THE STRUCTURE OF APOLIPOPROTEIN A-II IN DISCOIDAL HIGH DENSITY LIPOPROTEINS

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Running Title: Lipid-bound apoA-II structure

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It is well accepted that high levels of high density lipoproteins (HDL) reduce the risk of atherosclerosis in humans. Apolipoprotein (apo) A-I and apoA-II are the first and second most common protein constituents of HDL. Unlike apoA-I, detailed structural models for apoA-II in HDL are not available. Here, we present a structural model of apoA-II in reconstituted (r)HDL based on two well established experimental approaches; chemical cross-linking/mass spectrometry (MS) and internal reflection infrared (IR) spectroscopy. Homogeneous apoA-II rHDL were reacted with a cross-linking agent to link proximal lysine residues. Upon tryptic digestion, cross-linked peptides were identified by electrospray mass spectrometry. 14 cross-links were identified and confirmed by tandem mass spectrometry (MS/MS). IR spectroscopy indicated a belt-like molecular arrangement for apoA-II in which the protein helices wrap around the lipid bilayer rHDL disc. The cross-links were then evaluated on three potential belt arrangements. The data clearly refute a parallel model but support two anti-parallel models, especially a ‘double hairpin’ form. These models form the basis for understanding apoA-II structure in more complex HDL particles.

High density lipoproteins (HDL) have received a great deal of attention in recent years due to postulated roles in atheroprotective processes such as reverse cholesterol transport, anti-inflammation and anti-oxidation. Apolipoprotein (apo)A-I is the most abundant protein in HDL (at about 1 mg/ml in plasma) and clearly modulates many HDL atheroprotective functions. By contrast, there is much less known about the second most abundant protein, apoA-II. The average apoA-I:apoA-II molecular ratio in human plasma is 2:1, hinting that such an abundant protein should have important physiological functions. Murine studies with either human or mouse transgenes showed that apoA-II over-expression creates a more atherogenic lipoprotein profile (1-4). On the other hand, apoA-II knockout mice exhibited dramatically decreased HDL cholesterol levels (5). However, these studies have not provided a clearly recognized function for apoA-II.

There is evidence that a small amount of HDL in human plasma contains apoA-II as its only protein constituent (6). Moreover, this apoA-II-only HDL (LpA-II-HDL) dominates in patients with Tangier disease, presumably compensating for the lack of apoA-I in HDL (6). Recently, it was suggested that nascent LpA-II-HDL and LpA-I-HDL may fuse to form mature HDL particles in plasma (7;8). However, most circulating apoA-II is present in LpA-I/A-II mixed HDL particles. There is growing evidence that one role of apoA-II may be to modulate apoA-I structure, potentially modulating HDL function. Structural studies on LpA-I/A-II particles suggest that apoA-II can cause profound conformational changes in apoA-I (9;10). One
functional study compared the hydrolysis rates of LpA-I, LpA-II and LpA-I/A-II HDL by endothelial lipase (EL), an important enzyme in physiological regulation of HDL levels (11). The lipid hydrolysis rate was found to be highest in the mixed particles but lower in rLpA-I HDL and almost undetectable in LpA-II particles. The fact that apoA-II facilitates hydrolysis of lipids in mixed particles but not in A-II rHDL suggests that apoA-II can affect apoA-I conformation to stimulate EL activation. Unfortunately, little information exists on the structural interactions between apoA-I and apoA-II and understanding of apoA-II structure has not kept pace with recent advancements for apoA-I (12).

In human plasma, almost all apoA-II exists as a homodimer, consisting of a single S-S bridge formed across the Cys residue at position 6 of each polypeptide (13). Monomeric apoA-II is 77 residues long with three assigned putative amphipathic helices. ApoA-II was recently crystallized with the lipid surrogate, β-octyl glucoside (BOG) (14). The resulting structure indicated that the apoA-II/BOG complex was composed of eight apoA-II homodimers winding around themselves in a circular spiral-like arrangement in a head-to-tail manner. However, the relationship of this structure to physiological lipid containing particles is not clear. ApoA-II containing rHDL particles containing 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) have been subjected to limited proteolysis analyses (15). The data indicated that lipid-bound apoA-II is more resistant to proteolysis than the lipid-free form and has an entirely different cleavage pattern, indicating significant conformational changes. However, the conformational details of apoA-II structure in native like HDL particles are not known.

In this study, we have used two independent and powerful approaches to gain structural information on apoA-II in native-like HDL particles. The results indicate that apoA-II adopts a “belt-like” orientation similar to that proposed for apoA-I and apoE (16;17). We propose one of the first detailed models for the three-dimensional arrangement of apoA-II in rHDL particles.

Experimental Procedures

ApoA-II purification and preparation of rHDL particles. Human apoA-II isolation and purification from normolipidemic subjects was carried out as reported previously for apoA-I (9;18). In brief, after HDL isolation by ultracentrifugation and delipidation, fractions corresponding to apoA-II and apoA-I were collected from a Q-Sepharose fast flow anionic exchange column. The isolated proteins were then passed over a Superdex 200 gel filtration column (Amersham Biosciences, Piscataway, NJ) prior to the particle reconstitution reaction. The Bio-bead/cholate removal method was used for the preparation of reconstituted HDL (rHDL) particles of apoA-II as has been reported for apoA-I (19-21). The dimeric (i.e. physiological) form of apoA-II was used. Phospholipid, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC, Avanti Polar Lipids, Birmingham, AL) was used as the sole lipid component in particle reconstitution (Table 1). Particles that are designated as A-I-POPC-rHDL and A-II-POPC-rHDL were reconstituted with starting molar ratios of 1:78 apoA-I: POPC and 1:58 apoA-II: POPC respectively (Table 1). The rHDL particles that were subjected to infrared spectroscopic analysis were prepared incorporating the anionic analog of POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-[phosphor-L-serine] (POPS) in a 1:9 molar ratio with POPC as required by the technique (Table 1). These particles which contain 10 mole % of anionic lipids are designated as A-I-POPC/PS-rHDL and A-II-POPC/PS-rHDL and were prepared with 1:78 and 1:58 starting protein to total lipid ratios (Table 1). Once prepared, the rHDL was repurified using a tandem gel filtration column setup (Superdex 200 and Superose 6, Amersham Biosciences, Piscataway, NJ) equilibrated in phosphate buffered saline (PBS, pH 7.8) to remove any unreacted lipids and proteins.

