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Reaction of Human Lecithin:Cholesterol Acyltransferase with Micellar Substrates Is Independent of the Phase State of the Lipid†

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ABSTRACT: Micellar complexes with different phosphatidylcholine (PC) compositions were prepared by the dialysis of PC–cholesterol dispersions with cholate in the presence of human apolipoprotein A-I (apo A-I). The complexes isolated by gel filtration had molecular weights around 200 000, two apo A-I molecules per particle, PC to apo A-I molar ratios from 91 to 123, and cholesterol to apo A-I molar ratios from 6 to 11. The phase-transition behavior of these complexes was examined by fluorescence polarization of diphenylhexatriene: the complexes containing dimyristoyl-PC had a transition temperature (T_m) of 32 °C, the complexes with dipalmitoyl-PC had a T_m of 45 °C, and those prepared with palmitoyl-oleoyl-PC were mostly present in the liquid-crystalline state in the temperature range investigated (55–7 °C). The initial velocities of the enzymatic reaction with purified human lecithin:cholesterol acyltransferase decreased in the order palmitoyloleoyl-PC > dipalmitoyl-PC > dimyristoyl-PC, at saturating micellar substrate levels. Arrhenius plots of the reaction rates from 15 to 41 °C were linear, and the activation energies ranged from 20 to 30 kcal/mol. These results indicate a marked dependence of the enzymatic reaction rates on the nature of the acyl donor, a dependence which is not related to the phase state of the bulk lipid in the micellar complexes.

Lecithin:cholesterol acyltransferase (LCAT,[1] EC 2.3.1.43) is the enzyme responsible for the esterification of cholesterol and for the transformations of high-density lipoproteins (HDL) is plasma (Glomset, 1972). So far, the substrate specificity of LCAT has been investigated by using native lipoproteins or synthetic vesicles of phosphatidylcholines (PC) and cholesterol with added apolipoproteins. The human enzyme shows a marked preference for the 2-acyl position and for long-chain unsaturated fatty acids of natural and synthetic PCs in the transesterification reaction (Glomset, 1972; Sgoutas, 1972). When saturated PCs are used as acyl donors in the LCAT reaction, their order of reactivity depends on the type of substrate particle used (vesicle or HDL) and on the physical state of the lipid (Soutar et al., 1974; Yokoyama et al., 1977).

Although vesicle substrates have the advantage over native lipoproteins of being chemically defined, they present several problems: instability in the presence of apolipoproteins (Jonas et al., 1977, 1980), different apolipoprotein binding affinities and stoichiometries on stable vesicles (Yokoyama et al., 1980), a limited capacity for cholesterol ester storage (Chajek et al., 1980; Janiak et al., 1979), and major morphological differences from the natural LCAT substrates. In fact, previous observations with vesicles containing dilauroyl-PC and dimyristoyl-PC (DMPC) while the temperature was changed (Soutar et al., 1974) may have included the effects of the spontaneous transformation of vesicles into discoidal complexes of lipid and apolipoprotein. Even with PC vesicles which do not form spontaneously micellar complexes with apolipoprotein A-I (apo A-I), the extent and strength of apo A-I binding to the vesicle, and hence, LCAT activation, may be affected by the nature of the PC. Reports that optimal LCAT activity is observed at PC to cholesterol molar ratios of 4/1 in vesicle

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[1] Abbreviations: LCAT, lecithin:cholesterol acyltransferase; HDL, high-density lipoprotein(s); PC, phosphatidylcholine; apo A-I, apolipoprotein A-I, the major protein component of HDL; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; POPC, palmitoyloleoylphosphatidylcholine; DPH, 1,6-diphenyl-1,3,5-hexatriene; T_m, gel to liquid-crystal phase transition temperature; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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substrates (Fielding et al., 1972), again, may be due to the differences of apo A-I binding when vesicles contain different cholesterol ratios. Yokoyama et al. (1980) found that egg PC vesicles bound five apo A-I molecules per vesicle with a dissociation constant $K_d = 9 \times 10^{-7}$ M, whereas vesicles containing 4/1 egg PC to cholesterol mole ratios bound 10 apo A-I molecules with a $K_d = 3 \times 10^{-7}$ M.

Recently we prepared discoidal, micellar complexes of various PCs with cholesterol and apo A-I of very similar size and morphology to nascent HDL (Matz & Jonas, 1982a). We demonstrated, using egg PC complexes, that the discoidal complexes are about 4 times more reactive with LCAT than vesicular substrates of equivalent chemical composition (Matz & Jonas, 1982b).

