Interaction of Lipoprotein Lipase with Phospholipid Vesicles: Effect on Protein and Lipid Structure†

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ABSTRACT: The interaction of lipoprotein lipase (LpL) and a nonhydrolyzable phosphatidylcholine, 1,2-ditetradecyl-*rac*-glycero-3-phosphocholine (C_{14}-ether-PC), has been studied by several physical methods. Analysis of the circular dichroic spectrum of LpL gave the following fractional conformation: 35% α-helix, 30% β-pleated sheet, and 45% remaining structure. No significant change in the circular dichroic spectrum of LpL was observed on addition of C_{14}-ether-PC vesicles. The quenching of LpL fluorescence by acrylamide and iodide ion was decreased only slightly by addition of C_{14}-ether-PC vesicles. Addition of LpL to sonicated C_{14}-ether-PC vesicles containing entrapped carboxyfluorescein caused the release of <15% of the vesicle contents in 20 min, indicating that the enzyme did not disrupt the structure of the lipid. In contrast, >80% of the vesicle contents were released with the addition of apolipoprotein A-I to an identical vesicle preparation. The temperature dependence of the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene incorporated into C_{14}-ether-PC vesicles was not significantly altered by the addition of LpL. When LpL is added to vesicles, the bilayer structure of the vesicles is not disrupted as observed by freeze–fracture electron microscopy. However, at low ionic strength (0.1–0.25 M NaCl) significant aggregation of intact vesicles is observed by light scattering and electron microscopy. Vesicle aggregation is prevented and reversed by 1 M NaCl and by heparin. These data demonstrate that LpL binds to the surface of a lipid interface, without dramatic changes in lipid bilayer or protein structure.

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 Hamosh & Hamosh (1983), Quinn et al. (1983), and Jackson (1983)]. LpL has four fractional sites: (1) a glycosaminoglycan binding site that immobilizes LpL to endothelial cell surfaces; (2) a binding site for the physiological activator of LpL catalysis, apolipoprotein C-II; (3) an active (or catalytic) site; and (4) a lipoprotein interfacial lipid-binding site. Although the detailed molecular organization of the lipoprotein substrate for LpL has not been elicited, the major lipid component of the surface of the lipoprotein is PC (Morrisett et al., 1977). LpL is thought to hydrolyze triacylglycerol molecules within the lipoprotein surface. Miller and Small (1983) estimated that the surface concentration of triacylglycerol in triacylglycerol-rich lipoproteins is 2–4%; the maximum solubility of triacylglycerol in PC is <5% by weight (Demel et al., 1982; Gorrissen et al., 1982; Hamilton et al., 1983; Demel & Jackson, 1985).

A variety of systems, including emulsions (Bengtsson & Olivecrona, 1980), monolayers (Jackson et al., 1980; Vainio et al., 1983), and PC vesicles (Shirai et al., 1981), have been used as models for LpL interactions with the lipoprotein surface. LpL penetrates egg yolk PC monolayers, increasing surface pressure, with a critical pressure for penetration >40 mN m^{-1} (Vainio et al., 1983) and binds to PC vesicles (Shirai et al., 1981; McLean & Jackson, 1985) and monolayers of 1,2-didecanoylglycerol (Jackson et al., 1980) in the absence and presence of apolipoprotein C-II. The effect of LpL on the molecular organization of lipid bilayers and the effect of lipid on structural features of the LpL molecule have not been described. The purpose of this study was to examine the interactions of LpL with a model substrate interface consisting of ether-PC bilayer vesicles that are not hydrolyzed by the enzyme.

Materials and Methods

Materials

Lipoprotein lipase was purified from bovine skim milk by a modification of the procedure of Kinnunen (1977) as described previously (McLean & Jackson, 1985). The isolated enzyme had a molecular weight of 55 000 as determined by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (Socorro & Jackson, 1985). C_{14}-ether-PC was a kind gift of Dr. R. Demel (State University of Utrecht, Utrecht, The Netherlands). Acrylamide (electrophoresis purity >99.9%) was from Bio-Rad; L-α-di[1-^{14}C]palmitoylphosphatidylcholine (sp act. 100 mCi/mmol) was obtained from New England Nuclear (Boston, MA). Heparin (porcine intestinal mucosal,

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[1] Abbreviations: LpL, lipoprotein lipase; PC, phosphatidylcholine(s); C_{14}-ether-PC, 1,2-ditetradecyl-*rac*-glycero-3-phosphocholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; CD, circular dichroism; apoA-I, apolipoprotein A-I; apoC-II, apolipoprotein C-II; DPH, 1,6-diphenyl-1,3,5-hexatriene; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin.

