ABSTRACT
Spherical high-density lipoprotein (sHDL), a key player in reverse cholesterol transport, is the most abundant form of HDL and associated with cardiovascular diseases. Small angle neutron scattering with contrast variation was used to determine the solution structure of protein and lipid components of reconstituted sHDL. Apolipoprotein A1, the major protein of sHDL, forms a hollow structure that cradles a central compact lipid core. Three apoA1 chains can be arranged within the low resolution structure of the protein component as one of three possible global architectures: (i) a helical dimer with a hairpin (HdHp); (ii) three hairpins (3Hp); or (iii) an integrated trimer (iT) where the three apoA1 monomers mutually associate over a portion of the sHDL surface. Cross-linking and mass spectrometry analyses help discriminate amongst the three molecular models and are most consistent with the HdHp overall architecture of apoA1 within sHDL.

INTRODUCTION
Epidemiological studies firmly establish that circulating levels of high density lipoprotein (HDL) cholesterol and apolipoprotein A1 (apoA1), the major protein constituent of HDL particles, are inversely associated with atherosclerotic heart disease risk (1-3). Moreover, genetic studies further confirm a strong mechanistic link between HDL and apoA1 and cardiovascular disease (4-8). Defined by its buoyant density characteristics, HDL represent a heterogeneous group of particles with varied lipid composition and protein content that participate in diverse biological functions ranging from lipid transport to innate immune functions. For example, HDL serves as an acceptor of cholesterol from peripheral tissue macrophages and promotes lipid transport through delivery of cholesterol to the liver and steroidogenic tissues (9-11). HDL also mediates systemic anti-inflammatory and anti-oxidant functions (12-14), and HDL associated proteins can play critical host defense functions (15). ApoA1 represents nearly three quarters of the protein content of HDL by mass, and plays a central functional role in facilitating the
numerous biological activities of HDL. Typically present at 2 to 4 molecules per particle depending upon the degree of HDL maturation, apoA1 serves as the fundamental structural element of the particle (16,17) and is critical for specific interactions with proteins involved in HDL biogenesis (18,19), maturation and remodeling (20,21), and recognition by target organ receptors (22,23).

Because it can be generated in relatively homogenous form, most structural studies of HDL have focused on reconstituted nascent HDL, a particle comprised of two molecules of apoA1 associated with phospholipid and free cholesterol (16,17). Early small angle neutron scattering (SANS) and small angle X-ray scattering (SAXS) studies of nascent HDL particles were reported nearly 3 decades ago, and are consistent with an outer protein layer relative to a central lipid core (24,25). Current structural models of nascent HDL have an anti-parallel apoA1 chain orientation, and posit that the protein exists either as a circumferential double belt shape around a central bilayer lipid core comprised of phospholipid and free cholesterol (16,17,26-31), or as a superhelical shape wrapping around a predominantly micellar lipid core (32,33). The anti-parallel organization of apoA1 molecules in nascent HDL is supported by numerous biophysical investigations including mass spectrometry/ cross-linking (34,35), fluorescence energy resonance transfer (FRET) (36,37) and electron spin resonance (ESR) transfer studies (37).

Spherical HDL (sHDL) particles are more mature forms of HDL, whereby large accumulations of neutral lipids, predominantly cholesteryl esters, are carried as cargo by the particles. Despite sHDL being the most abundant circulating form of HDL, relatively few studies have experimentally addressed its structure in detail owing to its size and compositional heterogeneity. Indeed, analytical ultracentrifugation (38), nondenaturing gradient gel electrophoresis (39), immunoaffinity chromatography (40), and two-dimensional electrophoresis (41) techniques all reveal heterogenous sHDL preparations containing typically 3 or 4 apoA1 per particle. The ability to form chemically and physically defined reconstituted sHDL by incubation of reconstituted nascent HDL with lecithin:cholesterol acyltransferase (LCAT), the plasma enzyme responsible for the maturation of nascent HDL into spherical particles (42,43), has permitted some structural studies into the overall architecture of apoA1 on the sHDL surface. Early characterization studies using circular dichroism and fluorescence spectroscopy suggest that the apoA1 in reconstituted nascent HDL and sHDL have similar alpha-helical contents, with sHDL being more resistant to chaotropic denaturants (42). Further, monoclonal antibody studies to different apoA1 epitopes suggest the overall structure of apoA1 is comparable in reconstituted sHDL and HDL isolated from human plasma (42). However, differences in fluorescence emission (36), particle charge (44), proteolytic sensitivity (45) and 13C NMR studies (46) between reconstituted nascent HDL and sHDL have also been reported, suggesting at a minimum, the presence of localized structural changes of the apoA1 in nascent versus sHDL. Despite these observations, recent protein cross-linking / mass spectrometry-based studies of reconstituted nascent HDL of various sizes and monodisperse preparations of sHDL demonstrated for the most part similar apoA1 inter-chain cross-links within the different particles, suggesting that within the resolution limits of protein cross-linking analysis, apoA1 apparently adopts a relatively consistent general structural framework in HDL particles irrespective of shape, size and the number of apoA1 molecules present (47). Thus, whether apoA1 undergoes a global reorganization during maturation from a nascent to spherical form remains unknown. Indeed, despite the importance of HDL to both cholesterol metabolism and cardiovascular disease, the overall conformation of apoA1 within sHDL forms has not yet been directly visualized. Because of the lack of direct experimental data on the shape of apoA1 within sHDL, some investigators have produce computational models by means of course grained molecular dynamics simulations for sHDL particles containing either two (48,49), or three (50)
truncated apoA1 chains. The relevance of these hypothetical models remains unclear until they are more fully tested against structural data for apoA1 in sHDL.

Small angle scattering (SAXS for X-Ray and SANS for neutron) is a powerful tool for investigation of macromolecular complexes in solution, providing information on their overall dimensions, morphology and composition (51-53). SANS with contrast variation yields low resolution structures yet is particularly useful in revealing the organization of a multi-component system because it combines structural and compositional information in a way that is difficult to attain by other approaches (32,54). During the contrast variation experiment, the scattering length density of solvent is adjusted (by varying the D_2O/ H_2O ratio) to match that of a component within a complex (e.g. protein, lipid, DNA or RNA), reducing the scattering intensity observed for that component. SANS with contrast variation, especially when coupled with selective deuteration of individual components, can facilitate triangulation of the location of individual components within macromolecular complexes. For example, SANS with contrast variation was the first method to correctly predict the subunit organization of eukaryotic chromosomes (55), the structural orientation of protein and DNA within the nucleosome (56), the organization of rhodopsin within retinal rod outer segment (57), as well as to triangulate the location of various proteins and RNA within the ribosome (58,59). Herein we use the combination of contrast variation SANS and selective deuteration of apoA1 to directly visualize the low resolution structure of the protein and lipid core components individually within biologically active reconstituted sHDL.