In this paper, we use discoidal complexes of DMPC, dipalmitoyl-PC (DPPC), and palmitoyloleoyl-PC (POPC) containing from 6 to 11 mol % of cholesterol, each with two apo A-I molecules per complex, to examine the effect of the phase state of the lipid on the activity of LCAT, in the absence of interfering effects due to apo A-I binding and activation.

Experimental Procedures

Apolipoprotein A-I Preparation. The procedure for the isolation of human apo A-I from pooled, fresh serum was similar to that described by Edelstein et al. (1972). The purity of the apolipoprotein (~98%) was examined by polyacrylamide gel electrophoresis in sodium dodecyl sulfate under reducing conditions. Before use, apo A-I preparations stored at −20 °C in lyophilized form were dissolved in 3 M guanidine hydrochloride and were extensively dialyzed against buffer.

Concentrations of apo A-I were determined from its extinction coefficient at 280 nm, $E^{1\%} = 1.15 \times 10^3$ cm^2 g^{-1} (Gwynne et al., 1974), and by the Lowry et al. (1951) procedure modified to include 1% sodium dodecyl sulfate (Markwell et al., 1978) for samples containing lipid.

LCAT Preparation. The enzyme was purified 10 000-fold from 1 L of pooled human serum by the procedures described previously (Matz & Jonas, 1982b). The enzyme was assayed during the course of the isolation by using micellar complexes of ^{14}C-labeled cholesterol·egg PC·apo A-I prepared by the cholate dispersion method (Matz & Jonas, 1982a). The [^{14}C]cholesterol ester products and residual [^{14}C]cholesterol were extracted from the reaction mixtures by the Folch et al. (1957) procedure and were separated by thin-layer chromatography on silica gel plates developed with a petroleum ether–diethyl ether–acetic acid (90:12:1.5 v/v) solvent system. The lipid spots were scraped into vials containing Beckman HP scintillationf fluid, and ^{14}C radioactivity was counted in a Beckman LS-100 counter. The purified enzyme was stored at 4 °C in 0.01 M Tris-HCl–5 mM EDTA, pH 7.6, buffer, after sterilization by passage through a 22-μm Millipore filter.

Lipids. The PCs (DMPC, DPPC, and POPC) of the L-α configuration and cholesterol were purchased from Sigma Chemical Co. All the PCs showed a single lipid spot upon chromatography on overloaded thin-layer plates of silica gel in a chloroform–methanol–water (65:35:1 v/v) solvent. Cholesterol was tested by using a cyclohexane–ethyl acetate (60:40 v/v) solvent system, which revealed the presence of a single lipid component. Radiolabeled cholesterol ([4-^{14}C]-cholesterol), obtained from New England Nuclear Co., was purified by thin-layer chromatography in the same system used for cholesterol purity determination. The sodium cholate (98% pure) purchased from Sigma Chemical Co. was used without any treatment.

Complex Preparation and Characterization. Ternary complexes of each PC with cholesterol and apo A-I were prepared by the sodium cholate dispersion and dialysis method described previously (Matz & Jonas, 1982a). The reaction mixtures contained 6 mg of PC and 5.7/1 PC to cholesterol and 1/1 PC to sodium cholate molar ratios. The PC to apo A-I molar ratio was 150/1, and about 6.5×10^5 [^{14}C]cholesterol cpm were present in a volume not exceeding 0.5 mL. Incubations and dialysis were performed in 0.01 M Tris-HCl, 0.15 M NaCl, 1 mM NaN_3, and 0.01% EDTA, pH 8.0, buffer at 4 °C for the POPC-, at 23 °C for the DMPC-, and at 37 °C for the DPPC-containing mixtures. After 3 days of dialysis with several changes of buffer, the reaction mixtures were centrifuged at 18 000 rpm for 90 min at 20 °C in order to remove lipid that had not formed micellar complexes. The recovery of [^{14}C]cholesterol and PC in the complexes, present in the supernatant, varied from 50 to 90% depending on the PC. Most of the apo A-I was recovered in the supernatant.

