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Interaction of Lipoprotein Lipase and Apolipoprotein C-II with Sonicated Vesicles of 1,2-Ditetradecylphosphatidylcholine: Comparison of Binding Constants†

Larry R. McLean* and Richard L. Jackson

Division of Lipoprotein Research, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0575, and Merrell Dow Research Institute, Cincinnati, Ohio 45215

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ABSTRACT: The interaction of lipoprotein lipase (LpL) and its activator protein, apolipoprotein C-II (apoC-II), with a nonhydrolyzable phosphatidylcholine, 1,2-ditetradecyl-*rac*-glycero-3-phosphocholine (C14-ether-PC), was studied by fluorescence spectroscopy. A complex of 320 molecules of C14-ether-PC per LpL was isolated by density gradient ultracentrifugation in KBr. The intrinsic tryptophan fluorescence emission spectrum of LpL was shifted from 336 nm in the absence of lipid to 330 nm in the LpL–lipid complex; the shift was associated with a 40% increase in fluorescence intensity. Addition of C14-ether-PC vesicles to apoC-II caused a 2.5-fold increase in intrinsic tryptophan fluorescence and a shift in emission maximum from 340 to 317 nm. LpL and apoC-II/C14-ether-PC stoichiometries and binding constants were determined by measuring the increase in the intrinsic tryptophan fluorescence as a function of lipid and protein concentrations; for LpL the rate and magnitude of the fluorescence increases were relatively independent of temperature in the range 4–37 °C. A stoichiometry of 270 PC per LpL for the LpL–lipid complex compares favorably with the value obtained in the isolated complex. The dissociation constant (K_d) of the complex is 4.3×10^{-8} M. For apoC-II, the stoichiometry of the complex is 18 PC per apoprotein, and the K_d is 3.0×10^{-6} M. These data suggest that LpL binds more strongly than apoC-II to phosphatidylcholine interfaces.

Lipoprotein lipase (LpL)[1] (EC 3.1.1.34) catalyzes the hydrolysis of tri- and diacylglycerols, phosphatidylcholines (PC), and phosphatidylethanolamines in plasma triacylglycerol-rich lipoproteins [for reviews, see Cryer (1981), Quinn et al. (1983), and Hamosh & Hamosh (1983)]. The triacylglycerol-rich lipoprotein substrate consists of a central core of neutral lipid, primarily cholesteryl esters and triacylglycerols, and a surface monolayer of lipids and various apolipoproteins (Morrisett et al., 1977). The primary lipid components of the surface monolayer are PC and cholesterol. One of the proteins associated with the surface monolayer of the lipoprotein substrate, apolipoprotein C-II (apoC-II), is required for maximal rates of hydrolysis of triacylglycerols and long-chain PC molecules by LpL.

Four functional sites on the LpL molecule have been defined: (1) a glycosaminoglycan binding site that anchors LpL to the endothelial cell surface, (2) an active (catalytic) site, (3) an apoC-II binding site, and (4) a lipid binding site that interacts with the surface of the lipoprotein substrate. Although the series of molecular events responsible for LpL catalysis remain speculative, several steps in the reaction pathway may be defined (Quinn et al., 1983). First, the enzyme binds to the substrate interface. Then, the active site of the enzyme binds a substrate molecule forming an interfacial Michaelis–Menten complex in which catalysis occurs. Finally, the products dissociate from the active site of the enzyme. One possible mechanism by which apoC-II enhances the activity of LpL is by promoting the binding of LpL to the substrate interface. Alternatively, LpL activation by apoC-II may occur subsequent to binding of LpL to the interface.