The overall shape observed for apoA1 and its orientation relative to the central lipid phase of sHDL provides structural insights into how the protein component of sHDL accommodates the dynamic changes that occur within the lipid core during particle maturation and remodeling. It also suggests how apoA1 within sHDL functions as a structural scaffolding to facilitate lipid transport functions of the particle during reverse cholesterol transport.

EXPERIMENTAL PROCEDURES

Preparation of Reconstituted Spherical HDL Particles. Human apolipoprotein A1 was purified from plasma from healthy volunteers as described with modifications. Briefly, HDL were isolated from fresh human plasma by density centrifugation from 1.07 g/ml to 1.21 g/ml with KBr. Then HDL proteins were precipitated using methanol/chloroform/cold ether sequentially before resuspension in 20 mM Tris, pH 8.5, 6 M urea. Human apoA1 in HDL was purified from HDL protein precipitates using ion-exchange chromatography (Q sepharose HP HiLoad 26/10 column from GE Healthcare). Human apoA1 was stored in PBS with 3M guanidine HCl at -80°C before use. Deuterated recombinant His-tagged human apo A1 (rhA1) was produced in E.coli BL21 pLys strain grown in minimal media as previously described (27). One liter of the minimal media consisted of M9 salts, 0.1% NH_4Cl, 10 mM of MgCl_2·6H_2O, 30 mg of Thiamine in 99.8% D_2O supplemented with trace elements which included 60 mg of CaCl_2·2H_2O, 60 mg of FeSO_4·7H_2O, 11.5 mg of MnCl_2·4H_2O, 8mg of CoCl_2·6H_2O, 7mg of ZnSO_4·7H_2O, 30mg of CuCl_2·2H_2O, 0.2 mg of H_3BO_3 2.5 mg of (NH_4)_6Mo_7O_24·4H_2O and 50mg of EDTA. Bacterial Cells were first grown in LB media containing 70% D_2O overnight at 37°C and then harvested by centrifugation at 3000 rpm for 30 minutes. Cells were resuspended in minimal media after being washed with PBS three times and grown to mid-log phase. Expression of rhA1 was induced by addition of 0.4 mM IPTG. Deuterated rhA1 was purified to homogeneity by Nickel-chromatography as described (27).

Reconstituted nascent HDL were prepared by the cholate dialysis method (42). For preparation of deuterated reconstituted nascent HDL, deuterated rhApoA1 was used. An initial ratio of 100:10:1, 1-palmitoyl-2-oleoyl-sn-glycero-3 phosphatidylcholine (POPC): cholesterol: apoA1 (mol:mol:mol) was used for generating the reconstituted nascent HDL, followed by gel filtration chromatography as previously described (27). Reconstituted spherical HDL were prepared by incubating either deuterated or
non-deuterated nascent HDL with isolated human LCAT and isolated human LDL at 37 °C for 24 h (42). Reconstituted spherical HDL were then isolated by sequential ultracentrifugation in the 1.07 < d < 1.21 g/ml density range and further purified by gel filtration chromatography on a Sephacryl S-300 column.

**Chemical Cross-linking and Mass Spectrometric Analyses.** Chemical cross-linking and mass spectrometric analyses were performed to determine the stoichiometry of apoA1 on spherical HDL. Briefly, bis(Sufosuccinimidyl) suberate (BS3) (from Pierce) was dissolved in PBS at a concentration of 8 mg/ml added to reconstituted spherical HDL (0.4 mg/ml apoA1) at a 200:1 molar ratio (BS3 vs. apoA1) and incubated for 5 minutes at 37ºC. The reaction was immediately quenched by the addition of 1 M Tris buffer to a final concentration of 100 mM at pH 7.4. To estimate the number of BS3 mol added to apoA1, the content of derivatizable lysine residues remaining on cross-linked delipidated apoA1 were assayed using the sensitive fluorogenic o-phthaldialdehyde (OPA) reagent and purified apoA1 as the standard (60). The mass of delipidated cross-linked apoA1 from sHDL was directly measured using MALDI-TOF mass spectrometry. Sinapic acid prepared in 50% acetonitrile with 0.1%TFA was mixed with cross-linked apoA1 (1:1 by volume) and ~ 0.75 ul of the mixture was spotted on a stainless MALDI plate. The spectra were acquired in the positive linear mode on an ABI 4800 proteomics analyzer mass spectrometer. Each spectrum was generated by averaging 3000 laser shots with the laser intensity at 5000. External calibration was performed using bovine serum albumin and monoclonal antibody against IKK as calibrates.

ApoA1 cross-linking within spherical HDL was performed as described (47). Briefly, sHDL (0.5 ug/ul) in PBS at pH 7.4 was incubated with BS3 (10:1 molar ratio) at 4°C for 24 hours. During the first hour the solution was mixed gently for every 15 minutes. The cross-linking reaction was terminated by addition of Tris HCl at a final concentration of 100 mM, and then stored at –80°C prior to analysis. Cross-linked monomer, dimer and trimer forms of apoA1 within sHDL were separated on a 12% SDS-PAGE gel before being transeluted into a 14 channel fraction collector using a minielutor apparatus (BioRad Inc.). The fractions containing monomer, dimer and trimer apoA1 (confirmed by MALDI-TOF analysis as outlined above) were dialyzed against 20 mM NH₄HCO₃ and then digested with Trypsin (Promega Corporation, Madison, WI) at a ratio of 500:1 (w:w). The tryptic digests were separated on a nano C18 column (75 mm X 10 cm, Proxeon) using a Easy nLC interfaced with LTQ MS (Thermo Scientific) at a flow rate of 300 nl/min and a gradient of 5-35% of acetonitrile in 0.1% formic acid in 45 minutes. The mass spectra were acquired in a data dependent mode with a mass scan range of 300-2000 daltons. The candidates of cross-linked peptides were searched by inputting masses of peptide list into a web-based search program, ([http://www.ms3d.org](http://www.ms3d.org)) and then the sequences of cross-linked peptides were confirmed by manually analyzing the MS/MS spectra. Only sequence confirmed cross-links were used.

**Compositional Analysis of Reconstituted sHDL.** The phospholipid composition of reconstituted spherical HDL was determined by microphosphorus analysis (27,32). The amount of protein in reconstituted spherical HDL was quantified by stable isotope-dilution LC/MS/MS analysis of multiple amino acids (Phe, Tyr, Lys) assuming the protein sequence of human apoA1. Calculated results of protein mass from all three amino acids agreed within +/- 3%. The free and total (following KOH saponification) cholesterol concentrations in reconstituted spherical HDL were determined enzymatically using commercial kits from Stanbio Laboratory and Wako Diagnostics. The amount of cholesterol ester in the sHDL preparations was calculated by subtraction of free cholesterol from total cholesterol.