The centrifuged complex preparations were subsequently eluted through a Bio-Gel A-5 m (1.8 × 45 cm) column, equilibrated with the buffer in which the complexes were prepared. The column was calibrated with horse ferritin (1.3×10^6 daltons, Stokes radius 79 Å; Siegel & Monty, 1966), apo A-I·DMPC complexes (2.3×10^5 daltons, Stokes radius 49 Å; Jonas & Krajnovich, 1977), bovine serum albumin (6.7×10^4 daltons, Stokes radius 32 Å; Jonas & Weber 1970), and human apo A-I (2.8×10^4 dalton, Stokes radius 28Å; Jonas & Krajnovich, 1977). Stokes radii for all the marker particles, except ferritin, were calculated from rotational relaxation times. Ferritin and serum albumin (defatted) were obtained from Sigma Chemical Co. Five to seven fractions at the peak of protein (monitored by the absorbance at 280 nm) and lipid (monitored by ^{14}C radioactivity) elution were pooled and were concentrated about 4-fold by Amicon pressure cell (10 PA) filtration, using type PM 10 membranes.

The composition of the concentrated complexes was determined by the absorbance at 280 nm and the Markwell et al. (1978) assay for protein content, and by the Chen et al. (1965) procedure for phospholipid content. Cholesterol was determined from ^{14}C radioactivity by using a specific activity of 460 cpm/nmol, determined on the original stock solution of cholesterol from the cpm and dry weight of cholesterol.

The lipid phase of the complexes was examined by means of 1,6-diphenyl-1,3,5-hexatriene (DPH) (Aldrich Chemical Co.) fluorescence polarization (Jonas & Krajnovich, 1978; Jonas & Mason, 1981). The fluorescent probe was dissolved in tetrahydrofuran and introduced into a 2×10^{-4} M solution of the complexes (in terms of PC). The final molar ratios of PC to DPH were in excess of 500/1; the tetrahydrofuran (about 5 μL in 2 mL of sample) was removed by gentle flushing with N_2. Fluorescence polarization of DPH, incorporated into the complexes, was measured with an SLM Series 400 polarization instrument by using 360-nm exciting light, and Corning Glass 3-74 filters in the path of the fluorescent light, over a temperature range from 55 to 7 °C.

The isolated complexes were stored at 4 °C until use. They were shown to retain their Bio-Gel A-5 m column elution properties, original composition, and reactivity with LCAT for at least 1 month.

Enzymatic Reaction. Prior to reaction with LCAT, the complexes were changed into 0.01 M Tris-HCl, 0.01% EDTA, and 1 mM NaN_3, pH 8.0, buffer by dialysis. The reaction mixtures contained complexes, representing 10 nmol of cholesterol, 2 mg of defatted bovine serum albumin, 4 mM β-mercaptoethanol, and buffer to a 0.5-mL volume. After equilibration for 30 min to 1 h at the desired temperature, the enzymatic reaction was initiated with 0.1 mL of the purified

LCAT preparation containing around 3 units of enzyme activity (1 unit = 1 nmol of cholesterol ester formed/h, using the micellar egg PC·cholesterol·apo A-I substrates described previously) (Matz & Jonas, 1982b). The assay procedure was as described above.

Reaction rates for the conditions used in these experiments were shown to be proportional to the enzyme concentration. Also the amount of cholesterol transformed changed linearly with time for about 1 h, provided that the extent of cholesterol transformation did not exceed 30–40%. The reaction of the enzyme with the complexes was investigated from 15 to 41 °C, a temperature range in which the enzyme is stable (Soutar et al., 1974). In these experiments, LCAT concentration was varied in order to obtain linear reaction rates with all complexes; 0.02, 0.04, and 0.2 mL of LCAT solution were used with POPC, DPPC, and DMPC complexes, respectively. In addition, the reaction mixtures contained 10 nmol of cholesterol in complexes, 2 mg of defatted bovine serum albumin, 4 mM β-mercaptoethanol, and buffer to a total volume of 0.5 mL.

Results

Characterization of the Micellar Complexes. The elution profiles of PC complexes with cholesterol and human apo A-I from the calibrated Bio-Gel A-5 m column are shown in Figure 1. The DMPC- and DPPC-containing complexes elute at very similar positions, with peak maxima between fractions 40 and 41 of this column (panels A and B, respectively, of Figure 1). The POPC complexes show a protein peak with a maximum at fraction 40 and a shoulder at fraction 32; the lipid elutes in a broad peak which can be resolved into two components also eluting at fractions 40 and 32. Thus, there are probably two types of complexes with POPC: a smaller one corresponding in size to those formed with the saturated PCs and a much larger complex which was not investigated further in this work. A portion of the pooled fractions of the POPC sample was rechromatographed on the same column. The elution peak had a maximum at fraction 40–41 and was essentially symmetrical and very similar to the elution profiles for the DMPC- and DPPC-containing complexes. From other work in our laboratory (J. Wetterau and A. Jonas, unpublished experiments), we have established that, depending on the reaction conditions (proportion of reactants and temperature), large and/or small micellar complexes can be formed with various PCs and apo A-I. We have also shown that whereas the larger complexes can be transformed into the smaller ones, the latter are more stable species which cannot give rise to the larger complexes.

