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Molecular Mechanism of Apolipoprotein E Binding to Lipoprotein Particles*

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Abstract

The exchangeability of apolipoprotein (apo) E between lipoprotein particles such as very low density lipoprotein (VLDL) and high density lipoprotein (HDL) is critical for lipoprotein metabolism but, despite its importance, the kinetics and mechanism of apoE-lipoprotein interaction are not known. We have used surface plasmon resonance (SPR) to monitor in real time the reversible binding of apoE to human VLDL and HDL₃; biotinylated lipoproteins were immobilized on a streptavidin-coated SPR sensor chip and solutions containing various human apoE molecules at different concentrations were flowed across the surface. Analysis of the resultant sensorgrams indicated that the apoE3/lipoprotein interaction is a two-step process. After an initial interaction, the second slower step involves opening of the N-terminal helix bundle domain of the apoE molecule. Destabilization of this domain leads to more rapid interfacial rearrangement as is seen when comparing the lipoprotein binding of apoE4 relative to that of apoE3. The resultant differences in interfacial packing seem to underlie the differing abilities of apoE4 and apoE3 to bind to VLDL and HDL₃. The measured dissociation constants for apoE binding to these lipoprotein particles are in the micromolar range. C-terminal truncations of apoE to remove the lipid binding region spanning residues 250–299 reduces binding to both types of lipoprotein but the effect is less with HDL₃; this suggests that protein-protein interactions are important for apoE binding to this lipoprotein while apoE-lipid interactions are more significant for VLDL binding. The two-step mechanism of lipoprotein binding exhibited by apoE is likely to apply to other members of the exchangeable apolipoprotein family.

There is great interest in understanding the structure-function relationships of apolipoprotein (apo) E because of its pronounced anti-atherogenic properties (1). ApoE protects against the development of atherosclerosis, in part, by binding to lipoprotein particles and mediating their clearance from the plasma compartment via interaction with cell surface receptors, notably those of the low density lipoprotein (LDL) receptor family (2;3). ApoE also promotes the cellular uptake of lipoprotein particles by binding to cell surface heparan sulfate proteoglycans (4). The endocytosis of apoE-containing lipoprotein particles reduces plasma cholesterol levels and, thereby, the risk for developing cardiovascular disease (1).

Human apoE is a 299 residue single polypeptide chain that folds into two tertiary structure domains; the N-terminal region (residues 1–191) forms a helix bundle while the C-terminal region is folded separately into some unknown structure (3;5;6). ApoE binds readily to lipoprotein particles such as very low density lipoprotein (VLDL) and high density lipoprotein

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(HDL) and forms both exchangeable and non-exchangeable pools (7;8). However, the mechanisms by which apoE associates with and dissociates from lipoprotein particles are not known. Human apoE exhibits polymorphism and the two common isoforms apoE3 and apoE4 (which differ by the mutation C112R) partition differently between HDL and VLDL particles (9); this effect occurs because of variations in the interactions between the N- and C-terminal domains so that apoE4 binds preferentially to VLDL whereas apoE3 prefers HDL (6). This differential lipoprotein binding leads to variations in lipoprotein profile such that apoE4 is associated with higher plasma cholesterol levels and increased risk of cardiovascular disease relative to individuals with apoE3 (10;11). The molecular basis for these isoform effects on lipoprotein binding is not well understood.

To address some of the unresolved issues about apoE-lipoprotein interaction, we have used surface plasmon resonance (SPR) to study the reversible binding of apoE to VLDL and HDL particles in real time. SPR has been employed successfully to measure the binding of apoE to proteoglycans (12–15) and members of the LDL receptor family (16), and of enzymes to lipoprotein particles (17;18). The present results show that apoE binds by a two-step mechanism to HDL and VLDL.

