Discoidal Complexes of A and C Apolipoproteins with Lipids and Their Reactions with Lecithin:Cholesterol Acyltransferase*  

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Micellar, discoidal complexes of human apolipoproteins A-I, A-II, C-I, C-II, C-III-1, and C-III-2 with egg phosphatidylcholine (egg-PC) and cholesterol were prepared by the cholate dialysis method. The complexes, isolated by gel filtration, had similar lipid and protein contents by weight, on the average: 1.77:0.083:1.0, egg-PC/cholesterol/apolipoprotein (w/w). The diameters of the discs, visualized by electron microscopy and estimated by gel filtration, ranged from 100 to 200 Å. The α-helix content of the apolipoproteins in the complexes was from 50–72%, and their fluorescence properties indicated nonpolar, but quite varied environments for the tryptophan residues in the various complexes.

Initial reactions of purified human lecithin:cholesterol acyltransferase with the complexes, adjusted to equal egg-PC concentrations, indicated that all the apolipoproteins activate the enzyme from 6-fold to 400-fold over control vesicles of egg-PC and cholesterol. In decreasing order of reactivity were the complexes with A-I, C-I, C-III-1, C-III-2, C-II, and A-II. These results indicate that aside from lipid-binding capacity and high amphipathic α-helix content, other structural features are required for optimal enzyme activation by apolipoproteins.

Concentration and temperature dependence experiments gave similar apparent $K_v$ values, markedly different apparent $V_{max}$, and very similar activation energies (about 19 kcal/mol), for the various complexes. These observations suggest that the rate-limiting enzymatic step of the reaction is common to all the complexes but that the activated enzyme levels differ from complex to complex. We propose that enzyme activation occurs upon binding to complexes via apolipoproteins. Addition of excess (5-fold) free apolipoprotein A-I or A-II to complexes resulted in the exchange of bound for free apolipoproteins and in loss of reactivity with the enzyme.

Initial investigations of the properties of lecithin:cholesterol acyltransferase (EC 2.3.1.43) indicated that HDL$^1$ were the preferred lipoprotein substrates of this enzyme (1).

After the isolation of HDL apolipoproteins, several laboratories examined their effects on the activity of lecithin:cholesterol acyltransferase, using synthetic vesicular substrates prepared with PC and cholesterol. Fielding et al. (2) demonstrated that apo-A-I activates lecithin:cholesterol acyltransferase in such a system, whereas apo-A-II does not activate the enzyme, but inhibits the effect of apo-A-I. Subsequent studies, employing vesicular systems and lecithin:cholesterol acyltransferase purified to various extents, confirmed that apo-A-I is the best activator of lecithin:cholesterol acyltransferase, and indicated that apo-C-I also activates lecithin:cholesterol acyltransferase but to a lesser extent than apo-A-I (3–5). Other HDL apolipoproteins had little effect on the enzyme activity, but were inhibitory when apo-A-I or apo-C-I were included in the system (3–5).

Although the apo-A-I activator role for the lecithin:cholesterol acyltransferase reaction is clearly established, the mechanism of this activation is not completely understood. It has been shown that fragments of apo-A-I and some synthetic amphipathic peptides, either related or unrelated to apolipoproteins, can activate lecithin:cholesterol acyltransferase to various extents, provided they bind to lipid (3, 6–9). Similarly, activation by apo-A-I depends directly on the binding of apolipoprotein to the lipid surface (4, 6, 10). On the basis of these observations, however, it cannot be decided whether the amphipathic peptides and polypeptides have requirements for lecithin:cholesterol acyltransferase activation other than a high content of amphipathic α-helix plus lipid-binding capacity. Also it is not yet established whether activation affects the lipid or the enzyme itself. The present report addresses these important questions.

In recent work on the substrate specificity of lecithin:cholesterol acyltransferase, we used micellar discoidal particles of apo-A-I containing cholesterol and a variety of phosphatidylcholines (11–14). These synthetic substrates are superior to the vesicular systems because of their high reactivity with the enzyme, their capacity to store high levels of cholesterol esters, and their stability and similarity to nascent HDL (11, 12). We showed that the lecithin:cholesterol acyltransferase reaction is not affected by the phase state of the lipid or by the PC/cholesterol molar ratio of the particles (13, 14). In addition, we observed that apo-A-I, even if α-helical and bound to a PC (dipalmitoyl-PC), which normally reacts well with the enzyme, can lose its activating capacity; by changing the size of the complexes, we induced structural apo-A-I changes at the lipid interface which resulted in a marked loss of enzymatic activity. These results have led us to propose that the lecithin:cholesterol acyltransferase reaction occurs at the apolipoprotein-PC interface.