It is evident from the elution profiles and the corresponding Stokes radii, given in Table I, that the sodium cholate method consistently gives rise to complexes of PC·cholesterol·apo A-I of very similar sizes and molecular weights, since their compositions and densities are comparable (1.9×10^5 to 2.5×10^5 daltons) with all PCs used (Matz & Jonas, 1982a). In previous work, we confirmed the molecular weights of micellar complexes estimated from gel filtration, by using sedimentation equilibrium analysis (Jonas et al., 1977; Matz & Jonas, 1982a).

Figure 2 shows the lipid-phase behavior of the complexes as reported by the fluorescence polarization of DPH in the temperature range from 55 to 7 °C. The complexes containing DMPC and DPPC undergo a gel to liquid-crystal phase transition in this temperature range with considerable broadening and shift of the midpoint of the transition (T_m) to higher temperatures, relative to multilamellar vesicles of those two lipids. Note that T_m values for the DMPC and

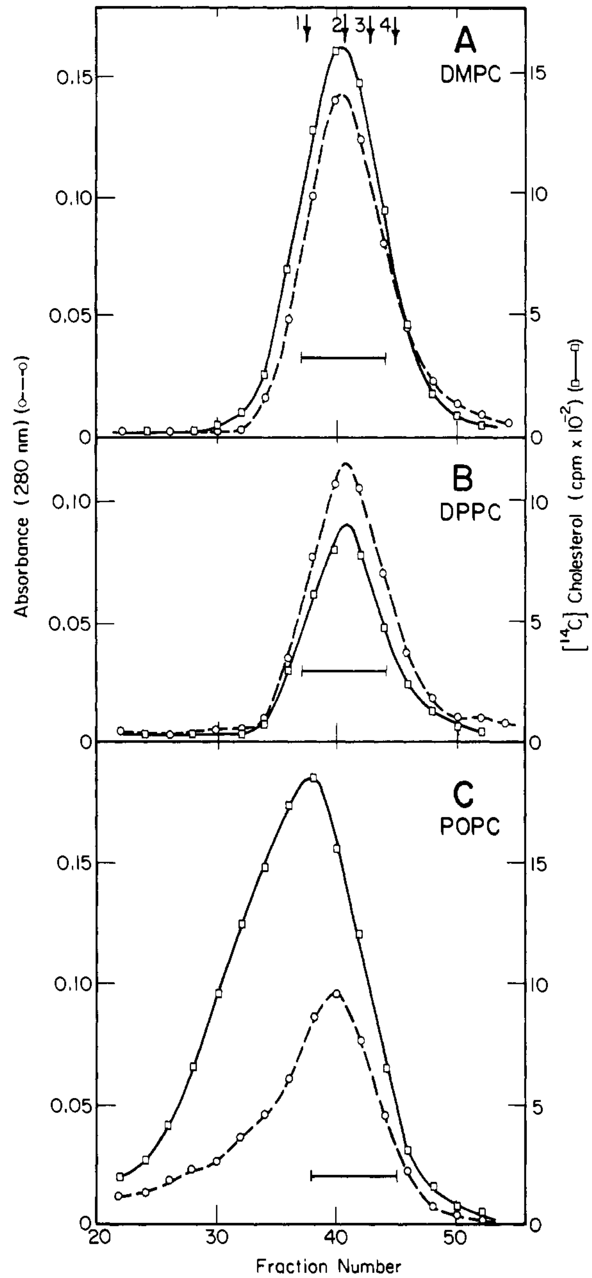

FIGURE 1: Elution patterns of phosphatidylcholine, cholesterol, and apo A-I micellar complexes from a Bio-Gel A-5 m (1.8 × 45 cm) column. Protein elution was monitored by the absorbance at 280 nm (O) and lipid elution by [^{14}C]cholesterol cpm (□). The bar across each peak indicates the fractions that were pooled and used for complex characterization and for enzymatic reactions. Panels A, B, and C correspond to complexes containing DMPC, DPPC, and POPC, respectively. The elution positions of calibrating markers are shown in panel A by arrows: (1) ferritin; (2) apo A-I·DMPC micellar complex; (3) bovine serum albumin; (4) human apo A-I. The excluded and total volumes of the column were at fractions 20 and 52, respectively.