EXPERIMENTAL PROCEDURES

Materials

HDL₃ and VLDL were purified by sequential density ultracentrifugation (19;20) from a pool of fresh human plasma obtained by combining several single units from normolipidemic individuals. Full-length human apoE3, apoE4 and their 22kDa (residues 1–191), 12kDa (residues 192–299) and 10kDa (residues 222–299) fragments were expressed and purified as described previously (21–23). The C-terminal truncation variants (Δ 251–299, Δ 261–299 and Δ 273–299) of apoE3 and apoE4 were made as described before (24;25). The apoE preparations were at least 95% pure as assessed by SDS-PAGE. In all experiments, the apoE sample was freshly dialyzed from a 6M GdnHCl and 1% β -mercaptoethanol (or 5mM DTT) solution into a buffer solution before use.

Biotinylation of HDL particles

HDL₃ and VLDL were dialyzed into phosphate-buffered saline (pH 7.4) prior to biotinylation. The EZ-link sulfo-NHS-LC-biotinylation kit from Pierce Chemical Co. (Rockford, IL) was used for attaching biotin molecules through a 2.24 nm spacer arm to lysine residues on the surface of HDL particles. HDL₃ and VLDL, each at 1.0 mg protein/ml, were mixed with a freshly made 10 mM sulfo-NHS-LC-biotin solution at a 10 fold molar excess of biotin. The lipoproteins were incubated under nitrogen at 4°C overnight before dialysis against Tris-buffered saline (TBS, pH 7.4) to remove unreacted sulfo-NHS-LC-biotin. The degree of biotinylation of the particles was determined using conditions recommended by Pierce. Briefly, solutions containing biotinylated lipoproteins were added to a mixture of HABA reagent [2-(4'-hydroxyphenyl) azobenzoic acid] and immunopure avidin (Pierce Chemical Co.). Because of its higher affinity for avidin, biotin, from the biotinylated lipoproteins, displaced avidin-bound HABA. Therefore, the absorbance at 500 nm of the HABA-avidin complex was reduced. The change in absorbance was used to calculate the level of biotin incorporated into the lipoprotein particles. This procedure yielded an average degree of labeling of 1 biotin molecule per lipoprotein particle.

Surface Plasmon Resonance (SPR)

Studies of the binding of apolipoproteins (association and dissociation) to HDL₃ and VLDL were performed with a Biacore 3000 SPR instrument (Biacore, Uppsala, Sweden) using SA sensor chips (Biacore, Uppsala, Sweden). This chip is designed to bind biotinylated ligands

through a high-affinity capture process. Prior to immobilization of HDL₃ or VLDL on the sensor chip, the streptavidin surface was conditioned with three consecutive 1-minute injections of 1 M NaCl in 50 mM NaOH (50 µl/minute). The biotinylated HDL₃ or VLDL was then immobilized onto the surface through the quasi-covalent biotin-streptavidin interaction by exposing the surface to the biotinylated lipoprotein solutions in running buffer (50 mM Tris-buffered saline (TBS) pH 7.4) until 2500–3000 and 5000–7000 response units (RU) of biotinylated HDL₃ or VLDL, respectively, were bound to the surface. This was achieved by a 10 µl injection of biotinylated HDL₃ or VLDL (1.0 mg protein/ml) at a flow rate of 2 µl/minute at room temperature. After 5 minutes, the chip was washed with degassed TBS to remove unattached lipoprotein. A 50 µg/ml human apoE3 solution was flowed over the chip at 20 µl/min for 2 minutes to block any remaining hydrophobic surface areas and reduce subsequent apoE binding to non-lipoprotein sites; the chip was then washed with TBS until the SPR signal reached a steady background value. The surface of the immobilized HDL₃ or VLDL was then exposed to a 4 minutes injection of apoE dissolved in degassed TBS at a flow rate of 20 µl/min to monitor association and then TBS alone was passed over the sensor surface to monitor apoE dissociation from the immobilized lipoprotein particles. For these experiments, two flow cells were monitored simultaneously with flow cells 1 and 2 containing immobilized biotinylated VLDL and HDL₃, respectively. A sensor chip lacking immobilized lipoprotein could not be used as a reference cell because apoE bound more to this surface than to a lipoprotein-coated chip. The apolipoproteins were dialyzed from 6 M GdnHCl containing 5 mM DTT into TBS, filtered (Ultrafree – MC centrifugal filter devices, 0.1 µm filter unit, Millipore, Bedford, MA) and degassed before serial dilutions (2.5 to 50 µg/ml) were made just prior to injection. The sensor chip was washed two times with 20 µl TBS between each injection of apolipoprotein. The chips were used for 2 days in repetitive experiments. Regeneration of the sensor chip surface was not possible since the lipoproteins were directly immobilized via biotin-streptavidin interaction. The apoE sensorgrams were independent of flow rate in the range 10–40 µl/min indicating that the apoE binding at 20 µl/min was not limited by mass transport (diffusion) effects. Steady state binding isotherms and K_d values of the binding to HDL₃ and VLDL were obtained by generating sensorgrams at different apoE concentrations. The sensorgrams were analyzed with the BIA evaluation software version 4.1 (Biacore, Uppsala, Sweden). The response curves of various apolipoprotein (analyte) concentrations were fitted to the two-state binding model described by the following equation (26;27).