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[1] Abbreviations: LpL, lipoprotein lipase; PC, phosphatidylcholine; C14-ether-PC, 1,2-ditetradecyl-*rac*-glycero-3-phosphocholine; [^{14}C]DPPC, L-α-dipalmitoyl[1-^{14}C]phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; apoC-II, apolipoprotein C-II; T_c, lipid phase transition temperature; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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A variety of model systems have been used to examine the interaction between LpL and a lipid interface. LpL binding to lipid–water interfaces has been demonstrated in monolayers (Jackson et al., 1980), emulsions (Fielding & Fielding, 1976; Bengtsson & Olivecrona, 1980, 1982, 1983; Posner et al., 1983), and phosphatidylcholine vesicles (Shirai et al., 1981a,b). However, the dissociation constant for LpL binding to a PC interface has not been reported. The complex structure of lipoproteins makes selection of a suitable model system for studies of LpL binding and activity relatively difficult. PC bilayer vesicles have been used in numerous studies of lipase–lipid interactions and apolipoprotein–lipid interactions because of their well-defined structures and similarities to cell membranes and the lipoprotein surface monolayer. The primary purpose of the present study was to measure the binding of LpL and apoC-II to the same model substrate. Ditetradecylphosphatidylcholine- (C14-ether-PC-) sonicated vesicles were chosen as a model substrate because the ether linkage prevents hydrolysis of the lipid by LpL and the sonicated vesicles are bilayer structures with a well-defined phase transition at an experimentally accessible temperature (T_c = 27 °C; Shirai & Jackson, 1982; Jain et al., 1982).

Materials and Methods

Materials. 1,2-Ditetradecyl-*rac*-glycero-3-phosphocholine (C14-ether-PC) was a generous gift from R. Demel (State University of Utrecht, The Netherlands); L-α-dipalmitoyl[1-^{14}C]phosphatidylcholine (100 mCi/mmol) was obtained from New England Nuclear. Heparin (porcine intestinal mucosal, 176 USP units/mg) was obtained from Sigma Chemical Co. Heparin–Sepharose CL-4B was prepared as described by Iverius (1971) with modification (Matsuoka et al., 1980). Human apoC-II was isolated from very low density lipoproteins (Jackson et al., 1977). Standard buffer was 0.15 M NaCl, 10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.01% sodium azide, pH 7.40.

Purification of Lipoprotein Lipase. LpL was purified from bovine skim milk by a modification of the procedure of Kinnunen (1977). Briefly, skim milk (32 L) was adjusted to 0.4 M NaCl by addition of the solid salt; 300 mL of packed, heparin–Sepharose CL-4B was added. Following incubation with stirring for 2 h at 4 °C, the gel was collected and washed on a scintered glass filter with 3.5 L each of the following: 0.4 M NaCl–20 mM Tris-HCl, pH 7.4; 0.4 M NaCl, 0.2% Triton N-101, and 20 mM Tris-HCl, pH 7.4; 0.4 M NaCl–20 mM Tris-HCl, pH 7.4. The gel was transferred to a glass column and washed with 1 L each of 0.4 M NaCl–20 mM Tris-HCl, pH 7.4, and 0.75 M NaCl–20 mM Tris-HCl, pH 7.4. The enzyme was eluted with 2 M NaCl–20 mM Tris-HCl, pH 7.4. Fractions giving a single protein staining band with Coomassie blue in sodium dodecyl sulfate–3–20% polyacrylamide gradient gels (Socorro & Camejo, 1979) with a 20-μg loading were pooled and stored at −70 °C in 50% glycerol.

Preparation of Lipid Vesicles. Lipids were dissolved in chloroform, dried under nitrogen, and lyophilized for 15 min. The dry lipid was dispersed in standard buffer and sonicated for 15 min at 40 °C in a Heat Systems Model W-225R sonicator equipped with a tapered microtip; titanium particles were removed by centrifugation for 5 min at 2000 rpm. Vesicles were prepared daily. The vesicles are largely unilamellar and of diameters 250–300 Å as judged by negative-stain electron microscopy (L. R. McLean and W. J. Larsen, unpublished results).

Density Gradient Ultracentrifugation. Sonicated vesicles of C14-ether-PC (1 mg/mL) containing 1 μCi of L-α-dipalmitoyl[1-^{14}C]phosphatidylcholine (DPPC) were prepared as described above. In a typical experiment, LpL (0.4 mg) in standard buffer was mixed with heparin (2 mg) in a total volume of 3 mL. The LpL solution was mixed with 2 mL of the sonicated vesicles. Samples of the sonicated vesicles and of LpL alone were diluted to 4 mL. The samples were incubated at room temperature for 60 min. A step gradient of KBr was prepared by layering 4 mL of d = 1.16 g/mL KBr in standard buffer, 5 mL of d = 1.083 g/mL KBr in standard buffer, and 4 mL of sample into 14 × 95 mm polyallomer centrifuge tubes. The samples were subjected to ultracentrifugation in a Beckman SW-40 Ti rotor at 39 000 rpm for 50 h at 4 °C. The contents of each tube were fractionated from the bottom of the tube with a capillary pipet attached to a Gilson Minipuls 2 pump; 1.2-mL fractions were collected. Densities were determined on an Abbe refractometer (Bausch & Lomb). Fractions were assayed for LpL activity with Triton X-100 emulsified trioleoylglycerol as substrate (Matsuoka et al., 1980); phospholipid was determined by radioactivity.

