Published in final edited form as:

Biochim Biophys Acta. 2012 March ; 1821(3): 448–455. doi:10.1016/j.bbalip.2011.12.010.

The “Beta-Clasp” model of apolipoprotein A-I - a lipid-free solution structure determined by electron paramagnetic resonance spectroscopy

Jens O. Lagerstedt[‡], Madhu S. Budamagunta[¶], Grace S. Liu[§], Nicole C. DeValle[§], John C. Voss[¶], and Michael N. Oda^{§,1}

[‡]Department of Experimental Medical Science, Lund University, S-221 84 Lund, Sweden

[§]Children's Hospital Oakland Research Institute, Oakland, CA 94609-1673

[¶]Department of Biochemistry and Molecular Medicine, University of California, Davis, CA 95616

Abstract

Apolipoprotein A-I (apoA-I) is the major protein component of high density lipoproteins (HDL) and plays a central role in cholesterol metabolism. The lipid-free / lipid-poor form of apoA-I is the preferred substrate for the ATP-binding cassette transporter A1 (ABCA1). The interaction of apoA-I with ABCA1 leads to the formation of cholesterol laden high density lipoprotein (HDL) particles, a key step in reverse cholesterol transport and the maintenance of cholesterol homeostasis. Knowledge of the structure of lipid-free apoA-I is essential to understanding its critical interaction with ABCA1 and the molecular mechanisms underlying HDL biogenesis. We therefore examined the structure of lipid-free apoA-I by electron paramagnetic resonance spectroscopy (EPR). Through site directed spin label EPR, we mapped the secondary structure of apoA-I and identified sites of spin coupling as residues 26, 44, 64, 167, 217 and 226. We capitalize on the fact that lipid-free apoA-I self-associates in an anti-parallel manner in solution. We employed these sites of spin coupling to define the central plane in the dimeric apoA-I complex. Applying both the constraints of dipolar coupling with the EPR-derived pattern of solvent accessibility, we assembled the secondary structure into a tertiary context, providing a solution structure for lipid-free apoA-I.

1. Introduction

1.1 Apolipoprotein A-I and cholesterol mobilization

Exchangeable apolipoproteins are central elements of lipid metabolism and homeostasis, a key aspect of whole body physiology. Exchangeable lipoproteins participate in this essential metabolic function by transporting lipids between peripheral tissues and the liver/intestines. Apolipoprotein A-I (apoA-I) is an exchangeable apolipoprotein, the principal protein component of high density lipoprotein (HDL), and a primary determinant of HDL structure, composition, and stability.

© 2012 Elsevier B.V. All rights reserved.

¹Address correspondence to: Michael N. Oda, Children's Hospital Oakland Research Institute, 5700 Martin Luther King Jr. Way, Oakland CA 94609; Tel. 510 450-7652; Fax. 510 450-7920; moda@chori.org.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

HDL is the primary mediator of reverse cholesterol transport (RCT) [1], wherein excess cholesterol in peripheral tissues is conveyed to the liver by lipoproteins for excretion into the intestine as bile [2]. A significant portion of HDL's anti-atherosclerotic nature is due to its ability to mediate mobilization of cholesterol from macrophages in the arterial wall through RCT [3].

A key process during RCT is the transition of lipid-free apoA-I to a spherical HDL complex. This is a two-step event. First, lipid-free / lipid-poor apoA-I acquires phospholipid and cholesterol from the ATP-binding cassette transporter A1 (ABCA1) [4,5] to generate nascent HDL and second, cholesterol on nascent HDL is esterified by lecithin:cholesterol acyltransferase (LCAT) to yield cholesteryl ester [6,7] and mature spherical HDL.

1.2 ApoA-I structure

ApoA-I must be structurally adaptive to accommodate the acquisition of phospholipid and cholesterol. An understanding of apoA-I structure will provide insight into how apoA-I's conformational dynamics enable a fundamental aspect of RCT, namely the biogenesis of HDL through its interaction with ABCA1.

Most of our understanding of apoA-I structure is relative to apoA-I on nascent HDL. The molecular details of the belt model have provided insight into how apoA-I mediates HDL's biological activity. But the conformation of lipid-free apoA-I has been elusive due in large measure to its dynamic nature. Early investigation into the structure of full length lipid-free apoA-I had been limited to a general investigation of apoA-I's secondary structure content via circular dichroism (CD) [8,9] or fourier transform infrared spectroscopic (FTIR) [10]. Lipid-free apoA-I's susceptibility to protease cleavage provided initial insight into the possible arrangement of these secondary structural elements [11]. Chemical cross-link mass spectroscopy gave a more detailed view of this arrangement [9]. More recent studies have yielded higher resolution models of lipid-free apoA-I based on X-ray crystallography [12] or deuterium-proton exchange [13]. However, the validity of the X-ray crystallographic model of Ajees, et al. [12] has been called into question [14,15] and remains to be verified. Thus deuterium-proton exchange presents the highest resolution map for the distribution of secondary structural elements in apoA-I [13]. Here we report the solution structure of lipid-free apoA-I as determined by electron paramagnetic resonance (EPR) spectroscopy analysis, which provides a residue-specific description of the in solution structure of the protein.

A defining paradox of exchangeable apolipoproteins is that they maintain their solubility in the presence and absence of lipid. Unlike most lipid-binding proteins, in the absence of lipid they avoid self-aggregation and precipitation. Our model of lipid-free apoA-I, which we term the "beta clasp", provides a possible mechanism whereby aggregation is avoided. We have determined that the helical bundle of lipid-free apoA-I arranges around four strategically positioned β -strands. We hypothesize that these β -strands serve as a proxy for lipids, binding the hydrophobic face of apoA-I's amphipathic helices. This coordinates the orientation of apoA-I's helices, preventing excessive exposure of hydrophobic regions and thereby avoiding self aggregation events.

2. Materials and Methods

2.1 Materials

Thio-specific nitroxide spin label (MTS; (1-Oxyl-2,2,5,5-tetramethylpyrroline-3-methyl) methanethiosulfonate) was received as a kind gift from Dr K Hideg (University of Pecs, Hungary).

2.2 Production of recombinant and spin-labeled apoA-I protein

Cys-substitutions within apoA-I cDNA (S6C to K238C) were created using either primer-directed PCR mutagenesis or by the megaprimer PCR method [16]. The mutations were verified by dideoxy automated fluorescent sequencing. The proteins were expressed and purified as described [17]. Nitroxide spin label was covalently attached to specific sites by reacting proteins with MTSL probe as described [18]. In brief, 8 mg of protein was sequentially incubated with 100 μ M Tris-(2-carboxyethyl)phosphine and 300 μ M MTS spin label on a Ni²⁺-chelated HiTrap column (GE Healthcare) under denaturing conditions (3 M guanidine-HCl), then extensively washed with PBS (20 mM phosphate, 500 mM NaCl) and eluted by imidazole. Protein purity (>95%) was confirmed by SDS-polyacrylamide gel electrophoresis analysis.