The equilibrium constants of each binding step are K₁ = k_{a1}/k_{d1} and K₂ = k_{a2}/k_{d2} and the overall equilibrium binding constant is calculated as K_a = K₁ (1 + K₂) and K_d = 1/K_a. In this model, the analyte (A) binds to the ligand (HDL₃ or VLDL) (B) to form an initial complex (AB) and then undergoes subsequent binding or conformational change to form a more stable complex (AB_x). A further check of the two-state binding mechanism was obtained by variation of the contact time for association between the apoE and the lipoprotein particle. For a two-state reaction, an increase in the contact time between the analyte and the ligand decreases the dissociation rate since more of the stable AB_x complex is formed. For the apolipoproteins, binding responses in the steady-state region of the sensorgrams (R_{eg}) were also plotted against apolipoprotein concentration (C) to determine the overall equilibrium binding affinity. The data were subjected to nonlinear regression fitting (Prism 4, GraphPad Inc.) according to the following equation;

$$R_{\text{eq}} = C R_{\text{max}} / (C + K_d) \quad (\text{Eq. 2})$$

R_{max} is the maximum binding response and K_d is the dissociation constant. This SPR approach for measuring K_d is validated by the fact that monitoring the binding of apoE3 and apoE4 to VLDL by ultracentrifugation yielded similar K_d values (23).

RESULTS

Kinetics and mechanism of apoE binding to VLDL and HDL₃

Fig. 1 shows the immobilization of biotinylated HDL₃ and VLDL on a streptavidin-coated sensor chip when a solution of the lipoprotein is flowed across the surface. It is apparent that there is rapid binding to give a steady state level. Flowing buffer solution alone across the chip washes away a small amount of weakly bound lipoprotein but the remainder is essentially irreversibly bound by the biotin-streptavidin interaction. In multiple experiments, the amounts of HDL₃ and VLDL immobilized by this procedure were 2633 ± 508 and 5893 ± 1247 RU (mean $\pm$ SD), respectively. It should be noted that these RU levels for bound HDL₃ and VLDL cannot be simply interpreted in terms of relative masses of immobilized lipoprotein because of different refractive index properties due to different lipid/protein stoichiometries. The immobilization of HDL₃ or VLDL in this fashion permits evaluation of the binding of apoE which is difficult to monitor in solution because of problems, particularly in the case of HDL₃, in readily separating the lipoproteins and unbound apoE. Another advantage of the SPR method is that labeling the apoE molecules is not required and the association/dissociation reactions can be monitored in real time. A potential problem in the SPR experiment is steric restriction of apoE binding by the proximity of the sensor chip surface, but, because the HDL₃ and VLDL particles are attached to the surface by a 2.24nm spacer arm, protein molecules can access the lipoprotein surface. For example, LCAT (17) and CETP (28) have been shown to bind to HDL in similar SPR experiments.