Determination of number of protein molecules per rHDL particle. The cross-linker 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride dissolved in water at 10 mg/ml, Pierce, Rockford, IL) was added to A-I-POPC-rHDL and A-II-POPC-rHDL in PBS at a 1 mg/ml protein concentration. The protein to cross-linker molar ratios used were either 1:50 or 1:500. The sample solutions were incubated at 4 ºC for 24 h then subjected to PAGE analysis.

Cross-linking and tryptic digestion of apoA-II. Freshly prepared bis(sulfosuccinimidyl) suberate (BS3) cross-linker (6.5 mg/ml in PBS,
pH 7.8, Pierce, Rockford, IL, USA) was added to homogeneous A-II-POPC-rHDL with 1 mg/ml protein concentration, at a protein to BS3 molar ratio of 1:10. The BS3 solution preparation and addition to the protein was done within 1 min to minimize hydrolysis of the cross-linker as was described previously (20;22). Samples were incubated at 4 °C for 24 h. The samples were lipid extracted using standard chloroform/methanol extraction procedures. The cross-linked protein was resolubilized in 3M guanidine in 5mM ammonium bicarbonate, dialyzed into 5mM ammonium bicarbonate and concentrated by ultrafiltration (YM-10, Millipore Corporation, Bradford, MA, USA). The protein samples were digested using 2.5 w/w % sequencing grade trypsin (Promega, USA) and incubated at 37 °C for ~12 h, followed by a second aliquot of trypsin at the same ratio for 2 additional hours. The samples (50 μg protein aliquots) were lyophilized and stored at -20 °C until used in the mass spectrometry analysis.

Tryptic peptide analysis by nano LC and mass spectrometry. Peptide mass detection was carried out on Applied Biosystems MDS Sciex QStar® XL mass spectrometer equipped with a nano spray ion source following separation of the peptides on a nano-flow high performance liquid chromatography (HPLC) from LC Packings. Five pmols of the peptides generated from trypsin digestion of the cross-linked apoA-II were injected onto a C18 Trap cartridge (from LC Packings, 300 μm id and 1 mm length) and washed with 0.1% tri-fluoro acetic acid/0.1% formic acid in water flowing at 20 μL/min for 20 min. This was followed by eluting the peptides onto a C18 nano analytical column (75 μm id and 15 cm in length from LC Packings) for separation using a gradient of acetonitrile from 5% to 40% at a flow rate of 250 nL/min over 90 min. The gradient was generated using solvent A (0.1% formic acid/0.01% tri-fluoro acetic acid/2% acetonitrile in HPLC grade water) and solvent B (0.1% formic acid/0.01% trifluoro acetic acid/2% water/10% isopropanol in acetonitrile). Under these conditions, most peptide masses were well separated as seen by the total ion chromatogram (TIC). The mass spectrometer was set to acquire MS and MS/MS data in an automated fashion using the information dependent acquisition (IDA) functionality of the Analyst® QS software. Each MS spectrum acquired in 1 s was followed by acquisition of four MS/MS spectra at 3 s each of the four most intense ions after satisfying the dynamic exclusion criteria. The dynamic exclusion criteria allowed for generating an exclusion list of peptide masses already fragmented for a period of 60 sec with a mass tolerance of 100 ppm for match of peptide mass. Upon completion of the LC/MS/MS run, a peptide mass list was generated using the Applied Biosystems Analyst QS 1.1 software. The mass list was subjected to analysis by GPMAW (ChemSW, Inc.) to assign putative sequence identity of individual peptides with or without a modification by a cross-linker (19;20;22). The putative amino acid sequence identity of the inter peptide cross-linked masses were assigned by mass mapping of experimental masses with theoretically constructed list of all possible intra/inter-molecularly cross-linked masses for apoA-II (19;20;22). A map was considered positive if the experimental and theoretical masses came within 10 ppm with the correct number of Lys residues available for the cross-linker formation (19). This deviation is a considerable improvement compared to our previous cut off of 50 ppm (19;20;22), a result of hardware and technique improvements. Confirmation of peptide identities were provided by MS/MS analysis.

Particle characterization by circular dichroism (CD) spectroscopy. CD spectra of rHDL particles that contained POPC only, POPC/PS as well as free proteins (apoA-II and apoA-I) in 5 mM Standard Tris Buffer were measured on a J-810 spectrometer (Jasco, Easton, MD). A homemade demountable cell composed of two UV CaF2 windows separated by a 100 μm Teflon spacer clamped in a brass holder was used in the sample measurements. Such a set up for the cell was required due to use of a relatively high protein concentration of 0.225 mg/ml. A scan rate of 20 nm/minute, a bandwidth of 0.2 nm with a time response of 2 s were used to obtain CD spectra as an average of 8 scans. The final spectra were obtained by subtracting a background spectrum of the same buffer. The fractional secondary structure of the proteins in solution was estimated from the CD spectra by use of the SELCON 3 method, which is part of the CD-Pro software package (23).

Fluorescence studies. Fluorescence measurements of POPC, POPC/PS rHDL
particles as well as free proteins (apoA-II and apoA-I) were performed on a Fluoromax-3 spectrofluorometer (Jobin Yvon Inc., Edison, NJ) on 0.225 mg/ml samples in a 1 mm quartz cuvette. The excitation wavelengths were 275 nm (Tyr excitation for apoA-II) and 295 nm (Trp excitation for apoA-I). The emission spectra for both were recorded from 300 to 450 nm. In all experiments, a background spectrum of the buffer was subtracted from the corresponding sample spectra.

Infrared spectroscopy. Polarized attenuated total internal reflection Fourier transform infrared (PATIR-FTIR) spectroscopy was performed on A-II-POPC/PS-rHDL and A-I-POPC/PS-rHDL. We included an rHDL particle as a positive control which had been analyzed previously (16). The control particle contained apoA-I with (DMPC)/1,2-dimyristoyl-sn-glycero-3-[phosphor-L-serine] (DMPS). The protein: total lipid ratio for these particles (A-II-DMPC/PS-rHDL) was 1:78 with 10% DMPS in the lipid mixture as for other particles that were subjected to PATIR-FTIR analysis. The PATIR-FTIR measurements were carried out using a Bio-Rad FTS-6000 spectrometer equipped with an MCT detector. The instrument was interfaced to a lipid film balance by means of a horizontal 50x10x2 mm germanium crystal internal reflection element. A lipid monolayer composed of 80 mol% DMPC and 20 mol% DMPS was spread at room temperature at the air-water interface in the film balance and applied flat onto the crystal. Adsorption of the HDL particles and their horizontal orientation was facilitated by Ca++ ions in the buffer bridging the anionic lipid in the monolayer (20 mole%) and in the complexes (10 mole %) (16). All spectra were collected in the rapid scan mode as 512 co-added interferograms, with a resolution of 2 cm-1, triangular apodization, and one level of zero filling. Baseline spectra were recorded immediately prior to the addition of 20 μg of protein in the form of lipoprotein complexes to the continuously stirred buffer subphase composed of 30 mM HEPES, 1 mM CaCl2, in D2O at pH 7.4.