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The abbreviations used are: HDL, high density serum lipoproteins; apo-A-I, apo-A-II, apo-C-I, apo-C-II, apo-C-III-1, apo-C-III-2, apolipoproteins isolated from human lipoproteins (the A apolipoproteins are major components of HDL, whereas the C apolipoproteins are important components of very low density lipoproteins); PC, phosphatidylcholine; apo-A-I-Dns, apo-A-I covalently labeled with 6-dimethylaminonaphthalene-1-sulfonate fluorescent label.

The work described here relies on the unique properties of the micellar, discoidal complexes to examine the differences in lecithin:cholesterol acyltransferase activity by the HDL apolipoproteins, bound to lipid in a manner closely related to the apolipoprotein-lipid interactions in lipoproteins; we show that all the HDL apolipoproteins can activate lecithin:cholesterol acyltransferase but to very different extents, and that the activation by apolipoproteins is primarily due to the activation of enzyme molecules in the system and not to lipid activation.

EXPERIMENTAL PROCEDURES

Preparations—Human apo-A-I and apo-A-II were prepared essentially by the method of Edelstein et al. (16). The C apolipoproteins were isolated by DEAE chromatography using well established procedures (17, 18). Purity of the apolipoproteins was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and amino acid analysis. All the apolipoproteins appeared to be >85% pure by these criteria. They were stored in lyophilized form at −20 °C, and were dissolved in 0.9 mM guanidine hydrochloride prior to dialysis into 0.1 M Tris-HCl, 0.15 M NaCl, 1 mM NaN3, 0.01% EDTA, pH 8 buffer. This buffer was used throughout the preparation and characterization of the complexes with lipid.

Complexes were prepared using egg-PC, cholesterol, and sodium cholate obtained from Sigma Chemical Co. The purity of these lipids and of [4,4'-C]cholesterol (New England Nuclear Co.) was tested by thin layer chromatography in the usual buffers (11, 13, 14). Each reaction mixture contained from 2.5 to 4.6 mg of egg-PC and from 0.14- to 0.23 mg of cholesterol (including the radiolabeled cholesterol) to give a PC/cholesterol molar ratio of 10:1. To these lipids, dissolved in 2:1 chloroform/methanol (v/v), 7.3 × 10−6 to 1.21 × 10−5 cpm of [14C]cholesterol were added, and a working specific activity was determined. After thorough evaporation of organic solvent under N2, 0.2 ml of buffer was added and the lipids were dispersed by vortexing. Sodium cholate (a 30 mg/ml stock solution in buffer), was added next to give final amounts of 1.6-2.6 mg/sample, i.e. a PC/cholate molar ratio of 1:1. After a 4-h incubation at 4 °C, the stock apolipoprotein solutions (from 3.3 to 7.2 mg/ml), were added to the corresponding lipid dispersions to give final weight ratios of 2.79±0.1381.01.54, egg-PC/cholesterol molar ratios of 2:1 (v/v), per 0.25 ml of reaction mixture. The organic phase with added cold carrier cholesterol and cholesteryl oleate (Sigma Chemical Co.) was dried under N2; [14C]cholesterol and [14C]cholesteryl esters were separated by thin layer chromatography, and were quantitated by scintillation counting as described previously (12, 13).

Interaction of Free Apolipoproteins with Complexes—Compared with egg-PC-cholesterol-apolipoprotein vesicles of egg-PC plus cholesterol were incubated with apo-A-I, apo-A-I-Dns, or apo-A-II in reaction mixtures which contained ~50 μg of complex apolipoprotein and an approximately equivalent amount of free apolipoprotein in 0.8 ml of solution. Incubations were carried out at 37 °C 1 h prior to addition of 8.0 ml of NaBr solution of density 1.185 g/ml. The samples were then centrifuged at 45,000 rpm, 15 °C, for 2 h, and were fractionated into 1-m1 fractions for analysis. Absorbance at 250 nm indicated the presence of protein and light-scattering vesicles; [14C]cholesterol counts/min identified complex and vesicle lipids; Dns fluorescence, with 340-nm excitation and 500-nm emission wavelengths, was used to monitor apo-A-I-Dns; and intrinsic fluorescence was used to identify apo-A-II by its fluorescence maximum at 305 nm, and Trp fluorescence with maxima between 330 and 333 nm to localize apo-A-I.