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grade I, Sigma) was used to prepare heparin–Sepharose CL-4B (Iverius, 1971). All other reagents were ACS grade. Human apolipoprotein A-I (apoA-I) was isolated from plasma high-density lipoproteins, and human apolipoprotein C-II (apoC-II) was isolated from very low density lipoproteins (Cardin et al., 1984). Standard buffer was 0.15 M NaCl, 10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.01% sodium azide, pH 7.40. The purity of the lipids was established by thin-layer chromatography. PC vesicles were prepared and incubated with LpL to form LpL–C_{14}-ether-PC vesicle complexes as described previously (McLean & Jackson, 1985).

Methods

Circular Dichroic (CD) Spectra. Spectra were recorded on a Cary 61 spectropolarimeter operated at 10 °C. The instrument was calibrated with *d*-10-camphorsulfonic acid. All measurements were made at optical density readings that maintained the dynode voltage at <0.8 kV. Spectra were run in triplicate in a buffer of 1.4 M NaCl, 30% glycerol, and 10 mM Tris-HCl, pH 7.40. The high salt concentration allowed higher concentrations of enzyme to be used; under these conditions, LpL activity remains high (>90% of initial activity) and binding to lipid vesicles is unaffected (McLean & Jackson, 1985). Fluorescence measurements on the samples diluted to 50 μg of LpL/mL were made in parallel. After each set of spectra, the spectrum of a blank corresponding to the sample without LpL was measured. Mean residue ellipticities, $[\theta]$, were calculated in units of deg cm^2/dmol from the equation $[\theta] = \theta_{obsd} M_r/10lc$, where θ_{obsd} is the observed ellipticity in degrees (corrected for the blank), M_r is the mean residue molecular weight (115; Iverius & Ostlund-Lundqvist, 1976), l is the cell path length in centimeters, and c is the protein concentration in grams per milliliter. The far-UV CD data were digitized at 1-nm intervals and analyzed by fitting the individual data points to standard ellipticity values obtained from proteins of known secondary structure (Chen et al., 1974) by nonlinear regression (Marquardt, 1963).

Fluorescence Spectra. Fluorescence spectra and light-scattering measurements were recorded on a Perkin-Elmer MPF-44A spectrofluorometer. Blank values were subtracted with a Perkin-Elmer differential corrected spectra unit or by hand. Temperature was maintained within 0.2 °C with a recirculating water bath. For polarization measurements, 1,6-diphenyl-1,3,5-hexatriene (DPH) was added to sonicated vesicles to give a final molar ratio of probe to PC of 1:100. The vesicles were incubated at room temperature until the fluorescence of DPH reached a maximal value. Polarization measurements were made as described previously (Cardin et al., 1982).

Flourescence Quenching. Fluorescence quenching of solutions of tryptophan and of LpL in the presence and absence of C_{14}-ether-PC vesicles was measured by progressive addition of small aliquots of 8 M acrylamide in standard buffer. Fluorescence intensities were measured at 25 °C with excitation at 295 nm and emission monitored at the wavelength of maximum fluorescence of the sample. Corrections due to absorptive screening by acrylamide ($\epsilon_{295} = 0.23$; Parker, 1968) were made according to $F_{cor} = F_{obsd}$ antilog (0.115[acrylamide]). Quenching with KI was measured by using a stock solution of 3 M KI and 0.1 mM sodium thiosulfate in standard buffer. The ionic strength was maintained constant by addition of 3 M KCl. The solutions were equilibrated at 25 °C for 5 min before each measurement of fluorescence intensity. Flourescence intensities remained constant for at least 30 min, and >90% of the LpL activity was recovered at the end of each experiment.

Fluorescence quenching was analyzed according to the Stern and Volmer (1919) equation $F_0/F = 1 + K_{sv}[Q]$, where F_0 and F are the fluorescence intensities in the absence and presence of quencher at concentration [Q], and K_{sv} is the Stern–Volmer quenching constant. Protein fluorescence quenching data were fitted to two modified Stern–Volmer expressions: the first (Eftink & Chiron, 1984) included an exponential term V, $F_0/F = (1 + K_{sv}[Q]) \exp(V[Q])$, and the second (Lehrer, 1971) assumed a fraction (f_a) of accessible fluorophores, $F_0/F = (1 - f_a) + f_a(1 + K_{sv}[Q])$. Data were fitted to the models by nonlinear regression (Marquardt, 1963) and were compared by Hamilton's R (Hamilton, 1964).