**Cholesterol Efflux Assay.** Cholesterol efflux assays were performed as described (32). Briefly, cholesterol loaded RAW264.7 cells in 24 well plates were loaded with 0.3 µCi/ml [³H] cholesterol overnight in 1% FBS DMEM. The next day, ABCA1 expression was induced with
8-Br-cAMP in DGGB (DMEM supplemented with 50mM glucose, 2mM glutamine and 0.2% BSA) for 16 h at 37 °C. The day after induction, plasma HDL (5µg/ml protein final) or reconstituted spherical HDL (5µg/ml apoA-I final) in 0.5ml of serum free DMEM with or without 8-Br-cAMP were added to each well. After a 6 h incubation at 37°C, the medium was removed, centrifuged and 250µl was counted as a measure of cholesterol released into the media. The cells were extracted with hexane/isopropanol (3:2) and radioactivity counted as a measure of cholesterol retained within the cell. The percent cholesterol efflux was calculated as the radioactivity in the medium/total radioactivity (medium radioactivity plus cell radioactivity). The control was performed using the same procedure without addition of sHDL.

**SR-B1 Specific Binding of Spherical HDL.** Spherical HDL made with bacterial ApoA1 was iodinated using the iodine monochloride method as described by Brown and Goldstein (61). 293T cells were transfected with vector or SR-B1 using lipofectamine-2000 according to manufacturer’s instructions. Binding of the radiolabeled reconstituted spherical HDL to SR-B1 was determined by incubating 125I-sHDL with either vector transfected or SR-B1 transfected cells for 1.5 hours at 4ºC(32). The cells were washed twice with 250 mM NaCl, 25 mM Tris (pH 7.4) and once with 250 mM NaCl, 25 mM Tris (pH 7.4) containing 2 mg/mL BSA, then solubilized in 1 mL 0.1 M NaOH at room temperature for 20 minutes. Cell associated radioactivity was subsequently determined with a Gamma4000 spectrometer (Beckman Coulter, Fullerton, California, USA). Specific binding was calculated as total binding minus binding in the presence of a 20-fold excess of unlabeled sHDL.

**SANS Experiments.** Small angle neutron scattering experiments were performed on sHDL samples prepared as described above. SANS data were collected at controlled temperature (6-8°C) both on the beam line D22 in Grenoble, France and KWS-2, the outstation of the Forschungszentrum Juelich at FRMII in Munich, Germany. All experiments showed consistent results on multiple preparations. Following analysis, the samples were recovered and used for chemical, biochemical and biological assays. Scattering data were obtained as a function of the scattering angle and corrected for background, and subtraction of incoherent scattering and empty cell before being radially averaged and calibrated in absolute units. All samples and buffers were recorded with neutron beams of λ= 4.5 or 7 Å. The scattering neutron density was determined as the macroscopic cross-section I(q) in units of cm⁻¹ versus the momentum transfer q=(4π/λ)sinθ, where 2θ is the scattering angle. To cover a wide range of q and to ensure good merging statistics the scattering experiments were performed at 2, 4 and 7 m detector-to-sample distance covering a range of 0.007 < q < 0.45. The buffer was subtracted from the scattering curve, and the resulting scattering curves were processed using the program PRIMUS (62). The experimental radii of gyration of scattering contrast, R₉, of the samples were calculated from the Guinier range, (R₉q)² ~ 1, of the scattering curve:

\[
I_{\text{corr}}(q) = I_{\text{corr}}(0) \exp(-R_9^2 q^2/3) \tag{1}
\]

The molecular weight calculation of apoA1 was based on equation 2 using the I_{corr}(0) of the scattering curve:

\[
I_{\text{corr}}(0)/c = 10^{-3} /N_A (N_A \Sigma b - \rho^0 V/M)^2 M \tag{2}
\]

The factor 10⁻³ is required in order to express protein concentration c in the usual way as mg/ml, Nₐ is Avogadro’s number, the term in parentheses is the excess scattering length sum of protein atoms, calculated from its composition, ρ₀ is scattering length density of the solvent, V is protein or lipid volume calculated from its partial specific volume, and M is the molar mass in g/mol. (63)

**Low Resolution Structure Determination.** Low resolution structures of protein and lipid components were generated from their respective corrected intensities, I_{corr}, by using the program DAMMIN (64). The corrected sample
intensities were first fitted in the specified q-ranges (protein component: 0.007 – 0.35 Å⁻¹; lipid component: 0.007 – 0.24 Å⁻¹) by using the program GNOM (65). GNOM (which, uses the Indirect Fourier Transformation (66)) reads one-dimensional experimental scattering intensities (considering errors) and evaluates a distance distribution function \( p(R) \). Then the program DAMMIN produces low resolution structures (with maximal extension \( D_{\text{max}} \)) by using the \( p(R) \) function. The final structure obtained is consistent with the experimental data. Two qualitative criteria for these fits were checked for each contrast level: (1) a smooth behavior of \( p(R) \), and (2) a good agreement between the radii of gyration \( (R_g) \) calculated by GNOM and determined by the Guinier approximation (equation 1). All samples of different contrast level fulfilled these criteria in a satisfactory manner. The output of the GNOM program was used as input data for the program DAMMIN to build the low resolution structures. The DAMMIN default parameters were used for the modeling process. The final models of the protein and lipid components are the average of at least ten outputs of the program DAMMIN using the program DAMAVER (64). The low resolution structure obtained from the 12% \( D_2O \) scattering intensities (protein component of sHDL), was used to build the first cartoon-like model of the protein architecture. This model was further optimized by improving the goodness-of-fit of the calculated scattering intensities versus the experimental data. This was achieved by gradually adjusting the shape of the three apoA1 chains in the model and comparing the calculated scattering intensities with those obtained experimentally.

High Resolution Models. In order to identify possible arrangements and plausible conformations of the three chains of ApoA1 that can fill the SANS shape of the protein component of sHDL, initially we built straight-line 3D tube-like models made that match the length of a full ApoA1 chain. Next, we bent the tubes to follow the shape of the SANS low resolution structure of the protein and created three different combinations designated in the main text as the HdHp, 3Hp and iT models. Then, using these tube-like models we constructed all atom models of the three possible topologies of the apoA1 trimer (HdHp, 3Hp and iT). The all atom models follow accurately the same conformation of the individual chains found in their tube-like counterparts. The apoA1 chains were modeled in an all-alpha-helix conformation and oriented with the hydrophobic face of the amphipathic \( \alpha \)-helix towards the lipid core and with the hydrophilic face towards outside similarly with the orientation of the amino acid residues found in previous models of nascent HDL (26,32). These models are very crude and their geometry was not optimized. That is, energy minimizations or molecular dynamics simulations were not performed, neither in gas phase nor in solution. Thus, these models do not contain accurate information about the solvent accessibility of individual residues. The all atom models of the ApoA1 trimer were built for the purpose of performing the SANS and cross-link analyses presented in the main text and in Supplemental Table I. We calculated the scattering intensity given off by the all atom models and the goodness-of-fit, with the experimental scattering data listed in Table I, with the program CRYSON (67). For the cross-link analysis we performed free rotations of lysine residues within 25 Å from each other for all models. The distances between lysine residues found to be involved in cross-links were measured between the N atoms of the side chains of the two residues. These distances are listed in Supplemental Table I.