2.3 Electron paramagnetic resonance (EPR) analysis

EPR measurements were carried out in a JEOL X-band spectrometer fitted with a loop-gap resonator [19,20]. Aliquots (5 μ l) of purified lipid-free apoA-I (1.8 mg/ml, ~60 μ M spin-labeled protein) were placed in sealed quartz capillaries and loaded in the resonator. Spectra were acquired at room temperature (20–22°C) from a single 2-min scan over a field of 100 G at a microwave power of 2 mW and a modulation amplitude optimized to the natural line width of the individual spectrum (0.5 – 1.5 G). To facilitate spectra integration for spin normalization, all samples were also examined the presence of sodium-dodecyl sulfate (SDS) (at a final concentration of 2%) to diminish tertiary and quaternary interactions (i.e., to eliminate signal broadening due to strong immobilization or dipolar coupling). Spectra obtained in the presence of SDS were double integrated and normalized. Molecular accessibility of spin-labeled side chains to CrOx and O₂ was determined using successive power saturation scans as described [21]. $\Pi_{1/2}$ values (which also were used to calculate the contrast function (Φ)) were calculated using software provided by C. Altenbach).

2.4 Molecular modeling

Chimera (UCSF) [22] was used to construct the model of lipid-free apoA-I. A general structure for apoA-I based on the secondary structure map was generated in Pymol [23]. The model was refined in Chimera, wherein solvent accessibility and spin coupling defined the arrangement of secondary structural elements. The final model was rendered in Pymol [23].

3. RESULTS

3.1 Spin labeling of apoA-I

Site-directed spin-labeling of apoA-I followed by EPR spectroscopy was used to analyze the structure of lipid-free apoA-I. Here 104 novel apoA-I substitution variants of apoA-I were created, wherein residues were individually cysteine substituted and labeled with a thiol-specific paramagnetic nitroxide spin-label, MTSL (Fig. 1A) providing a probe that reports on both main chain and side chain dynamics and has remarkably little effect on the native protein fold [24,25]. The positions were selected to complement our previous EPR analyses of apoA-I's lipid-free structure [18,26] and together they provide structural information on over 95% of the apoA-I protein. The newly analyzed residues are primarily located in the extreme N-terminus (residues 6 to 13), the central domain (residues 100 to 180) and the C-terminal domain. The spin-labeled apoA-I variants were examined by EPR spectroscopy, as described in *Experimental Procedures*.

3.2 Side chain mobility and Molecular accessibility of lipid-free apoA-I residues

EPR spectroscopic analysis of nitroxide-labeled proteins provide information on mobility and molecular accessibility of the labeled residue (see Fig. 1 for overview). The inverse

central linewidth (δ^{-1}) is a function of the central peak-to-peak width and provides a measure of the local steric constraints imposed upon a residue. The collision frequency between the nitroxide on the labeled side chain and the polar (chromium oxalate (CrOx)) or nonpolar (oxygen (O₂)) quenching agents provide a quantitative measure of the local chemical environment of the labeled residues. This is represented in two values, the accessibility parameter (Π) of the two quenchers and the contrast function (θ), which is the logarithmic ratio of the accessibility parameter values of the polar (Π_{CrOx}) and nonpolar (Π_{O_2}) quenchers. These values were calculated for all residues analyzed and used to generate a topological map of apoA-I, whose periodicity is indicative of lipid-free apoA-I's secondary structure distribution. Specifically, plots of Π and θ parameters as a function of residue number allow for identification of secondary structure patterns where α -helical structures typically display a periodicity of 3.6 whereas β -strand structures exhibit an alternating periodicity (Fig. 2).

3.3 The extreme N-terminus of lipid-free apoA-I - residues 6 to 20

The accessibility parameters, Π_{CrOx} and Π_{O_2} , for residues 6 to 20 exhibit a periodicity of approximately 3.6 consistent with an α -helical secondary structure. This pattern is more pronounced in the 14 to 19 region as compared to the upstream residues (6 to 13). These findings are supported by δ^{-1} and θ values, which both exhibit a pattern indicative of α -helical structure. From this, we conclude that residues 14 to 19 form a stable α -helix that is transiently inclusive of residues 6 to 13. It can also be noted that the overall mobility is high, in particular in the 6 to 14 region ($\delta^{-1}_{\text{average}}$ is 0.4), which indicates that the extreme N-termini is not embedded in the core of the structure but rather is solvent accessible.

3.4 The N-terminal domain of lipid-free apoA - residues 20 to 98

Previously, we had performed EPR analysis of most of the residues in this region [26] except for residues 66, 79, and 81. In this current study we examined these residues. As expected, spectral and accessibility information from these positions do not alter our previous conclusions regarding the secondary structure assignment in this domain. We continue to assert that the distribution of α -helices are to positions 26 to 34, 36 to 42, 44 to 51, 55 to 65, 67 to 85 and 92 to 98. These domains are interconnected by short segments of non-structured amino acids or by single residues that provide a kink in the helical rod (e.g., P66). The polar accessibility parameter (Π_{CrOx}), as well as both the contrast function and the inverse central linewidth (δ^{-1}) of residues 20 to 25 indicate this region assumes a β -strand conformation.

3.5 The central domain of lipid-free apoA-I - residues 99 to 188

Our current study is the first EPR analysis of the central domain region of the lipid-free protein. To enable a complete description of this region's secondary structure we determined the EPR spectral properties of 74 newly generated nitroxide labeled single cysteine substitutions. The accessibility parameters, Π_{CrOx} and Π_{O_2} , for residues 102 to 115 indicate that this region assumes an α -helical conformation. This is partly supported by the mobility data (δ^{-1}). The low mobility seen for residues 110 to 115, which partially interrupts the helical pattern of δ^{-1} , may be due to additional tertiary contacts. Interestingly, the parameter values for 102 to 115 helix appears to be out of phase with the 92 to 98 helix. However, because bending or kinking within the helix may alter its periodicity, we cannot exclude the possibility that residues 92 to 115 may form a single helix. The next helical region identified starts at residue 130 and extends to residue 148. This is also substantiated by the helical pattern exhibited by the two accessibility parameters, Π_{CrOx} and Π_{O_2} , and partly by the mobility parameters, δ^{-1} and θ . Of note is that the side chains of the first ten residues of this region (130–139) display a high mobility suggesting little tertiary contacts. Finally, we determined that residues 158 to 188 form one contiguous helix. The presence of helical

structure in this region is supported by both mobility and accessibility data (including the contrast function values).

