Specificity of the lipid-binding domain of apoC-II for the substrates and products of lipolysis

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Abstract Functional similarities between colipase and apolipoprotein C-II (apoC-II) in activating lipases suggest that apoC-II may, like colipase, preferentially interact with interfaces containing the substrates and products of lipolysis. To test this hypothesis, the binding of a peptide comprising residues of the cofactor implicated in lipid binding, apolipoprotein C-II(13-56), and, to a lesser extent, apoC-II, to monomolecular lipid films was characterized. The lipids used were a diacylphosphatidylcholine, a diacylglycerol, and a fatty acid. The peptide had an affinity for the argon-buffer interface and for all lipids consistent with a dissociation constant of <10 nM. Changes in surface pressure accompanying peptide binding were comparable to those reported for native apoC-II and indicate peptide miscibility with each of the lipids tested. The capacity of the surfaces to accommodate the peptide decreased with increasing lipid concentration in the interface, indicating competition between lipid and peptide for interfacial occupancy. At a lipid acyl chain density of 470 pmol/cm^2, or 35 Å^2 per acyl chain, a lower limit of peptide adsorption was reached with all lipids. The limiting level of adsorption to phosphatidylcholine was only 1 pmol/cm^2 compared with 6–7 pmol/cm^2 for fatty acid and diacylglycerol. Similar results were obtained with apoC-II. The difference in the extent of protein adsorption to lipid classes suggests that the distribution of apoC-II among lipoproteins will depend on their lipid composition and surface pressure.—Dahim, M., W. E. Momsen, M. M. Momsen, and H. L. Brockman. **Specifity of the lipid-binding domain of apoC-II for the substrates and products of lipolysis. *J. Lipid Res.* 2001.** 42: **553–562.**

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There are multiple families of lipases in mammals such as the acid lipases, the classical pancreatic-type lipases, and the carboxylester lipases. Three prominent members of the classical lipase family are pancreatic triacylglycerol lipase (PTL), lipoprotein lipase (LPL), and hepatic lipase. These show marked structural similarities and are derived from a common ancestral gene (1). Two of these, PTL and LPL, are notable because neither can function properly in vivo without the assistance of a protein cofactor. The cofactor for PTL, colipase, is a 10 kD protein. Without colipase, PTL is inhibited by phospholipids and bile salts (2), which are abundant constituents of the intestine (3). The cofactor for LPL is apolipoprotein C-II (apoC-II), a 79-amino acid peptide (4). Triacylglycerol-rich lipoproteins isolated from individuals lacking apoC-II or having apoC-II with certain mutations are resistant to hydrolysis by LPL in the absence of exogenously added apoC-II (5–9). The third member of the lipase family, hepatic lipase, shows activation by apolipoprotein E, but this cofactor is not essential for activity (10).

Functionally, the enhancement of PTL catalysis in interfaces by colipase is analogous to lowering the apparent K_m for a classical enzyme reaction. The mechanistic basis for the action of colipase has been studied extensively, and two major roles for the cofactor have been proposed. As recently reviewed (11), one well-established role is to help anchor PTL to interfaces via protein-protein interactions. Second, in the presence of diacylglycerol or fatty acid, colipase adsorption to phospholipid-rich interfaces is greatly enhanced, and physical data suggest that the nonphospholipids are concentrated around colipase (12). This lateral substrate concentrating effect should increase the availability of substrate to PTL. Additional work has shown that the creation of this colipase-lipid nanodomain helps anchor PTL to phospholipid-rich interfaces via PTL-lipid interactions (11, 13).

The mechanism by which apoC-II enhances LPL activity is known with less certainty (14). Two studies (15, 16) reported that the primary effect of apoC-II on lipoprotein lipase activity was to decrease the apparent K_m for triacylglycerol with minor affects on V_m. The substrate used was triacylglycerol-rich lipoproteins isolated from a patient with an apoC-II deficiency. However, it was later reported that the primary effect of apoC-II on lipoprotein lipase activity toward apoC-II-deficient chylomicrons was to in-

Abbreviations: apoC-II, apolipoprotein C-II; [^{14}C]apoC-II(13-56), [^{14}C]apolipoprotein C-II(13-56); PTL, pancreatic triacylglycerol lipase; LPL, lipoprotein lipase; SOPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DOG, 1,2-dioleoylglycerol; DA, 13,16-*cis,cis*-docosadienoic acid.

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crease the apparent V_m (17). As with the PTL-colipase system, the mechanism for the activation of LPL toward chylomicrons involves protein-protein interaction between the cofactor and the lipase and apoC-II binding to the lipid surface (18). Thus, like colipase, apoC-II on the lipoprotein surface appears not to participate in catalysis directly but to activate LPL.

Whereas the functional domains of colipase comprise the hydrophobic and hydrophilic faces of the wedge-shaped protein, the functional domains of apoC-II appear separated along the peptide sequence (14). A peptide composed of residues 51-79 can restore LPL activity toward apoC-II-deficient lipoproteins but it cannot bind to very low density lipoprotein (17). However, another peptide composed of residues 44-79 complexes with 1,2-dimyristoyl-*sn*-glycerol-3-phosphocholine and is capable of restoring the triacylglycerol hydrolyzing activity of LPL in phosphatidylcholine-rich monomolecular films to the same extent as the native cofactor. These and other observations of binding and LPL activation led to the conclusion that residues 44-50 are part of the lipid-binding region of the cofactor and that LPL activation resides in the C-terminal residues at positions >50 (17).