Fluorescence Spectra and Titrations. Fluorescence spectra were recorded on a Perkin-Elmer MPF-44A spectrofluorometer. Blanks were subtracted with a Perkin-Elmer differential corrected spectra unit. The instrument was equipped with a recirculating water bath to maintain temperature constant to ±0.2 °C. Vesicles were prepared in standard buffer; LpL was added in standard buffer; apoC-II was added in a 6 M guanidine hydrochloride solution in which it is primarily monomeric (Mantulin et al., 1980). The highest concentration of guanidine hydrochloride was <0.2 M in the titrations with apoC-II; this concentration has no detectable effect on the structure of PC vesicle bilayers (Chen et al., 1980; Reijngoud & Phillips, 1982).

Binding constants for C14-ether-PC and LpL or apoC-II were calculated from the difference in the intrinsic fluorescence of protein in the presence and absence of lipid (ΔF) as a function of lipid (L) and protein (P) concentrations by using an independent-sites model for binding (Hille et al., 1981):

$$K_d = (P - X)(L/N - X)/X \quad (1)$$

where K_d is the dissociation constant of the complex (concentration X) and N is the stoichiometry of the complex (lipid:protein molar ratio). Values of X were obtained from the relationship: $\Delta F = CX$, where C is the molar fluorescence intensity difference constant. Values of C under various experimental conditions were established by using high ratios of lipid to protein where the fluorescence difference is proportional to protein concentration. Under these conditions, $\Delta F = CP$. The titration data were fit to eq 1 by nonlinear regression according to the model of Marquardt (1963). Starting parameters for N and K_d were estimated from titrations of C14-ether-PC with LpL and apoC-II by using an inverse transform of eq 1 (Hille et al., 1981). Convergence was judged by either a reduction of the residuals to $<10^{-5}$ (μM concentration units) or correspondence of the parameters in consecutive iterations to four significant places. Maximum values for ΔF in titrations with protein were obtained from the relationship: $\Delta F = C(L/N)$, since, when $P = L/N$, all of the protein is complexed to lipid.

Analytical Methods. Protein was measured by the method of Lowry et al. (1951) with bovine serum albumin as standard. Phospholipid concentrations were determined by analysis of inorganic phosphorous (Bartlett, 1959).

Results

LpL Stability. To establish optimal conditions for the measurement of LpL binding to lipid, the effects of lipid and heparin on the fluorescence spectra of LpL and on LpL activity

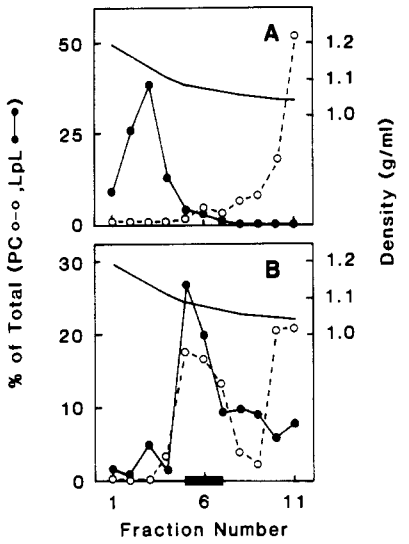

FIGURE 1: Ultracentrifugation of LpL, C-14-ether-PC vesicles, and LpL mixed with C-14-ether-PC vesicles. (A) A total of 2 mg of C-14-ether-PC vesicles (○) or 0.4 mg of LpL (●) was subjected to ultracentrifugation separately. (B) A total of 2 mg of C-14-ether-PC vesicles and 0.4 mg of LpL was mixed and centrifuged. Samples were prepared in standard buffer and centrifuged in a KBr density gradient as described under Materials and Methods. The distribution of LpL was obtained by activity measurements using a trioleoylglycerol emulsion substrate, densities were determined by refractive index, and PC was measured by radioactivity. Fractions 5–7 were pooled as indicated by the bar, dialyzed against standard buffer, and used for fluorescence measurements (Figure 2).