Rate of Carboxyfluorescein Leakage. The release of 6-carboxyfluorescein entrapped in lipid vesicles was followed as described by Chen (1977) and Weinstein et al. (1977) with modification. Sonicated vesicles containing entrapped 6-carboxyfluorescein were prepared as follows. Five milligrams of C_{14}-ether-PC was dried under nitrogen and then dispersed in 2 mL of standard buffer containing 150 mg of 6-carboxyfluorescein, pH 7.4. After sonication (McLean & Jackson, 1985), the vesicles were applied to a column (1.5 × 30 cm) of Bio-Rad A-15m and eluted with standard buffer. Fractions of 1 mL were collected, and the fractions within the included volume of the column were used for leakage experiments. PC vesicles (50–100 μg) containing entrapped carboxyfluorescein were diluted to 1.4 mL of standard buffer containing 500 μg of heparin. Base line leakage was established as <0.2%/15 min by recording fluorescence continuously at 492-nm excitation and 525-nm emission. Then, 50 μg of protein was added, the solution was mixed for 30 s, and the fluorescence was recorded continuously for 20 min. Finally, 100 μL of 10% Triton N-101 (w/v) was added with stirring. The percent leakage = 100(fluorescence in the presence of protein − blank fluorescence)/(fluorescence in the presence of Triton N-101). Fluorescence intensities were all corrected (linearly) for dilution.

Freeze–Fracture Electron Microscopy. C_{14}-ether-PC vesicles were prepared as described above. LpL was added to the vesicles to a weight ratio of 2:1 PC:LpL. The sample was then incubated for 4 h at 25 °C. All samples were equilibrated at room temperature for 6 h prior to freezing. The final concentration of glycerol was 25%. Samples mounted in Balzers mirror image sample holders were frozen from 20 °C in Freon 12 that was cooled in a liquid nitrogen bath. Frozen samples were fractured with a mirror image device in a Balzers BAF 301 freeze-etching device equipped with an electron beam apparatus for platinum and carbon shadowing and a quartz-crystal monitor for regulating shadow thickness. Fracturing was performed at −117 °C without etching. Platinum films were approximately 2 nm thick. Replicas were floated from the sample holders onto water and cleaned by flotation on $NaHClO_3$ solution for 30 min. Then, the replicas were rinsed on water and picked up on 400-mesh copper grids. Electron micrographs were taken with a JEOL 100CX electron microscope at 40000×.

Analytical Methods. LpL was assayed for hydrolytic activity in the presence of apolipoprotein C-II in a Triton X-100 emulsified trioleoylglycerol substrate as described previously (Matsuoka et al., 1980). Protein was determined by the method of Lowry et al. (1951). Phospholipid concentrations were obtained by analysis of inorganic phosphorus (Bartlett, 1959).

RESULTS

Effect of Lipid on LpL Structure. The far-UV CD spectra of LpL alone and LpL mixed with C_{14}-ether-PC vesicles are

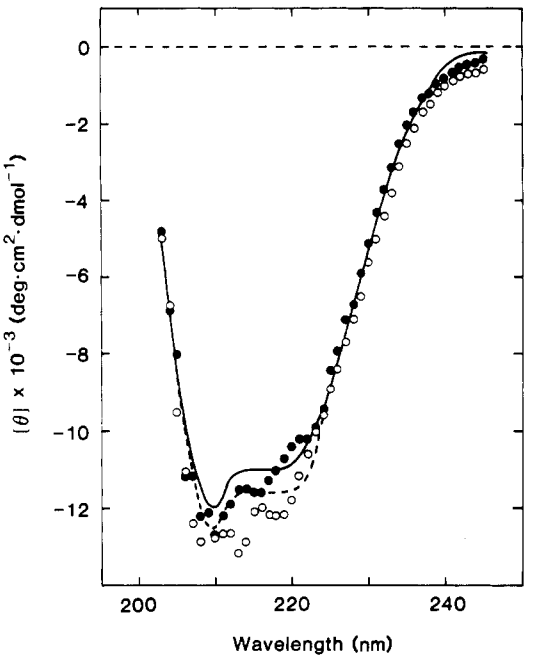

FIGURE 1: Far-UV circular dichroic spectra of LpL in the absence and presence of C_{14}-ether-PC vesicles. The symbols show the experimental data at 1-nm intervals: (○, ---) LpL (300 μg) in 1.5 mL of buffer containing 30% glycerol, 1.4 M NaCl, and 10 mM Tris-HCl, pH 7.4; (●, —) LpL (300 μg) plus C_{14}-ether-PC vesicles (400 μg) in the same buffer. The curves were obtained by fitting the experimental data to standard ellipticity values as described under Materials and Methods. Each point is the average of three experiments. Spectra were recorded at 10 °C with a 2-mm path-length cuvette.