DPPC complexes are 32 and 45 °C, respectively, whereas the T_m values for the pure lipids are 24 and 41 °C, respectively (Jonas & Mason, 1981). The complexes with POPC exist mostly in the liquid-crystalline state but enter into a broad transition at the lower temperatures of the measured range.

From previous work, it is known that the presence of cholesterol in micellar complexes broadens and shifts the transition beyond the effect of the apolipoprotein alone (Jonas & Krajnovich, 1978; Matz & Jonas, 1982a). Since apo A-I·DMPC complexes have T_m values of 26–27 °C (Jonas et al., 1977; Jonas & Mason, 1981), the increase to 32 °C, in the

Table I: Properties of Isolated Complexes of Phosphatidylcholines, Cholesterol, and Human Apo A-I[a]

PC component	PC/apo A-I[b] (mol/mol)	Chol/apo A-I[b] (mol/mol)	Stokes[c] radii (Å)	apo A-I/complex[d] (mol/mol)	T_m (°C)
DMPC	123 ± 18	7.8 ± 0.8	49 ± 3	2.00	32 ± 2
DPPC	91 ± 14	6.1 ± 0.6	47 ± 3	2.15	45 ± 2
POPC	94 ± 14	10.8 ± 1.1	49 ± 3	2.21	

[a] All these properties for two independent complex preparations were essentially indentical. Only one of the experiments is reported here. [b] Phosphatidylcholine was determined by the Chen et al. (1965) method, apo A-I by the Markwell et al. (1978) assay, and cholesterol from the specific activity (460 cpm/nmol) and cpm. [c] Stokes radii were determined by gel filtration on a calibrated Bio-Gel A 5-m column (see Figure 1). Stokes radii of 47 and 49 Å correspond to complex molecular weights of 2.1 × 10^5 and 2.3 × 10^5, respectively. [d] Apo A-I content per particle was calculated from the complex composition and molecular weights of 2.3 × 10^5 for the DMPC and POPC complexes and 2.1 × 10^5 for the DPPC complex.

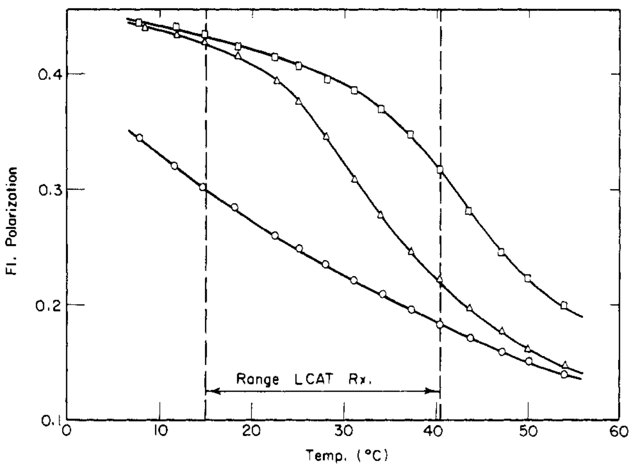

FIGURE 2: Fluorescence polarization of DPH as a function of temperature in isolated, micellar complexes of phosphatidylcholine, cholesterol, and apo A-I. Complexes containing DMPC (△), DPPC (□), and POPC (○) had around 500 PC molecules per DPH probe.

present case, is due to the presence of cholesterol. Interestingly, the effect of cholesterol in DPPC complexes is much less marked; the T_m in the presence and absence of cholesterol is near 45 °C (Jonas & Mason, 1981).

Table I summarizes the chemical composition of the complexes in terms of moles of PC and cholesterol per mole of apo A-I. It also gives Stokes radii estimated from the gel filtration experiments and the calculated moles of apo A-I per complex. The gel to liquid-crystal T_m values are included for the DMPC and DPPC complexes.