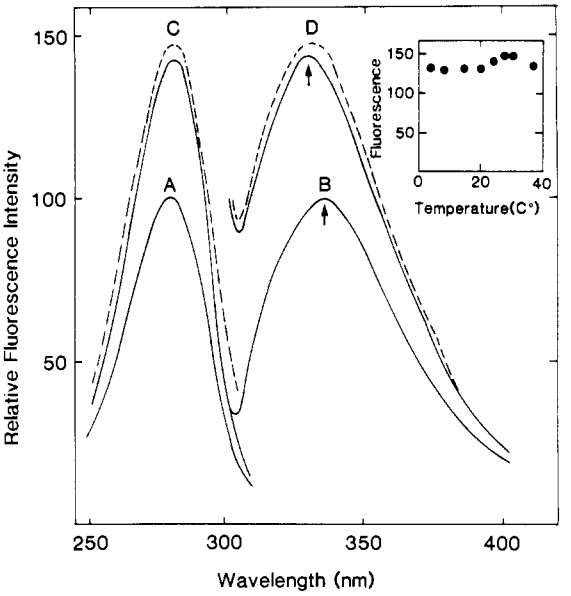

FIGURE 2: Fluorescence excitation and emission spectra of LpL in the presence and absence of C-14-ether-PC vesicles at 25 °C: (A) excitation (emission 336 nm) spectrum of 0.05 mg of LpL in 1.5 mL of standard buffer; (B) the corresponding emission (excitation 282 nm) spectrum; (C) excitation (emission 330 nm) spectrum of 0.05 mg of LpL plus 0.2 mg of C-14-ether-PC vesicles in 1.5 mL of standard buffer (solid line) or LpL–C-14-ether-PC complexes isolated by ultracentrifugation as described under Figure 1 (dotted line); (D) the corresponding emission (excitation 282 nm) spectra. All samples contained 0.25 mg of heparin. Measurements were made on a Perkin-Elmer MPF-44A spectrofluorometer with subtraction of the appropriate blanks with a differential corrected spectra unit as described under Materials and Methods. The arrows indicate the peak emission maxima of LpL and the LpL–C-14-ether-PC complexes. The inset gives the temperature dependence of the fluorescence of 0.05 mg of LpL plus 0.2 mg of C-14-ether-PC vesicles in 1.5 mL of standard buffer (excitation 282 nm, emission 330 nm).

were examined. Incubations were performed at 37 °C and the substrate was Triton X-100 emulsified trioleoylglycerol. In the absence of lipid or heparin, LpL activity decreased by more than 76% of the initial value in 30 min. In the presence of C14-ether-PC vesicles at a weight ratio of four PC per LpL, there was only a 15% decrease in activity after 30 min of incubation. Heparin (100 μg/mL) stabilized LpL activity such that only 2% of the activity was lost in 60 min. Fluorescence spectra of LpL in the presence of heparin or C14-ether-PC vesicles were unchanged for >60 min at 37 °C. In addition, the fluorescence spectra of the enzyme in the absence or presence of several concentrations of C14-ether-PC were not affected by the addition of heparin, suggesting that heparin had little effect on LpL binding to C14-ether-PC vesicles. On the basis of these results, heparin was included in all samples to maintain enzyme activity and fluorescence intensities constant.

Isolation of LpL–C14-ether-PC Complex. The stabilizing effect of C14-ether-PC vesicles on LpL activity suggested that LpL interacts with a PC interface to form a stable complex. To test this hypothesis, LpL and C14-ether-PC vesicles were incubated at 24 °C for 60 min and then subjected to ultracentrifugation in a salt gradient of KBr to separate free and bound protein. In control experiments, LpL and C14-ether-PC vesicles centrifuged alone were present at the bottom and top of the gradient, respectively (Figure 1A). When mixed, LpL and C14-ether-PC form a complex that bands at a density between 1.07 and 1.09 g/mL (Figure 1B). Fractions 5–7 were pooled and dialyzed against standard buffer, and the content of protein and lipid was determined. On the basis of a M_r of 48 300 for LpL (Iverius & Ostlund-Lindqvist, 1976), the stoichiometry of the complex is 320 ± 35 (three experiments) molecules of PC per molecule of LpL.