shown in Figure 1 [cf. Olivecrona et al. (1982)]. Both sets of spectra were recorded within 60 min of preparation of the samples, which were maintained in an ice–water bath prior to recording the spectra at 10 °C. Only a small change in the CD spectrum of LpL is observed following the addition of C_{14}-ether-PC vesicles. The data are best fit by the following fractional conformations: LpL alone contains 35% α-helix, 30% β-pleated sheet, and 45% remaining structure; LpL in the presence of C_{14}-ether-PC vesicles contains 32% α-helix, 24% β-pleated sheet, and 44% remaining structure. After 2 h of incubation at 10 °C, little change in the CD spectrum of the LpL–C_{14}-ether-PC vesicle mixture was observed (α-helix = 30%; β-pleated sheet = 29%; remaining structure = 41%). In parallel fluorescence experiments with the same samples diluted to 50 μg of protein/mL, the fluorescence spectrum of the LpL–C_{14}-ether-PC vesicle mixture was blue-shifted 6 nm and was 1.45 times as intense as LpL alone, consistent with binding of LpL to the vesicles (McLean & Jackson, 1985). Under all conditions used in the present experiments, LpL is bound to the vesicles. At the completion of the experiments, the LpL activity was >90% of the initial activity.

Fluorescence quenching of LpL in the presence and absence of C_{14}-ether-PC vesicles is shown in Figure 2. Quenching of tryptophan was used as a basis of comparison; the Stern–Volmer quenching constants (K_{sv}) for tryptophan were 16.5 M^{-1} with acrylamide [cf. 17.7 M^{-1}; Eftink & Ghiron (1984)] and 11.4 M^{-1} with KI [cf. 11.6 M^{-1}; Lehrer (1971)]. Protein fluorescence quenching data were fit to the Stern–Volmer equation and to two modified Stern–Volmer equations (Eftink & Ghiron, 1984; Lehrer, 1971). No significant improvement in the fit of the data (at $p < 0.05$) was obtained with the modified models. Therefore, the classical Stern–Volmer constant was used. Only a small blue shift (<1 nm) was observed with quenching by acrylamide. In the absence of PC vesicles, the constant for LpL quenching by acrylamide is 2.7

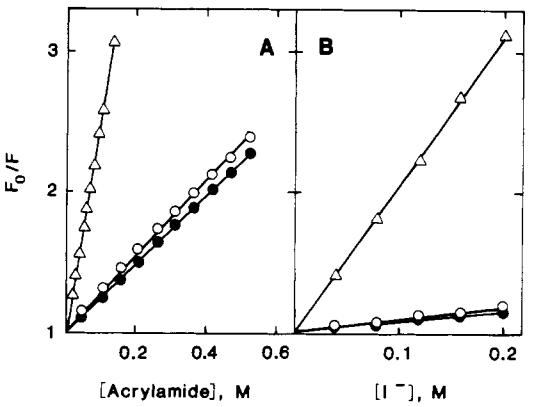

FIGURE 2: Fluorescence quenching of tryptophan and LpL in the presence and absence of C_{14}-ether-PC vesicles. Data shown are the average of two to three experiments. (A) Quenching by acrylamide of tryptophan (12 μg) in standard buffer (△) or of lipoprotein lipase (50 μg) in the absence (○) and presence (●) of 200 μg of C_{14}-ether-PC vesicles in 1.5 mL of standard buffer. (B) Quenching by KI under conditions identical with (A), except that standard buffer also contained a constant potassium concentration of 0.2 M by addition of KCl and KI. The lines are based on fitting data to the Stern–Volmer equation.