The sensorgram obtained when a 50 μ g/ml apoE3 solution was flowed across the sensor chip with immobilized VLDL is shown in Fig. 2A. The data are corrected for bulk refractive index effects and fitted using a two-state binding model (equation 1). The kinetic data were not fitted well by a 1:1 Langmuir binding model as reflected by larger values of the goodness of fit parameter (χ^2) (data not shown). It follows that the binding of apoE3 to VLDL particles involves either a sequential two-step process or some conformational change (14;26;27). The binding of apoE3 to HDL₃ is also best described by the two-state binding model (data not shown). The deconvoluted curves in Fig. 2A show the initial binding (AB) and subsequent binding or conformational changes (AB_x). Because of uncertainties about the valences of the lipoprotein particles (the HDL₃ and VLDL particles are heterogeneous and do not contain a discrete number of binding sites), it is difficult to interpret the kinetics of association in detail. However, inspection of the deconvoluted curves in Fig. 2A reveals that at the end of the 4 minute association phase of the experiment, the size of the AB pool of bound apoE3 is approximately twice that of the AB_x pool. It is also apparent that in the dissociation phase (after 4 minutes), apoE3 molecules in the AB pool dissociate more readily than those in the AB_x pool. Analyzing several sensorgrams, the value for the dissociation rate constant k_{d1} for the AB pool is in the range of $2-9 \times 10^{-2} \text{ s}^{-1}$ while the value of k_{d2} for the AB_x pool is about fifty times smaller. These k_d values correspond to halftimes of dissociation of about 14s and 10 min for the AB and AB_x pools, respectively. The rate of dissociation of apoE3 from the VLDL surface is dependent upon the length of time the apoE3 is in contact with the particle. As shown in Fig. 2B, progressively shortening the injection (contact) time from 240s to 60 and 30s enhances apoE3 dissociation from the VLDL surface. The fitting data indicate that the fraction of apoE3 in the AB pool approximately doubles to 0.25 as the contact time is decreased to 30s.

The value of k_{d1} for the AB pool is not affected by the change in contact time whereas k_{d2} increases by about 40% as the contact time is decreased to 30s. These results are consistent with the second step in the two-step mechanism of apoE3 binding to VLDL involving a conformational change in the bound apoE molecules.

Influence of apoE domain structure on lipoprotein binding

Fig. 3 shows a typical series of sensorgrams for binding of apoE at different concentrations to immobilized lipoprotein. Fitting of sensorgrams of this type with the two-state binding model yields a maximal binding value (R_{max}) for each concentration of apoE3. Fig. 4A shows a steady-state binding isotherm for WT apoE3 to VLDL plotted using such R_{max} values and fitted to a one-site binding model (equation 2). The data in Fig. 4A yield a K_d value of $20 \pm 5 \mu\text{g apoE3/ml}$ ($0.6 \pm 0.1 \mu\text{M}$, mean $\pm$ SEM) for the binding of human apoE3 to VLDL₃. This K_d value indicates that the apoE3/VLDL interaction is of moderate affinity and is in agreement with the value of $23 \pm 4 \mu\text{g/ml}$ reported previously by us using an ultracentrifuge method (23). Fig. 4A compares the isotherms obtained for WT human apoE3 and its separate N- (residues 1–191) and C-terminal (residues 192–299 and 222–299) domains binding to VLDL; the K_d and B_{max} values derived from these isotherms are listed in Table 1. It is apparent that removal of the C-terminal domain to form 22kDa apoE3 gives rise to a weakly hyperbolic binding isotherm so that the errors in the K_d and B_{max} determinations are large (Table 1). Neither the amount of apoE3 that binds (B_{max}) nor the binding affinity (K_d) are altered significantly by removal of the C-terminal domain although there is a trend towards a higher K_d value for 22kDa apoE3, especially when considered on a molar basis (Table 1). In contrast, the isolated apoE 10 and 12 kDa C-terminal fragments exhibit clearly saturable binding to VLDL (Fig. 4A); the K_d values are lower than that for WT apoE3 (Table 1) indicating higher binding affinity. Interestingly, the presence of the so-called hinge region (residues 192–221) (3) in 12 kDa apoE does not change the binding from that found with the isolated C-terminal 10 kDa apoE domain. Inspection of Fig. 4B and the K_d and B_{max} values listed in Table 1 indicates that the pattern of lipoprotein binding of the apoE3 domains is similar with VLDL and HDL₃.