Reaction of Complexes with LCAT. The reactivity of the three complexes with LCAT, under identical reaction conditions, at 37 °C, is represented in Figure 3. The reactivity of POPC complexes is 10–50-fold higher than that of complexes with saturated PCs, as expected from the preference of the enzyme for unsaturated fatty acyl transfer from the 2 position of the PC. Among the saturated PC complexes, DPPC, which is predominantly in the gel state at 37 °C, reacts better (about 4-fold) with LCAT than DMPC complexes, which are in the liquid-crystalline state at this temperature. The relative order of reactivity of these complexes is similar to that previously determined with egg PC, DPPC, and DMPC micellar complexes containing 20 mol % cholesterol (Matz & Jonas, 1982b). However, the present differences in reactivity cannot be attributed exclusively to the different PC compositions because the PC to cholesterol molar ratios vary from 15.7/1 to 8.7/1 in the complexes. It remains to be established in future work what the precise effect of PC to cholesterol molar ratios is on the reaction of micellar complexes with LCAT.

The reaction rates of the complexes, as a function of temperature, are expressed in terms of nmoles of cholesterol re-

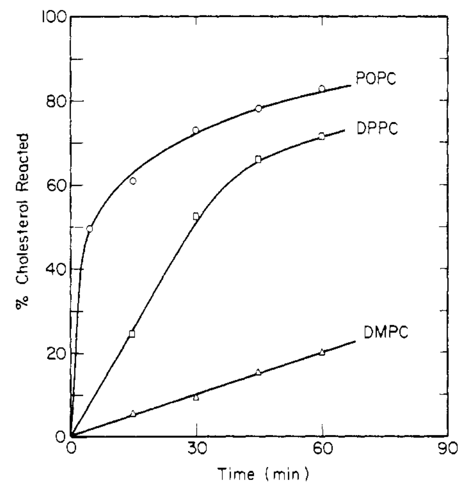

FIGURE 3: Reaction of LCAT with isolated micellar complexes of phosphatidylcholine, cholesterol, and apo A-I. Complexes containing DMPC (△), DPPC (□), and POPC (○) at equal substrate concentrations in terms of cholesterol concentration (2 × 10^{-5} M) were reacted at 37 °C with 3 units of enzyme.

Table II: Temperature Dependence of the LCAT Reactions with Micellar Complexes[a]

	PC component of complexes		
	DMPC	DPPC	POPC
initial velocity (nmol of Chol ester/h) at			
15.2 °C	0.034	0.390	10.5
20.4 °C	0.195	0.735	19.5
26.0 °C	0.315	1.58	44.1
30.8 °C	0.805	3.90	68.1
35.0 °C	1.48	7.95	102
40.5 °C	3.81	16.2	105
activation energy (kcal/mol)	29.5	28.0	20.0

[a] For each complex, the amount of enzyme was varied in order to obtain measurable and linear reaction rates for the temperature range observed. The initial velocities are calculated for equal enzyme concentrations, assuming a linear dependence of reaction rates on enzyme concentration.

acted per hour and are summarized in Table II. These data are presented in the form of an Arrhenius plot in Figure 4. The plots are linear in the temperature range from 15 to 41 °C. The only point that deviates significantly from a straight line can be attributed to the nonlinear time dependence of the fast reaction rate of POPC complexes at 41 °C. Evidently the physical state of the lipid domain of the complexes (gel for DPPC, liquid crystal for POPC, and mixed for DMPC complexes) has no influence on the activation energies of the enzymatic reactions. The activation energies, calculated from the slopes of the Arrhenius plots, are shown in Table II.

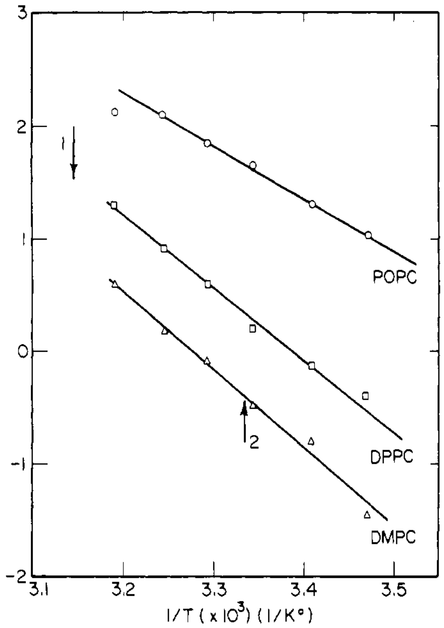

FIGURE 4: Arrhenius plots of the initial velocity of the LCAT reaction with isolated, micellar complexes of phosphatidylcholine, cholesterol, and apo A-I. The complexes contained DMPC (△), DPPC (□), and POPC (○). The arrows indicate the T_m's for DMPC-containing (2) and DPPC-containing (1) complexes.