Recently, the direct involvement of residues 44-50 in lipid binding has been challenged. It was reported (19) that apoC-II(39-62) does not bind to large unilamellar vesicles of 1,2-dimyristoyl-*sn*-glycerol-3-phosphocholine but binds to and activates LPL both in solution and when the LPL is already bound to lipids. On the basis of this evidence, it was proposed that this peptide interacts directly with LPL, rather than with the lipid, contradicting the role in lipid binding proposed earlier for residues 44-50. Moreover, a recent determination of the structure of apoC-II(44-79) in the presence of sodium dodecyl sulfate micelles (20) shows that residues 50-58 and 67-75, but not 44-50, form amphipathic helices in the presence of the anionic detergent. The helix composed of residues 67-75 was proposed to be the major lipid-binding determinant of apoC-II. However, natural mutations in this region result in nonfunctional apoC-II that is still found associated with chylomicrons and very low density lipoprotein in vivo (9), suggesting, as did the functional studies (14), that residues toward the carboxy terminus of apoC-II(44-79) are involved in LPL binding and activation rather than lipid binding.

Although apoC-II(44-79) can associate with lipids under some conditions, this peptide does not completely account for the interactions of native apoC-II with lipids. The peptide restores LPL-catalyzed triacylglycerol hydrolysis in monolayers but is not as effective as the native cofactor in restoring the LPL activity against phospholipids (21). Likewise, apoC-II(30-78) activates LPL-catalyzed hydrolysis of triacylglycerol more than apoC-II(43-78) and progressively shorter peptides (22). Indirectly, generation by anionic detergent of the amphipathic helices observed in the structure of apoC-II(44-79) required 4–7 times as much sodium dodecylsulfate as was needed for helix formation in other, apolipoprotein-derived model peptides (20). As noted by the authors, this indicates that the interactions of the detergent-induced amphipathic helices of apoC-II(44-79) at residues 50-58 and 67-75 with the detergent are relatively weak.

The possible involvement of residues in the N-terminal part of apoC-II, i.e., residues between positions 1 and 43, in lipid binding was predicted by Segrest and co-workers, who showed by helical wheel analysis that residues 14-39 could potentially form a class A_2 amphipathic helix (23). More recently, synthetic apoC-II(13-39) was shown to bind to small, unilamellar vesicles of phosphatidylcholine (24). The involvement of residues 13-39 in lipid binding is further supported by naturally occurring mutants of apoC-II. Two point mutants in which a polar or charged residue is replaced by a nonhydrophobic residue, lys-19-thr and glu-38-lys, show no functional defect (9, 25). However, a point mutation causing substitution of the hydrophobic residue trp 26 by arg results in serum apoC-II deficiency and chylomicronemia (26). It has been noted that this cofactor should be synthesized and excreted. Hence, its absence in serum has led to the suggestion that it must be rapidly cleared, owing to an inability to bind to lipoproteins (9). Overall, these studies support the idea that residues 13-39, as well as part or all of the residues between 44 and 58, contribute to the affinity of native apoC-II for lipids.

Specific roles for the substrates and products of lipolysis in regulating the interaction of apoC-II with lipoprotein surfaces have not been described. However, regulation of LPL location, activity, and interaction with apoC-II by free fatty acids is well documented (14). There is also evidence that fatty acids may affect lipoprotein morphology and the exchange of surface constituents among lipoprotein classes (27–30). On the basis of the functional similarities between colipase and apoC-II it is possible that, like colipase, preferential apoC-II-lipid interactions may regulate the affinity or extent of cofactor binding to specific lipoproteins and the availability of substrate to LPL. As a first step to evaluating these possibilities, we have studied the interaction of a radio-labeled peptide, [^{14}C]apoC-II(13-56), and, to a lesser extent, [^{14}C]apoC-II with monolayers of different lipids. The lipids used were 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC), 1,2-dioleoylglycerol (DOG), and 13,16-*cis,cis*-docosadienoic acid (DA). These lipid classes were chosen because they are constituents of lipoproteins (31–34). The particular lipids used are all fluid at ambient temperature, a requirement for the interaction of apoC-II with phospholipids (35). The results show that at saturation [^{14}C]apoC-II(13-56) and [^{14}C]apoC-II bind to lipid monolayers with high affinity and to an extent that depends on both the lipid class used and its interfacial concentration. This supports the hypothesis that apoC-II, like colipase, may bind to lipoprotein surfaces in a manner regulated by lipid class as well as surface pressure.

MATERIALS AND METHODS

DOG was from Sigma (St. Louis, MO), DA was from NuChek Prep, Inc. (Elysian, MN), and SOPC was from Avanti Polar Lip-

ids (Alabaster, AL). Inorganic [^{32}P]phosphate, [^{14}C]1,2-dioleoyl-*sn*-glycero-3-phosphocholine, and [^{14}C]formaldehyde were from NEN (Boston, MA). Human apoC-II was purchased from Athens Research & Technology, Inc. (Athens, GA). Purification of water and preparation of solvents, buffer, and lipid solutions have been described (12). [^{14}C]1,2-Dioleoylglycerol was converted from [^{14}C]1,2-dioleoyl-*sn*-glycero-3-phosphocholine using Type V phospholipase C from *B. cereus* supplied by Sigma (St. Louis, MO) (36).

Secondary structure predictions of apoC-II were carried out at NPS@ (Network Protein Sequence Analysis, http://pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_server.html). The algorithms used were DPM, DSC, Gor4, HNNC, PHD, Predator, SIMPA96, and SOPMA.