Interestingly, the three helical regions of the central domain appear to be separated by β -strands. Residues 120 to 129 and residues 150 to 158, both exhibit alternating accessibility parameter values typical for β -strand structure (periodicity of 2). This observation is largely supported by both the mobility parameter and the contrast function for both regions. We therefore conclude these amino acids adopt a β -strand conformation.

3.6 The C-terminal domain of lipid-free apoA-I - residues 189 to 238

We have previously examined the C-terminal domain of lipid-free apoA-I [18] and have refined the analysis by analyzing an additional 19 residues in this region. Residues 189 to 199 exhibit non-structured random EPR spectral properties, consistent with a random coil. Although the nonpolar accessibility parameter (Π_{O2}) bears an alternating pattern of amplitude, this is not consistent with both nonpolar accessibility (Π_{CrOx}) and side chain mobility. Moreover, these residues bear a very high degree of motional freedom ($\delta^{-1}_{average}$ is 0.47 for residues 190 to 198; as opposed to the $\delta^{-1}_{average}$ of 0.30 for the more ordered residues 170 to 182). Following the random coil is a short α -helix (residues 200 to 205) followed by a short random coil segment (residues 206 to 212). EPR parameter data in the downstream region (residues 214 to 220) indicate that these residues assume a β -strand structure, consistent with our previous report [18]. Following the β -strand, the values of accessibility and mobility parameters for residues 224 to 231 suggests this region assumes a helical structure, which is supported by the mobility data for the same region. Residues 221 to 223 and 232 to 238 (amino acid residues 239 to 243 have not been analyzed) do not display any structured character and are thus assigned a random coil secondary structure.

3.7 Sites of spin coupling

Lipid-free apoA-I is predominantly dimeric at the protein concentration used in these experiments (60 μ M) and its oligomeric properties have been extensively investigated [27,28,29]. We took advantage of apoA-I's self-associating properties and looked for sites within apoA-I wherein spin coupling was observed. Because apoA-I forms an anti-parallel dimer, sites of spin coupling define the central plane of the complex [18]. This provides a means of aligning the secondary structures within apoA-I into a tertiary context. In our previous studies we determined that residues 26, 44, 64, 167, 217 and 226 exhibit dipolar coupling [18,26]. From this we devised the alignment of apoA-I secondary structure elements (Fig. 3). The concentration dependence of these associations was evaluated (Supplemental Fig. 1) and confirmed that spin coupling is indeed a product of dimer formation and that the structure of the unit cell (dimer) within higher order forms of the protein is not measurably affected.

4. Discussion

ApoA-I plays several vital roles in HDL's ability to mobilize cholesterol. It must serve as an adaptable scaffold to maintain the integrity of the HDL particle, activate LCAT for the generation of a cholesteryl ester core, and serve as a substrate for cholesterol transporters and receptors such as ABCA1 and SR-B1, respectively. While lipid-bound cholesteryl ester laden apoA-I is the preferred substrate for SR-B1, the optimal substrate of ABCA1 is lipid-free / lipid-poor apoA-I [30,31]. The ability of an HDL particle to participate in cholesterol mobilization via ABCA1 is likely related to its ability to serve as a source of lipid-free / lipid poor apoA-I [32,33,34,35], emphasizing the importance of lipid-free apoA-I in cholesterol metabolism. Because circulating levels of lipid-free apoA-I is less than 1% of the

total circulating apoA-I [36], structural studies of apoA-I have focused mainly on the lipid-bound species of the protein.

Denaturation studies of apoA-I indicate that the molecule is loosely folded and relatively flexible in comparison to globular proteins [37,38]. The structural heterogeneity of apoA-I presents a challenge to crystallizing the full-length protein. Because its dynamic nature is central to apoA-I's mechanism of mediating HDL formation and cholesterol mobilization, the utility of a ground state conformer (as would be described in a crystallographic analysis) is in question. Thus EPR of site-directed spin-labels has proven valuable in that it is able to identify regions of disorder at high resolution within lipid-free apoA-I. Furthermore the EPR approach describes apoA-I at physiological concentrations in solution for both its lipid-free and lipid-bound states. The abundant dynamic, chemical, and spatial information provided by EPR complements information derived by other methods. Examination of an apoA-I proteolytic fragment (residues 1 to 195) by circular dichroism (CD) spectroscopy indicates the C-terminal domain consists of mostly random coil [28,39]. Studies from our laboratory have identified specific regions of disordered and ordered residues within the apoA-I C-terminus [18], suggesting that while the apoA-I molecule has been characterized as conformationally dynamic, regions of conformational disorder are localized to specific segments of the apoA-I protein. In light of the fact that other exchangeable apolipoproteins are known to exist as bundles of amphipathic α -helices [40,41,42] it is reasonable to consider that apoA-I may adopt a similar organization. A wide array of techniques have been applied to enhance our resolution of apoA-I's lipid-free structure. These methodologies include: analysis of synthetic peptides [43,44,45] domain deletion/swapping mutants [46,47,48], or spectroscopic analysis of the whole protein [38,42,44] and specific locales [49,50,51]. While extensive effort has gone into determining apoA-I's lipid-free structure, many studies have yielded contradictory results. Consequently, the predominant tertiary structure of lipid-free apoA-I is a matter of debate and a variety of conformations have been proposed. Deletion mutant analysis and limited proteolysis studies suggest that apoA-I exists as a monomeric two-helix, helical hairpin [52] or a four-helix bundle [11], while spectroscopic and calorimetric analysis suggests a molten globular conformation [53]. Interestingly, no existing model of apoA-I depicts the placement or role of β -strands within the protein, despite the consistent observation of β -strand domains in global secondary structure analysis. Our data indicate that lipid-free apoA-I consists of approximately 53% α -helix, 12% β -strand and 35% random coil. This data is in close agreement with previous analyses of apoA-I secondary structure content as determined by circular dichroism (55% α -helix, 8% β -strand, and 37% random coil [9]; 58% α -helix, 8% β -strand, and 34% random coil [8]) and FTIR (44% α -helix, 16% β -strand, 17% bend and turn, and 23% random coil [10]).