The tertiary structure domains in the apoE molecule are influenced by polymorphism. ApoE3 is the most prevalent isoform and has normal functionality whereas the C112R mutation to yield apoE4 induces abnormal behavior (2). Introduction of the arginine residue at position 112 destabilizes the N-terminal helix bundle domain (29;30) and alters the interaction between the N- and C-terminal domains (3;6). The altered domain-domain interactions are known to underlie the differing preferences of apoE3 and apoE4 for binding to HDL and VLDL (9;23; 25;31;32). To examine what effect, if any, the apoE3/apoE4 polymorphism has on the mechanism and kinetics of lipoprotein interaction, the binding of these isoforms to VLDL and HDL₃ was compared by SPR. Isotherms of the type shown in Fig. 4 were generated and the resultant K_d and B_{max} values are listed in Table 1. In the case of VLDL, the K_d values are the same indicating that the binding affinity for apoE3 is essentially unaltered by the C112R mutation. However, the B_{max} for apoE4 is approximately twice that for apoE3, consistent with prior observations that apoE4 partitions more to VLDL when added to plasma (9). In the case of HDL₃, the situation is reversed with the B_{max} for apoE3 being the highest (Table 1). As mentioned earlier, the apoE3-HDL₃ binding isotherm is quasi-linear which leads to large uncertainties in the derived K_d values and, because of this, a significant difference in binding affinity could not be detected. Similar considerations apply to the binding data for 22kDa apoE3 and 22kDa apoE4 because the binding to both VLDL and HDL₃ was not readily saturable and the binding isotherms were quasi-linear. However, it is apparent from the B_{max} values in Table 1 that the isolated 22kDa N-terminal domains of apoE3 and apoE4 do not discriminate between HDL₃ and VLDL like the intact apoE3 and apoE4 molecules do.

The sensorgram in Fig. 2A shows that when apoE3 binds to VLDL the kinetics of binding are such that at the end of the association phase (240s), the AB pool is greater than the AB_x pool. The sensorgram and deconvolution curves in Fig. 5A demonstrate that the kinetics are different for apoE4 binding to VLDL; in this case, the AB_x pool is greater than the AB pool at 240s. This more rapid shift to the AB_x pool for apoE4 compared to apoE3 is associated with higher values for the rate constants, k_{d1} and k_{d2} (equation 1). Similar differences in kinetics are seen when apoE3 and apoE4 bind to HDL₃ (data not shown). The data in Fig. 5B demonstrating that the AB and AB_x pools for 22kDa apoE4 binding to VLDL at 240s are of similar size indicates that the presence of both structural domains is required for the relatively rapid transition to the AB_x state seen with apoE4. Consistent with the concept, the AB_x pool observed when 12kDa apoE binds to VLDL is smaller than the AB pool at 240s (Fig. 5C). The 10kDa apoE C-terminal domain shows similar behavior (data not shown).