A peptide composed of residues 13-56 of apoC-II, apoC-II(13-56), was synthesized by the Mayo Protein Core Facility (Rochester, MN) using solid phase methods on an ABI 433A Peptide Synthesizer (PE Biosystems, Foster City, CA) and following procedures recommended by the manufacturer. The peptide was purified by reverse-phase high performance liquid chromatography (HPLC) on a Jupiter C18 column (250 × 21.1 mm, 15μ; Phenomenex, Torrance, CA) in 0.1% TFA. The integrity of the peptide was verified by electrospray ionization mass analysis on a Perkin/Elmer Sciex API 165 Mass Spectrometer (PE Biosystems, Foster City, CA.). The molecular weight of chemically synthesized apoC-II(13-56), determined by electrospray ionization mass analysis, was 5103.0, compared with a calculated value of 5103.7. The pI of apoC-II(13-56), calculated using EMBL Services (http://www-heidelberg.de/Services/index.html) is 7.02, compared with 4.36 for native apoC-II. Protein concentration was determined by absorbance at 280 nm using extinction coefficients of 2.12 ml $mg^{-1}cm^{-1}$ and 1.4 ml $mg^{-1}cm^{-1}$ for apoC-II and apoC-II(13-56), respectively, (ExPASy Molecular Biology Server, proteomic tools, http://www.expasy.ch/).

To introduce a radioactive label into the peptide, reductive methylation (37) was carried out in the presence of guanidine hydrochloride. This denaturant has been shown to have a minimal effect on the derivatization reaction while maintaining protein solubility (38). To 5.2 mg of peptide in 4 ml of 1 mM potassium phosphate, pH 6.6, 50 mM NaCl, 3.0 M guanidine hydrochloride was added 250 μl of 0.12 M $NaBH_3CN$ in H_2O. After 5 min, 365 μl of [^{14}C]formaldehyde (21.1 μCi, 53.0 μCi/μmol) was added, and the mixture was stirred for 24 hours at 25°C. The sample was transferred to a Spectra/Por Dispo-Dialyzer, MWCO 500 (Spectrum, Rancho Dominguez, CA) and dialyzed extensively against four changes of guanidine hydrochloride solution (500 ml of 0.05, 200 ml of 0.1, 200 ml of 0.2, and 15 ml 4.0 M, respectively). The resulting radio-labeled peptide, [^{14}C]apoC-II(13-56), had a specific radioactivity of 15.3 μCi/μmol (34.0 dpm/pmol) and was used in all experiments, unless otherwise noted. The extent of labeling corresponds to the average modification of 0.3 lysine residues per molecule of peptide. ApoC-II was radio-labeled in a similar manner to give modification of 0.13 lysine residues per molecule of the cofactor.

The experimental details for the measurement of the adsorption of proteins to lipid monolayers have been described previously (13, 39). Briefly, a cylindrical Teflon trough (surface area = 19.5 cm^2, volume = 20 ml) was filled with a buffer consisting of 5.65 mM KH_2PO_4, 3.05 mM K_2HPO_4, and 80 mM NaCl, pH 7.0. Temperature was held at 24°C. Lipid films were spread from a hexane/ethanol (95:5) solution until the desired surface pressure was reached. After allowing the lipid monolayer to stabilize, inorganic [^{32}P]phosphate and protein solutions were injected sequentially into the stirred aqueous phase supporting the lipid monolayer. Surface pressure was monitored until the monolayer was collected on one side of a hydrophobic filter paper disk, and the extent of protein adsorption was determined. As described, inorganic [^{32}P]phosphate was used to correct the measured protein adsorption for the amount of protein in the aqueous subphase, which adheres to the paper (40).

RESULTS

Selection of peptide

As described in the Introduction, the N-terminal part of apoC-II has been implicated in its binding to lipoproteins and model interfaces. To better understand what residues of the cofactor might be involved in lipid association, the secondary structure of human apoC-II was predicted by a collection of algorithms as described in Materials and Methods. The results of that analysis are shown in **Fig. 1A** and vary with the algorithm used. However, between residues 11 and 54 most predict two helical regions separated by a linker region of 2–4 residues around residue 41. On the basis of these predictions and previous studies described in the Introduction, apoC-II(13-56) was chosen to constitute a model peptide that encompasses the putative helices, has equal numbers of charged and uncharged amino acid side chains, and begins and ends with a semipolar residue.

ApoC-II(13-56) has 12 hydrophobic residues according to the hydrophobicity scale of Wimley and White (41). Using helical wheel analysis, Segrest and co-workers (23) predicted that each of the two putative helices in the selected peptide should be a class A_2 amphipathic helix. This can be illustrated with a helical net diagram (Fig. 1B) of apoC-II(13-56), which arbitrarily projects the entire peptide as an α-helix. The figure shows that the hydrophobic residues fall into two sets, corresponding approximately to the helices defined in Fig. 1A. Note that the hydrophobic faces of these helices are approximately 180° out of phase. This implies that if the peptide assumed a completely helical conformation at interfaces, all of the hydrophobic residues could not occupy a common plane with their hydrophobic faces similarly oriented. However, alignment of the hydrophobic faces could occur as a consequence of the nonhelical linker region predicted in the vicinity of residue 41 (Fig. 1A). The presence of an interface should provide a strong driving force for such a conformational rearrangement. That the hydrophobic faces can be brought into phase in a common plane by nonhelix values of phi and psi angles involving two or more residues in the linker region was demonstrated by model building (not shown). Fig. 1B also shows that all of the five lysine residues in the two putative helices lie at the boundary between the hydrophilic and hydrophobic faces, and that all but one of the acidic residues lie more in the center of the hydrophilic faces (the "snorkel" hypothesis) (23). Thus, assuming proper alignment of the hydrophobic faces of the two helices, apoC-II(13-56), should be surface active.