RESULTS & DISCUSSION

Chemical, Biochemical and Biological Activity Characterization of Reconstituted sHDL. Monodispersed and relatively homogeneous preparations of reconstituted sHDL were prepared using the methods initially reported by KA Rye and colleagues (43). Preparations of non-deuterated and deuterated sHDL were generated from reconstituted nascent HDL produced with either human apoA1 or deuterated recombinant human apoA1 as outlined in Methods. Following the final isolation step of gel filtration chromatography, the sHDL preparations were initially characterized by
native non-denaturing gradient gel electrophoresis and dynamic light scattering to confirm the relative homogeneity of the preparation. More extensive characterization studies, including those shown in Supplemental Figure 1, were then repeated on samples recovered following SANS analyses to ensure that the properties of the particles did not change during the course of the experiment. sHDL particle preparations studied by SANS and cross-linking / mass spectrometry were initially characterized to confirm homogeneous mono-dispersed particles with similar composition and biological activity to sHDL isolated from human plasma (Supplemental Fig. 1). Reconstituted sHDL particle preparations containing either human plasma derived apoA1 (h-sHDL) or deuterated recombinant apoA1 (d-sHDL) were each shown to have a hydrodynamic diameter of approximately 93 Å as monitored by equilibrium native non-denaturing gradient gel electrophoresis and dynamic light scattering (Supplemental Fig. 1A and 1C). Chemical compositional analyses of both non-deuterated and deuterated sHDL preparations showed similar lipid composition (phospholipid/free cholesterol/cholesterol ester) and apoA1 content (Supplemental Fig. 1B), and cross-linking studies and SDS/PAGE analysis suggested three molecules of apoA1 per sHDL particle (Supplemental Fig 1D). This result was further confirmed by independent MALDI-TOF mass spectrometry analyses of delipidated cross-linked apoA1 recovered from sHDL, with the bulk of protein detected possessing mass close (within ~0.2% predicted) to that anticipated for a trimeric apoA1 particle plus the estimated added mass provided from cross-linking/quenching agents (Supplemental Fig. 1E). Analyses of the biological activity of the sHDL preparations used for SANS showed cholesterol efflux activity when incubated with cholesterol loaded macrophages similar to that observed with HDL3 isolated from human plasma (Supplemental Fig. 1F), and sHDL preparations showed saturable and specific binding to the HDL receptor, scavenger receptor-B1 (Supplemental Fig. 1G). Collectively, these results demonstrate that the reconstituted sHDL particles studied by SANS and cross-linking / mass spectrometry in the present studies have chemical composition, overall stoichiometry, particle size and biological activity similar to HDL3 isolated from human plasma, and appeared both mono-dispersed and of homogeneous.

SANS Analyses of sHDL are Consistent with a Peripheral Trimeric ApoA1 that Cradles an Inner Spheroidal Compact Lipid Core. SANS experiments were performed at multiple levels of contrast on sHDL preparations generated using either non-deuterated or deuterated apoA1 (Fig. 1). Samples demonstrated mono-dispersed characteristic, without detectable trace of aggregation. The molecular weight of the protein within sHDL measured by SANS was consistent with a trimer (8.1 to 8.6 x 10^4 amu). The radius of gyration (R_g) at each contrast level and the overall particle dimensions are listed in Table I. SANS with contrast variation studies permitted determination of the individual low resolution structures of the protein and lipid components of sHDL. The scattering signal of the sHDL protein component was enhanced through the incorporation of deuterated apoA1 into the reconstituted sHDL preparations and measurement at the average matching point of the lipid (12% D_2O). Figure 1A, left panel, shows the experimental sHDL scattering intensity data from 12% D_2O solution and the superimposed calculated scattering curve for the low resolution structure. The maximum value of the scattering vector, q, is 0.44 Å^-1, which corresponds to a resolution in real space of 14.5 Å. The middle panel of Figure 1A shows the distance distribution function, p(R), which is used to construct the low resolution structure of the protein component of sHDL (Fig. 1A, right). To determine the low resolution structure of the lipid component of sHDL, particles reconstituted with non-deuterated apoA1 were measured at 42% D_2O contrast level, the matching point of the protein component of sHDL. Figure 1B, left panel, shows the experimental data and the calculated scattering curve obtained from the low resolution shape envelop of the lipid (right panel). The p(R) function of the lipid component is shown in the middle panel. Finally, the Stuhrmann plot (68) (where the square of R_g is plotted against the inverse of the difference...
between the matching point of the whole molecule (18% D\textsubscript{2}O and each contrast level) produces a straight-line with positive slope (Fig. 1C), indicating two structural features: (i) the centers of gravity of the protein and lipid components within sHDL coincide; and (ii) the component with higher scattering length density (protein) is located at the periphery of the particle. Goodness-of-fit ($\chi^2$) analyses for the predicted scattering curves from the low resolution SANS structures of the protein and lipid components of sHDL versus the experimental scattering intensities show excellent agreement for both protein and lipid components. Overlapping views of the low resolution structures of the protein and lipid components of reconstituted sHDL in two different orientations are shown in Figure 1C (right). The shape of the combined protein and lipid structures is spheroidal. It is evident from the superposition that the overall architecture of the protein component of sHDL is a hollow structure (orange beads) that cradles the non-spherical compact lipid core (green beads), and the lipid component located inside the particle is only partially surrounded by the outer protein wrapper.

Proposed Possible Global Arrangements of ApoA1 Chains in sHDL. In contrast with nascent HDL, whose structure has been extensively investigated, there are relatively few experimental studies on sHDL structure to date (29,47). Only one of the recent studies obtained geometrical constrains amongst apoA1 molecules within sHDL particles using cross-linking (47), and the other only produced EM images of an entire particle (29). Of note, no studies to date directly visualize the shape of apoA1 in sHDL in sufficient detail to determine the overall architecture of protein within the spherical particle. Recent mass spectrometry studies by Silva et al (47) reported that cross-links in reconstituted sHDL preparations are for the most part the same as those observed in nascent HDL. However, among the cross-links identified seven were only observed in reconstituted sHDL (Supplemental Table I), and not in nascent HDL preparations. In that study, several hypothetical arrangements of three apoA1 molecules were proposed to explain the identified cross-links within sHDL. The first arrangement posited was a planar circular belt consisting of three apoA1 molecules, all in anti-parallel orientation. In the second arrangement, one apoA1 was hypothesized to exist as a hairpin and the other two apoA1 molecules were arranged in a planar circular anti-parallel double belt configuration. In a third arrangement, the three apoA1 molecules were hypothesized to be associated in a clever symmetrical structure retaining the anti-parallel apoA1 chain orientation forming a “trefoil” (47).