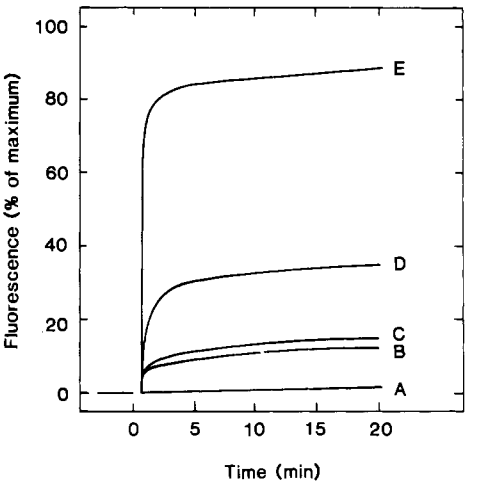

FIGURE 3: 6-Carboxyfluorescein leakage from C_{14}-ether-PC vesicles in the presence of LpL, bovine serum albumin, or apoA-I. The leakage of 6-carboxyfluorescein from C_{14}-ether-PC vesicles (50 μg in 1.5 mL of standard buffer) was followed continuously at 25 °C after addition at zero time of 50 μL of (A) standard buffer, (B) bovine serum albumin (1 mg/mL), (C) LpL (1 mg/mL), (D) apoA-I (1 mg/mL), or (E) apoA-I (10 mg/mL). After 20 min of incubation, 100 μL of a 10% solution of Triton N-101 was added to give the 100% fluorescence intensity. Data have been corrected (linearly) for dilution.

± 0.1 M^{-1} (n = 33) and by KI is 0.99 ± 0.03 M^{-1} (n = 12). Addition of C_{14}-ether-PC vesicles decreases the quenching rate somewhat: for acrylamide, K_{sv} = 2.4 ± 0.1 M^{-1} (n = 22), and for KI, K_{sv} = 0.80 ± 0.03 M^{-1} (n = 12). Addition of the phospholipid did not affect the K_{sv} values of free tryptophan.

Effect of LpL on Lipid Vesicle Structure. In Figure 3, the effect of LpL and apoA-I on the permeability of C_{14}-ether-PC vesicle bilayers to 6-carboxyfluorescein is shown. In the absence of protein, leakage of 6-carboxyfluorescein is <0.2% in 15 min at 25 °C. Addition of LpL to the vesicles increases the rate of leakage over the control value somewhat; the effect of LpL is similar to that of bovine serum albumin and considerably less than that of apoA-I at equivalent weight ratios. The leakage of entrapped marker increases with increasing ratios of LpL to C_{14}-ether-PC vesicles up to a ratio of 1:1 by weight. Increasing the weight ratio to 2:1 has no effect on leakage of dye in the presence of LpL. Slow leakage of 6-carboxyfluorescein from 10 mol % cholesterol–egg PC vesicles

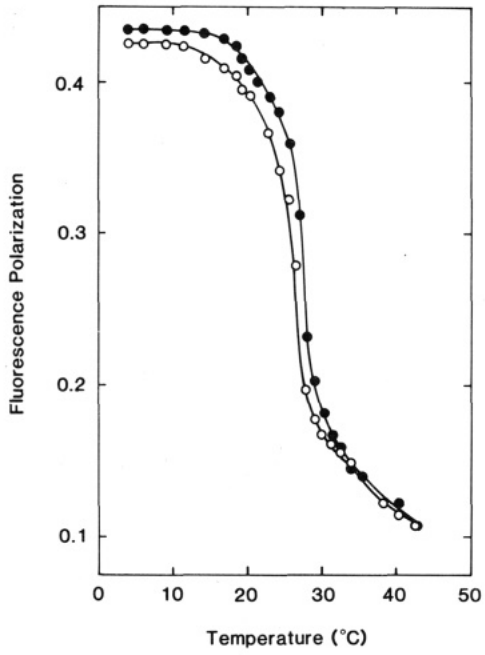

FIGURE 4: DPH fluorescence polarization of C_{14}-ether-PC vesicles in the presence and absence of LpL. C_{14}-ether-PC vesicles were labeled with DPH at a molar ratio of probe to PC of 1:100. The fluorescence polarization values of the samples were measured as a function of temperature: (○) C_{14}-ether-PC vesicles (225 μg) in 1.5 mL of standard buffer; (●) C_{14}-ether-PC vesicles (225 μg) plus LpL (100 μg) in 1.5 mL of standard buffer.

following addition of LpL in the absence of apoC-II has also been demonstrated (Fugman et al., 1984). In contrast, addition of apoA-I to an identical population of vesicles results in rapid leakage of dye: at a weight ratio of apoA-I:PC of 10:1, >90% of the entrapped dye is released in <2 min [similar to data of Kanellis et al. (1980) and Segrest et al. (1983)].