RESULTS

The complexes of egg-PC-cholesterol-apolipoproteins isolated by gel filtration were examined in detail in terms of their chemical composition, size, and spectral properties of the bound apolipoproteins. These properties are summarized in Table I; they correspond to discrete particles with similar chemical compositions containing approximately 1.81 weight ratios of egg-PC/apolipoprotein, and roughly 11:1 egg-PC/cholesterol molar ratios. The Stokes diameters determined by gel filtration range from 100-196 Å for complexes with different apolipoproteins. The appearance of electron micrographs agree quite well with those from gel filtration. All these
particles appeared as discs on electron micrographs (not shown); occasional round images corresponded in diameters to the discs and probably represented discs lying flat on the grid. In any case, the diameters of the round particles did not exceed 200 Å, which is considerably smaller than the diameters of small unilamellar vesicles of egg-PC viewed by electron microscopy (19). The particle dimensions are smallest for the apo-A-I and apo-A-II complexes, and increase for apo-C-I, apo-C-III, to apo-C-II containing complexes.

Circular dichroic spectra of the complexes indicated high contents of α-helix in the bound apolipoproteins, ranging from 50 to 72%. The errors due to protein determinations are large (±15%), but it is clear that amphiphilic helices must stabilize these complexes prepared by the cholate dialysis method. The intrinsic fluorescence spectra (uncorrected) had maxima ranging from 320-336 nm for the Trp-containing complexes, and 305 nm for the apo-A-II complexes which only contain Tyr. The observed wavelength maxima are consistent with previously reported values in the presence of phosphatidylcholine dispersions (9, 28). Fluorescence polarization values of Trp-containing complexes were measured at 25 °C; they ranged from 0.096 for apo-C-II to 0.122 for apo-A-I complexes. These values indicate significant local rotational motions of the Trp residues, since the limiting polarization for an exciting wavelength of 280 nm is 0.21 (29). The fluorescence properties indicate variable molecular environments of Trp residues for all the complexes.

Kinetics of the Lecithin-Cholesterol Acyltransferase Reaction with Complexes—The complexes and a vesicle control sample were adjusted to equal egg-PC concentrations and their reaction rates were measured. The initial rates of reaction for all the complexes, and greater than for vesicles without any apolipoprotein. The vesicle control eliminates the possibility that 0.15 M NaCl added to the buffer increases the rate of enzymatic reaction, particularly for the low substrate concentrations, and results in nonlinear double reciprocal plots. A likely explanation is an increased stability of substrate-enzyme complexes at higher salt concentrations, an effect that becomes more pronounced as the saturation of substrate particle with enzyme decreases. The results for apo-A-I complexes (Table II) agree closely with previously reported kinetic constants (12, 14). The other complexes have apparent $K_m$ values only 2- or 3-fold lower than that for apo-A-I complexes; on the other hand, the apparent $V_{max}$ values differ by 10- to 300-fold.
TABLE

### Kinetic parameters of the reaction of lecithin:cholesterol acyltransferase with egg-PC-cholesterol-apo-particle complexes

<table>
<thead>
<tr>
<th>Apolipoprotein complex</th>
<th>Relative reaction rates*</th>
<th>Apparent $K_{m}$</th>
<th>Apparent $V_{max}$</th>
<th>Activation energy $kcal/mol$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo-A-I</td>
<td>100</td>
<td>4.76 (±0.60) × 10^{-2}</td>
<td>17.9 ± 1.2</td>
<td>18.0</td>
</tr>
<tr>
<td>Apo-A-II</td>
<td>1.5</td>
<td>0.012</td>
<td>17.8</td>
<td>19.4</td>
</tr>
<tr>
<td>Apo-C-I</td>
<td>12.1</td>
<td>2.47 (±0.31) × 10^{-2}</td>
<td>1.59 ± 0.10</td>
<td>19.4</td>
</tr>
<tr>
<td>Apo-C-II</td>
<td>3.0</td>
<td>0.030</td>
<td>17.8</td>
<td>19.9</td>
</tr>
<tr>
<td>Apo-C-III-1</td>
<td>5.4</td>
<td>1.78 (±0.97) × 10^{-2}</td>
<td>0.189 ± 0.030</td>
<td>19.9</td>
</tr>
<tr>
<td>Apo-C-III-2</td>
<td>4.0</td>
<td>1.72 (±0.93) × 10^{-2}</td>
<td>0.064 ± 0.012</td>
<td>19.9</td>
</tr>
</tbody>
</table>

*Initial velocities were normalized to equal enzyme concentrations and were expressed relative to the apo-A-I complexes (100%).

