
<td>330</td>
</tr>
<tr>
<td>Affi-Gel blue</td>
<td>242</td>
<td>6,832</td>
<td>24.1</td>
<td>93.5</td>
<td>330</td>
</tr>
<tr>
<td>DE52</td>
<td>13.5</td>
<td>3,159</td>
<td>234</td>
<td>50.7</td>
<td>3,305</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>1.71</td>
<td>1,279</td>
<td>748</td>
<td>20.5</td>
<td>10,245</td>
</tr>
</tbody>
</table>

*CE, cholesterol ester.

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FIG. 1 (left). Initial velocities of lecithin cholesterol acyltransferase reaction with micellar complexes of apo A-I-PC-cholesterol and with small, unilamellar vesicles of egg-PC-cholesterol with added apo A-I*. The reactions were carried out at 37 °C using the same reaction conditions (see "Experimental Procedures"), identical amounts of substrate PC and cholesterol, and comparable amounts of apo A-I (see Table I). In these experiments, the enzyme was saturated with substrate. Percentage of cholesterol converted to cholesterol ester in: apo A-I-egg-PC-cholesterol (○); apo A-I-DPPC-cholesterol (○); apo A-I-DMPC-cholesterol (○), and small, unilamellar vesicles of egg-PC-cholesterol with added apo A-I (△).

FIG. 2 (center). Kinetics of lecithin cholesterol acyltransferase reaction with micellar (○) and vesicular (△) complexes of apo A-I-egg-PC-cholesterol as a function of cholesterol concentration. The stoichiometry of the substrates was 1:76:1, apo A-I/egg-PC/cholesterol (molar ratio). Initial velocities were measured as described under "Experimental Procedures" in a range of cholesterol concentrations from 5 to 60 μM with 7.2 units of enzyme activity in 0.5-ml reaction volumes. The Vmax are 2.17 and 7.14 nmol of cholesterol ester formed/h, and the Km are 47.6 and 23.8 μM cholesterol, for the vesicular and micellar complexes, respectively.

FIG. 3 (right). Density gradient ultracentrifugation of micellar complexes before (A) and after (B) reaction with lecithin cholesterol acyltransferase. Protein was detected by the fluorescence of the Dns probe at 500 nm; cholesterol and cholesterol esters were measured by scintillation counting after extraction and separation by TLC.

TABLE II

Kinetics of enzymatic reaction with micellar and vesicular substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>PC*</th>
<th>Cholesterol*</th>
<th>Apo A-I*</th>
<th>Vmax*</th>
<th>Maximum* cholesterol converted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small vesicles, egg-PC-cholesterol + apo A-I</td>
<td>320</td>
<td>81</td>
<td>3.4</td>
<td>26.5</td>
<td>37</td>
</tr>
<tr>
<td>Micellar complex, egg-PC-cholesterol-apo A-I</td>
<td>321</td>
<td>81</td>
<td>3.4</td>
<td>135.5</td>
<td>99</td>
</tr>
<tr>
<td>Micellar complex, DPPC-cholesterol-apo A-I</td>
<td>316</td>
<td>80</td>
<td>2.6</td>
<td>95.8</td>
<td>99</td>
</tr>
<tr>
<td>Micellar complex, DMPC-cholesterol-apo A-I</td>
<td>314</td>
<td>80</td>
<td>2.3</td>
<td>22.8</td>
<td>(67)</td>
</tr>
</tbody>
</table>

*Phosphatidylcholine, cholesterol, and apo A-I contents of the substrates in terms of their concentrations in the reaction mixture.

*Initial velocity.

*Extrapolated to infinite time (using a reciprocal time plot). Extrapolation for the DMPC-containing complex was based on the last two time points.

*CE, cholesterol esters.

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continued toward 100% conversion of the cholesterol. With the small vesicle substrate, on the other hand, the enzymatic reaction stopped after 37% conversion of cholesterol into cholesterol esters.

The kinetics of the lecithin cholesterol acyltransferase reaction with micellar or vesicular substrates (apo A-I-egg-PC-cholesterol) as a function of cholesterol concentration is shown in a double reciprocal plot in Fig. 2. For the chosen conditions, the enzymatic reaction follows Michaelis-Menten kinetics with both substrates. The Vmax are 2.17 and 7.14 nmol of...
cholesterol ester formed/h, and the $K_m$ are 47.6 and 23.8 $\mu$M cholesterol, for the vesicular and micellar complexes, respectively. Thus, under identical reaction conditions, the enzymatic reaction is more efficient and the lecithin cholesterol acyltransferase is about four times more active with the micellar than with the vesicular substrates.

Fig. 3 shows the density gradient isolation of apo A-I-Dns-egg-PC-cholesterol micellar complexes before and after conversion of 80% of the cholesterol to cholesterol esters, in the presence of BSA. The isolated product had the composition and properties given in Table III and contained apo A-I as the sole protein, as indicated by polyacrylamide gel electrophoresis in sodium dodecyl sulfate.