Adsorption of [^{14}C]apoC-II(13-56) to the argon-buffer interface

The surface activity of [^{14}C]apoC-II(13-56) was first tested at the argon-buffer interface, i.e., in the absence of

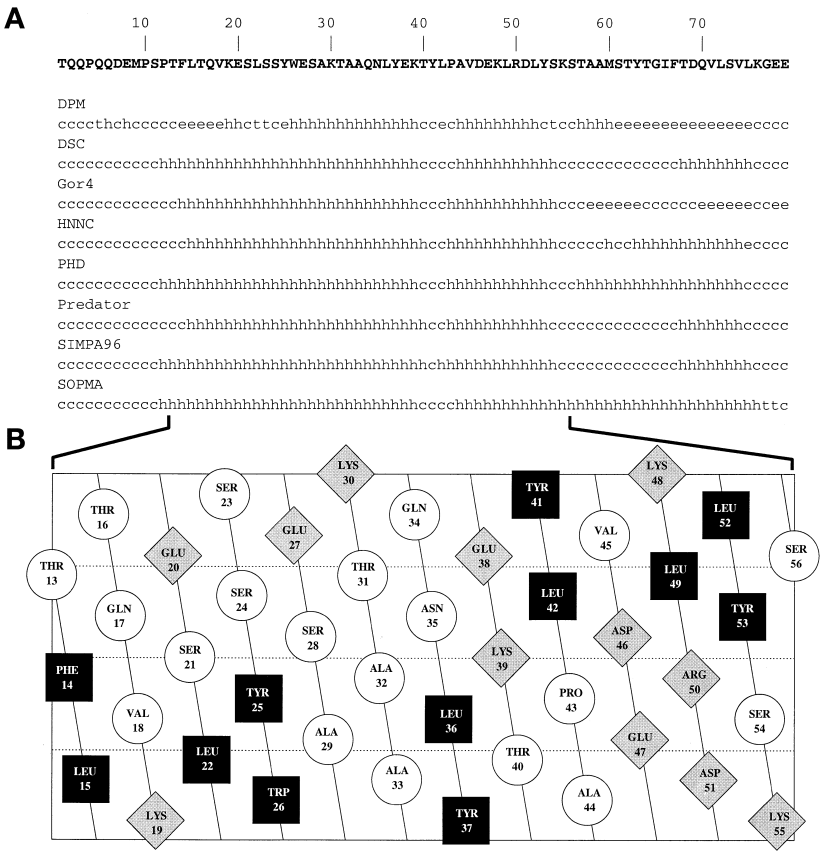

Fig. 1. Sequence analysis of apolipoprotein C-II (apoC-II). A: Secondary structure prediction using different methods (Materials and Methods). Abbreviations: c, coil; e, β-sheet; h, α-helix; c, turn. B: Helical net projection of apoC-II(13-56) sequence. Key: neutral residues, open circles; charged residues, gray diamonds; hydrophobic residues, filled squares.

lipid. As shown in **Fig. 2** (open squares), 11 nM [^{14}C]apoC-II(13-56) increases the surface pressure from an initial value of 0 to 17 dyn/cm over a period of 10 h. Lower aqueous concentrations were not used because, even in the stirred system, equilibration times become so long that evaporation of the aqueous phase was significant. Increasing the peptide concentration to 62 nM caused the surface pressure to plateau in a much shorter time at a final value of about 22 dyn/cm (Fig. 2, open triangles). The dependence of the final surface pressure on the concentration of peptide in the aqueous phase is shown in **Fig. 3**, filled circles. The essentially constant value of 21.5 ± 0.4 dyn/cm above 100 nM (solid line) indicates peptide saturation of the interface. After equilibration of the surface pressure, the interface was collected and the surface concentration of adsorbed peptide was determined as described in Materials and Methods. As shown in Fig. 3 (squares), in the range of [^{14}C]apoC-II(13-56) concentrations over which the final surface pressure was essentially constant (filled circles), the values of the surface concentration (squares) were also constant, although more scattered, at an average of 12.1 ± 2.6 pmol/cm^2 (Fig. 3, dotted line). At low peptide concentrations, for which incubation times were very long, somewhat higher values were obtained, possibly as a consequence of annealing during the long incubations required for equilibration at low peptide concentrations. Overall, the surface pressure and adsorption data in Figs. 2 and 3 indicate that an [^{14}C]apoC-II(13-56) concentration of >100 nM will rapidly saturate the surface with the peptide. These data also imply that the dissociation constant for the peptide-surface interaction is ≤10 nM.

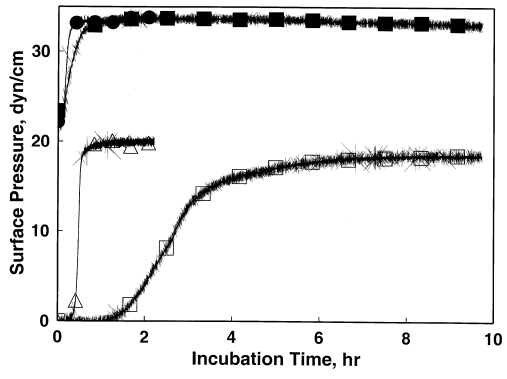

Fig. 2. Time course for the adsorption of [^{14}C]apoC-II(13-56) to interfaces. Adsorption was measured in the absence (open symbols) or presence of a 1,2-dioleoylglycerol (filled symbols) monolayer. [^{14}C]apoC-II(13-56) concentrations were 11 nM (□)/13.5 nM (■) and 62 nM (Δ)/66.6 nM (●).