Influence of C-terminal residues on apoE binding to VLDL and HDL₃

The data in Fig. 4 and Table 1 showing that the C-terminal 10kDa and 12kDa domains are capable of higher affinity, saturable, binding to VLDL and HDL₃ are consistent with prior reports (3;31;33) of the importance of the region spanning residues 250–299 for lipid binding. To further explore the contributions of this part of the apoE molecule to binding to VLDL and HDL₃, we employed apoE3 and apoE4 variants with residues 273–299, 261–299 and 251–299 deleted. The physical properties of these engineered apoE3 and apoE4 molecules have been characterized previously (24;25). The isotherms in Fig. 6A show that C-terminal truncation reduces the level of apoE4 binding to VLDL; in particular the B_{max} values for the 1–260 and 1–250 variants are significantly reduced. The results also show that removing residues 273–299 has a relatively small effect and that residues 261–272 make a marked contribution to binding (cf. (24;31)). These C-terminal truncations in apoE3 have similar effects on VLDL binding (Fig. 6B); the hyperbolic nature of the binding isotherms is reduced for the C-terminal truncation variants and saturation is not evident in the concentration range covered. The data in Fig. 6C, D show that the C-terminal truncations cause smaller reductions in the binding of apoE3 and apoE4 to HDL₃ compared to the situation with VLDL (Fig. 6A, B). Since residues in the region 250–299 promote lipid interactions, their small contribution to the binding of apoE3 and apoE4 to HDL₃ implies that protein-lipid interactions are not a major factor in this case.

DISCUSSION

This SPR study gives the first direct measurements of real-time apolipoprotein association with and dissociation from lipoprotein particles. The fact that the sensorgrams for apoE interaction with VLDL and HDL₃ are best fitted to a two-state model indicates that some sequential binding or conformational events occur. Given that apoE can exist in two lipid-bound states on spherical lipid particles (22;34) with the N-terminal four-helix bundle in either open or closed conformations (3;35;36), it is likely that this conformational change underlies the two-step kinetics seen when apoE binds to VLDL or HDL₃. A model incorporating this concept is presented in Fig. 7. On this basis, the second AB_x phase of binding for apoE3 or apoE4 corresponds, at least in part, to the rate of helix bundle unfolding. The fact that the isolated 12kDa C-terminal domain of apoE also exhibits a small AB_x phase (Fig. 5C) indicates that other conformational changes besides helix bundle opening occur when apoE binds to VLDL or HDL₃. Also, the C-terminal domain is not essential for initiating binding of apoE to a lipoprotein particle because the 22kDa N-terminal fragments of apoE3 and apoE4 can bind to some extent (Table 1).

The isotherms depicted in Fig. 4 together with the binding constants listed in Table 1 show clearly that the isolated 10kDa and 12kDa apoE C-terminal domains can bind in a saturable,

high affinity, fashion to VLDL and HDL₃. Consistent with the extreme C-terminal part of the apoE molecule being important for lipid binding, deletion of residues in the range 250–299 reduces lipoprotein association (24;25;31;33;37). The finding (Fig. 6) that such truncations cause bigger reductions in binding of apoE3 and apoE4 to VLDL than to HDL₃ implies that lipid interactions play a bigger role in the former case. This effect might be expected because, compared to HDL₃, a larger fraction of the VLDL particle surface contains exposed phospholipid molecules. In the case of HDL₃ a large fraction of the particle surface is covered by the resident apolipoproteins so that apoE binding is more likely to involve protein-protein interactions.