The effect of LpL on the polarization of DPH incorporated into C_{14}-ether-PC bilayer vesicles is shown in Figure 4. In the absence of LpL, the midpoint of the phase transition, T_m, for sonicated C_{14}-ether-PC vesicles is 26.7 °C. This value compares favorably with previous data (Shirai & Jackson, 1982; Jain et al., 1982). Since the polarization values measured above and below T_m are similar to those obtained for DPPC vesicles (Cardin et al., 1982), the local environment of the DPH probe in C_{14}-ether-PC vesicles is similar to that in DPPC vesicles in corresponding gel and liquid-crystalline phases. Addition of LpL to the sonicated vesicles increases T_m slightly to 27.3 °C. The increase in T_m is accompanied by an increase in fluorescence polarization of the probe at all temperatures below 30 °C. No significant difference in polarization was observed when the C_{14}-ether-PC bilayer was in the liquid-crystalline phase (above 30 °C).

The light-scattering intensity of C_{14}-ether-PC vesicles in the presence and absence of various proteins is shown in Figure 5. Addition of bovine serum albumin to the samples had no effect on the light-scattering intensity of the samples (Figure 5D). Addition of apoA-I decreased the scattering (Figure 5E), consistent with the formation of smaller micellar structures as previously observed near the phase-transition temperature of saturated PC vesicle bilayers (Morrisett et al., 1977). LpL increased the light scattering of vesicles to an extent dependent upon the NaCl concentration of the medium. At 1 M NaCl, LpL addition had no effect on light scattering (Figure 5D). An ~1.5× increase in light scattering was observed on addition of LpL to vesicles at 0.4 M NaCl (Figure 5C); a 3-fold increase occurred at 0.1 M NaCl (Figure 5B). To test the reversibility of the light-scattering increase, 5 M NaCl was

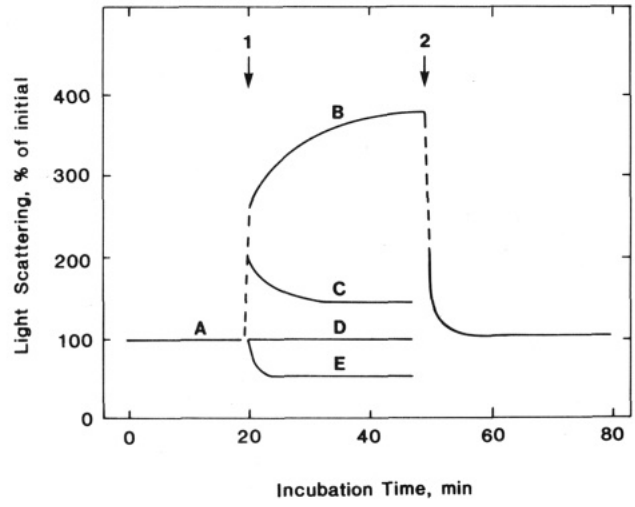

FIGURE 5: Effect of LpL, BSA, and apoA-I on the light scattering of C_{14}-ether-PC vesicles. Light scattering at a 90° angle was monitored at an excitation and emission wavelength of 400 nm at 25 °C. C_{14}-ether-PC vesicles (50 μg) were prepared in 1.5 mL of standard buffer (A). At arrow 1, 50 μL of each of the following proteins was injected: (B) LpL (1 mg/mL), final NaCl concentration 0.1 M; (C) LpL (1 mg/mL), 0.4 M NaCl; (D) LpL (1 mg/mL), 1 M NaCl or BSA (1 mg/mL), 0.1 M NaCl; (E) apoA-I (1 mg/mL), 0.1 M NaCl. At arrow 2, sample B was adjusted to 1 M NaCl by addition of a 5 M NaCl solution or to 0.5 mg of heparin/mL. Data were recorded continuously and corrected for dilution.