Fluorescence Spectra of LpL and PC Complexes. In the absence of lipid, the emission maximum of LpL is 336 nm and the excitation maximum is 282 nm [Figure 2; cf. Vainio (1983)]. Addition of C14-ether-PC vesicles to LpL at a PC:LpL weight ratio of 4 results in an ~40% enhancement of the fluorescence intensity and a shift in the emission maximum to 330 nm. The fluorescence spectrum of complexes of LpL and C14-ether-PC isolated in KBr density gradients is similar to that of LpL mixed with C14-ether-PC vesicles (compare dotted and solid lines in Figure 2, curves C and D). The magnitudes of the fluorescence enhancements and blue shifts are relatively independent of temperature in the range 4–37 °C (Figure 2, inset). Furthermore, 1 M NaCl had no effect on the fluorescence spectrum of the complexes or the LpL–PC vesicle mixture.

The increased fluorescence intensity observed with the addition of lipid vesicles reaches a maximum value during the 30-s mixing time and remains constant for at least 60 min. In titration experiments, fluorescence intensities were measured for 1–5 min after mixing, over which time the intensity was constant. At several points during the titration, the samples were incubated for 5–30 min without change in fluorescence intensity. Since the time scale of the fluorescence increase was short compared to the time scale of the measurements, it was assumed that the system was at equilibrium.

Fluorescence Spectra of ApoC-II and PC Complexes. The emission maximum of apoC-II in the absence of lipid is 340 nm and in the presence of C14-ether-PC vesicles (weight ratio 1:1) is 317 nm (Figure 3). Addition of vesicles results in a 2.5-fold increase in the intrinsic fluorescence of the protein. At 25 °C the increase in fluorescence intensity develops rapidly (in <30 s) and is constant for at least 15 min. At lower temperatures the fluorescence develops more slowly. At 16

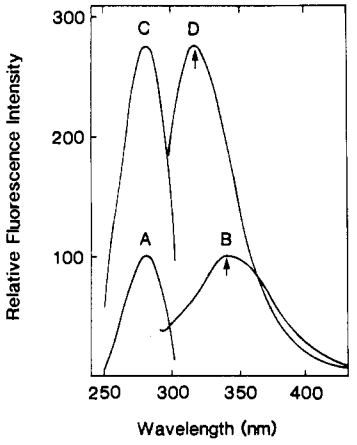

FIGURE 3: Fluorescence excitation and emission spectrum of apoC-II in the absence and presence of C-14-ether-PC vesicles at 25 °C: (A) excitation (emission 340 nm) spectrum of 0.05 mg of apoC-II in 1.5 mL of standard buffer; (B) the corresponding emission spectrum (excitation 280 nm); (C) excitation (emission 317 nm) spectrum of 0.05 mg of apoC-II plus 0.05 mg of C-14-ether-PC vesicles; (D) the corresponding emission (excitation 280 nm) spectrum. Measurements were made as described in Figure 2.

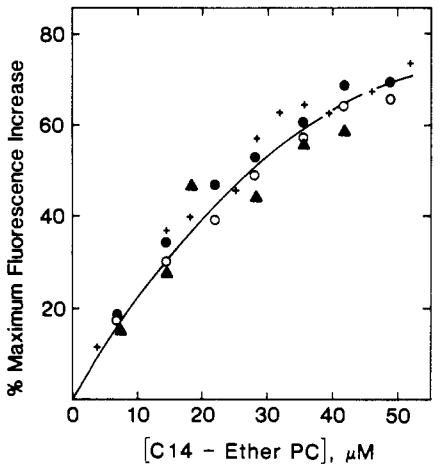

FIGURE 4: Lipid binding to LpL as a function of temperature. In a total volume of 1.5 mL, 0.25 mg of LpL in standard buffer was titrated with C-14-ether-PC vesicles (0.5 mg/mL). The fluorescence intensity (excitation 280 nm, emission 335 nm) was measured after each addition of vesicles following a 30-s mixing interval. The fluorescence intensity measurements were made 1–5 min after each addition. The temperatures of incubation and measurement were 37 (▲), 27 (+), 20 (○), and 16 °C (●). The maximal fluorescence increase was obtained from the y intercept of a plot of 1/fluorescence vs. 1/[lipid]. The line was fitted to all of the data by using the best fit parameters obtained by nonlinear regression analysis as described under Materials and Methods.