The highly symmetric protein models proposed for the three apoA1 molecules based on cross-linking studies (47) clearly do not fit the experimental SANS shape visualized for the protein component of sHDL. However, combining SANS studies with contrast variation and complementary structural information from alternative biophysical approaches such as cross-linking/mass spectrometry data can provide synergistic information that when considered in combination, impose considerable constraints on the possible permutations through which the apoA1 may be oriented within sHDL. We note that the SANS resolution for the protein component of sHDL (14 Å) is sufficient to resolve the location of the apoA1 chains within the low resolution structure of sHDL because the average distance between two apoA1 chains (outer residues) in a belt configuration is about 14 Å. Thus, the low resolution structure of the protein within sHDL visualized by SANS creates a puzzle in how to arrange three apoA1 chains in sHDL preparations. Following other investigators (e.g. Silva et al(47)) we produced computer-generated three-dimensional cartoon-like models that have all-atom representation, which makes them suitable for distance analysis. We do not claim that these cartoons give detailed information about the relative orientation of amino acid residues in apoA1 within sHDL, but we believe that taking into account the spatial constraints imposed by
the primary and secondary structure of apoA1 in combination with its low resolution structure resolved by SANS, the cartoon-like model of the protein is a fair description of the overall architecture of the apoA1 trimer in sHDL, and thus is appropriate for the MS cross-linking analysis. In this spirit, all protein structures considered included maximal retention of a predominantly anti-parallel orientation for the apoA1 chains in 5/5 helix registry, a necessity given the previously observed crystal structure of lipid free truncated mutant (residues 44-243, exon 4) apoA1 (69), and the large numbers of reported FRET, ESR, and cross-links studies on HDL (17,27,35,47,70-72).

While it is not immediately recognizable how individual chains of apoA1 might be oriented within the protein low resolution structure, given the length of an apoA1 polypeptide chain, further analyses suggest only a handful of possible global categories of apoA1 configurations that can fit within the structural confines dictated by the protein shape envelop. Figure 2 shows three general categories of possible arrangements of the apoA1 trimer superimposed on the protein envelop determined by SANS while simultaneously maintaining apoA1 chain anti-parallel orientation. In Figure 2A the three protein chains (colored red, blue and green) are arranged as a combination of a helical dimer and a folded-back hairpin. We call this arrangement the "Helical-dimer-Hairpin" (HdHp) model. The HdHp model for the apoA1 trimer predicts that the apoA1 hairpin folds back bringing its N-terminus close to its helix 5 (h5) domain. Other predicted distinct features are specific contacts/interactions of the hairpin with the dimer, and the fact that the hairpin h5 domain is not aligned with the dimer h5 domains. Another possible global arrangement of the apoA1 trimer within the protein envelope is shown in Figure 2B as three distinct apoA1 hairpins. While multiple configurations of this global arrangement are possible, we chose to orient two hairpins (red and blue) such that they interact through their h5 domains (given the cross-linking data for apoA1 in sHDL, see below), while the third hairpin (green) has the same orientation as in the HdHp structure in Figure 2A. This model is called the three-Hairpins (3Hp) model, because the apoA1 dimer is replaced by two apoA1 monomers that occupy the same volume of the SANS envelope. Further, and it can be expected that cross-links that have been reported to discriminate between a double belt versus hairpin models for apoA1 of nascent HDL (34,37,73), will also discriminate between models HdHp and 3Hp. Finally, we explored the possibility of a third global arrangement whereby the model topologically resembles in part the trefoil model(47), because all three apoA1 monomers associate mutually over a portion of their protein chains. Because the protein envelop visualized by SANS does not share the same symmetry properties with the trefoil model, we call the apoA1 trimeric model that somewhat simulates the trefoil model yet fits within the SANS shape the "integrated-Trimer" (iT) model (Figure 2C; note that the iT model has only one node instead of two where all three apoA1 chains join together, and the actual conformation of the individual apoA1 chains are very different from those in the trefoil model). In the trefoil model all apoA1 monomers are aligned with their h5 domains (47).

Table II shows the goodness-of-fit for each of the hypothetical orientations of apoA1 posited versus the actual experimental SANS data. We used the 3D tube-representations shown in Figure 2 for each model to generate an all atom model for the protein that was made mostly of a continuous α-helical ribbon. These models do not contain information about the solvent accessibility of individual amino acids, but the orientation of the amino acid residues was consistent with that found in previous models of nascent HDL whereby hydrophobic α-helical surfaces are oriented toward the lipid core, and predominantly hydrophilic α-helical apoA1 side chains are oriented toward the aqueous phase (26). The scattering intensities at various scattering angles are shown in Figure 3A (blue line) for the HdHp model superimposed on the experimental data (black open circles). Also shown is the calculated scattering curve corresponding to the low resolution SANS structure (solid orange line). The agreement
among all three curves is excellent, as indicated by the goodness-of-fit (calculated $\chi^2 = 1.192$ and 1.483, respectively; Tables I and II).

Comparison of the goodness of fit of the calculated scattering curves from each of the three global models of apoA1 architecture (HdHp, 3Hp, or iT) versus the actual experimental SANS scattering data reveals that the HdHp and 3Hp models most closely fit the experimental data, while the iT model fits less well (Table II). The all-atom structure for the HdHp model is shown in Figure 3B superimposed with the low resolution structure of the protein (semitransparent orange beads). Figure 3C shows the all-atom model HdHp in three different orientations that emphasize the helical conformation of the dimer, while Figure 3D shows how the all-atom model of the protein wraps around the low resolution structure of the lipid core (semitransparent green beads). The SANS analysis of the three possible overall architectures of the apoA1 trimer in sHDL cannot on its own discriminate between the three global models, but it can select specific conformations of apoA1 that fit the low resolution structure of sHDL. Consequently, the subset of possible conformations can be further analyzed for compliance with geometrical constraints derived from cross-linking studies, and the combined analysis can thus be used to identify the most probable architecture(s) of the apoA1 trimer that satisfy all arrays of biophysical constraints revealed so far.