Recently, Chetty and colleagues reported a model of apoA-I consisting of approximately 50% α -helix and 50% random coil (Fig. 2), that was determined by hydrogen-deuterium exchange mass spectroscopic analysis [13]. The Chetty model depicts residues 179 to 243 as random coil, whereas our current analysis identifies three regions of ordered structure within these residues. The differences in the secondary structure arrangement of the Chetty model versus our model may be the result of the protein concentration at which the hydrogen-deuterium exchange was performed, (2.5 μ M, compared to 60 μ M for EPR analysis). As self-association of apoA-I only occurs at concentrations greater than 0.1 mg/ml (3.7 μ M) [28,29], it is very likely that apoA-I of the Chetty model is monomeric. The C-terminal domain of apoA-I is the site of self association [54,55] and, when monomeric, apoA-I may adopt a significantly different conformation. Similarly, apoA-I's self-association may stabilize the presence of β -strand.

4.1 The “beta clasp” model of apoA-I - the central β -strands

The “beta clasp” model of lipid-free apoA-I is composed of a series of α -helices stabilized by the presence of four β -strands (Fig. 4A). In this model there is a loop centered at position 139, which is also observed in the “looped-belt” model for apoA-I on 9.6 nm discoidal reconstituted HDL (rHDL) particles [56] (Fig. 4B). In the “beta clasp” model residue 139 exhibits a high mobility indicative of a loop residue and is flanked by two β -strands (residues 102 to 115 and 130 to 148). We propose that this pairing of β -strands leads to the formation of the loop centered at residue 139. This is a key element in apoA-I’s structure, as it is also present on discoidal rHDL wherein it is hypothesized to form a pore by which LCAT gains access to the acyl chains of the particle lipid bilayer [56,57]. During apoA-I’s association with lipid, this region transitions from β -strand to α -helix [56], eliminating a stabilizing β -sheet from the center of the protein (Fig. 4A). This may facilitate the partitioning of nearby α -helices into a lipid environment and the opening of the molecule into its extended lipid-bound conformation. This hypothesis is supported by the observation of Tanaka and colleagues who found impaired lipid binding with the Δ 190–243 deletion mutant of apoA-I [58]. Interestingly, they also observed that a proline substitution mutation (S55P/ Δ 190–243) restored lipid binding activity to apoA-I, suggesting that the proline substitution destabilizes the Δ 190–243 apoA-I protein and allows the apoA-I molecule to more readily respond to the presence of lipid.

The tertiary fold of lipid-free apoA-I has also been examined by chemical cross-link mass spectroscopy [9] and is largely consistent with the findings reported here. As expected, many of the cross-links involve lysine residues positioned on flexible loops (as identified by EPR) or on regions of secondary structure that are closely flanked by disordered segments. Thus it is particularly informative to consider the intramolecular cross-link between K23 and K59, as it involves two sites residing along segments of fixed secondary structure. In our model, the alpha carbons of these two positions are separated by 10.5Å, falling within the 11.4Å constraint of the cross-linking reagent. Other intramolecular pairs involving more flexible regions are also consistent, such as the NT amine and position K96, K94–K208, K96–K195, and K96–K208. The K96–K226 is difficult to reconcile with our model with the placement of the C-terminal segment as shown (Fig. 4), however this domain is separated from the protein by a flexible loop and could, relative to the helical bundle, reside on a surface more proximal to position K96. The only intramolecular pair reported by Silva and colleagues that is not easily reconciled with our model is the cross-link observed between K118 and K140. In our model, the segment containing position K140 is extended away from the main helical bundle. However, the beta strands need not necessarily be positioned in this manner relative to the helical bundle and could very well fold over the protein bringing residues K118 and K140 into greater proximity. Likewise the intermolecular cross-links identified by Silvia and co-workers are consistent with an antiparallel association of apoA-I proteins as we present here and in earlier studies [18,26].

4.2 The role of the N- and C-terminal β -strands

Similar to the central β -strands, we hypothesize that the N- and C-terminal β -strands (position 20 to 25 and 214 to 220) (Fig. 4A) play a stabilizing role. But instead of stabilizing the central region of the protein they maintain the N- and C-termini in a compact conformation [59]. We hypothesize the N-terminal β -strand (residues 20 to 25) preserves the stable association of the N-terminus with the remainder of the protein. We have observed that the arginine substitution mutant apoA-I_{Iowa} (G26R), which is associated with amyloid formation [60,61], leads to the extension of the β -strand at residues 20 to 25 to a much longer β -strand comprising residues 20 to 56 [62]. This increase in β -strand content increases the stability of the N-terminal domain’s structure and interferes with lipid association and HDL formation [62], leading to enhanced particle catabolism [60]. The role

of the N-terminal β -strand in particle stability is further supported by the observation that deletion of the first 43 residues of apoA-I results in the formation of an extended “lipid-bound like” like conformation [48,63,64]. We hypothesize that the N-terminal β -strand is a conformational linchpin. Similarly, we believe that the C-terminal β -strand (residues 214 to 220) maintain the position of the C-terminus and also prevents the C-terminal lipid sensing random coil from inappropriately responding in the absence of lipid [18]. This was intimated by C-terminal deletion mutants (Δ 190–243 [65], Δ 212–233, and Δ 213–243 [66]), all of which are significantly impaired in lipid binding ability. During lipid association, the C-terminal β -strand, which is critical to apoA-I self-association, transitions to an α -helical conformation [28]. This transition from β -strand to α -helix disrupts apoA-I’s self-recognition and may be an important step in the lipidation process. This supposition is supported by the finding that apoA-I valine substitution mutations at positions L211, L214, L218, and L219 are dramatically impaired in phospholipid association [55]. The sensitivity of apoA-I lipidation to the seemingly conservative substitution of one hydrophobic residue with another, while perplexing, may be explained by the differential effect of leucine and valine on β -strand stability. Valine is the most frequently observed amino acid in β -sheet structures [67] and while primary and tertiary structural context is a substantial determining factor in β -strand formation [68], the presence of valine contributes significantly to β -sheet stability compared to leucine at the same position (-0.41 kcal/mol; [69]). We hypothesize that the presence of valine stabilizes the C-terminal β -strand (residues 214 to 220) and prevents its conversion to α -helix upon lipid association, thereby interfering with subsequent conformational events necessary for lipid-binding (opening of the C- and N-terminal contact and disruption of the intermolecular contact between apoA-I molecules).

4.3 The molecular “trigger” to apoA-I’s response to lipid

The presence of a random coil at position 189 to 199 was reported previously [18], wherein we hypothesized the transition of this region to α -helix during lipid binding is a key regulatory event. The transition of random coil structures to α -helical structures during lipidation may be a common trait amongst apolipoproteins. A 4 kDa cyanogen bromide fragment from *Manduca sexta* apolipoprotein III’s C-terminal domain assumes a random coil conformation in the absence of detergent and phospholipid [70], which then adopts an α -helix conformation in the presence of detergent or specific phospholipids. Similarly, NMR and circular dichroism (CD) spectroscopic analysis of residues 35 to 53 of human apolipoprotein C-I shows that this region of the protein undergoes a transition from random coil to α -helix upon interaction with dodecylphosphocholine [71]. CD analysis of canine apolipoprotein E reveals a similar scenario, in which the molecule undergoes a significant random coil to α -helix conformational transition upon lipid association [72].