From the polarized absorption spectra, dichroic ratios were evaluated using integrated areas of characteristic absorption bands as determined by linked analysis of sets of parallel and perpendicular spectra. These dichroic ratios were then converted to order parameters S(Rg) as described (16,24). An order parameter of S = 1.0 indicates a uniform orientation perpendicular to the membrane surface, while a value of S = -0.5 indicates a uniform orientation parallel to the membrane. An order parameter of S = 0.0 may indicate an isotropic distribution, a uniform orientation at the magic angle (θ = 54.7° relative to the membrane normal), or any distribution for which \( \langle \cos^2 \theta \rangle = 1/3 \).

Results

Generation and characterization of A-II-POPC-rHDL particles. We hypothesized that apoA-II, like apoA-I, adopts a specific conformation in discoidal rHDL and therefore exhibits sequence specific interactions with itself and with other apoA-II molecules on the particle (19,20,25). We elected to generate apoA-II containing rHDL particles that contain POPC, a synthetic lipid with an acyl chain composition commonly found in cellular membranes and lipoproteins. At a starting ratio of 58 mol POPC: 1 mol apoA-II, we were able to reconstitute A-II-POPC-rHDL that exhibited a slightly larger hydrodynamic diameter than previously well characterized A-I-POPC-rHDL (Fig. 1, A) (20,26,27). Despite our best efforts, we were unable to eliminate a minor secondary band which ran a few angstroms smaller than the major band at ~102 Å. The number of protein molecules per particle was determined by cross-linking with EDC which can bridge an acidic amino acid (Asp or Glu) with a Lys residue (28,29). When cross-linked at a high ratio of protein to EDC, the A-II-POPC-rHDL sample exhibited a single band ~70 kDa corresponding to the mass of four dimeric apoA-II molecules (Fig. 2). At lower cross-linker ratios, bands corresponding to 1, 2, 3 and 4 molecules of apoA-II were apparent indicating incomplete cross-linking. No other condition yielded MW bands higher than 4 cross-linked molecules of apoA-II. A similar analysis for an A-I-POPC-rHDL particle exhibited a single band ~70 kDa corresponding to the mass of four dimeric apoA-II molecules. A similar analysis for an A-I-POPC-rHDL particle yielded the expected 2 molecules of apoA-I per particle (Fig. 2). Component analysis on the rHDL (after isolation from unreacted protein and lipid) indicated that the A-II-POPC-rHDL contained ~190 POPC molecules per particle compared to ~156 POPC molecules for A-I-POPC-rHDL (Table 1). The increased lipid...
in the apoA-II particle likely accounts for its larger apparent diameter (Fig. 1, A).

Cross-linking and mass spectrometry analyses: Cross-linking experiments for structural analyses were performed with BS\(^3\), a homo-bifunctional cross-linker capable of linking Lys residues within its spacer arm length of 11.4 Å. A molar ratio of 1:10 protein: cross-linker was used based on previous work demonstrating that linkages formed under these conditions occur within a single rHDL particle, but not between two rHDL particles (19;20). After cross-linking, the particles were delipidated, digested with trypsin and analyzed by electrospray MS. In theory, there can be four types of cross-links for apoA-II as illustrated in Fig 3: Type I intrapeptide cross-links occur between two Lys residues within the same tryptic peptide. Type II, joins two peptides from the same apoA-II monomer, but separated by at least one tryptic cleavage site; Type III, joins two peptides within the same dimeric apoA-II molecule, but on different strands; Type IV, joins two peptides from different apoA-II molecules. We identified 5 Type I intra-peptide cross-links (Table 2). The same set of Type I cross-links was identified from two independent particle preparations. We also identified nine long-range, inter-peptide cross-links (Table 3). These consisted of two peptides separated by at least a single tryptic cleavage site. Due to the small size of apoA-II, we were unable to assign most of these cross-links to the scheme illustrated in Fig 3, i.e. they can be Types II, III or IV. The exception is the cross-link Lys 46-Lys 46 which must be either Type III or IV, but not II. MS/MS sequencing of these long range cross-links indicated that each putative sequence had more than 50% of expected theoretical fragment ions, unambiguously confirming the sequences of the assigned peptides. An example analysis is shown in Fig. 4. Interestingly, we found no evidence for either Lys 3 or Lys 23 modification by BS\(^3\). However, unmodified peptides in this region were clearly identified in the spectra e.g. two copies of peptide 4-23 linked by an S-S bond found at m/z 1146.5349 (charge state +4) corresponding to MW 4582.1084.