Adsorption of [^{14}C]apoC-II(13-56) to individual lipids

To determine how the presence of lipid regulates the interaction of [^{14}C]apoC-II(13-56) with interfaces, similar experiments were performed in which the peptide was injected under preformed lipid monolayers of a diacylglycerol (DOG), a fatty acid (DA), or a phosphatidylcholine

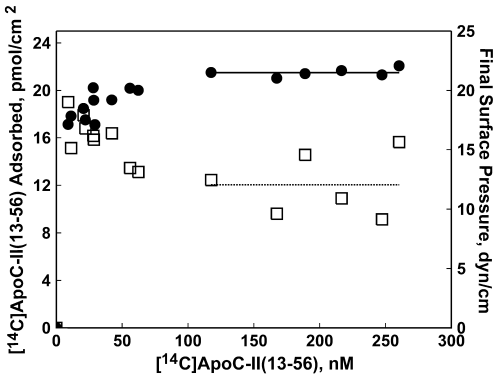

Fig. 3. [^{14}C]apoC-II(13-56) interaction with the argon-buffer interface. Final surface pressure (●) and [^{14}C]apoC-II(13-56) surface concentration (□) were measured following equilibration of the interface with peptide added to the aqueous phase. The solid and dotted lines indicate averages of surface pressure and surface concentration values over the range shown.

(SOPC). The lipids were spread to an initial surface pressure of 22 dyn/cm. This initial surface pressure was chosen to be significantly below the collapse surface pressures of the lipids [30.6, 38.3, and 47.6 dyn/cm, respectively, (42)] but comparable to the maximum pressure exerted by [^{14}C]apoC-II(13-56) in the absence of lipid (Fig. 3). In this way, surface pressure increases should reflect the relative affinities of the peptide for the different lipid classes, i.e., the ability of the peptide to do work against the lipid monolayers. A triacylglycerol (collapse surface pressure ~11 dyn/cm) was not used in this study because it can be expelled from the surface simply as a consequence of peptide adsorption, even in the absence of any interaction with the protein.

As illustrated for DOG in Fig. 2, the surface pressure reached a constant value within 1 h, even at a low concentration of peptide. Moreover, at all concentrations of peptide above 25 nM in the aqueous phase, the surface pressure of the DOG monolayer increased to a constant value

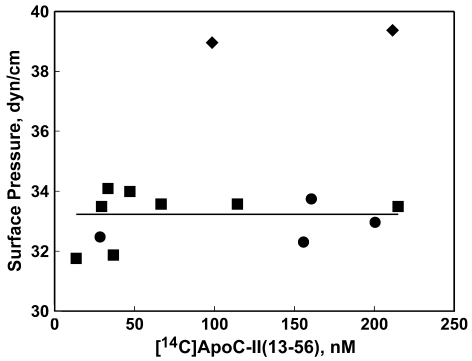

Fig. 4. Surface pressure of lipid monolayers following interaction with [^{14}C]apoC-II(13-56). Lipids, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC) (●); 13,16-*cis,cis*-docosadienoic acid (DA) (◆); and 1,2-dioleoylglycerol (DOG) (■), spread to a initial surface pressure of 22 dyn/cm, and [^{14}C]apoC-II(13-56) was injected at different concentrations into the aqueous phase. The solid line indicates the average value obtained using DOG over the range shown.

of 33.2 ± 0.9 dyn/cm (**Fig. 4**, solid line), which exceeds the collapse surface pressure of DOG, 30.6 dyn/cm. The invariance of the final surface pressure with aqueous-phase peptide concentration indicates that saturation has been achieved. Importantly, the higher surface pressures attained in the presence of lipid (Fig. 4), as compared with its absence (Fig. 3), indicate at least partial miscibility of the peptide and lipid in the interface. At all values of peptide concentration >25 nM, the surface concentration of [^{14}C]apoC-II(13-56) was constant at 6.8 ± 0.5 pmol/cm^2 (**Fig. 5**, solid line), a value substantially lower than in the absence of lipid. This indicates that the peptide and DOG compete, at least partially, for occupancy of the interface rather than residing in parallel planes.

Proteins that contain amphipathic helices can form complexes with lipids in a bulk aqueous phase (43, 44). As a control to test for this possibility, a monolayer stability test was performed. Monolayers of radio-labeled DOG were spread to 22 dyn/cm and incubated for 1 h with and without 250 nM apoC-II(13-56) in the stirred subphase. The monolayers were then collected and analyzed for DOG content. Recovery of DOG in the absence and presence of protein was identical. Thus, apoC-II(13-56) does not catalyze significant dissolution of the DOG monolayers during the time course of our experiments.

Because the presence of DOG clearly did not decrease the affinity of [^{14}C]apoC-II(13-56) for the interface, more limited comparisons were done with SOPC and DA initially spread to a surface pressure of 22 dyn/cm (Fig. 4). With DA, [^{14}C]apoC-II(13-56) raised the surface pressure to 39 dyn/cm, a value above the collapse surface pressure of the fatty acid. This indicates, as with DOG, interaction of the peptide with the lipid in the interface. The maximal surface concentration of peptide adsorbed to DA (Fig. 5, diamonds) was comparable to that obtained with DOG, 6–7 pmol/cm^2. For SOPC the surface pressure increased to about 33 dyn/cm (Fig. 4, circles), a value comparable to that for DOG but below that obtained with DA. This indicates interaction of the peptide with SOPC. This final surface pressure is, however, well below the collapse surface pressure of 47.6 dyn/cm for SOPC. In sharp contrast to the data obtained with DOG or DA, the highest

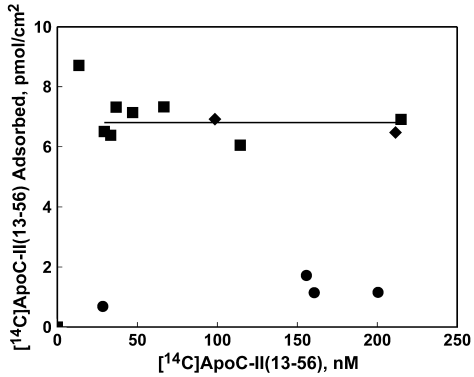

Fig. 5. Adsorption of [^{14}C]apoC-II(13-56) to lipid monolayers. Symbols and conditions were as for Fig. 4. The solid line indicates the average value obtained using DOG over the range shown.

surface concentration of peptide adsorbed to SOPC was only 1–2 pmol/cm^2 (Fig. 5, circles). This suggests a much more direct competition between SOPC and [^{14}C]apoC-II(13-56) for interfacial occupancy than with DOG or DA.