Discussion

We have previously described the preparation of micellar complexes containing various PCs, cholesterol, and apo A-I, by the cholate dialysis method (Matz & Jonas, 1982a). We also showed that micellar complexes containing egg PC are much better substrates for LCAT than vesicles of essentially the same composition (Matz & Jonas, 1982b). The difference in the reactivity of the two types of substrates was attributed to the 4-fold higher content of bound apo A-I in the micellar complexes than in the corresponding vesicles.

In the present investigation, we prepared micellar complexes with very similar apo A-I contents (about two apo A-I molecules per particle) and sizes (49-Å Stokes radii, corresponding to 2×10^5 molecular weights). In these complexes, the apo A-I activator of LCAT is an integral part of the complex and remains tightly associated with it regardless of the nature and physical state of the PC–cholesterol phase. This is in contrast to vesicular substrates, which may have affinities for apo A-I depending on the composition and physical state of the lipid domains. Yokoyama et al. (1980) demonstrated higher stoichiometry and binding affinity of apo A-I for egg PC vesicles, with 4/1 molar ratios of PC to cholesterol, than for pure egg PC vesicles. Some PC vesicles can spontaneously break up into micellar complexes, particularly at the gel to liquid-crystal T_m of the lipid. Numerous publications, from our laboratory (Jonas et al., 1977; Jonas & Mason, 1981) and others (Pownall et al., 1978; Tall et al., 1977; Swaney, 1980a, b), have demonstrated the ability of DMPC and DPPC liposomes to "lyse" in the presence of apo A-I into micellar products, with optimal reaction rates at the T_m of the PC. Thus, temperature effects on the LCAT reaction with vesicular substrates may have superimposed apo A-I binding and particle structural changes upon the temperature effects on the catalytic reaction itself. Here, we have examined the temperature effects on the LCAT reaction by using the micellar ternary substrates where the structure, and the tight binding of apo A-I, is preserved over a very broad set of conditions, including temperatures from nearly freezing to apo A-I denaturation (at temperatures greater than 70 °C) (Tall et al., 1977).

The fluorescence polarization results shown in Figure 2 indicate that in the temperature range of the enzymatic reaction (15–41 °C) the DMPC-containing complexes undergo the gel to liquid-crystal phase transition (T_m = 32 °C), the DPPC complexes are mostly in the gel state (T_m = 45 °C), and the POPC complexes exist predominantly in the liquid-crystalline state. Thus, any major influence of the phase state of the lipid on the rate-determining step of the LCAT reaction should be detectable in this temperature range. The Arrhenius plots (Figure 4) show a linear dependence of log v_0 vs. $1/T$ for each of the complexes examined. The saturated PCs have essentially identical slopes, i.e., very similar activation energies for the enzymatic reaction (28 and 30 kcal/mol); POPC complexes have a lower activation energy of 20 kcal/mol.

The LCAT reaction involves many steps, including the binding and activation of the enzyme on the substrate particle, the formation of catalytic complexes with PC and cholesterol, possibly going through a covalent acyl-enzyme intermediate, and finally the release of products and free enzyme. Since the individual steps of this complicated reaction have not yet been quantitatively investigated, with the exception of the report that the phospholipase and cholesterol acyltransferase reactions of this enzyme have very similar kinetics (Aron et al., 1978), the rate-limiting step is not known. Awaiting further experimental information, we can nevertheless state that the rate-limiting step of the LCAT reaction is not sensitive to the phase state of the bulk lipid as had been previously suggested in experiments with vesicular substrates (Soutar et al., 1974). The mechanism of LCAT activation by apo A-I is not yet understood, but it may involve enzyme and/or substrate activation. In the latter case, it is reasonable to assume that activated lipid would be in contact with the apolipoprotein, in the boundary layer. This boundary lipid is known not to participate in the phase changes of the bulk lipid of micellar complexes of apo high-density lipoprotein with DMPC (Tall et al., 1977).