Molecular orientation of apoA-II on discoidal rHDL particles. In order to use the cross-link distance constraints listed in Tables 2 and 3 to propose models for apoA-II structure on the edge of discoidal rHDL, it was necessary to determine the orientation of the apoA-II backbone. Based on previous success with apoA-I and apoE rHDL, PATIR-FTIR spectroscopy was chosen for this purpose (16;17). As noted in Experimental Procedures, this technique requires the incorporation of a small amount of anionic lipid into the particles in order to sequester and align them during the measurements. Table 1 shows that the anionic lipid (POPS) containing particles (POPC/PS) exhibited a similar composition to the particles that contain only POPC. When analyzed by native PAGE (Fig. 1), the POPC/PS particles appeared to migrate faster than the POPC particles with an apparent diameter of ~100 nm. However, gel filtration chromatography indicated that the POPC/PS particles exhibited the exact same retention volume as the POPC particles, indicating that the two particles were of similar hydrodynamic diameter. The example of the apoA-I containing particles is shown in Fig. 1, C. It is likely that the faster migration of POPC/PS particles on the native gel was due to the increased negative charge imparted by the added anionic lipid. ApoA-II particles prepared with and without anionic lipids (A-II-POPC/PS-rHDL and A-II-POPC-rHDL) exhibited similar circular dichroism spectral signatures (Fig 5A) indicating similar overall secondary structural contents. Analysis of the spectra indicated that A-II-POPC-rHDL and A-II-POPC/PS-rHDL had 79 ± 5% and 75 ± 5% α-helical contents respectively. Fluorescence emission spectra of A-II-POPC/PS-rHDL and A-II-POPC-rHDL particles were also similar (Fig 5B). Based on these data, we concluded that PS inclusion minimally affected particle structure. Similar CD and fluorescence analyses carried out on apoA-I particles indicated that A-I-POPC/PS-rHDL and A-I-POPC-rHDL were similar in structure as well (data not shown). PATIR-FTIR spectroscopy was performed on three different rHDL particles: A-I-POPC/PS-rHDL, A-I-POPC/PS-rHDL, and A-I-DMPC/PS-rHDL. The particles studied as a control, A-I-DMPC/PS-rHDL, were homogeneous as seen by native PAGE and contained ~216 lipid molecules per particle (data not shown). First, we examined A-I-DMPC/PS-rHDL particles and verified that modifications to the instrumentation made since these particles were last examined did not change the results (Table 4) (16). Second, we examined A-I-
POPC/PS-rHDL particles to determine the effect of longer lipid acyl chains on the results. Although there was a trend towards less order and greater variability with longer chain lipids, the orientational relationship between protein and lipid components was unchanged (Table 4). Third, we examined A-II-POPC/PS-rHDL particles. Amide I and methylene stretching bands from A-I-POPC/PS-rHDL and A-II-POPC/PS-rHDL particles are compared in Figure 6. In both particles, the amide I absorption maximum was located at 1643 cm\(^{-1}\), and both spectra could be fitted with similar components. Likewise, antisymmetric and symmetric methylene stretching bands at ~2924 and ~2854 cm\(^{-1}\) from both particles were similar. Most importantly, there was no difference in protein and lipid order parameters (Table 4).

Discussion

This study produced two principal findings. The first was a set of peptide cross-links between specific Lys residues in the native structure of well-defined apoA-II rHDL particles. Second, infrared spectroscopy on similar particles indicated that apoA-II adopts a belt-like orientation in which apoA-II wraps around a lipid bilayer parallel to the particle surface. These data, combined with the geometric constraints inherent to the particle phospholipid bilayer, provide sufficient information to propose and test a series of structural models for the apoA-II particles. The results of the IR measurements are discussed first, followed by an analysis of potential structural models using the cross-links.

The order parameter data in Table 4 show that apoA-II adopts a belt molecular arrangement that is quite similar to that adopted by apoA-I. Thus, apoA-I (16), apoE (17) and apoA-II all adopt a similar general orientation with respect to the lipid bilayer. The results obtained for apoE differed from apoA-I suggesting that certain apoE regions may associate in the plane of the membrane, possibly perturbing the lipid packing characteristics of the particle. These conclusions were based on an observed decrease in the magnitude of the order parameters for the lipid acyl chains (from -0.45 for \(\Delta(1-43)\)apoA-I-DMPC/PS to -0.35 apoE-DMPC/PS). In this study, we used the more physiological lipid POPC. The reduced lipid order parameter compared to DMPC-rHDL was most likely due to the introduction of unsaturated acyl chains. This is illustrated by comparing the lipid order parameters for the apoA-I particles with each of these lipids in Table 4. An acyl chain tilt of about 26 ° for POPC with respect to DMPC could also account for an order parameter near 0.33, but this degree of a tilt in such a small particle seems unlikely on geometric grounds. In any case, the lipid order parameter for the A-II-POPC-rHDL particles was comparable to the A-I-POPC-rHDL particles. Thus, unlike apoE, apoA-I and apoA-II do not seem to perturb lipid packing and have overall similar backbone orientations.

A model for apoA-II rHDL. Based on theoretical considerations from studies of apoA-I rHDL disc studies, we approached the modeling of apoA-II with the following basic assumptions: 1) the particles are composed of a patch of phospholipid bilayer and apoA-II is limited to the bilayer edge, 2) the bilayer edge is covered by two continuous or discontinuous amphipathic helical strands of apoA-II (disulfide linked) with the hydrophobic faces of the amphipathic helices facing the lipids, 3) all four molecules of apoA-II on the particle adopt identical conformations, and 4) the protein backbone is oriented predominantly parallel to the plane of the bilayer. Given these assumptions, we envisioned three general models for apoA-II conformation in the particles (Fig. 7). In the parallel model, all four molecules are oriented in the same direction making head-to-tail (or in an alternate version, head-to-head) contacts. Figure 7 shows only two apoA-II molecules for clarity, but additional molecules can be added to the ends with no impact on the cross-linking parameters. In the anti-parallel model, the monomeric constituents of each apoA-II molecule are rotated 180 ° with respect to the disulfide linkage and proceed in opposite directions. Note the extensive intermolecular contacts in this model. An additional antiparallel model exhibits the same rotation about the disulfide linkage, but postulates that each apoA-II strand doubles back on itself. We call this the double hairpin model.

We tested these models with the distance constraints derived from the cross-linking data in Tables 2 and 3. We began with the P-Q dimer of apoA-II as determined from the crystal in association with the detergent BOG (Fig. 7, top) (14). While not derived from true phospholipid-bound apoA-II, Kumar and colleagues proposed...
that this pair may be suggestive of a lipid-bound form of apoA-II. In this structure, two monomeric constituents of each apoA-II molecule, lying parallel to each other, wrap around other apoA-II molecules with intermittent detergent molecules bound at an orientation normal to that of the helical segments. The degree of curvature of the P-Q dimer, being at the outer edge of the structure, is relatively consistent with the curvature of a 96 Å apoA-I/phospholipid disc. To generate the antiparallel and double hairpin models, the parallel P-Q dimer structure was graphically manipulated and a relative distance measuring system was established based on measurements made in the P-Q dimer structure. The 14 intra- and inter-peptide cross-links were then tested within each model. In many cases, we were unable to determine if a particular interpeptide crosslink was formed within the same apoA-II strand, i.e. Type II (Fig. 3), or was formed between two separate strands, i.e. Types III or IV. Therefore, each cross-link was evaluated for both possibilities. Given the length of two Lys side chains and the cross-linker spacer arm, we previously established that the β-carbons of two Lys residues must reside within about 20 Å in order to form a BS3 crosslink (20). Therefore, distances < 20 Å for a given cross-link were considered possible for each model while larger distances were considered not possible. The results of this analysis are shown in Table 5. For the parallel model, only 9 out of 14 cross-links could fit the data. Many of these included short range cross-links that equally fit all three models. The fits were much more plausible for the antiparallel model with only 2 cross-links that were inconsistent with the data. The best fit came for the double hairpin model with all but one cross-link judged to be plausible. In addition, the distances for most of the cross-links were substantially shorter for this model than for the anti-parallel version.