°C, >2 h are required to obtain >95% of the maximum fluorescence intensity. For this reason only a limited number of experimental measurements on binding of C14-ether-PC vesicles to apoC-II were practical at low temperatures (<25 °C). No significant differences in binding constants or stoichiometrics were obtained at 16 or 25 °C.

Binding of LpL and ApoC-II to C14-ether-PC Vesicles. The increase in fluorescence intensity of LpL as a function of added C14-ether-PC vesicles is shown in Figure 4. At each temperature examined (16–37 °C), the titrations are indistinguishable. Furthermore, the binding constants and stoichiometries of the LpL–C14-ether PC complex are independent of temperature on the basis of the independent sites model calculations described under Materials and Methods.

The binding of LpL and apoC-II to C14-ether-PC vesicles was compared by titrating vesicles with protein. Data for LpL

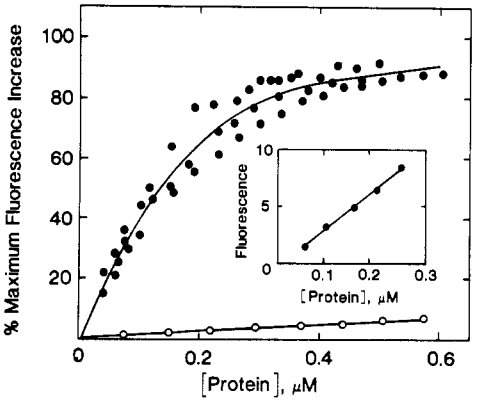

FIGURE 5: Titration of C-14-ether-PC vesicles with LpL (●) or apoC-II (○) at 25 °C. Aliquots of 5 μL of LpL (0.29 mg/mL) in standard buffer, 50% glycerol, and 5 mg of heparin/mL or 1 μL of apoC-II (1 mg/mL) in 6 M guanidine hydrochloride were added to 0.05 mg of C-14-ether-PC vesicles in 1.5 mL of standard buffer. The difference in fluorescence intensity (excitation 280 nm, emission 335 nm) was measured in two samples of protein, one of which was titrated with standard buffer and the other with C-14-ether-PC vesicles. After subtraction of the appropriate blanks, the fluorescence difference was calculated as a function of added protein. The maximum fluorescence increase was obtained by fitting the data to an independent sites binding equation as described under Materials and Methods. The inset gives an example of the data used to calculate the molar fluorescence difference intensity constant (C). The lines are the best fit by nonlinear regression analysis.

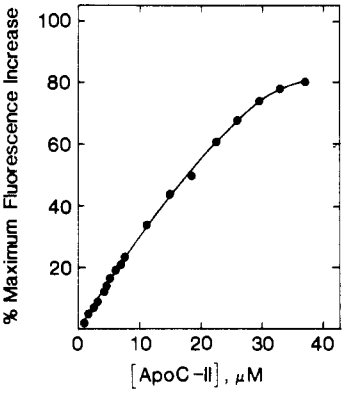

FIGURE 6: ApoC-II titration of C-14-ether-PC vesicles. Experimental conditions were identical with those in Figure 5 except that the concentration of vesicles was 0.7 mg in 2 mL of standard buffer and the titrant was at a concentration of 10 mg of apoC-II/mL in 6 M guanidine hydrochloride. The line is in the best fit by nonlinear regression analysis.

and apoC-II binding to C14-ether-PC vesicles at protein concentrations from 0 to 0.6 μM and at 25 °C are shown in Figure 5. For LpL, the stoichiometry of the complex, N, is 270 ± 30, and K_d is 0.043 ± 0.006 μM. These values are similar to those obtained in the lipid titrations (Figure 4, average K_d = 0.070 μM, N = 220). In contrast, apoC-II binds much less tightly to vesicles of the same composition. Since minimal binding of apoC-II to vesicles was observed at low concentrations of apoC-II, protein concentrations from 0 to 40 μM were used to estimate K_d = 3.0 ± 0.5 μM and N = 18 ± 0.3 (Figure 6).