**Determined from Fig. 2. Initial velocities were normalized to equal enzyme concentrations. Complexes with apo-A-II and apo-C-II gave unreliable results due to their low reactivities with lecithin:cholesterol acyltransferase. The error limits were determined from the errors in initial velocity measurements.

Fig. 3. Arrhenius plots of the initial velocity of the enzymatic reactions versus absolute temperature for apolipoprotein-lipid complexes. The reactions were performed in the same buffer as in Fig. 2 using 0.20 ml of enzyme preparation for the apo-A-II, apo-C-II, and apo-C-III-1 complexes, and 0.10 ml of enzyme for the apo-C-1 complexes. Normalized initial velocities were used in the calculation of log $V_{max}$ data points above 35 °C were neglected in the calculation of activation energies (Table II). Results for the apo-A-I complexes have been published earlier (14).

Fig. 4. Effect of the addition of free apo-A-I-Dns on the reaction of apolipoprotein-lipid complexes with lecithin:cholesterol acyltransferase. All the reaction mixtures contained 4.04 × 10^{-6} M egg-PC and essentially equivalent amounts of complex apolipoprotein (approximately 0.018 mg/ml). The reactions were performed in the buffer described in Fig. 2. All initial velocities are normalized for 0.05 ml of stock enzyme. The corresponding results of the experiments where free apo-A-I was used in place of apo-A-I-Dns are indicated by 3, apo-A-I complexes with added apo-A-I-Dns; x, apo-C-I complexes with added apo A-I; E, vesicles with added apo-A-I; CE, cholesteryl esters; Ves, vesicles.
effects are more gradual with unlabeled apo-A-I. When an amount of apo-A-I equivalent to that present in the apolipoprotein complexes was added, only 10 and 54% inactivation of apo-A-I and apo-C-I complexes was observed, respectively; with a 5-fold excess of apo-A-I, the inactivation was 97 and 66%, respectively. Maximum vesicle activation with labeled and unlabeled apo-A-I was identical, suggesting that the marked deactivating effects of excess apo-A-I-Dns are due not to the lipid bound, but rather to the free apolipoprotein.

Fig. 5 shows an analogous experiment where apo-A-II was added to apo-A-I and apo-C-I complexes and to vesicles which initially contained enough apo-A-I-Dns for maximum activation (i.e., 0.003 mg/ml apo-A-I-Dns). Similar to free apo-A-I-Dns and apo-A-I at high concentrations, apo-A-II has a deactivating effect on the enzymatic reaction. Interestingly, in all cases, the deactivation of vesicles is more gradual than the inactivation of apo-A-I and apo-C-I complexes.

In order to assess the physical basis of these effects of added apolipoproteins, we prepared appropriate mixtures of complexes with free apo-A-I-Dns or apo-A-II. After equilibration of the mixtures at 37 °C, we separated complexes from free apolipoproteins by ultracentrifugal flotation at d = 1.180 g/ml. A typical separation and analysis of centrifugal fractions is shown in Fig. 6, where apo-A-I complexes were equilibrated with an equivalent amount of free apo-A-I-Dns. Note the equal distribution of apo-A-I-Dns among the complexes and the free apolipoprotein fractions. Table III summarizes the results for apo-A-I and apo-A-II complexes incubated with free apo-A-I-Dns or apo-A-II. In general, we observed that original protein/lipid stoichiometry of the complexes is preserved, but that apolipoprotein exchange occurs in all cases. Note the presence of apo-A-I-Dns and of added apo-A-II in the complex fractions and of displaced apo-A-I and apo-A-II in the bottom fractions.