As stated above, the crystal structures of both lipid-free and detergent bound apoA-II clearly indicate that apoA-II can adopt the parallel model, at least under crystallization conditions. But our analysis shows that 5 experimentally derived cross-links are simply not consistent with this model in true lipid bound apoA-II particles in solution. There are also several other compelling arguments against the parallel model. First, our pilot experiments indicated that even low protein to cross-linker ratios are quite capable of linking all four apoA-II molecules in a particle. This observation is difficult to rationalize given the limited end-to-end molecular interactions apparent in Figure 7, (top), especially since there are no Lys residues present in the C-terminal 22 amino acids that could participate in cross-linking. Second, if the parallel model is correct, then none of our observed cross-links would be capable of joining two apoA-II molecules together. It is possible that we were unable to detect some cross-links due to poor ionization etc., but given the ease at which apoA-II can be cross-linked on a particle; we find it highly unlikely that we would be unable to detect any intermolecular cross-links. In contrast, intermolecular cross-links are extremely plausible in the antiparallel model in which there are ample opportunities for intermolecular contact. Similarly, the double hairpin model puts clusters of Lys resides in close opposition for forming potential intermolecular cross-links. Third, if the region from 3-27 adopts a continuous amphipathic helix as suggested by some secondary structure prediction algorithms, the Cys residues at position 6 would be predicted to be in the opposite docking interface in the parallel model (Fig. 8) (30). By contrast, in the antiparallel and double hairpin models, the Cys residues would be in an ideal arrangement to form the linkage.

One cross-link that does not strictly fit either the double hairpin or antiparallel models is Lys 46-46. Interestingly, the crystal structure shows that the region between residues 29 and 39 represents a random coil break in an otherwise helical region. It is possible that this region could be “looped off” of the particle in solution. This would have the effect of bringing residues 29 and 39 much closer together. In this case, it could be envisioned that the Lys46-46 cross-link could be plausible in the anti-parallel model and slightly more extensive unfolding in this region might make this cross-link plausible in the double hairpin model as well. Indeed, the crystal structure used to generate these models puts apoA-II about 10% more helical than we measured in our CD analysis on particles in solution. One interesting feature of the double hairpin model is that it provides an attractive rationale for the formation of the intrapeptide link between residues 30 and 39. This link is at the
limit of the cross-linker range in both the parallel and antiparallel models, but is more likely when the hairpin turn puts them into closer opposition. Limited proteolysis of lipidated apoA-II indicated that this region is highly cleavable (15), consistent with a potential turn sequence. Moreover, there is a proline present at position 32, which could be a turn initiator.

In conclusion, our data strongly indicates that apoA-II adopts a belt-like structure in POPC-rHDL particles. Our data are most consistent with the double hairpin model. However, the models proposed in Fig. 7 are based on a crystal structure in which the proteins were under non-native conditions. There are likely differences in conformation and secondary structure in the truly lipid-bound forms in solution. Thus, the theoretical distances and the putative fits to the data listed in Table 5 should be considered approximations only. Further studies using cross-linking agents that are either not limited to Lys residues or have shorter spacer arm lengths will be required to unambiguously distinguish between the antiparallel and double hairpin models. Nevertheless, this study provides a detailed and testable models for lipid-bound apoA-II. This information will be extremely useful for future studies aimed at understanding the structural interactions between apoA-I and apoA-II in more complex forms of HDL. These studies will be critical for evaluating the possibility that apoA-II may function indirectly by modulating apoA-I conformation to affect HDL metabolism in vivo.

Acknowledgements

We thank Dr. Timothy A. Keiderling (University of Illinois at Chicago) for generous assistance with CD and fluorescence measurements.
References

1. Warden, C. H., Hedrick, C. C., Qiao, J. H., Castellani, L. W., and Lusis, A. J. (1993) Science 261, 469-472

2. Castellani, L. W., Navab, M., Lenten, B. J. V., Hedrick, C. C., Hama, S. Y., Goto, A. M., Fogelman, A. M., and Lusis, A. J. (1997) Journal of Clinical Investigation. 100, 464-474

3. Hedrick, C. C., Castellani, L. W., Wong, H., and Lusis, A. J. (2001) J Lipid Res 42, 563-570

4. Escola-Gil, J. C., Marzal-Casacuberta, A., Julve-Gil, J., Ishida, B. Y., Ordenez-Llanos, J., Chan, L., Gonzalez-Sastre, F., and Blanco-Vaca, F. (1998) J Lipid Res. 39, 457-462

5. Allayee, H., Castellani, L. W., Cantor, R. M., de Bruin, T. W., and Lusis, A. J. (2003) Circ. Res 92, 1262-1267

6. Bekaert, E. D., Alaupovic, P., Knight-Gibson, C., Norum, R. A., Laux, M. J., and Ayrault-Jarrier, M. (1992) Biochim. Biophys. Acta 1126, 105-113

7. Clay, M. A., Pyle, D. H., Rye, K. A., and Barter, P. J. (2000) J Biol Chem 275, 9019-9025

8. Hime, N. J., Drew, K. J., Wee, K., Barter, P. J., and Rye, K. A. (2006) J. Lipid Res. 47, 115-122

9. Durbin, D. M. and Jonas, A. (1997) Journal of Biological Chemistry. 272, 31333-31339

10. Boucher, J., Ramsamy, T. A., Braschi, S., Sahoo, D., Neville, T. A., and Sparks, D. L. (2004) J. Lipid Res. 45, 849-858

11. Jahangiri, A., Rader, D. J., Marchadier, D., Curtiss, L. K., Bonnet, D. J., and Rye, K. A. (2005) J Lipid Res 46, 896-903