**Geometrical Constraints Analysis of ApoA1 Chain Cross-Linking Data Coupled with SANS Discriminates Amongst the Overall Architectures Posited for ApoA1 in sHDL.** Construction of crude all-atom models (HdHp, 3Hp, and iT) for the apoA1 trimer allowed us to perform a geometry constraints analysis of the apoA1 chains by comparing the experimentally reported cross-links for sHDL (35,47) and those newly determined in our sHDL preparations with those predicted by the models. A summary of this comparison is given in Table II, and more detailed description in Supplemental Table I, which lists the individual cross-links identified in our sHDL preparation (93 Å particle, S93) and those predicted by the three proposed architectures of apoA1 trimer in sHDL (i.e. the HdHp, 3Hp, and iT models). Recently, Silva et al (47) identified 23 cross-links in sHDL particles 93 Å in diameter (S93) that contain three apoA1 chains. Inter-chain and intra-chain designations for cross-links observed in tryptic digests of cross-linked S93 by Silva et al were based upon results previously observed with nascent HDL (which contains 2 apoA1) and a smaller more compact trimeric sHDL form. Thus, cross-linked monomer vs. multimeric apoA1 forms were not separated prior to mass spectrometry analysis within S93 sHDL by Silva et al (ref (47) and WS Davidson, personal communication). Among the 23 cross-links identified in S93 by Silva et al, 14 were designated as intra-chain and 7 as inter-chain cross-links. Two cross-links were not assigned as either intra- or inter-chain. We performed similar mass spectrometry / cross-linking analyses of sHDL (S93) preparations but added the step of electro-eluting various cross-linked apoA1 bands from sHDL preparations (monomer vs dimer vs trimeric forms confirmed by both SDS PAGE and MALDI-TOF mass spectrometry (Supplemental Methods), and performed in-solution tryptic digestion/MS analyses on the isolated cross-linked proteins. This allowed for direct assignment of detected cross-links as either intra- or inter-chain. We performed similar mass spectrometry / cross-linking analyses of sHDL (S93) preparations but added the step of electro-eluting various cross-linked apoA1 bands from sHDL preparations (monomer vs dimer vs trimeric forms confirmed by both SDS PAGE and MALDI-TOF mass spectrometry (Supplemental Methods), and performed in-solution tryptic digestion/MS analyses on the isolated cross-linked proteins. This allowed for direct assignment of detected cross-links as either intra- or inter-chain cross-links within sHDL (Supplemental Table I). It is remarkable that all three all-atom models for the apoA1 trimer in sHDL preparations satisfy the majority of identified cross-links, though the HdHp model appears superior to both 3Hp and iT models. For example, the HdHp model satisfies 23, the 3Hp model satisfies 19, and the iT model satisfies 21 cross-links (Table II). None of the models satisfies cross-link K208-K208 identified by the same group in a 96 Å nascent HDL particle (47), but is not predicted by any of the proposed nascent HDL models (e.g. the Double Belt, the Hairpin, the Solar Flares and the Double Super Helix models) unless it arises from a particle-particle interaction (27,32). The previously reported inter-molecular cross-link Lys182-Lys238 was suggested to perhaps represent apoA1 monomers that had undergone a shift from a 5/5 to a 5/2 helix registry (47). All protein
models proposed here contain this cross-link in a 5/5 registry, but predict that it is intra-chain. An intra-chain designation for this cross-link is in agreement with cross-link MS analyses of electro-eluted apoA1 forms examined in the present study (Supplemental Table I). The two cross-links observed by Silva et al in sHDL that were not assigned as either intra- or inter-chain (K182-K239 and N_T-K118) were assigned in this study as inter- and intra-chain cross-links, respectively, based upon cross-link MS analyses of electro-eluted proteins (Supplemental Table I). In the molecular models K182-K239 is readily accommodated as an intra-chain cross-link and inter-chain distances as small as 40 Å are noted, suggesting that an inter-chain cross-link is also plausible based on the models. The distance corresponding to N_T-K118 in all models was < 30 Å for both intra- and inter-chain distances in all models (Supplemental Table I).

While it is remarkable that all three all atom models for the apoA1 trimer in sHDL preparations satisfy the majority of identified cross-links, there are still significant differences in the cross-links predicted by the models that make the HdHp model appear superior. For example, a more detailed analysis of data in Supplemental Table I shows that the 3Hp model can be ruled out as a possible architecture of the apoA1 trimer because it cannot accommodate most of the inter-chain cross-links due to its specific topology (all three apoA1 chains self associate, and only few inter-chain cross-links would be predicted). As a consequence, four inter-chain cross-links (K40-K239, K118-K140, K59-K208 and K77-K195) are not accommodated by this model. On the other hand, the iT model has all three chains associated with each other and thus does not accommodate some of the intra-chain cross-links (K12-K94 and N_T-K106) seen in the HdHp model. Taken together, the combined SANS and MS/cross-link data point to the HdHp model as the most plausible global topology of apoA1 within sHDL.

Comparison of currently visualized SANS shape of apoA1 within sHDL versus prior reported models obtained by molecular dynamics simulation, and electron microscopy studies.

In the absence of having direct structural data for the overall shape of protein within sHDL, other investigators have attempted to construct purely theoretical models of the particle by computer simulations. It therefore may be of interest to compare the low resolution (~14Å) shape envelop of the protein component within reconstituted sHDL as visualized by SANS in the present study, with the previous hypothetical models obtained by simulation. Three molecular dynamics simulations of sHDL have been reported to date (48-50). Despite the fact that sHDL typically contains three or more apoA1 chains, these computational models predominantly focus on simulations of sHDL with only two apoA1 chains (48-50). In general, the molecular dynamic simulation studies of sHDL start with the protein in a discoidal belt organization at the beginning of the simulation, and have suggested that the overall belt shape of apoA1 becomes more disordered and contorted, with pronounced “kinks” or zigzags in an out-of-plane conformation (48,49). The protein conformations in the simulations reported are at marked contrast to the SANS shape observed in the present investigation. It should also be noted that prior simulation models of sHDL are also reported to be at marked variance(49) with the three apoA1-containing trefoil structure suggested by Silva and colleagues (47). It seems likely that one major reason marked differences exist between the experimentally observed SANS shape (which contains three apoA1) is because only two apoA1 chains are used in the molecular dynamics simulations. It is also worth noting, however, that all simulations and the SANS shapes directly visualized have some similarities. At a very gross level, the topological organization of protein within the particle toward the exterior, and a neutral lipid core interior, is a clear similarity. It is interesting to note that the simulations of a sHDL particle have suggested that the N- and C-terminal helices of the apoA1 chains are more flexible and mobile than the central region of the apoA1 chains (49), a finding consistent with the conformational alterations we propose for apoA1 within a maturing nascent HDL particle to form sHDL (see discussion below, and Figure 4A, top).
During the course of review of this manuscript, Zhang et al published a methodology paper for optimized negative staining electron microscopy (EM) of multiple lipoprotein particles, including some sHDL preparations (29). While both cryo-EM and negative staining EM images of sHDL were shown, no structural models were presented and the low resolution permitted few details of the overall shape of apoA1 within the particles. Zhang and colleagues did note, however, that the images of sHDL show “contiguous high densities near the particle edge and center”, which presumably corresponds to the protein within sHDL (29). This description is consistent with the low resolution structure of apoA1 within sHDL visualized by SANS.