The composition of the reacted complex clearly shows the parallel disappearance of PC and cholesterol, the formation of cholesterol esters, and little associated lysophosphatidylcholine (from TLC analysis). The total fluorescence of apo A-I in the reacted sample was about 20% lower than in the original complex but the amount of radioactivity associated with the complexes remained the same. The changes in fluorescence intensity may be due to static fluorescence quenching of the chromophore in an altered protein conformation. The density of the reacted complex is slightly increased in agreement with the increase in protein mass, relative to the total lipid.

Rotational relaxation times determined by the Perrin method, from the fluorescence polarization of the Dns chromophore in media of different viscosities (21), and from the fluorescent lifetimes of the probe, show a marked decrease for the reacted complexes. This decrease in rotational relaxation time and in Stokes radius is due to the small decrease in mass of the particles (loss of PC, as lysophosphatidylcholine) but most importantly to the change in shape.

The change in shape is evident in the electron micrographs of negatively stained samples. Three different samples reacted with the enzyme were negatively stained and 20 to 40 grid squares were examined per grid. No stacking and no discoidal particles were detected in the samples reacted with the enzyme, as contrasted to the original micellar complexes which always had the characteristic stacked, lamellar structures (see Fig. 4). The particles in both types of complexes appear heterogeneous and have dimensions from 45 (width) $\times$ 130-180 (diameter) $\AA$ for the discoidal substrates and 120-160 (diameter) $\AA$ for the products. In the latter case, the particles have a circular cross-section, but it is impossible to tell from the electron micrographs alone if they are spherical or oblate-ellipsoidal.

### DISCUSSION

From the results shown in Figs. 1 and 2 and in Table II, it is evident that the micellar complexes of apo A-I-egg-PC-cholesterol are far better substrates for lecithin cholesterol acyltransferase than vesicles of essentially the same chemical composition. The differences in initial velocities and in kinetic parameters are due to the different physical form of the substrates: a discoidal micelle versus a single bilayer vesicle.

The small vesicles have 30 to 40% of their PC in the internal monolayer of the vesicles (22), i.e. not accessible to the enzyme. All the cholesterol is potentially available because of the relatively rapid transbilayer diffusion of this lipid (23). Nevertheless, because the enzymatic reactions were carried out under conditions where the enzyme is saturated or ap-

### TABLE III

*Properties of micellar complexes before and after reaction with lecithin cholesterol acyltransferase*

<table>
<thead>
<tr>
<th>Complexes before enzyme</th>
<th>Complexes after enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stoichiometry$^a$ of apo A-I/egg-PC/C/CE$^a$</td>
<td>1.80:20:0 (molar ratio) 1.65:4:16 (molar ratio)</td>
</tr>
<tr>
<td>Density</td>
<td>1.092 g/ml 1.101 g/ml</td>
</tr>
<tr>
<td>Apo A-I-Dns fluorescent lifetime$^b$</td>
<td>11.3 ns 11.2 ns</td>
</tr>
<tr>
<td>Rotational relaxation$^b$ time</td>
<td>576 ns 287 ns</td>
</tr>
<tr>
<td>Stokes radius$^b$</td>
<td>57 $\AA$ 47 $\AA$</td>
</tr>
<tr>
<td>EM shape</td>
<td>Discoidal Spheroidal</td>
</tr>
<tr>
<td>EM dimensions</td>
<td>45 (width), 130-180 (diameter) $\AA$ 120-160 (diameter) $\AA$</td>
</tr>
</tbody>
</table>

$^a$Apo A-I was determined by the Markwell *et al.* (12) procedure; phospholipid was measured by the method of Chen *et al.* (13); and cholesterol and cholesterol esters were determined from counts/min after separation on TLC plates.

$^b$C, cholesterol; CE, cholesterol esters; EM, electron micrograph.

$^c$Determined at 25 °C in the phase mode.

$^d$Calculated from the rotational relaxation times by assuming spherical shapes.

**Fig. 4.** Electron micrographs of negatively stained preparations of micellar complexes of apo A-I-egg-PC-cholesterol before (left) and after (right) reaction with lecithin cholesterol acyltransferase. Magnification is $\times$ 240,000.
proaching saturation with substrate a small difference in the amount of the available substrate should not be significant. Rather, the difference in the initial reaction rates must be sought in the activity of the enzyme.

Small, unilamellar vesicles of egg-PC and cholesterol (20 mol %) have been shown to bind a maximum of 1 apo A-I/270 to 300 egg-PC molecules (16, 17); the micellar complexes, on the other hand, contain 1 apo A-I/80 egg-PC molecules. That is, under the same conditions, the micellar complexes have 3 to 4 times more apo A-I complexed with lipid than the vesicles. Since the concentration of apo A-I in our experiments is well above that required for the optimal activation of vesicles (1 apo A-I/500 egg-PC molecules) (24), and since unbound apo A-I does not affect the activity of the enzyme in the vesicle assay system (24), we conclude that the 4- to 5-fold higher rate of the enzymatic reaction with the micellar substrates is largely due to the increased activity of lecithin cholesterol acyltransferase in the presence of higher levels of lipid-bound apo A-I. In addition, there is the possibility that apo A-I may adopt a conformation that makes it a better activator of the enzymatic reaction when present in micellar complexes than when it is bound to vesicles.