12. Davidson, W. S. and RG, D. S. (2005) Curr. Opin. Lipidol. 16, 295-300

13. Gillard, B. K., Chen, Y. S., Gaubatz, J. W., Massey, J. B., and Pownall, H. J. (2005) Biochemistry 44, 471-479

14. Kumar, M. S., Carson, M., Hussain, M. M., and Murthy, H. M. K. (2002) Biochemistry 41, 11681-11691

15. Massey, J. B., Hickson-Bick, D. L., Gotto, A. M., Jr., and Pownall, H. J. (1989) Biochim. Biophys. Acta 999, 121-127

16. Koppaka, V., Silvestro, L., Engler, J. A., Brouillette, C. G., and Axelsen, P. H. (1999) J. Biol. Chem. 274, 14541-14544
17. Schneeweis, L. A., Koppaka, V., Lund-Katz, S., Phillips, M. C., and Axelsen, P. H. (2005) *Biochemistry* **44**, 12525-12534

18. Lund-Katz, S. and Phillips, M. C. (1986) *Biochemistry* **25**, 1562-1568

19. Davidson, W. S. and Hilliard, G. M. (2003) *J Biol Chem* **278**, 27199-27207

20. Silva, R. A., Hilliard, G. M., Li, L., Segrest, J. P., and Davidson, W. S. (2005) *Biochemistry* **44**, 8600-8607

21. Jonas, A. (1986) *Methods in Enzymology* **128**, 553-582

22. Silva, R. A., Hilliard, G. M., Fang, J., Macha, S., and Davidson, W. S. (2005) *Biochemistry* **44**, 2759-2769

23. Sreerama, N. and Woody, R. W. (2000) *Anal.Biochem.* **287**, 252-260

24. Axelsen, P. H. and Citra, M. J. (1996) *Prog.Biophys.Mol.Biol* **66**, 227-253

25. Maiorano, J. N., Jandacek, R. J., Horace, E. M., and Davidson, W. S. (2004) *Biochemistry* **43**, 11717-11726

26. Panagotopulos, S. E., Horace, E. M., Maiorano, J. N., and Davidson, W. S. (2001) *J.Biol.Chem.* **265**, 22123-22129

27. Jonas, A., Wald, J. H., Toohill, K. L., Krul, E. S., and Kezdy, K. E. (1990) *J.Biol.Chem.* **265**, 22123-22129

28. Pliszka, B., Redowicz, M. J., and Stepkowski, D. (2001) *Biochem.Biophys.Res Commun.* **281**, 924-928

29. Kruip, J., Chitnis, P. R., Lagoutte, B., Rogner, M., and Boekema, E. J. (1997) *J Biol Chem* **272**, 17061-17069

30. De Coen, J. L., Deboeck, M., Delcroix, C., Lontie, J. F., and Malmendier, C. L. (1988) *Proc.Natl.Acad.Sci.U.S.A* **85**, 5669-5672

31. Sparks, D. L. and Phillips, M. C. (1992) *J.Lipid Res.* **33**, 123-130

32. Koppaka, V. (2001) *Cell Mol.Life Sci.* **58**, 885-893
Footnotes

1 This work was supported by a RO1 (HL67093) grant from the NHLBI to WSD and an Ohio Valley Affiliate Postdoctoral Fellowship from the AHA to RAGDS. LAS and PHA were supported by NIH grant R01-HL68186.

2 Abbreviations used: apo, apolipoprotein; BOG, β-octyl glucoside; BS³, bis (sulfosuccinimidyl)suberate; CD, circular dichroism; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DMPS, 1,2-dimyristoyl-sn-glycero-3-[phosphor-L-serine]; EDC, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride; HDL, high density lipoproteins; HMW, high molecular weight; HPLC, high performance liquid chromatography; LC, liquid chromatography; LMW, low molecular weight; MS, mass spectrometry; MS/MS, tandem mass spectrometry; IDA, information dependent acquisition; IR, infrared spectroscopy; PAGE, poly acrylamide gel electrophoresis; PATIR-FTIR, Polarized Attenuated Total internal Reflection Fourier Transform infrared; PBS, phosphate buffered saline; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine; POPS, 1-palmitoyl-2-oleoyl-sn-glycero-3-[phosphor-L-serine]; PS, anionic lipid variants of DMPC or POPC; ppm, parts per million; rHDL, reconstituted HDL; TIC, total ion chromatogram.

3 Human apoA-II is most commonly found in circulation as a disulfide linked homodimer of 77 a.a. apoA-II polypeptides. For the purposes of this manuscript, we use the term molecule to refer to this apoA-II homodimer. We refer to each polypeptide constituent of this molecule as strands.
Figure Legends

Figure 1. **PAGE analysis of A-I-rHDL and A-II-rHDL particles.** rHDL particles were generated either with POPC only or with a 9:1 mixture of POPC/PS as required for different experiments. Panel A: native PAGE analysis (8-25% Phast gel) showing the apparent hydrodynamic diameters of A-I-POPC-rHDL (Lane 1), A-I-POPC/PS-rHDL (Lane 2), apoA-II-POPC-rHDL (Lane 3) and apoA-II-POPC/PS-rHDL (Lane 4). Panel B: SDS PAGE analysis (8-25% Phast gel) showing apoA-I (lane 6) and apoA-II (lane 7) in POPC-rHDL particles prior to cross-linking. High Molecular Weight markers are shown in Lanes 5 (GE Healthcare, Amersham HMW standards, Cat # 17-0445-01). Hydrodynamic diameters corresponding to the HMW standards were taken from the literature (31). The Low Molecular Weight standards are shown in Lane 8 (GE Healthcare, Amersham LMW standards, Cat # 17-0446-01). Both gels were stained with Coomassie blue. Panel C: FPLC traces of A-I-POPC-rHDL (top) and A-I-POPC/PS-rHDL (bottom).

Figure 2. **SDS PAGE analysis of cross-linked A-I-POPC-rHDL and A-II-POPC-rHDL particles.** The number of protein molecules per rHDL particle was determined by cross-linking the particles with EDC in a 1:50 protein to cross-linker ratio (lanes 1, 4) and at a 1:500 ratio (lanes 2, 5). Low molecular weight standards (see Fig. 1) are shown in lanes 3 and 6. The gels were stained with Coomassie blue.