The Protein and Lipid Organization in sHDL: a General Structural Motif for Lipid Transport.

The present study provides the first direct visualization of the individual structural components of sHDL, the major form of HDL in plasma, by means of SANS with contrast variation and selective isotopic deuterated protein sample. Low resolution images of the protein and lipid components were obtained individually to resolve global time-averaged conformations of each within the whole particle. The overall conformation noted shows how the protein component of sHDL provides structural support, much like a cradle or catcher’s mitt, within which the lipid core is carried. This is a highly unusual configuration for known macromolecular complexes and may represent a structural motif replicated in other lipoprotein complexes involved in lipid transport. It is easy to envision how this structural arrangement can enable the lipoprotein to facilitate its lipid cargo-carrying function, while also allowing for dynamic flexibility of a constantly changing particle dimension during cholesterol acceptor and lipid delivery roles. It is intriguing that this sort of global protein and lipid configuration is somewhat similar to observations reported by Weisgraber and colleagues during analysis of apolipoprotein E / diphosphatidylcholine particles in solution using small angle X-ray scattering (74). In that study ellipsoidal particles with apolipoprotein E organized in a twisted hairpin conformation were noted, with proposed packing of the phospholipid core being micellar.

The low resolution structures observed for sHDL, and previously for nascent HDL with contrast variation SANS (32) suggest a hypothetical path for HDL maturation and reversible remodeling (Figure 4A). During maturation, cholesterol ester (CE) molecules formed by LCAT at the HDL surface partition into the particle core (Figure 4A, top). Concurrently, the particle changes from a more elliptical nascent HDL shape to a spherical form, consistent with results observed with contrast variation SANS analyses of both nascent HDL and sHDL (Fig. 4A). As can be seen in Fig. 4A (bottom), maturation of the HDL particle is predicted to result in a dramatic change in one of the N and C termini of the helical dimeric apoA1 (folding back upon itself) probably induced by the addition of a third apoA1 chain in the more mature sHDL. This refolding of apoA1 double chain is suggested based upon the remarkable overlap in SANS shapes noted between nHDL and sHDL whereby the helical dimeric apoA1 within nascent HDL closely overlaps with the helical component of the HdHp conformation of sHDL (Fig. 4A, bottom). As noted above, previous simulation studies of sHDL have suggested that the N- and C-terminal regions of apoA1 are more dynamic (49). Similarly, cross-linking studies point toward the N- terminus of apoA1 within sHDL folding back and coming in closer proximity to the central region of the apoA1 polypeptide chain (Supplemental Table I).

ApoA1 is an exchangeable apolipoprotein (75,76) and can convert between lipid-poor and lipid-associated states (77,78). ApoA1 exchangeability and its predilection for forming both highly dynamic amphipathic \( \alpha \)-helices and salt bridges seem to be imprinted in its chemical composition (32,33). A hypothetical mechanism for apoA1 inter-exchangeability on the surface of the sHDL particle is suggested by the present studies. While this mechanism may be important for sHDL remodeling in plasma at the body temperature, the rate of apoA1 exchange should
be much lower during the SANS experiment when samples have been analyzed at 8 °C. For example, assuming that the activation energy barrier for exchange is about 20 kcal/mol, the rate constant at 8 °C is about thirty times smaller than at 37 °C). Figure 4B is a cartoon showing a hypothetical mechanism of how each of the apoA1 of sHDL may be inter-exchangeable through a transient rearrangement of apoA1 conformations posited between the global architectures in the HdHp and iT models. It is conceivable that an apoA1 hairpin chain, following modest rearrangement (Fig. 4B, left), may begin annealing into the apoA1 dimer of HdHp forming a 3-way node around helix 5 domain. This annealing process might be driven by concomitant salt bridge breakage (from intra-chain) and formation (in inter-chain) of the annealing apoA1 monomer while simultaneously, the dimer gradually unzips at one end to permit association leading to a transient integrated trimer-like form (Fig. 4B, center). This intermediate configuration may then rearrange into another dimer/hairpin combination (Fig. 4B, right) by a reversal of the annealing process. This mechanism would make it possible for apoA1 chains in the dimer to exchange partners by incorporating the hairpin, thus making all apoA1 polypeptide chains interchangeable on the surface of sHDL. We hypothesize that breakage and formation of a large number of salt bridges within the apoA1 dimer and hairpin may serve as a primary driving force behind the annealing of the dimer-hairpin and dissolution of the annealed trimer, allowing for facile inter-exchangeability of apoA1 within a dynamic sHDL particle. Thus, in the above hypothetical model to explain apoA1 inter-exchangeability, inter-chain salt bridges are replaced by intra-chain salt bridges, and vice versa, with little or no energetic penalty.

In summary, we applied SANS with contrast variation and isotopic protein deuteration to the structural interrogation of biologically competent reconstituted sHDL. The low resolution structure of sHDL analyzed by SANS at 42% D₂O indicates that the lipid phase of sHDL is a slightly elongated spheroid encased within the protein component that wraps around it, providing a scaffolding upon which lipids can associate. The protein and lipid organization revealed, may serve as a general structural motif for lipoproteins that mediate lipid transport, and help explain the inter-exchangeability of apoA1 within HDL.

ACKNOWLEDGEMENTS

We are grateful to Dr. Giuseppe Zaccai (Institut de Biologie Structurale (CEA-CNRS-UJF), Grenoble, France) for helpful discussions and insightful comments on the manuscript. We thank also Dr. John W. Crabb for (Cole Eye Institute, Cleveland Clinic, Cleveland, OH 44195) for generous access to use of the ABI 4800 MALDI-TOF mass spectrometer used to analyze molecular weight of delipidated cross-linked apoA1 of sHDL. This study was supported by National Institutes of Health grants P01 HL098055, P01 HL076491-05S28, and P01 HL087018-02001. Computational resources were provided by the Ohio Supercomputer Center and the National Center for Supercomputer Applications (NCSA, University of Illinois).
REFERENCES


FIGURE LEGENDS

Figure 1. Small Angle Neutron Scattering Results. (A) Neutron scattering results for sHDL in 12% D₂O. Left panel: The scattering intensity as a function of the scattering vector q. The experimental data are plotted with open circles and have error bars attached. The experimental scattering curve displays oscillations with maxima at approximately 0.1, 0.2, and 0.25 Å⁻¹. The orange solid line is the scattering intensity calculated from the low resolution structure obtained by deconvoluting the experimental data; Middle panel: The distance distribution function, p(R), plotted as a function of the distance between scattering centers. The p(R) obtained by deconvoluting the experimental scattering intensities of sHDL in 12% D₂O with the program GNOM. Right panel: Low resolution structure of the protein component of sHDL obtained from the p(R) function (middle panel) with the program DAMMIN. (B) Neutron scattering results for sHDL in 42% D₂O. Left panel: The scattering intensity (open circles) for sHDL in 42% D₂O. The error bars are very small and only slightly visible at the center of the open circles. The green solid line is the scattering intensity produced by the low resolution structure of the lipid. Middle panel: The p(R) function for the lipid component of sHDL. Right panel: Low resolution structure of the lipid component of sHDL obtained from the p(R) function (middle panel). (C) Left panel: The Stuhrmann plot gives the dependence of Rg² (Rg, the radius of gyration) on the contrast level (X) in the SANS experiment. A linear graph (red line) indicates that the protein component is located on the outside and the lipid component is inside as shown by the overlap of the low resolutions structures for the 12% D₂O (orange) and 42% D₂O (green) in the Middle and Right panels.