The free energy released from the formation of α -helix from random coil in apoA-I likely contributes to overcoming the energetic demands of lipidation. For viral fusion proteins this type of conformational transition yields approximately -30 kcal/mol free energy [73,74]. Tanaka and colleagues observe comparable free energy gains for apoA-I lipid binding [75]. They hypothesize that a latent lipid binding domain resides at Y18, which is revealed after association of C-terminal residues with lipid. This is consistent with our mechanism of lipid binding, wherein the transition of the C-terminal β -strand (residues 214 to 220) to α -helix somehow destabilizes the N-terminal β -strand (residues 20 to 25), exposing the hydrophobic face of the amphipathic α helices. It is critical that this transition occur in the presence of lipid otherwise it may lead to self association / aggregation. Therefore we believe the position of the β -strands within apoA-I mediate a carefully orchestrated series of conformational changes that direct the association of apoA-I’s α -helices with lipid, leading to HDL formation.

Although lipid-free apoA-I has proven difficult to examine, we have successfully applied site-directed spin-labeling electron paramagnetic resonance spectroscopy to yield a model of apoA-I that is consistent with data generated from other physical techniques. Furthermore, the structural data have provided us insights into the probable mechanism by which lipid-free apoA-I maintains its solubility via the “beta clasp”. Variations on this mechanism may play a role in the transition of other exchangeable apolipoproteins into a lipid environment.

Highlights

> Electron paramagnetic resonance spectroscopy was used to map the structure of apoA-I. > ApoA-I structure bears 4 beta strands, which may serve to stabilize apoA-I's protein structure. > The tertiary structure of apoA-I was determined. > The central beta strands may organize the overall structure of apoA-I.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We dedicate this manuscript to the memory of Dr. Jack Oram. He was a dear friend, colleague, and mentor and will be greatly missed. This work was supported by grants from the Swedish Research Council (522-2008-3724), the Petrus and Augusta Hedlund foundation, the Crafoord Foundation, the Carl Trygger Foundation for Scientific Research, National Institutes of Health grants HL77268 and HL78615, and by the American Heart Association Scientist Development Grant 0235222N.

References

1. Miller NE, Miller GJ. Letter: High-density lipoprotein and atherosclerosis. *Lancet*. 1975; 1:1033. [PubMed: 48701]
2. Glomset JA. The plasma lecithins:cholesterol acyltransferase reaction. *J Lipid Res*. 1968; 9:155–167. [PubMed: 4868699]
3. Cuchel M, Rader DJ. Macrophage reverse cholesterol transport: key to the regression of atherosclerosis? *Circulation*. 2006; 113:2548–2555. [PubMed: 16735689]
4. Adorni MP, Zimetti F, Billheimer JT, Wang N, Rader DJ, Phillips MC, Rothblat GH. The roles of different pathways in the release of cholesterol from macrophages. *J Lipid Res*. 2007; 48:2453–2462. [PubMed: 17761631]
5. Mulya A, Lee JY, Gebre AK, Thomas MJ, Colvin PL, Parks JS. Minimal lipidation of pre-beta HDL by ABCA1 results in reduced ability to interact with ABCA1. *Arterioscler Thromb Vasc Biol*. 2007; 27:1828–1836. [PubMed: 17510466]
6. Glomset JA. The mechanism of the plasma cholesterol esterification reaction: plasma fatty acid transferase. *Biochim Biophys Acta*. 1962; 65:128–135. [PubMed: 13948499]
7. Fielding CJ, Shore VG, Fielding PE. A protein cofactor of lecithin:cholesterol acyltransferase. *Biochem Biophys Res Commun*. 1972; 46:1493–1498. [PubMed: 4335615]
8. Brubaker G, Peng DQ, Somerlot B, Abdollahian DJ, Smith JD. Apolipoprotein A-I lysine modification: effects on helical content, lipid binding and cholesterol acceptor activity. *Biochim Biophys Acta*. 2006; 1761:64–72. [PubMed: 16495141]
9. Silva RA, Hilliard GM, Fang J, Macha S, Davidson WS. A three-dimensional molecular model of lipid-free apolipoprotein A-I determined by cross-linking/mass spectrometry and sequence threading. *Biochemistry*. 2005; 44:2759–2769. [PubMed: 15723520]
10. Chen H, Sanyal G. Direct biophysical characterization of human apolipoprotein A-I in ISCOMs. *J Pharm Sci*. 1999; 88:1122–1126. [PubMed: 10564059]