Figure 3. **Types of potential cross-links within and between molecules of apoA-II on a discoidal particle.** Two apoA-II molecules are shown as thick and thin solid lines with each strand labeled (A, A’ in molecule 1, B, B’ in molecule 2). Hypothetical tryptic cleavage sites are shown as short vertical line segments across the strands. **Type I, an intra-peptide cross-link:** This cross-link can occur between two Lys residues on the same tryptic peptide within a single strand of apoA-II. **Type II, an inter-peptide, intra-strand cross-link:** This forms within the same strand of apoA-II, but across at least one tryptic cleavage site. **Type III, an inter-strand, intra-molecular cross-link:** This forms between two apoA-II strands that are already joined by the disulfide linkage. **Type IV, an inter-strand, inter-molecular cross-link:** This forms between two strands of two different apoA-II molecules. These are the only cross-links capable of producing the oligomeric species observed in Fig. 2.

Figure 4. **MS/MS sequencing evidence for the cross-link K39-K46.** Typical MS/MS fragment ions b and y, resulting from different amide bond fragmentations of the precursor ion (m/z=974.1807, charge state= +3) corresponding to the cross-linked peptide K39-K46 (MW 2919.5478). Note that 20 of 22 expected b and y fragment ions are clearly available in the spectrum. The m/z value that corresponds to charge state +1 is shown next to each observed fragment ion. Deaminated forms of the fragments ions (e.g.: b-NH3 and y-NH3) are also present, but are not labeled for clarity. The two panels represent high and low m/z ranges of the same spectrum.

Figure 5. **Circular dichroism and fluorescence spectra of A-II-rHDL particles in solution.** Panel A. CD spectra of A-II-POPC-rHDL (thick-solid), A-II-POPC/PS-rHDL (dotted) and free apoA-II (thin-solid) recorded in standard Tris buffer. The spectra were recorded in a 100 μm demountable cell with 0.225 mg/ml sample concentration. Panel B. Fluorescence spectra of A-II-POPC-rHDL (thick-soild), A-II-POPC/PS-rHDL (dotted) and free apoA-II (thin-solid) recorded with the same sample concentrations as were CD spectra in a 1 mm quartz cuvette. The fluorescence excitation wavelength was 275 nm that corresponds to the Tyr absorbance. In both CD and fluorescence measurements, the buffer spectra were subtracted from the corresponding sample spectra.

Figure 6. **Infrared spectra of A-I and A-II-POPC/PS-rHDL particles in the lipid methylene stretching and amide I regions.** Both apoA-I and apoA-II rHDL amide I bands are located at ~1643 cm⁻¹. The antisymmetric and symmetric vibrational bands of the methylene groups of the lipids are located at 2924 cm⁻¹ and 2854 cm⁻¹ respectively. Fitted component bands are shown as thin lines in each
spectrum. The dichroic ratios were then used to calculate the order parameters (see Experimental Procedures) summarized in Table 4.

Figure 7. Potential structural models of apoA-II on the edge of a discoidal rHDL particle. A) Parallel model: Two P-Q dimers taken from the crystal structure of apoA-II in association with the detergent BOG are shown (14) as if they had been taken off the edge of a rHDL particle and laid flat on the paper or screen. Only two apoA-II molecules are shown for simplicity (green and blue), but each particle contains four molecules total. The position of the β-carbon of each Lys residue is shown in red and numbered. The disulfide bond at Cys 6 of each strand is shown in black. B) Antiparallel model. Using the same crystal structure as for panel A, one monomer was rotated 180° centered on the disulfide linkage. C) Double hairpin model. Based on the antiparallel model in Panel B, the C-terminal 38-40 residues of each strand double back onto the N-terminal half of the strand with an intervening turn sequence. All models are drawn to the same relative scale. The maximum expected length of a BS3 cross-link (20 Å) is shown by the black bar, also drawn to scale.

Figure 8. Helical wheel diagram for amino acids 5-21 of apoA-II indicating potential orientations of Cys 6 for the parallel and antiparallel models. The helical wheel for residues 5-21 is shown in panel A with hydrophobic residues in blue, polar residues in grey and charged residues in red. The Cys residues (position number 2 in the selected sequence, but at position 6 in apoA-II) are shown in green. Hydrophobic and hydrophilic phases of the helical wheel are divided by a dashed line.
Table 1. Compositional analysis of rHDL particles.

| rHDLa       | Protein:PL  | Protein:PL  | Hydrodynamic | Protein  |
|-------------|------------|-------------|--------------|----------|
|             | (start)b   | (final)b    | diameter (Å)c| molecules/|
|             |            |             |              | particled|
| A-I-POPC-rHDL | 1:78       | 1:78±4      | 96 ± 1       | 2        |
| A-I-POPC/PS-rHDL | 1:78       | 1:84±4      | nd           | 2        |
| A-II-POPC-rHDL | 1:58       | 1:48±2      | 102 ± 1      | 4        |
| A-II-POPC/PS-rHDL | 1:58       | 1:51±2      | nd           | 4        |

a In cases where both lipids were used, POPC:PS molar ratio is 9:1.
b Final protein: phospholipid (PL) ratios are based on Lowry and the Phosphorus assays on purified rHDL particles.
c Average apparent particle diameters were calculated based on migration distance on a native gel (PAGE) using three independent particle preparations. Particles that contain anionic lipids (PS) were not subjected to hydrodynamic diameter calculations using this method because of charge effects due to the presence of the anionic lipid (see Results section).
d Number of protein molecules per rHDL particle is obtained by cross-linking followed by SDS-PAGE analysis (Fig. 2).

Table 2. Intra peptide (Type I) cross-links identified in an apoA-II rHDL particle cross-linked with BS3.

| Peptide Involved | X-link (K-K) | Experimental mass (Da) | Theoretical mass (Da) | Deviation (±ppm) |
|------------------|--------------|------------------------|-----------------------|------------------|
| 24-39            | 28-30        | 1953.0124              | 1953.0126             | 0                |
| 29-44            | 30-39        | 1991.0249              | 1991.0154             | 5                |
| 31-46            | 39-44        | 1978.9928              | 1978.9885             | 2                |
| 40-54            | 44-46        | 1948.0411              | 1948.0554             | 7                |
| 45-55            | 46-54        | 1421.8399              | 1421.8491             | 6                |

*All cross-links were identified in both experiments carried out on two independent sample preparations. The cross-link 30-46 was identified in one experiment.