Work published in the past on the acyl donor specificity of the lecithin cholesterol acyltransferase reaction indicated that differences among PC substrates were due to the degree of unsaturation of the acyl chain in the 2-position of the phosphatidyl choline (25), and to the physical state of the lipid preparation (2, 26). However, at that time, these authors were not aware that some of their vesicular lipid preparations could be transformed into micellar particles when exposed to apo A-I, at or above the gel to liquid-crystalline phase transition temperature of the lipid. For example, dipalmitoylphosphatidylcholine7 and DMPC (27) vesicles spontaneously form micellar particles with apo A-I above their transition temperatures. Thus, in several of the reported cases, lecithin cholesterol acyltransferase activity comparisons were made on distinct particles, micelles or vesicles, which we demonstrated here to have quite different activities. We observe the following decrease in the initial velocities of egg-PC, DPPC, and DMPC. An examination of the acyl donor specificity in the micellar particles was not an objective of this paper; nevertheless, these results suggest that a re-examination of this problem is in order and can now be made with uniform substrates, where the ability of apo A-I to bind to or penetrate a PC bilayer is not a factor as it may well be in a vesicular system (2, 26).

Another important observation we made concerns the extent of the enzymatic reaction. With the vesicular substrates, reaction stops when 37% of the cholesterol is converted into cholesterol esters, whereas with at least two of the micellar substrates the reaction proceeds to completion. Recently, Chajek et al. (28) have pointed out that vesicular substrates of lecithin cholesterol acyltransferase have a limited capacity for cholesterol esters in the absence of cholesterol ester acceptors and cholesterol ester transfer protein. The capacity of vesicles for cholesterol esters is apparently limited by their low solubility in PC bilayers (28, 29). Micellar complexes also contain a PC bilayer, but have in addition apo A-I in a state which is capable not only of stabilizing this discoidal structure but also spherical particles with a separate, neutral lipid phase in its center, as seen in reconstitution experiments (30). Thus, as cholesterol esters form in micellar particles by lecithin cholesterol acyltransferase action and exceed their solubility in the bilayer, they can be stored as a separate, internal phase in the core of these particles.

The products of the enzymatic reaction are, in fact, micellar particles containing the same amounts of apo A-I and total cholesterol, as the substrate particles. The PC content of the products is decreased in parallel with free cholesterol, and these components are replaced by equimolar amounts of cholesterol ester. On the basis of the chemical composition of the products (see Table III), it can be calculated that the average molecular weight of the products is 2.35 x 106 as compared to 2.55 x 106 (11) for the substrate. The loss of mass as lysophosphatidylcholine is reflected in the slightly increased density of the product particles. Information on the dimensions and the shape of the products was obtained from rotational relaxation time measurements (Table III) and from electron micrograph pictures (Fig. 4). Stacked discoidal particles 45 Å thick and 130 to 180 Å in diameter with a ρs = 530 ns were converted to free standing structures of circular cross-section 120 to 160 Å in diameter, with a ρs = 290 ns. Similar morphologic transformations by lecithin cholesterol acyltransferase action have been observed by Forte et al. (3) on synthetic systems, and by Norum et al. (4) on discoidal particles from patients with lecithin cholesterol acyltransferase deficiency.

Our product particles are probably not spherical because, for the observed electron micrograph dimensions, a sphere would have a volume of 1.44 x 106 Å3 as compared to a volume of 8.49 x 106 Å3 calculated from the dimensions of the substrate disc. Since an increase in volume is totally inconsistent with the observed loss in mass and markedly decreased ρs, the diameter observed for the product particle must be that of an oblate ellipsoid of lower asymmetry than the original particle. The ρs results support this point. For oblate ellipsoidal particles of similar mass (only ~8% difference) and with the same hydration, a 1.8-fold difference in the ρs represents approximately a 3-fold change in axial ratio (21). Starting with substrates with axial ratios of 4:1, the products would have axial ratios around 1.3:1. Considering the molecular weights and these axial ratios, the product particle diameters still appear too large; however, staining artifacts, for example, stain exclusion from the space between the flattened particles and the grid surface, may give rise to distorted dimensions. With higher cholesterol contents, it is very likely that discoidal substrates would give rise to spherical product particles, saturated with cholesterol esters.

REFERENCES

Apo A-I•Lipid Micellar Complex Reactions

Reaction of human lecithin cholesterol acyltransferase with synthetic micellar complexes of apolipoprotein A-I, phosphatidylcholine, and cholesterol.

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