Overall, the results described above show that the affinity of [^{14}C]apoC-II(13-56) was high at all interfaces tested and that there were large differences in the extent of peptide interaction with the different lipid classes. However, the absence of measurable saturation behavior in the experimentally accessible range of peptide concentrations (Figs. 4 and 5) did not give a clear indication of the relative strengths of the interactions. As recently reviewed (45), another common measure of the strength of the interaction of soluble proteins with insoluble monolayers is the ability of the excess protein to increase the surface pressure, i.e., lower the interfacial free energy, as a function of the initial surface pressure of the lipids comprising the monolayers. This technique was used in the present study at a saturating nominal peptide concentration of 250 nM.

Figure 6 shows the surface pressure changes (filled symbols) measured when [^{14}C]apoC-II(13-56) was injected beneath monolayers of SOPC, DOG, and DA at different initial surface pressures, i.e., lipid packing densities. The change in surface pressure decreases linearly with initial surface pressure from near 1.0 dyn/cm to the point of intercept with the abscissa. Notably, if overlaid (not shown) the data for DOG and SOPC are nearly superimposable, whereas those for DA are displaced approximately 6 dyn/cm higher at any given initial surface pressure. As a consequence of this displacement, the intercepts of the regression lines with the abscissa are moved by a similar amount. For DOG, SOPC, and DA the intercepts are, respectively, 36, 37, and 44 dyn/cm. Hence, this experiment indicates

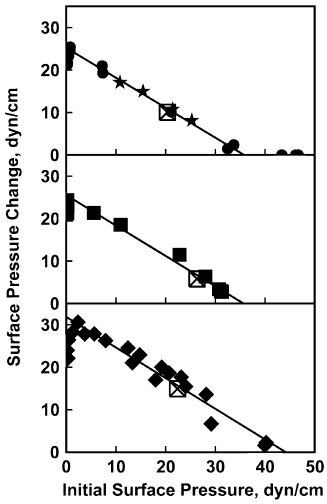

Fig. 6. Surface pressure changes following peptide adsorption to lipid monolayers. Monolayers of SOPC (top), DOG (center), or DA (bottom) were spread to different initial surface pressures, and [^{14}C]apoC-II(13-56) at 250 nM (-●-, -■-, -◆-), apoC-II(13-56) at 250 nM (★), or [^{14}C]apoC-II at 220 nM (⊠) was injected into the aqueous phase. The lines represent least square fits of the data obtained with [^{14}C]apoC-II(13-56) at initial surface pressures ≥1 dyn/cm.

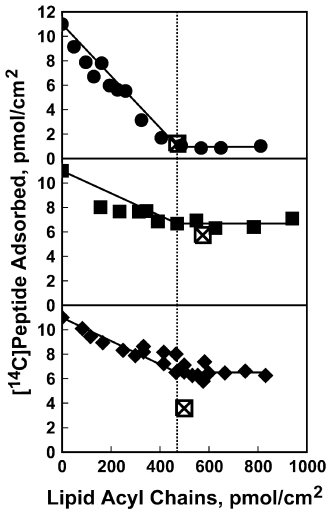

Fig. 7. Surface concentration of [^{14}C]apoC-II(13-56) and [^{14}C]apoC-II following interaction with lipid monolayers. Symbols and conditions were as for Fig. 6. To aid comparison, lipid concentrations are plotted as the concentration of lipid acyl chains in the monolayer. The dotted vertical line indicates a lipid acyl chain concentration of 470 pmol/cm^2. The horizontal line in each panel is the average of values obtained with lipid chain concentration ≥470 pmol/cm^2. Angled lines connect ends of horizontal lines to the value for [^{14}C]apoC-II(13-56) adsorption in the absence of lipid.

a greater ability of the peptide to do work against DA monolayers, compared with DOG and SOPC.

For each experiment shown in Fig. 6, the value of the surface concentration of [^{14}C]apoC-II(13-56) was determined after recording of the final surface pressure. The values are shown in **Fig. 7** as a function of the lipid concentration in the monolayer. To facilitate comparison of data obtained with both one- and two-chain lipids, the lipid concentrations are expressed as the concentration of acyl chains rather than molecules. Inspection of the three panels in the figure shows a single pattern of behavior. From a concentration of zero chains (no lipid) to about 470 pmol/cm^2 (vertical dotted line), adsorbed peptide decreases approximately linearly. However, at higher chain concentrations, peptide adsorption becomes constant. What is dissimilar among the experiments is the concentration of adsorbed peptide at lipid chain concentrations ≥470 pmol/cm^2. Whereas the values obtained with DOG and DA are nearly identical, 6.7 ± 0.3 and 6.5 ± 0.4 pmol/cm^2, peptide adsorbed to SOPC to only 1.0 ± 0.1 pmol/cm^2.