Figure 2. Possible Architectures of the apoA1 trimer in Spherical HDL. (A) Superposition of the 12% D₂O low resolution structure of sHDL (orange) with a cartoon representation of the apoA1 trimer made of a helical dimer (red/blue) and a hairpin (green): the Helical dimer / Hairpin model (HdHp). The three chains are gradient colored (dark N terminus, lighter color C terminus). The three panels depict different orientations of the HdHp model. (B) The architecture of the apoA1 trimer corresponds to three hairpins that fit the 12% D₂O low resolution shape: the 3Hp model. The hairpins red and blue interact with their helix₆ domains. The green hairpin retains the same orientation as the one in panels above. (C) In this architecture (integrated trimer, the iT model), the apoA1 trimer has each chain associated with each other producing three dimers. All chains interact all together with their helix 6 domains.

Figure 3. Comparison of Experimental Neutron Scattering Intensities with those obtained from the Low Resolution Structure and the Molecular Model of the ApoA1 Trimer. (A) The fit of scattering intensities produced by the 12% D₂O low resolution structure of sHDL (orange curve) and the molecular model (blue curve) of the apoA1 trimer (the HdHp model) to the experimental data. (B) The superposition of the molecular model of the apoA1 trimer (HdHp) with the 12% D₂O low resolution structure (semitransparent orange beads). The molecular model of the apoA1 trimer is shown as three chains, two of them in a helical conformation form a dimer and the third chain forms a hairpin. Because the molecular model does not include any local information about the conformation of the individual amino acid residues, the secondary structure of the chains was assigned to be α-helix for all residues. The three chains are gradient colored with red, blue and cyan. The superposition of the HdHp model with (C) the low resolution structure (orange) of the protein component (12% D₂O) of sHDL, and with (D) the low resolution structure of the lipid component (42% D₂O).

Figure 4. Hypothetical Mechanism of Conformational Change of ApoA1 in Nascent HDL during Maturation into Spherical HDL. (A) Left: Superposition of the low resolution structures of protein (orange) and lipid (green) components of nascent HDL (top), and one particular orientation of the Double Super Helix model of the protein component in nascent HDL (bottom). The two apoA1 chains are gradient colored with red and blue, and the putative LCAT binding loops (Solar Flares) are colored in yellow. Middle panel: The overlap of the middle domains of nascent HDL and sHDL apoA1 dimers. The overlapping suggests how the N/C termini of the apoA1 dimer in nHDL might swing and rearrange during particle maturation into what is found to be the dimer conformation in sHDL. The grey region at
the middle of the particle represents the growing core of neutral lipids that accumulate during maturation. **Right panel:** Superposition of the low resolution structures of protein (orange) and lipid (green) components of spherical HDL (top), and the resultant architecture of apoA1 trimer in spherical HDL (the HdHp model, bottom). (B) Hypothetical mechanism for apoA1 inter-exchange in sHDL: the hairpin and the apoA1 dimer are shown in different conformations that match the low resolution structure (12% D₂O) of the protein. The different configurations suggest that it is reasonable to expect that due to particle dynamics the hairpin can bend such that it aligns its h5-domain with the h5-domain of the dimer (dotted double arrow lines) as a preamble for annealing with the dimer. Thus, the hairpin can exchange with one of the apoA1 monomers of the dimer, through a transient integrated-trimer-like configuration (center), thus generating other arrangements of the helical dimer / hairpin combination (solid double arrow lines). This protein reorganization mechanism makes all apoA1 monomers equivalent from an exchange point of view, and can in principle lead to the integrated trimer (the iT model) as illustrated on the right side of the panel.
Table I. Radius of Gyration, Dimensions, and Goodness of Fit for the Low Resolution SANS Structures of Spherical Reconstituted HDL.

<table>
<thead>
<tr>
<th>sHDL % D₂O</th>
<th>R₉ (Å)</th>
<th>χ²</th>
<th>Size (Å)</th>
<th>L : W : H</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental curve</td>
<td>Low resolution structure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>39.1±0.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>47.3±0.08</td>
<td>45.2</td>
<td>1.483</td>
<td>111 : 96.6 : 88.7</td>
</tr>
<tr>
<td>42</td>
<td>31.0±0.03</td>
<td>30.9</td>
<td>2.887</td>
<td>88.4 : 68.2 : 60.3</td>
</tr>
<tr>
<td>90</td>
<td>34.8±0.49</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table II. Radius of Gyration, Dimensions, and Goodness of Fit Geometrical Constraints for the Molecular Models of ApoA1 Trimer in Spherical Reconstituted HDL.

<table>
<thead>
<tr>
<th>Model</th>
<th>R₉ (Å)</th>
<th>χ²</th>
<th>Satisfied X-links a</th>
</tr>
</thead>
<tbody>
<tr>
<td>HdHp</td>
<td>45.6</td>
<td>1.192</td>
<td>23</td>
</tr>
<tr>
<td>3Hp</td>
<td>45.6</td>
<td>1.183</td>
<td>19</td>
</tr>
<tr>
<td>iT</td>
<td>45.8</td>
<td>1.515</td>
<td>21</td>
</tr>
</tbody>
</table>

a Out of 23 reported by Silva et al (47).
Figure 1
Figure 2

A  Helical dimer / Hairpin (HdHp)

B  Three hairpins (3Hp)

C  Integrated trimer (iT)
Figure 4

A  
Nascent HDL  MATURATION  Spherical HDL

B  
Hypothetical Mechanism for Inter-exchangeability of ApoA1 in sHDL

Helical dimer/Hairpin (HdHp)  
Integrated trimer (IT)  
HdHp
The low resolution structure of ApoA1 in spherical high density lipoprotein revealed by small angle neutron scattering


J. Biol. Chem. published online February 3, 2011

Access the most updated version of this article at doi: 10.1074/jbc.M110.209130

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2011/02/03/M110.209130.DC1