**DISCUSSION**

The sodium cholate reconstitution method previously used with apo-A-I (11-14) is obviously effective in the formation of micellar, discoidal complexes with other apolipoproteins as well. All the complexes prepared in this work have similar protein and lipid contents by weight, but range in size from 100-200 Å-diameter particles. The smaller discs, formed with apo-A-I and apo-A-II, with molecular weights near 2 × 10^6 (11) contain on the average 2.5 and 4.2 apolipoprotein molecules/complex, respectively. These apolipoproteins appear to fit around the lipid bilayer disc in a peripheral ring. In the larger particles formed with the C apolipoproteins, there is an excess of protein, over that required to cover the exposed PC acyl chains. A similar protein excess was previously observed for larger egg-PC-cholesterol-apo-A-I complexes (up to 200 Å in diameter); this excess protein was thought to localize on the face of the disc (14). Therefore, it is possible that some apolipoprotein may be distributed on the faces of the discs formed with the C apolipoproteins.

Detailed work on apo-A-I and apo-A-II complexes with PC has shown that, depending on the ratio of reactants and the reaction conditions, particles of different sizes can be produced either by spontaneous or cholate-mediated reaction (27, 31, 32). Presumably variations of reaction conditions could also lead to different particle distributions containing the apo C proteins. However, for the purposes of this work, discoidal particle diameters are not an important factor, since we have already demonstrated that egg-PC-cholesterol-apo-A-I discs, ranging in size from 100 to 200 Å diameters, are equally reactive with lecithin:cholesterol acyltransferase (14). It is important to note that all the complexes investigated in this study have from 50 to 72% α-helical contents in their bound apolipoproteins, in good agreement with previously published data for these apolipoproteins either in dimyristoylphosphatidylcholine complexes or bound to egg-PC vesicles (9, 28).

In recent work, Yokoyama et al. (6), Pownall et al. (8), and Segrest et al. (33) have synthesized amphipathic, lipid-binding peptides unrelated to apolipoproteins. Several of these peptides exhibit significant activation of lecithin:cholesterol acyltransferase. On the basis of such findings, Yokoyama et al. (6) have proposed that any amphipathic peptide with sufficiently extensive α-helical regions would activate lecithin:cholesterol acyltransferase. This implies that a specific
related to apolipoprotein structural properties other than α-acyltransferase for the apolipoprotein complexes, from high-plexes have high α-helix contents in the lipid-bound apolipoproteins, but we also demonstrate that there is no correlation with 1ecithin:cholesterol acyltransferase activation is clear that 1ecithin:cholesterol acyltransferase. We find that all the complexes, results in complete inactivation. Complexes with apo-A-II and apo-C-III-1 undergo slight activation, at different stages of apo-A-I-Dns addition, but are finally inactivated in 5-fold excess apo-A-I-Dns. Egg-PC cholesterol vesicles with minimal initial reactivity go through a maximum in activity and then decrease more gradually than the discoidal complexes. In the latter case, maximal activity occurs when six apo-A-I-Dns molecules are present per vesicle (≈2,500 PC/vesicle), in good agreement with the previously reported apo-A-I/egg-PC vesicle stoichiometry by Yokoyama et al. (6). The mechanism of the observed deactivations and activations was suggested by ultracentrifugal separation of complexes from free protein, and analysis of the apolipoproteins, free or associated with complexes. In the apo-A-I complexes exposed to equivalent apo-A-I-Dns amounts, there was approximately 50% incorporation of labeled protein into the complexes with 50% of unlabeled apo-A-I appearing in the sediment. Experiments with apo-A-II complexes, where the added apo-A-I-Dns was equivalent to the bound apolipoprotein, showed that around 30% of apo-A-I-Dns appeared in complexes and the remainder sedimented. In all cases, the overall protein/lipid ratios in complexes remained constant, indicating an exchange rather than an addition of protein. On the basis of these results, the modest activation by apo-A-I-Dns seen with the apo-A-II and apo-C-III complexes could be due to the gradual incorporation of apo-A-I-Dns into the complexes counteracted by the inhibiting effect of free apo-A-I-Dns. The inhibition by free apo-A-I-Dns is most likely due to enzyme binding.

The more gradual effect of excess free apo-A-I-Dns on the vesicles than on the apo-A-I or apo-C-I complexes can be explained by the equilibration of the apolipoprotein with the vesicle surface which results in a “buffering” effect.