Adsorption of [^{14}C]apoC-II and apoC-II(13-56) to lipids

Because experiments of the type shown in Figs. 6 and 7 reflect directly the capacity of the monolayer to accommodate proteins and the work necessary to achieve a saturated surface, control experiments were performed using both apoC-II(13-56) and [^{14}C]apoC-II. ApoC-II(13-56) was injected under monolayers of SOPC to determine whether the radio-methylation of 0.3 lysine residues per peptide changed its adsorption behavior. As shown in Fig. 6, at initial surface pressures from 10 to 30 dyn/cm the

labeled and unlabeled peptides behaved identically. [^{14}C]apoC-II was used to determine whether the lipid class specificity of the peptide shown in Figs. 6 and 7 reflected qualitatively or quantitatively the properties of the native cofactor. The adsorption of [^{14}C]apoC-II (220 nM) was measured at approximately the lipid concentration, 470 pmol/cm^2, at which the binding of [^{14}C]apoC-II(13-56) became constant. Under these conditions the extent of binding is expected to be lower than in the absence of lipid. This is a consequence of the spacing apart of protein molecules by the lipid, and this separation should minimize nonspecific interactions or steric repulsion between the [^{14}C]apoC-II molecules either in the interface or the aqueous phase. As shown in Fig. 6, for each of the three lipids the surface pressure increase measured using [^{14}C]apoC-II falls on the line defined by the [^{14}C]apoC-II(13-56) binding to that lipid. Furthermore, the extent of adsorption of [^{14}C]apoC-II was essentially identical to that obtained with [^{14}C]apoC-II(13-56) when the monolayer was formed with SOPC or DOG (Fig. 7). With DA the extent of [^{14}C]apoC-II adsorption was only half that obtained with the peptide, but still three times that obtained with SOPC. Repeating the measurements with all lipids at lower [^{14}C]apoC-II concentrations did not change either the observed surface pressure changes or binding (not shown). Thus, [^{14}C]apoC-II concentration was saturating in these experiments. Overall, the results show that, with one exception, the model peptide, [^{14}C]apoC-II(13-56) behaves identically to the native cofactor in this system and that radio-labeling of the peptide does not measurably alter its surface activity.

Adsorption of [^{14}C]apoC-II(13-56) to a lipid mixture

The data in Fig. 7 show clearly that the extent of adsorption of [^{14}C]apoC-II and [^{14}C]apoC-II(13-56) to interfaces is highly dependent on the lipid class used. To determine whether the apparent preference for adsorption to lipids such as DOG and DA could be expressed in the presence of SOPC, adsorption of [^{14}C]apoC-II(13-56) to a mixed SOPC/DOG monolayer (60/40) was measured. In **Table 1** the results are compared with those for adsorption to SOPC or DOG at similar initial surface pressures. The data show similar final surface pressures for all experiments. With the mixed monolayer the adsorption of [^{14}C]apoC-II(13-56) was 2.7 pmol/cm^2. This value is greater than that for adsorption to SOPC alone but less than the value predicted by prorating the values obtained using the pure lipids on a mole fraction basis. However, if adsorption is predicted from surface pressure-area isotherms on an area fraction basis, attributing the loss of area from nonideal mixing to DOG, the predicted value falls to 3.4 pmol/cm^2, which is closer to the measured value.

TABLE 1. Adsorption of [^{14}C]ApoC-II(13-56) to mixed monolayers

Lipid	Initial Pressure	Final Pressure	[^{14}C]ApoC-II(13-56)
	dyn/cm	*dyn/cm*	*pmol/cm^2*
SOPC	21.2	31.3	1.7
SOPC/DOG (60/40)	22.5	32.7	2.7
DOG	22.8	34.3	6.7

DISCUSSION

Studies of colipase-lipid interactions have shown that fatty acids and diacylglycerol have a marked effect on the extent of adsorption of the cofactor to surfaces. This suggested that colipase causes lateral redistribution of those lipid classes to its vicinity (12), thereby regulating the adsorption of PTL to phospholipid-rich surfaces (13). The functional similarities between apoC-II and colipase suggested that apoC-II might also play a similar role in LPL-catalyzed lipolysis. As a first step to testing this hypothesis, a peptide, [^{14}C]apoC-II(13-56), was synthesized to model the lipid-binding determinants of apoC-II. The choice of these residues was made on the basis of prior functional and lipid-binding studies of peptides derived from apoC-II, predicted amphipathic helical regions of the cofactor (Fig. 1), and naturally occurring mutants.

Surface activity of apoC-II(13-56)

To determine whether the peptide was surface active, its affinity for the empty argon-buffer interface was measured. Based on both changes in surface pressure and direct binding measurements, the data show that the peptide has a substantial affinity for the argon-buffer interface. At an [^{14}C]apoC-II(13-56) concentration of 50 nM in the aqueous phase the surface pressure was about 20 dyn/cm (Fig. 3). This value is in the range of 18–24 dyn/cm reported earlier for native apoC-II at the same concentration (46–48). Thus, surface pressure changes measured in the absence of lipid show that [^{14}C]apoC-II(13-56) can account for the surface activity of the native cofactor. At saturation and in the absence of lipid, the concentration of [^{14}C]apoC-II(13-56) in the interface averaged 12.1 pmol/cm^2 (Fig. 3). By using the size and shape of α-helices (49), the measured concentration can be compared with the value of 21.0 calculated assuming, for simplicity, that all of the 44-residue peptide is α-helical at the interface and that all surface is occupied by peptide. This comparison shows that only 60% of the surface is occupied by the peptide at saturation, and implies that the peptide is not optimally packed, i.e., that adsorption is sterically limited by peptide shape. For a narrow linear peptide, the theoretical jamming limit of adsorption should be 50–60% of optimal (50), in good agreement with the data obtained.

The presence of lipids clearly enhanced peptide affinity for interfaces. This is indicated by the surface pressure increases that occurred following peptide introduction into the aqueous phase beneath lipid monolayers initially at surface pressures higher than that induced by the peptide in the absence of lipid (Fig. 4). Following peptide adsorption to DA, the surface pressure approached 40 dyn/cm; for DOG the increase was essentially independent of [^{14}C]apoC-II(13-56) concentration at an average of 33.2 ±

0.9 dyn/cm. Each of these surface pressures exceeds the collapse surface pressure of the lipid alone. The surface pressure increase for peptide adsorption to SOPC was virtually identical to that obtained with DOG and agrees with values for native apoC-II obtained in comparable studies (46). Moreover, with all three lipid classes the surface pressure increases induced by [^{14}C]apoC-II were identical to those obtained with [^{14}C]apoC-II(13-56) (Fig. 6). These results demonstrate lipid class specificity in protein binding. Furthermore, they support the use of the peptide as a model for the lipid-binding region of native apoC-II and imply that one or, possibly, both of the predicted amphipathic helices interact with lipid interfaces.