11. Roberts LM, Ray MJ, Shih TW, Hayden E, Reader MM, Brouillette CG. Structural analysis of apolipoprotein A-I: limited proteolysis of methionine-reduced and -oxidized lipid-free and lipid-bound human apo A-I. *Biochemistry*. 1997; 36:7615–7624. [PubMed: 9200714]
12. Ajees AA, Anantharamaiah GM, Mishra VK, Hussain MM, Murthy HM. Crystal structure of human apolipoprotein A-I: insights into its protective effect against cardiovascular diseases. *Proc Natl Acad Sci U S A*. 2006; 103:2126–2131. [PubMed: 16452169]
13. Chetty PS, Mayne L, Lund-Katz S, Stranz D, Englander SW, Phillips MC. Helical structure and stability in human apolipoprotein A-I by hydrogen exchange and mass spectrometry. *Proc Natl Acad Sci U S A*. 2009; 106:19005–19010. [PubMed: 19850866]
14. Schekman R. Editorial Expression of Concern for Multiple Articles. *Proc Natl Acad Sci U S A*. 2010; 107:6551.
15. U.R. Archive. Birmingham: University of Alabama at Birmingham; 2009. UAB Statement on Protein Data Bank Issues (<http://main.uab.edu/Sites/reporter/articles/71570/>).
16. Kammann M, Laufs J, Schell J, Gronenborn B. Rapid insertional mutagenesis of DNA by polymerase chain reaction (PCR). *Nucleic Acids Res*. 1989; 17:5404. [PubMed: 2548160]
17. Ryan RO, Forte TM, Oda MN. Optimized bacterial expression of human apolipoprotein A-I. *Protein Expr Purif*. 2003; 27:98–103. [PubMed: 12509990]
18. Oda MN, Forte TM, Ryan RO, Voss JC. The C-terminal domain of apolipoprotein A-I contains a lipid-sensitive conformational trigger. *Nat Struct Biol*. 2003; 10:455–460. [PubMed: 12754494]
19. Froncisz W, Hyde JS. The loop-gap resonator: A new microwave lumped circuit ESR sample structure. *Journal of Magnetic Resonance*. 1982; 47:515–521.
20. Hubbell WL, Froncisz W, Hyde JS. Continuous and stopped flow EPR spectrometer based on a loop gap resonator. *Review of Scientific Instrumentation*. 1987; 58:1879–1886.
21. Segrest JP, Jones MK, Klon AE, Sheldahl CJ, Hellinger M, De Loof H, Harvey SC. A detailed molecular belt model for apolipoprotein A-I in discoidal high density lipoprotein. *J Biol Chem*. 1999; 274:31755–31758. [PubMed: 10542194]
22. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem*. 2004; 25:1605–1612. [PubMed: 15264254]
23. Schrodinger, LLC. The PyMOL Molecular Graphics System, Version 1.3r1. 2010.
24. Guo Z, Cascio D, Hideg K, Kalai T, Hubbell WL. Structural determinants of nitroxide motion in spin-labeled proteins: tertiary contact and solvent-inaccessible sites in helix G of T4 lysozyme. *Protein Sci*. 2007; 16:1069–1086. [PubMed: 17473014]
25. Fleissner MR, Cascio D, Hubbell WL. Structural origin of weakly ordered nitroxide motion in spin-labeled proteins. *Protein Sci*. 2009; 18:893–908. [PubMed: 19384990]
26. Lagerstedt JO, Budamagunta MS, Oda MN, Voss JC. Electron paramagnetic resonance spectroscopy of site-directed spin labels reveals the structural heterogeneity in the N-terminal domain of apoA-I in solution. *J Biol Chem*. 2007; 282:9143–9149. [PubMed: 17204472]
27. Barbeau DL, Jonas A, Teng T, Scanu AM. Asymmetry of apolipoprotein A-I in solution as assessed from ultracentrifugal, viscometric, and fluorescence polarization studies. *Biochemistry*. 1979; 18:362–369. [PubMed: 217411]
28. Davidson WS, Hazlett T, Mantulin WW, Jonas A. The role of apolipoprotein AI domains in lipid binding. *Proc Natl Acad Sci U S A*. 1996; 93:13605–13610. [PubMed: 8942981]
29. Sparks DL, Frank PG, Braschi S, Neville TA, Marcel YL. Effect of apolipoprotein A-I lipidation on the formation and function of pre-beta and alpha-migrating LpA-I particles. *Biochemistry*. 1999; 38:1727–1735. [PubMed: 10026251]
30. Francis GA, Knopp RH, Oram JF. Defective removal of cellular cholesterol and phospholipids by apolipoprotein A-I in Tangier Disease. *J Clin Invest*. 1995; 96:78–87. [PubMed: 7615839]
31. Remaley AT, Stonik JA, Demosky SJ, Neufeld EB, Bocharov AV, Vishnyakova TG, Eggerman TL, Patterson AP, Duverger NJ, Santamarina-Fojo S, Brewer HB Jr. Apolipoprotein specificity for lipid efflux by the human ABCA1 transporter. *Biochem Biophys Res Commun*. 2001; 280:818–823. [PubMed: 11162594]

32. Mendez AJ, Oram JF. Limited proteolysis of high density lipoprotein abolishes its interaction with cell-surface binding sites that promote cholesterol efflux. *Biochim Biophys Acta*. 1997; 1346:285–299. [PubMed: 9219913]
33. Okuhira K, Tsujita M, Yamauchi Y, Abe-Dohmae S, Kato K, Handa T, Yokoyama S. Potential involvement of dissociated apoA-I in the ABCA1-dependent cellular lipid release by HDL. *J Lipid Res*. 2004; 45:645–652. [PubMed: 14729855]
34. Cavigiolio G, Geier EG, Shao B, Heinecke JW, Oda MN. Exchange of apolipoprotein A-I between lipid-associated and lipid-free states: a potential target for oxidative generation of dysfunctional high density lipoproteins. *J Biol Chem*. 2010; 285:18847–18857. [PubMed: 20385548]
35. Sacks FM, Rudel LL, Conner A, Akeefe H, Kostner G, Baki T, Rothblat G, de la Llera-Moya M, Asztalos B, Perlman T, Zheng C, Alaupovic P, Maltais JA, Brewer HB. Selective delipidation of plasma HDL enhances reverse cholesterol transport in vivo. *J Lipid Res*. 2009; 50:894–907. [PubMed: 19144994]
36. Asztalos BF, Cupples LA, Demissie S, Horvath KV, Cox CE, Batista MC, Schaefer EJ. High-density lipoprotein subpopulation profile and coronary heart disease prevalence in male participants of the Framingham Offspring Study. *Arterioscler Thromb Vasc Biol*. 2004; 24:2181–2187. [PubMed: 15388521]
37. Tall AR, Shipley GG, Small DM. Conformational and thermodynamic properties of apo A-I of human plasma high density lipoproteins. *J Biol Chem*. 1976; 251:3749–3755. [PubMed: 6462]
38. Sparks DL, Phillips MC, Lund-Katz S. The conformation of apolipoprotein A-I in discoidal and spherical recombinant high density lipoprotein particles. ¹³C NMR studies of lysine ionization behavior. *J Biol Chem*. 1992; 267:25830–25838. [PubMed: 1464597]
39. Ji Y, Jonas A. Properties of an N-terminal proteolytic fragment of apolipoprotein AI in solution and in reconstituted high density lipoproteins. *J Biol Chem*. 1995; 270:11290–11297. [PubMed: 7744765]
40. Wilson C, Wardell MR, Weisgraber KH, Mahley RW, Agard DA. Three-dimensional structure of the LDL receptor-binding domain of human apolipoprotein E. *Science*. 1991; 252:1817–1822. [PubMed: 2063194]
41. Breiter DR, Kanost MR, Benning MM, Wesenberg G, Law JH, Wells MA, Rayment I, Holden HM. Molecular structure of an apolipoprotein determined at 2.5-Å resolution. *Biochemistry*. 1991; 30:603–608. [PubMed: 1988048]
42. Wang G, Sparrow JT, Cushley RJ. The helix-hinge-helix structural motif in human apolipoprotein A-I determined by NMR spectroscopy. *Biochemistry*. 1997; 36:13657–13666. [PubMed: 9354635]
43. Epand RM, Surewicz WK, Hughes DW, Mantsch H, Segrest JP, Allen TM, Anantharamaiah GM. Properties of lipid complexes with amphipathic helix-forming peptides. Role of distribution of peptide charges. *J Biol Chem*. 1989; 264:4628–4635. [PubMed: 2925658]
44. Wald JH, Coormaghtigh E, De Meutter J, Ruyschaert JM, Jonas A. Investigation of the lipid domains and apolipoprotein orientation in reconstituted high density lipoproteins by fluorescence and IR methods. *J Biol Chem*. 1990; 265:20044–20050. [PubMed: 2123199]
45. Corijn J, Deleys R, Labeur C, Vanloo B, Lins L, Brasseur R, Baert J, Ruyschaert JM, Rosseneu M. Synthetic model peptides for apolipoproteins. II. Characterization of the discoidal complexes generated between phospholipids and synthetic model peptides for apolipoproteins. *Biochim Biophys Acta*. 1993; 1170:8–16. [PubMed: 8399330]
46. Holvoet P, Zhao Z, Deridder E, Dhoest A, Collen D. Effects of deletion of the carboxyl-terminal domain of ApoA-I or of its substitution with helices of ApoA-II on in vitro and in vivo lipoprotein association. *J Biol Chem*. 1996; 271:19395–19401. [PubMed: 8702626]
47. Sorci-Thomas MG, Curtiss L, Parks JS, Thomas MJ, Kearns MW. Alteration in apolipoprotein A-I 22-mer repeat order results in a decrease in lecithin:cholesterol acyltransferase reactivity. *J Biol Chem*. 1997; 272:7278–7284. [PubMed: 9054424]
48. Rogers DP, Roberts LM, Lebowitz J, Datta G, Anantharamaiah GM, Engler JA, Brouillette CG. The lipid-free structure of apolipoprotein A-I: effects of amino-terminal deletions. *Biochemistry*. 1998; 37:11714–11725. [PubMed: 9718294]