Discussion

In the present paper we have used a model system of ether phosphatidylcholine vesicles to directly compare the binding interactions of LpL and apoC-II with a lipid interface. The ether linkage in the lipid allows measurements of LpL–PC interactions in the absence of lipid hydrolysis. A similar system

has been used to study interactions between PC and phospholipase A_2 (Jain et al., 1982). The major experimental findings of the present study are that (1) LpL and C-14-ether-PC vesicles form stable complexes as judged by ultracentrifugation and intrinsic protein fluorescence spectra, (2) these complexes are not dissociated by heparin, 1 M NaCl, or KBr, (3) binding of LpL to the vesicles is independent of temperature in the range 4–37 °C, and (4) the dissociation constant for LpL (0.04 μM) and apoC-II (3 μM) interactions with C-14-ether-PC vesicles differs by 2 orders of magnitude.

Formation of stable complexes of LpL with triolein emulsions (Fielding & Fielding, 1976; Bengtsson & Olivecrona, 1980, 1982, 1983; Posner et al., 1983) and plasma lipoproteins (Jackson et al., 1979) has been demonstrated by ultracentrifugation. Addition of 1 M NaCl in most of these binding experiments has little or no effect on LpL binding, similar to the results reported here on binding of LpL to C-14-ether-PC vesicles. Heparin has been shown to have no effect on the equilibrium stoichiometry of LpL complexes with a trioleoglglycerol/bovine serum albumin emulsion in the presence of apoC-II (Posner et al., 1983). However, in contrast to LpL binding to C-14-ether-PC vesicles, the dissociation constant for LpL in the emulsion system is increased 1.8-fold by the addition of heparin.

The dissociation constants for LpL interactions with C-14-ether-PC vesicles are relatively independent of temperature over a range (4–37 °C) that includes the phase transition temperature of the vesicles (T_c = 27 °C; Shirai & Jackson, 1982; Jain et al., 1982). To our knowledge, this is the first data on the effect of temperature on LpL binding to an interface. The binding of another lipase, phospholipase A_2, to C-14-ether-PC vesicles is strongly temperature dependent, suggesting significant differences in the mechanism of binding of LpL and phospholipase A_2. From these data, it appears that differences in binding of LpL to the substrate interface do not account for the temperature dependence of PC hydrolysis (Shinomiya et al., 1984). However, the binding data must be interpreted with some caution in that the PC vesicles used for the binding experiments do not contain the carbonyl oxygen of the ester bond.

Taken together, the lack of effect of temperature (and the bilayer phase transition) and high salt concentrations on LpL binding to C-14-ether-PC vesicles suggests that the binding interaction is not solely electrostatic in nature and that phase boundaries formed in the PC bilayer near T_c (Phillips et al., 1975) do not influence the equilibrium interaction of LpL with the bilayer surface. In contrast, apoC-II binding to C-14-ether-PC vesicles is far slower at temperatures below T_c (16 °C) than near T_c (25 °C). The apoC-II binding data are consistent with observations on the rate of binding of other apolipoproteins to dimyristoyl-PC vesicles (Pownall & Massey, 1982), which show a strong temperature dependence of binding at $T < T_c$.

The dissociation constant for LpL binding to C-14-ether-PC vesicles is similar to that measured for LpL binding to trioleoglglycerol emulsions containing bovine serum albumin (0.076 μM; Posner et al., 1983). The apoC-II binding data are consistent with published binding constants measured with dipalmitoyl-PC vesicles (6.5 μM; Cardin et al., 1982) and trioleoylglycerol/PC emulsions (0.45–1.1 μM; Tajima et al., 1983). The differences in lipid binding constants of LpL and apoC-II suggest that LpL will preferentially bind to a PC interface in the presence of apoC-II. Several lines of evidence support the hypothesis that apoC-II does not increase the binding of LpL to lipid surfaces, including both kinetic (Fitzharris et al., 1981; Shirai et al., 1983; Shinomiya et al., 1983, 1984) and equilibrium (Jackson et al., 1980; Shirai et al., 1981b; Posner, 1983) studies. Furthermore, the association constant for LpL and a dansylated fragment of apoC-II is increased 40-fold on addition of lipid vesicles to a value (K_d = 0.05 μM; Voyta et al., 1983) similar to the K_d for LpL and C14-ether-PC vesicles. On the basis of these data, it is tempting to propose that the binding of LpL to lipid vesicles facilitates the binding of apoC-II.