The concentration and temperature dependence experiments of lecithin:cholesterol acyltransferase reaction rates with the various complexes provide some important information regarding the activation by apolipoproteins. The apparent kinetic constants are shown in Table II for apo-A-I, apo-C-I, and the apo-C-III complexes. Interestingly, the apparent $K_a$, which is a measure of the efficiency of product formation, is quite similar for all the complexes (the maximum difference is only about 3-fold), whereas the apparent $V_{max}$, a measure of activated enzyme concentration, varies by 10- to 300-fold. These results suggest that the Michaelis-Menten enzyme-substrate complex is similar for the different substrates; but that the number of activated lecithin:cholesterol acyltransferase molecules varies markedly, and is perhaps related to the initial binding of the enzyme to the particles, mediated by the apolipoproteins. Further support for this hypothesis comes from the temperature dependence experiments, and the essentially equal $E_a$ values for all complexes (Table II). We showed in the past that the $E_a$ values of the lecithin:cholesterol acyltransferase reaction with discoidal apo-A-I complexes depend markedly on the nature of the PC component and reflect the energy barrier of the rate-limiting catalytic step (13, 14); thus, the equivalence of $E_a$ values observed here indicates very similar thermal activation involving the enzyme with egg-PC, in all the complexes. It appears that the differences in reaction rates reflect not different catalytic steps, but rather the activation of the enzyme by apolipoprotein.

Further experiments were conducted to examine the effects of free apolipoproteins on the reaction of complexes with lecithin:cholesterol acyltransferase. As shown in Fig. 4, addition of apo-A-I-Dns to apo-A-I, and apo-C-I complexes to a maximum 5-fold excess, relative to apolipoprotein content in the complexes, results in complete inactivation. Complexes with apo-A-II and apo-C-III-1 undergo slight activation, at different stages of apo-A-I-Dns addition, but are finally inactivated in 5-fold excess apo-A-I-Dns. Egg-PC cholesterol vesicles with minimal initial reactivity go through a maximum in activity and then decrease more gradually than the discoidal complexes. In the latter case, maximal activity occurs when six apo-A-I-Dns molecules are present per vesicle (≈2,500 PC/vesicle), in good agreement with the previously reported apo-A-I/egg-PC vesicle stoichiometry by Yokoyama et al. (6). The mechanism of the observed deactivations and activations was suggested by ultracentrifugal separation of complexes from free protein, and analysis of the apolipoproteins, free or associated with complexes. In the apo-A-I complexes exposed to equivalent apo-A-I-Dns amounts, there was approximately 50% incorporation of labeled protein into the complexes with 50% of unlabeled apo-A-I appearing in the sediment. Experiments with apo-A-II complexes, where the added apo-A-I-Dns was equivalent to the bound apolipoprotein, showed that around 30% of apo-A-I-Dns appeared in complexes and the remainder sedimented. In all cases, the overall protein/lipid ratios in complexes remained constant, indicating an exchange rather than an addition of protein. On the basis of these results, the modest activation by apo-A-I-Dns seen with the apo-A-II and apo-C-III complexes could be due to the gradual incorporation of apo-A-I-Dns into the complexes counteracted by the inhibiting effect of free apo-A-I-Dns. The inhibition by free apo-A-I-Dns is most likely due to enzyme binding.

The more gradual effect of excess free apo-A-I-Dns on the vesicles than on the apo-A-I or apo-C-I complexes can be explained by the equilibration of the apolipoprotein with the vesicle surface which results in a “buffering” effect. Differences in the degree of inactivation by the lower levels (1-fold excess) of apo-A-I-Dns and unlabeled apo-A-I probably reflect
differences in affinity of the free apolipoproteins for the enzyme. We showed previously that apo-A-I-Dns self-associates less than apo-A-I in solution (34); in addition, the aromatic Dns label may increase directly the binding of apo-A-
I-Dns with enzyme.

Experiments performed with free apo-A-II added to apo-A-
I, apo-C-I, and vesicle-apo-A-I-Dns complexes, also show inactivation effects. These are probably due to apo-A-I1 ap-
pearance in complexes and liberation of lecithin:cholesterol acyltransferase-binding apolipoproteins. It should be noted that exchange of apo-A-I with apo-A-II has been reported to occur in lipoproteins (35) and synthetic lipid-apo-A-I complexes (36). We show, in addition, that apo-A-I-Dns can replace apo-A-II and C apolipoproteins in complexes, to varying extents, probably dependent on their relative affinities for the lipids.

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