49. Behling Agree AK, Tricerri MA, Arnvig McGuire K, Tian SM, Jonas A. Folding and stability of the C-terminal half of apolipoprotein A-I examined with a Cys-specific fluorescence probe. *Biochim Biophys Acta*. 2002; 1594:286–296. [PubMed: 11904224]
50. Tricerri MA, Behling Agree AK, Sanchez SA, Bronski J, Jonas A. Arrangement of apolipoprotein A-I in reconstituted high-density lipoprotein disks: an alternative model based on fluorescence resonance energy transfer experiments. *Biochemistry*. 2001; 40:5065–5074. [PubMed: 11305923]
51. Li HH, Lyles DS, Pan W, Alexander E, Thomas MJ, Sorci-Thomas MG. ApoA-I structure on discs and spheres. Variable helix registry and conformational states. *J Biol Chem*. 2002; 277:39093–39101. [PubMed: 12167653]
52. Rogers DP, Roberts LM, Lebowitz J, Engler JA, Brouillette CG. Structural analysis of apolipoprotein A-I: effects of amino- and carboxy-terminal deletions on the lipid-free structure. *Biochemistry*. 1998; 37:945–955. [PubMed: 9454585]
53. Gursky O, Atkinson D. Thermal unfolding of human high-density apolipoprotein A-I: implications for a lipid-free molten globular state. *Proc Natl Acad Sci U S A*. 1996; 93:2991–2995. [PubMed: 8610156]
54. Vitello LB, Scanu AM. Studies on human serum high density lipoproteins. Self-association of apolipoprotein A-I in aqueous solutions. *J Biol Chem*. 1976; 251:1131–1136. [PubMed: 175065]
55. Laccotripe M, Makrides SC, Jonas A, Zannis VI. The carboxyl-terminal hydrophobic residues of apolipoprotein A-I affect its rate of phospholipid binding and its association with high density lipoprotein. *J Biol Chem*. 1997; 272:17511–17522. [PubMed: 9211897]
56. Martin DD, Budamagunta MS, Ryan RO, Voss JC, Oda MN. Apolipoprotein A-I assumes a "looped belt" conformation on reconstituted high density lipoprotein. *J Biol Chem*. 2006; 281:20418–20426. [PubMed: 16698792]
57. Jones MK, Cate A, Li L, Segrest JP. Dynamics of activation of lecithin:cholesterol acyltransferase by apolipoprotein A-I. *Biochemistry*. 2009; 48:11196–11210. [PubMed: 19860440]
58. Tanaka M, Dhanasekaran P, Nguyen D, Nickel M, Takechi Y, Lund-Katz S, Phillips MC, Saito H. Influence of N-terminal helix bundle stability on the lipid-binding properties of human apolipoprotein A-I. *Biochim Biophys Acta*. 2011; 1811:25–30. [PubMed: 21040803]
59. Koyama M, Tanaka M, Dhanasekaran P, Lund-Katz S, Phillips MC, Saito H. Interaction between the N- and C-terminal domains modulates the stability and lipid binding of apolipoprotein A-I. *Biochemistry*. 2009; 48:2529–2537. [PubMed: 19239199]
60. Van Allen MW, Frohlich JA, Davis JR. Inherited predisposition to generalized amyloidosis. Clinical and pathological study of a family with neuropathy, nephropathy, and peptic ulcer. *Neurology*. 1969; 19:10–25. [PubMed: 4304452]
61. Genschel J, Haas R, Propsting MJ, Schmidt HH. Apolipoprotein A-I induced amyloidosis. *FEBS Lett*. 1998; 430:145–149. [PubMed: 9688527]
62. Lagerstedt JO, Cavigliolo G, Budamagunta MS, Oda MN, Voss JC. Mapping the structural transition in an amyloidogenic apolipoprotein A-I. *Biochemistry*. 2007 In Press.
63. Rogers DP, Brouillette CG, Engler JA, Tendian SW, Roberts L, Mishra VK, Anantharamaiah GM, Lund-Katz S, Phillips MC, Ray MJ. Truncation of the amino terminus of human apolipoprotein A-I substantially alters only the lipid-free conformation. *Biochemistry*. 1997; 36:288–300. [PubMed: 9003180]
64. Borhani DW, Rogers DP, Engler JA, Brouillette CG. Crystal structure of truncated human apolipoprotein A-I suggests a lipid-bound conformation. *Proc Natl Acad Sci U S A*. 1997; 94:12291–12296. [PubMed: 9356442]
65. Holvoet P, Zhao Z, Vanloo B, Vos R, Deridder E, Dhoest A, Taveirne J, Brouwers E, Demarsin E, Engelborghs Y, et al. Phospholipid binding and lecithin-cholesterol acyltransferase activation properties of apolipoprotein A-I mutants. *Biochemistry*. 1995; 34:13334–13342. [PubMed: 7577918]
66. Minnich A, Collet X, Roghani A, Cladaras C, Hamilton RL, Fielding CJ, Zannis VI. Site-directed mutagenesis and structure-function analysis of the human apolipoprotein A-I. Relation between lecithin-cholesterol acyltransferase activation and lipid binding. *J Biol Chem*. 1992; 267:16553–16560. [PubMed: 1644835]