Prior comparisons of apoE3 and apoE4 have attributed the differences in preference for binding to HDL₃ and VLDL to differences in binding affinity rather than binding capacity (22;23;32). The relatively low affinity and lack of saturability of binding of apoE3 and apoE4 to VLDL and HDL₃ indicated by the current SPR experiments makes detection of differences in binding affinity difficult. However, the binding parameters in Table 1 suggest that the variations in apoE isoform binding to VLDL and HDL₃ are, at least in part, a consequence of differences in ability to be accommodated on the lipoprotein particle surface. Since the second AB_x phase for apoE4 binding to VLDL is greater than that for apoE3 (cf. Fig. 2A and 5A), it follows from the model in Fig. 7 that the helix bundle in apoE4 opens more readily. This effect might be expected because the N-terminal domain of apoE4 is less stable than that of apoE3 (29;30). On this basis, apoE4 would require more interfacial area than apoE3 (where the helix bundle would be more likely to remain closed and out of contact with the surface – see step 1 in Fig. 7) and consequently favor binding to the larger VLDL particle. The smaller HDL₃ particle would more readily accommodate apoE3 molecules with only the C-terminal domain occupying interfacial area.

The fact that apoE binding to lipoprotein particles involves a two-step mechanism has physiological implications in that it explains the existence of exchangeable and non-exchangeable pools of apoE on the surface of lipoprotein particles. Thus, apoE molecules that are absorbed with the helix bundle domain closed (step 1 in Fig. 7) can exchange readily whereas those apoE molecules absorbed with the helix bundle open (step 2) do not exchange easily. Since the low density lipoprotein receptor (LDLR) binding site in apoE is not recognized when the helix-bundle is closed but only when it is open (3), it is expected that only molecules that achieve step 2 binding will act as effective ligands for the LDLR. This idea explains the fact that apoE in VLDL particles exists in two different conformations, one accessible and one inaccessible to the LDLR (38). Thus, apoE molecules bound with the helix bundle open are critical for apoE to achieve its major function of mediating the clearance of remnant lipoprotein particles from the circulation. Since apoE4 binds more than apoE3 to VLDL (Table 1) and it tends to bind in the helix bundle open conformation because the helix bundle is relatively unstable, it is expected that apoE4-VLDL will bind better than apoE3-VLDL to the LDLR. This difference in LDLR binding ability has been observed experimentally (39).

In summary, the application of SPR has provided new insights into the mechanism of apoE/ lipoprotein interaction and into the contributions of the apoE tertiary structure domains to binding. ApoE binds to VLDL and HDL₃ particles by a two-step mechanism with the rate being influenced by the stability and ease of unfolding of the N-terminal helix bundle domain. The mechanism and kinetics of lipoprotein interaction described here for apoE are likely to be relevant to the binding of other exchangeable apolipoproteins that have two-domain tertiary structures.

ABBREVIATIONS

apo

	apolipoprotein
B_{max}	saturation amount bound
HDL	high density lipoprotein
K_d	dissociation constant
LDLR	low density lipoprotein receptor
RU	resonance unit
SPR	surface plasmon resonance
VLDL	very low density lipoprotein
WT	wild type

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Fig. 1. Immobilization of VLDL and HDL₃ on an SPR sensor chip

Biotinylated VLDL or HDL₃ were immobilized on a pretreated streptavidin-coated sensor chip by flowing a 1mg/ml solution across the chip for 5 min at 2μl/min, and washing with tris-buffered saline at 100μl/min. RU, resonance units.

Fig. 2. Two-state binding of apoE3 to VLDL as detected by SPR measurement of the kinetics of association and dissociation
(A) The sensorgram (solid line) is for 50 μ g/ml apoE3 flowed at 20 μ l/min. The experimental data were fitted to the two-state binding model where $A + B \leftrightarrow AB \leftrightarrow AB_x$. The deconvoluted curves are the additive components of the fitted curve and show the initial binding (AB) and the subsequent binding or conformational change (AB_x). **(B)** The dissociation curves show the effect of increased injection or contact time on the stability of the apoE3/VLDL complex. The experimental dissociation data (dashed line) were fitted to a bi-exponential decay equation (solid line).

Fig. 3. Representative SPR sensorgrams of binding of human apoE to immobilized lipoprotein ApoE solutions at the indicated concentrations were flowed across the sensor chip and the experimental data were fitted with the two-state binding model, where $A + B \leftrightarrow AB \leftrightarrow AB_x$ (see Experimental Procedures).