The binding constants measured in the present study provide a quantitative estimate of the amount of LpL present at the surface of a C-14-ether-PC vesicle. Within the limitations imposed by the model system, the data suggest that binding of LpL to a substrate interface occurs at low bulk enzyme concentrations even in the absence of apoC-II. In a typical experiment [e.g., Shinomiya et al. (1983)], the K_d and N values for binding predict that 93–98% of the LpL is at the interface. Thus, it appears unlikely that apoC-II would bring more LpL to the interface. However, what happens subsequent to the binding step remains open to question since a lack of effect of apoC-II on binding to the interface does not rule out the possibility that apoC-II alters the interaction of substrate molecules with the active site of LpL. Further progress in understanding the role of substrate structure in LpL catalysis will require a more detailed description of the structure of LpL and its complexes with substrates and model substrates.

Registry No. C14-ether-PC, 69483-38-1; lipoprotein lipase, 9004-02-8.

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Short-Chain Phosphatidylethanolamines: Physical Properties and Susceptibility of the Monomers to Phospholipase A_2 Action†

Andreas Plückthun, Ronald Rohlfs, Florence F. Davidson, and Edward A. Dennis*,‡
Department of Chemistry, University of California at San Diego, La Jolla, California 92093
Received September 24, 1984

ABSTRACT: The homologous series of optically active short-chain phosphatidylethanolamines (PE) from dibutyryl-PE to dioctanoyl-PE was synthesized. In addition, two monomeric short-chain phospholipid analogues that are not degraded by phospholipase A_2 (1,2-bis[(butylcarbamyl)oxy]-*sn*-glycero-3-phosphocholine and the corresponding ethanolamine derivative) were synthesized. In contrast to the short-chain phosphatidylcholines (PC), short-chain PE's have defined solubilities in water. No break below the solubility limit was found in surface tension plots, suggesting that these compounds exist as monomers in aqueous solution. Only when a significant fraction of the molecules is negatively charged can they form micelles by themselves. Cobra venom phospholipase A_2 hydrolyzes monomeric short-chain PE's at about the same rate as short-chain PC's but hydrolyzes long-chain PC's much more rapidly than long-chain PE's. The hydrolysis of short-chain PE's is found to be activated by phosphocholine-containing compounds only in the presence of an interface; in its absence phosphocholine-containing compounds can act as competitive inhibitors. Possible explanations for this phenomenon are considered.

Synthetic phospholipids containing short fatty acid chains have unique properties that make them very useful as model membrane systems and as substrates for phospholipases. The physical chemical properties of short-chain phosphatidylcholines (PC)[1] have been previously investigated by Tausk et al. (1974a–c). These compounds are water soluble and form micelles above their cmc. Studies using short-chain phosphatidylcholines as substrates (Pieterson et al., 1974; Wells, 1972, 1974) or activators (Plückthun & Dennis, 1982b) have provided valuable information on the mechanism of action of phospholipase A_2 (Dennis, 1983), since their aggregation state can easily be varied. In particular, the poor hydrolysis of phosphatidylethanolamine (PE) by cobra venom phospholipase A_2 was found to be activated 10–20-fold by phosphocholine-containing compounds including dibutyryl-PC. Short-chain PC that is monomerically dispersed below its cmc is generally a poor substrate for phospholipases but does allow the use of the standard kinetic treatments for homogeneous enzymatic reactions. It is for this reason that we synthesized and studied the short-chain PE as substrate for phospholipase A_2, in particular to obtain kinetic information on the activation phenomenon (Plückthun & Dennis, 1982b). In addition, two nonhydrolyzable short-chain urethane analogues (with choline and ethanolamine head groups) were synthesized, and their effect on the enzymatic reaction was examined.

The physical properties of long-chain PE and PC are known to differ from one another in many respects, i.e., phase dia-

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[1] Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; TLC, thin-layer chromatography; cmc, critical micelle concentration; Triton, Triton X-100; HPLC, high-pressure liquid chromatography; CAPS, 3-(cyclohexylamino)propanesulfonic acid.

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