Affinity of apoC-II(13-56) for lipids

The dissociation constants reported for the binding of apoC-II and related peptides to phosphatidylcholines range from 3,000 to 6,500 nM (24, 51, 52). These are much greater than the K_d values of <10 nM for [^{14}C]apoC-II(13-56) and <<200 nM for [^{14}C]apoC-II binding to SOPC inferred from the data in this study. The reason for the much higher affinity of [^{14}C]apoC-II(13-56) and [^{14}C]apoC-II for lipid monolayers in the present study is not apparent. Although it could be related to the smaller radius of curvature of the bilayer systems used in some of the prior studies, the relatively flat monolayer interface is expected to result in slightly weaker, rather than much stronger, binding of amphipathic peptides (53). Another possibility is that the tight binding of a small amount of protein may have been overlooked in the earlier studies as a consequence of the weaker binding of a larger amount of protein. This idea is supported by the reported stoichiometry of 16-36 phosphatidylcholine molecules per protein molecule in bilayer studies (24, 52), compared with the value of ~235 that can be calculated for [^{14}C]apoC-II and [^{14}C]apoC-II(13-56) binding to SOPC monolayers in the present study (Fig. 7). Also, kinetic measurements of apoC-II transfer between vesicles (54) and from lipoproteins to emulsions (55) have identified slowly or nonexchangeable pools of the cofactor, indicating multiple modes of apoC-II-phosphatidylcholine interaction.

Extent of apoC-II(13-56) binding

Compared with results with SOPC, the more extensive binding of [^{14}C]apoC-II(13-56) and [^{14}C]apoC-II to DA and DOG (Fig. 7) is likely driven by the lower surface activity of the lipids and, possibly, by the shape of the lipids, i.e., relaxation of curvature stress. Type A amphipathic helices, like the putative helical domains of apoC-II(13-56) and apoC-II (Fig. 1), have a wedge shape, with the lysine residues making the side of the peptide in contact with water wider than the hydrophobic region (56). DOG and fatty acids, on the other hand, have the opposite shape. Thus, shape compensation could drive binding of the peptide to interfaces containing DOG and DA, much as type A helices compensate lytic effects of inverted wedge shaped, type L peptides (56). A similar mechanism has been invoked to explain the ability of non-bilayer forming lipids, like fatty acids and diacylglycerols, to induce the translocation of the amphipathic helical domain M of cytidine 5′-triphosphate (CTP):phosphocholine cytidyltransferase from the aqueous phase to lipid interfaces (57). The greater surface pressure changes observed with DA as compared with DOG for the same level of [^{14}C]apoC-II(13-56) binding suggest that, in addition to curvature, there may be charge interactions between the partly ionized fatty acid and the peptide.

With ⩾470 pmol/cm^2 of lipid chains in the monolayer, both [^{14}C]apoC-II and [^{14}C]apoC-II(13-56) bind to DOG to ~6 pmol/cm^2 and SOPC to ~1 pmol/cm^2 (Fig. 7). This observation, together with the identity of the accompanying surface pressure changes noted above, supports the idea that apoC-II(13-56) contains the residues of the native cofactor that interact with these lipids. In contrast, the extent of [^{14}C]apoC-II binding to DA is only half that of the peptide. This suggests that more of native [^{14}C]apoC-II than the residues in the region comprising the model peptide may interact with DA, giving it a bigger "footprint" on the surface. If so, this different interaction of apoC-II with fatty acids could contribute to their well-known effects in regulating LPL, such as the inability of apoC-II to activate LPL in the presence of fatty acids (14).

Relevance to lipoproteins

The experiments with mixed monolayers (Table 1) were conducted at an initial surface pressure of about 22 dyn/cm with final surface pressures reaching 31–34 dyn/cm. The range of surface pressures estimated for phospholipid stabilized emulsions and lipoproteins ranges from about 15 to 35 dyn/cm (58–61). Thus, the lipid class-based differences in the binding of [^{14}C]apoC-II and [^{14}C]apoC-II(13-56) reported in this work occur in a range of surface pressures found in lipoproteins. More important, this work suggests that surface pressure alone will not determine the extent and affinity of binding of apoC-II to lipoproteins. Both [^{14}C]apoC-II(13-56) and [^{14}C]apoC-II interact with lipid interfaces in a lipid class-specific manner. In vivo, how much apoC-II is associated with a given particle at any point in time will depend on the lipid composition of the lipoprotein interface and the amount of the cofactor available. It will also depend on what other apolipoproteins are present and their affinities for particular lipid classes. Phospholipids are a known surface component of lipoproteins. However, it has been recognized more recently that lipoproteins from fasted donors contain significant amounts of diacylglycerol (31, 32). For example, in high density lipoprotein subfractions 2 and 3 the mole ratios of diacylglycerol to phosphatidylcholine are 0.45–0.45 and this diacylglycerol is accessible on the lipoprotein surface (31). Free fatty acids are also associated with circulating lipoproteins, and their amounts increase dramatically during active lipolysis (14, 62). As a consequence of these competing, dynamic interactions among lipids and proteins, a simple role in vivo for the lipid class specificity of binding demonstrated in this study cannot be formulated. However, this specificity should, as previously suggested (34, 63), help regulate the distribu-

tion of apoC-II among lipoproteins and, ultimately, the activity of LPL.

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