Table 3. Inter peptide cross-links (Types II, III or IV) identified in an apoA-II rHDL particle X-linked with BS3.

| Peptides involved | X-link (K-K) | Experimental mass (Da) | Theoretical mass (Da) | Deviation (±ppm) |
|-------------------|--------------|------------------------|-----------------------|------------------|
| 24-30, 40-46      | 28-44        | 1886.9568              | 1886.9697             | 6.8              |
| 29-39, 40-46      | 30-44        | 2224.1532              | 2224.1625             | 4.2              |
| 29-39, 45-54      | 30-46*       | 2492.4152              | 2492.4099             | 2.1              |
| 29-39, 47-55      | 30-54        | 2405.3712              | 2405.3779             | 3.2              |
| 31-44, 45-54      | 39-46        | 2919.5358              | 2919.5478             | 4.1              |
| 31-44, 47-55      | 39-54        | 2832.5163              | 2832.5158             | 0.2              |
| 40-46, 47-55      | 44-54        | 2094.1470              | 2094.1610             | 6.6              |
| 40-46, 55-77      | 44-55        | 3537.8244              | 3537.7920             | 9.1              |
| 45-54, 45-54      | 46-46        | 2449.4373              | 2449.4404             | 1.2              |
Table 4. Order parameters derived for apoA-II and apoA-I rHDL particles.

| rHDL complex       | Amide I (overall) | Symmetric CH\textsubscript{2} stretch | N (number of experiments) |
|--------------------|-------------------|---------------------------------------|---------------------------|
| A-I-DMPC/PS-rHDL   | -0.16 ± 0.01      | -0.42 ± 0.02                          | 3                         |
| A-I-POPC/PS-rHDL   | -0.14 ± 0.07      | -0.34 ± 0.08                          | 3                         |
| A-II-POPC/PS-rHDL  | -0.13 ± 0.07      | -0.33 ± 0.05                          | 4                         |
| A-I-DMPC/PS-rHDL(h) | -0.19 ± 0.04     | -0.43 ± 0.05                          | ---                       |

\( ^a \) Component bands centered at 1671, 1645 and 1627 cm\(^{\text{-1}} \) were integrated and summed to determine order parameters for apoA-I amide I absorption; Components centered at 1652, 1630, and 1617 cm\(^{\text{-1}} \) were used for the apoA-II amide I absorption. The methylene stretching region from 3000-2800 cm\(^{\text{-1}} \) was fitted with four bands but only the symmetric CH\textsubscript{2} stretch component at 2854 cm\(^{\text{-1}} \) was used for order parameter calculations.

\( ^b \) The order parameters for A-I-DMPC/PS-rHDL(h) heterogeneous particles reported previously (32).
Table 5. Cross-link distance constraint compatibility with the different models in Figure 9.

| X-link (K-K) | Parallel model (estimated distance, Å) | Antiparallel model (estimated distance, Å) | Double hairpin model (estimated distance, Å) |
|--------------|----------------------------------------|------------------------------------------|-----------------------------------------|
|              | Intra-strand (I or II) | Inter-strand (III or IV) | Fit? | Intra-strand (I or II) | Inter-strand (III or IV) | Fit? | Intra-strand (I or II) | Inter-strand (III or IV) | Fit? |
| 28-30        | 5 | -- | Yes | 5 | -- | Yes | 5 | -- | Yes |
| 30-39        | 21 | -- | No | 21 | -- | No | 16 | -- | Yes |
| 39-44        | 10 | -- | Yes | 10 | -- | Yes | 10 | -- | Yes |
| 44-46        | 5 | -- | Yes | 5 | -- | Yes | 5 | -- | Yes |
| 46-54        | 12 | -- | Yes | 12 | -- | Yes | 12 | -- | Yes |
| 28-44        | 30 | 33 | No | 33 | 20 | Yes | 18 | 20 | Yes |
| 30-44        | 27 | 27 | No | 27 | 17 | Yes | 11 | 19 | Yes |
| 30-46        | 32 | 34 | No | 32 | 19 | Yes | 12 | 26 | Yes |
| 30-54        | 42 | 45 | No | 42 | 12 | Yes | 7 | 36 | Yes |
| 39-46        | 12 | 19 | Yes | 12 | 16 | Yes | 12 | 16 | Yes |
| 39-54        | 19 | 29 | Yes | 19 | 25 | Yes | 19 | 23 | Yes |
| 44-54        | 17 | 23 | Yes | 17 | 31 | Yes | 17 | 26 | Yes |
| 44-55        | 15 | 23 | Yes | 15 | 28 | Yes | 15 | 25 | Yes |
| 46-46        | -- | 12 | Yes | -- | 22 | No | -- | 25 | No |
| **Total potential fits** | **9/14** | | | **12/14** | | | **13/14** | |

a The measurements in Å were taken directly from the P-Q dimer of apoA-II in the BOG crystal structure (14). Since our method could not distinguish between intra and inter-strand cross-links, both possibilities were considered.
b The distances measured for the antiparallel and double hairpin models were made graphically at the same scale as the crystal structure and should be considered estimates.
c Peptides that were known to be intra-peptide due to their molecular weight were not considered for potential inter-strand cross-links.
d Potential fits were judged possible if the estimated distance between Lys residues for either an intra- or inter-strand cross-link fell within 20 Å (sum of the BS3 spacer arm length of 11.4 Å plus 8.6 Å for two Lys side-chains).
Figure 1
Figure 2

ApoA-I               ApoA-II

1 2 3       4 5 6

KDa

97 66 45 30 20 14.4
Figure 3

Type I

Type II

Type III

Type IV

Molecule 1

Molecule 1

Molecule 1

Molecule 1

Molecule 2

A

A'

A

A'

A

A'

A

A'

A

A'

A

A'

X-link

X-link

X-link

X-link
Figure 4

peptide 1

SPELQAEAKSYFEK

peptide 2

SKEQLTPLIK

Relative Abundance

m/z

0

100 150 200 250 300 350 400 450 500

m/z

550 600 650 700 750 800 850 900 950

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'

y

y'
Figure 5

A

Molar Ellipticity ([\theta] \times 10^{-4}) degcm^2dmol^{-1}

Wavelength (nm)

B

Counts \times 10^6

Wavelength (nm)
Figure 6
Figure 7
Figure 8
The structure of apolipoprotein A-II in discoidal high density lipoproteins
R. A. Gangani D. Silva, Lumelle A. Schneeweis, Srinivasan C. Krishnan, Xiuqi Zhang, Paul
H. Axelsen and W. Sean Davidson

J. Biol. Chem. published online January 30, 2007

Access the most updated version of this article at doi: 10.1074/jbc.M610380200

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