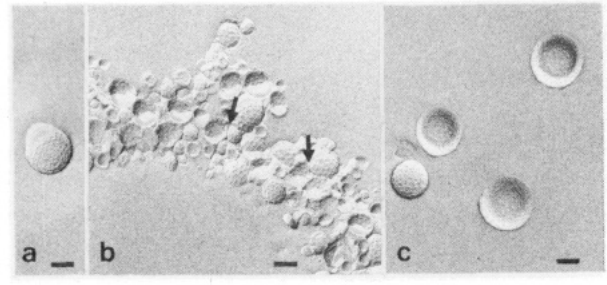

FIGURE 6: Freeze–fracture electron microscopy of C_{14}-ether-PC vesicles with and without LpL. (a) C_{14}-ether-PC vesicles in 0.25 M NaCl (500 μg of PC/mL); (b) C_{14}-ether-PC vesicles (500 μg of PC/mL) plus LpL (250 μg/mL) in 0.25 M NaCl; (c) sample B in 1 M NaCl. The final glycerol concentration was 25%. Magnification: (a and b) 30840×; (c) 31480×; bar = 0.1 μm.

added to the LpL–vesicle system in 0.1 M NaCl to bring the final NaCl concentration to 1 M. The increase in NaCl concentration resulted in a rapid return of the light-scattering intensity to the base line value. Similarly, addition of heparin (0.5 mg/mL) rapidly decreased the light-scattering intensity to the base line value. No difference in the fluorescence emission spectrum of LpL plus vesicles (excitation 280 nm) was observed when the NaCl concentration was increased from 0.1 to 1.0 M or after heparin was added.

Figure 6 illustrates freeze–fractured C_{14}-ether-PC vesicles in the absence (a) and presence of LpL at 0.25 M NaCl (b) and at 1 M NaCl (c). High NaCl concentrations (1 M) have no effect on the vesicle structure in the absence of LpL. In the presence and absence of LpL, the vesicles are largely intact, spherical, and single-shelled. The average vesicle diameter is 25–40 nm. The structure and size of individual vesicles do not appear to be affected by the addition of LpL (Figure 6a,c). The most striking difference among the three preparations is the extent of aggregation of the vesicles which appear to remain intact (see arrows). In the absence of LpL, the vesicles appear as individual particles. Although quantitative estimates for the number of aggregated vesicles when LpL is added at 0.25 M NaCl are difficult owing to the plane of fracture,

virtually all fractured vesicle aggregates contain at least 20 vesicle profiles. At 1 M NaCl, vesicles appear as disperse as those in solutions lacking LpL; infrequently, vesicles occur in loosely aggregated groups of two or three.

Discussion

In this and a previous paper (McLean & Jackson, 1985) we have used a model system of ether-PC vesicles to study interactions between lipoprotein lipase and a lipid interface. Since the ether linkage prevents the hydrolysis of the lipid by LpL and no significant structural differences between hydrated bilayers of acyl- and alkyl-PCs have been observed (Schwartz et al., 1976; Hauser, 1981), this lipid offers a system in which the effects of binding of LpL to a model lipoprotein interface may be examined. The molecular interaction between LpL and C_{14}-ether-PC vesicles differs from that observed between PC vesicles and serum apolipoproteins which also bind to lipoprotein interfaces (Morrisett et al., 1977). With regard to the effect of lipid on protein structure, the mean residue ellipticity (222 nm) of several plasma apolipoproteins (which form amphipathic helices in the presence of PC) is increased significantly by addition of PC vesicles (Morrisett et al., 1977; Segrest et al., 1974), and a corresponding large decrease in K_{sv} is observed (Pownall & Smith, 1974). In contrast, the increase and blue shift in LpL fluorescence following binding to C_{14}-ether-PC vesicles are not accompanied by significant secondary structural changes that can be observed by CD spectroscopy. This fluorescence increase is not the result of aggregation of the vesicles: the fluorescence spectra at 0.1 and 1 M NaCl are identical even though the light scattering measured at 400 nm is increased at low salt. Thus it appears that at least one of the tryptophan residues of LpL is transferred to a more hydrophobic environment. In addition, only a small decrease ($\sim$1.2×) in the Stern–Volmer quenching constants is observed for acrylamide and iodide quenchers on addition of vesicles to LpL. These data on LpL do not rule out the possibility than an amphipathic helix in LpL is responsible for binding to the lipid interface; only a small portion of the protein may bind to lipid, while the CD and fluorescence quenching measurements report on several regions of the protein molecule.