Fig.4. Isotherms describing the binding of human apoE3 and its tertiary structure domains to VLDL (A) and HDL₃ (B)
 The maximal response (RU_{max}) was derived by fitting sensorgrams obtained over a range of apoE3 concentrations (cf. Fig. 3) to the two-state binding model. These R_{max} values are plotted as a function of apoE3 concentration and fitted to a one-site binding model. (■) WT apoE3; (▲) 22 kDa N-terminal domain (residues 1–191); (◆) 12 kDa C-terminal domain (residues 192–299); (▼) 10 kDa C-terminal domain (residues 222–299).

Fig. 5. SPR sensorgrams of binding of WT human apoE4 (A) and its N-terminal (residues 1–191) (B) and C-terminal (residues 192–299) (C) tertiary structure domains to immobilized VLDL. The sensorgrams for total binding (solid line) are for 50 $\mu\text{g/ml}$ apoE4 or fragment flowed at 20 $\mu\text{l/min}$ and the initial binding (AB) and subsequent binding (AB_x) contributions were obtained by deconvolution, as described in the legend to Fig. 2.

Fig. 6. Influence of C-terminal truncation on the binding of apoE3 and apoE4 to VLDL and HDL₃. (A) apoE4 binding to VLDL. (B) apoE3 binding to VLDL. (C) apoE4 binding to HDL₃. (D) apoE3 binding to HDL₃

The binding isotherms were obtained and analyzed as described in the legend to Fig. 4. (■) WT apoE; (▲) apoE variant (1-272); (▼) apoE variant (1-260); (◆) apoE variant (1-250).

Fig. 7. Model of the two-step binding mechanism of apoE to a spherical VLDL or HDL₃ particle Initial binding (Step 1) is rapid and readily reversible, and occurs through amphipathic α -helices in either the C-terminal domain or the N-terminal helix bundle domain. Step 2 involves the subsequent opening of the helix bundle whereby hydrophobic helix-helix interactions are converted to helix-lipid interactions. Step 2 is relatively slow and less readily reversible.

Table 1
Binding constants for apoE interaction with VLDL and HDL₃

ApoE	K_d^a		B_{max}^b	
	$\mu\text{g/ml}$	μM	RU	relative
<u>VLDL</u>				
WT E3	20 ± 5	0.6 ± 0.1	80 ± 9	1
WT E4	29 ± 10	0.9 ± 0.3	173 ± 27 ^d	2.2
22kDa E3	44 ± 39	2.0 ± 1.8	100 ± 49	1.2
22kDa E4	34 ± 12	1.5 ± 0.5	67 ± 12	0.8
12kDa E	2.0 ± 0.6 ^c	0.2 ± 0.05	55 ± 3 ^d	0.7
10kDa E	3.0 ± 0.5 ^c	0.3 ± 0.05	68 ± 2	0.8
<u>HDL₃</u>				
WT E3	136 ± 100	4 ± 3	204 ± 116	1
WT E4	31 ± 11	0.9 ± 0.3	111 ± 19	0.5
22kDa E3	38 ± 28	1.7 ± 1.3	79 ± 31	0.4
22kDa E4	47 ± 10	2.1 ± 0.5	115 ± 14	0.6
12kDa E	1.2 ± 0.2 ^c	0.1 ± 0.02	59 ± 1	0.3
10kDa E	5.3 ± 1.4 ^c	0.5 ± 0.1	50 ± 3	0.2

^aThe K_d and B_{max} values are listed as mean ± SEM and are derived from representative binding isotherms of the type shown in Fig. 4.

^bThe B_{max} values for the VLDL and HDL₃ binding experiments cannot be compared directly because different amounts of lipoprotein are immobilized on the SPR chip.

^cSignificantly different ($p < 0.05$) to the K_d for WT apoE3.

^dSignificantly different to the B_{max} for WT apoE3.