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Lipoprotein Lipase Catalyzed Hydrolysis of Water-Soluble *p*-Nitrophenyl Esters. Inhibition by Apolipoprotein C-II†

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ABSTRACT: Bovine milk lipoprotein lipase (LpL) catalyzes the hydrolysis of the water-soluble esters *p*-nitrophenyl acetate (PNPA) and *p*-nitrophenyl butyrate (PNPB). The same protein and same active site are involved in hydrolysis of water-soluble *p*-nitrophenyl esters and emulsified trioleoylglycerol since (a) trioleoylglycerol hydrolysis and PNPB hydrolysis activities coelute from the heparin–Sepharose affinity column used to purify LpL and (b) LpL-catalyzed hydrolyses of trioleoylglycerol and PNPB are inhibited to equal extents by phenylmethanesulfonyl fluoride. The effect of apolipoprotein C-II (apoC-II) on the LpL-catalyzed hydrolysis of PNPA and PNPB has been determined. ApoC-II inhibits hydrolysis of both esters, with a maximum extent of inhibition of 70–90%. Inhibition of the LpL-catalyzed hydrolysis of PNPB is specific for apoC-II, since apolipoproteins A-I, C-I, and C-III-2 have little effect on this reaction, and is partial noncompetitive in form. K_I values for apoC-II inhibition of the LpL-catalyzed hydrolysis of PNPA and PNPB are in the range 0.26–0.83 μM. The effect of apoC-II on the temperature dependences of LpL-catalyzed hydrolysis of both esters and on NaCl inhibition of LpL-catalyzed PNPB hydrolysis is consistent with a change in rate-determining step when LpL and apoC-II interact. These results indicate not only that there is an interaction between apoC-II and LpL in aqueous solution in the absence of a lipid interface but also that this interaction conformationally modulates the active site of the enzyme.

Lipoprotein lipase (LpL)[1] is an enzyme that catalyzes the hydrolysis of triacylglycerols of chylomicrons and VLDL to produce 2-acylglycerols and fatty acids (Cryer, 1981, for review). LpL also catalyzes the hydrolysis of the *sn*-1 ester bond of phosphatidylcholine and phosphatidylethanolamine (Groot & Van Tol, 1978; Groot et al., 1978; Scow & Egelrud, 1976; Vogel et al., 1971). A protein constituent of VLDL and chylomicrons, apoC-II, is an activator of in vitro LpL-catalyzed hydrolysis of triacylglycerols in VLDL (Fitzharris et al., 1981; Matsuoka et al., 1981) and lipid emulsions (Krauss et al., 1973; Fielding, 1973; Ekman & Nilsson-Ehle, 1975; Schrecker & Greten, 1979; Bengtsson & Olivecrona, 1979; Havel et al., 1973; LaRosa et al., 1970) and of phosphatidylcholine in sonicated vesicles (Bengtsson & Olivecrona, 1980; Muntz et al., 1979; Stocks & Galton, 1980). In addition, severe hypertriglyceridemia in human subjects has been correlated with the absence of plasma apoC-II (Breckenridge et al., 1978; Cox et al., 1978; Yamamura et al., 1979). The molecular dynamic events of apoC-II activation of LpL catalysis are to date poorly defined.

Egelrud & Olivecrona (1973) have shown that bovine milk LpL catalyzes the hydrolysis of water-soluble esters such as PNPA. We have initiated a study of the purified bovine milk LpL-catalyzed hydrolysis of the water-soluble esters PNPA and PNPB. The use of water-soluble *p*-nitrophenyl esters as LpL substrates offers several experimental advantages. One is that at neutral and alkaline pH one of the products of LpL-catalyzed hydrolysis of *p*-nitrophenyl esters is *p*-nitrophenoxide, which strongly absorbs light at 400 nm, allowing for continuous monitoring of the reaction time course. Another advantage is that the kinetic parameters V_{max} and K_m are more easily interpreted for LpL-catalyzed hydrolysis of water-soluble

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[1] Abbreviations: apoA-I, apoC-I, apoC-II, and apoC-III-2, apolipoproteins A-I, C-I, C-II, and C-III-2, respectively; BSA, bovine serum albumin; DMF, dimethylformamide; DPPC, dipalmitoylphosphatidylcholine; LpL, lipoprotein lipase; PNPA, *p*-nitrophenyl acetate; PNPB, *p*-nitrophenyl butyrate; PNP, *p*-nitrophenoxide; PMSF, phenylmethanesulfonyl fluoride; HDL, high-density lipoproteins; VLDL, very low density lipoproteins; V_i, initial velocity; V_{max}, maximal velocity; K_m, Michaelis constant; k_{cat}, catalytic rate constant.

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