Significant changes in bilayer structure have been observed following addition of apolipoproteins: the polarization of DPH as a function of temperature in DMPC bilayers is altered (Rosseneu et al., 1979; Cardin et al., 1982), and apoA-I added to lipid bilayer vesicles containing entrapped carboxyfluorescein causes a rapid release of the vesicle contents (Figure 2; Kanellis et al., 1980; Segrest et al., 1983). In contrast, the effects of LpL on C_{14}-ether-PC vesicle physical properties (DPH polarization and carboxyfluorescein leakage) are minimal. Freeze–fracture electron micrographs of C_{14}-ether-PC vesicles with and without LpL also show that LpL does not disrupt the bilayer structure of the vesicles. Rather, vesicle aggregation is observed at low salt concentrations and is fully reversed by increasing the concentration of NaCl to 1 M. This reversibility demonstrates that fusion does not cause the increase in light scattering. Since LpL remains bound to C_{14}-ether-PC vesicles at 1 M NaCl (McLean & Jackson, 1985), these data suggest that LpL does not cause a reorganization of lipid structure in C_{14}-ether-PC vesicles. Rather, LpL binds to the vesicle surface and maintains an intact vesicle bilayer structure without causing dramatic changes in the secondary structure of the enzyme.

The aggregation of the lipid vesicles in the presence of LpL at low salt concentrations appears to be the result of interactions between two or more LpL molecules that are bound to separate lipid vesicles. The observation that NaCl reverses vesicle aggregation suggests that the putative LpL–LpL interaction is largely ionic. This ionic site may also be a heparin-binding site, since heparin reverses aggregation of the lipid vesicles. From our data, it is not possible to determine the aggregation number of LpL at the vesicle surface. In buffers with high salt concentrations, LpL monomers (Iverius & Ostlund-Lindqvist, 1976), dimers (Garfinkel et al., 1983; Olivecrona et al., 1985), and higher aggregates (Olivecrona et al., 1982, 1984) have been reported. In addition, LpL appears to bind to the endothelial cell surface (Garfinkel et al., 1983) and to heparin and lipid droplets (Olivecrona et al., 1985) as a dimer. The formation of a dimer on the lipid vesicle surface is not inconsistent with our data; such a dimer may serve as a bridge between the endothelial cell surface and the lipoprotein substrate. In the lipid vesicle model system, heparin may bind to the endothelial cell surface receptor-binding site on the LpL, mimicking the in vivo complex. In the absence of heparin, at low salt concentrations, the heparin-binding site on the vesicle-bound LpL dimer may be available for binding to an LpL molecule on another lipid vesicle, resulting in vesicle aggregation.

Acknowledgments

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Registry No. LpL, 9004-02-8; ^{14}C-ether-PC, 18656-38-7.

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Acylation of Cellular Proteins with Endogenously Synthesized Fatty Acids†

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ABSTRACT: A number of cellular proteins contain covalently bound fatty acids. Previous studies have identified myristic acid and palmitic acid covalently linked to protein, the former usually attached to proteins by an amide linkage and the latter by ester or thio ester linkages. While in a few instances specific proteins have been isolated from cells and their fatty acid composition has been determined, the most frequent approach to the identification of protein-linked fatty acids is to biosynthetically label proteins with fatty acids added to intact cells. This procedure introduces possible bias in that only a selected fraction of proteins may be labeled, and it is not known whether the radioactive fatty acid linked to the protein is identical with that which is attached to the protein when the fatty acid is derived from endogenous sources. We have examined the distribution of protein-bound fatty acid following labeling with [^{3}H]acetate, a general precursor of all fatty acids, using BC_3H1 cells (a mouse muscle cell line) and A431 cells (a human epidermoid carcinoma). Myristate, palmitate, and stearate account for essentially all of the fatty acids linked to protein following labeling with [^{3}H]acetate, but at least 30% of the protein-bound palmitate in these cells was present in amide linkage. In BC_3H1 cells, exogenous palmitate becomes covalently bound to protein such that less than 10% of the fatty acid is present in amide linkage. These data are compatible with multiple protein acylating activities specific for acceptor protein fatty acid chain length and linkage. The enzyme(s) that link(s) palmitate to protein by amide linkage preferentially use(s) fatty acid generated by fatty acid synthetase in BC_3H1 cells, but apparently has (have) equal access to exogenously and endogenously derived palmitate in A431 cells.

Covalent modification of proteins with fatty acid has been described for a number of specific cellular and viral proteins, including the vesicular stomatitis virus G protein (Schmidt & Schlesinger, 1979), calcineurin B (Aitken et al., 1982), the transferrin receptor (Omary & Trowbridge, 1981), pp60 src (Sefton et al., 1982), the catalytic subunit of cAMP-dependent protein kinase (Carr et al., 1982), and cytochrome b_5 reductase (Ozols et al., 1984). The linkage of fatty acid to acyl proteins

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