67. Levitt M. Conformational preferences of amino acids in globular proteins. *Biochemistry*. 1978; 17:4277–4285. [PubMed: 708713]
68. Minor DL Jr, Kim PS. Context is a major determinant of beta-sheet propensity. *Nature*. 1994; 371:264–267. [PubMed: 8078589]
69. Minor DL Jr, Kim PS. Measurement of the beta-sheet-forming propensities of amino acids. *Nature*. 1994; 367:660–663. [PubMed: 8107853]
70. Narayanaswami V, Kay CM, Oikawa K, Ryan RO. Structural and binding characteristics of the carboxyl terminal fragment of apolipoprotein III from *Manduca sexta*. *Biochemistry*. 1994; 33:13312–13320. [PubMed: 7947739]
71. Buchko GW, Rozek A, Zhong Q, Cushley RJ. Sequence-specific ¹H NMR assignments and secondary structure of a lipid-associating peptide from human ApoC-I: an NMR study of an amphipathic helix motif. *Pept Res*. 1995; 8:86–94. [PubMed: 7655189]
72. Chen GC, Guo LS, Hamilton RL, Gordon V, Richards EG, Kane JP. Circular dichroic spectra of apolipoprotein E in model complexes and cholesterol-rich lipoproteins: lipid contribution. *Biochemistry*. 1984; 23:6530–6538. [PubMed: 6529567]
73. Dieckmann GR, McRorie DK, Lear JD, Sharp KA, DeGrado WF, Pecoraro VL. The role of protonation and metal chelation preferences in defining the properties of mercury-binding coiled coils. *J Mol Biol*. 1998; 280:897–912. [PubMed: 9671558]
74. Kozlov MM, Chernomordik LV. A mechanism of protein-mediated fusion: coupling between refolding of the influenza hemagglutinin and lipid rearrangements. *Biophys J*. 1998; 75:1384–1396. [PubMed: 9726939]
75. Tanaka M, Dhanasekaran P, Nguyen D, Ohta S, Lund-Katz S, Phillips MC, Saito H. Contributions of the N- and C-terminal helical segments to the lipid-free structure and lipid interaction of apolipoprotein A-I. *Biochemistry*. 2006; 45:10351–10358. [PubMed: 16922511]

FIGURE 1. EPR spectroscopic resolution of protein structure

Electron paramagnetic resonance spectroscopy can provide a quantitative measure of the local environment (steric and chemical) of the spin-labeled (nitroxide) amino acid side-chain, as well as the distances between two spin-labeled side-chains (intra- or intermolecular). MTSL (methane thiosulfonate spin label) is used to covalently modify a thiol-group within the target protein (A), thereby introducing a stable unpaired electron. The shape of the EPR spectra (B), the derivative of microwave absorption as a function of the applied magnetic field strength, reports on the relative mobility of the spin labeled side chain. Examples of spectra that indicate low (K94, left) and high (A95, right) mobility are shown. Residues that exhibit high solvent mobility (A95, right) are likely to reside within a random coil and exhibits the sharpest spectral line shapes due to its higher degree of motional freedom, whereas more broadened spectra (appearing more attenuated and rounded) are exhibited by residues in a more ordered environment such as alpha helix (K94, left). The inverse values of the peak-to-peak distances (arrows) are used to provide a semi-quantitative measure of the mobility (inverse spectral linewidth or δ^{-1}), which gives insight into conformational state of the protein at that residue and the degree of steric hinderance. EPR power saturation experiments (C) with hydrophobic (O₂) or hydrophilic (CrOx) relaxation agents gives a measure of the chemical milieu proximal to the labeled side-chain (N₂ is used for background subtraction). Dotted lines indicate the applied power (mW) at the maximal central line amplitude. The analysis provide the accessibility parameters (Π) for the two relaxers, which are used to calculate a polarity index, or contrast function (Φ). Dipolar interaction of two (or more) spin-labels at a distance shorter $>20 \text{ \AA}$ results in

characteristically broadened spectra (**D**), and can be used to determine intra- or intermolecular distances.

FIGURE 2. EPR analysis of apoA-I's lipid-free secondary structure

The inverse central line width (δ^{-1} ; Gauss $^{-1}$), accessibility parameter data for the hydrophilic relaxer (Π_{CrOx}), hydrophobic relaxer (Π_{O_2}), and contrast value (Φ) are plotted as a function of residue number (A). These were jointly used to determine secondary structure. A periodicity of 3.6 residues per turn was used to identify the regions displaying α -helical character, indicated by yellow sinusoids. Dashed lines indicate regions where the helical structure pattern is less obvious in the mobility score analysis. A periodicity of 2 was used to identify β -strand structure (shown in blue). The linear representation of the resulting secondary structure model is compared to the secondary structure distribution defined by hydrogen-exchange/mass-spectroscopy experiments [13], and by X-ray crystallization [12] (B). α -helical structure is shown in yellow, random coil in green and β -strands in blue. Sites displaying intermolecular interaction are indicated with arrows.

FIGURE 3. Arrangement of apoA-I's secondary structural elements

ApoA-I assumes higher order associations at concentrations greater than 0.1 mg/ml. Because of this, specific nitroxide labeled locations within apoA-I elicit spin coupling due to their proximity to their counterpart on a paired molecule. Six locations within apoA-I were identified as exhibiting spin-coupling characteristics (residues 26, 44, 64, 167, 217, 226; red circles) (A). These sites of spin coupling define a central plane for the dimer and provide insight into how these secondary structures align into a tertiary context (B). The α-helices (tan) and β-strands (blue) are drawn to relative scale so that the position of turns (green) can be defined accurately.

FIGURE 4. The “Beta Clasp” model of lipid-free apoA-I

The combination of solvent accessibility, mobility (geometric constraint) and spin coupling was used to conceive the model. Whereas apoA-I typically is presented as a predominantly α -helical protein, this model (A), notably bears four anti-parallel β -strands, potentially arrayed in a single β -sheet (blue). This is surrounded by amphipathic α -helical bundle (tan) from the N-terminal, central, and C-terminal region of apoA-I. We hypothesize the β -strands serve as a hydrophobic core, which stabilizes the amphipathic α -helices in the absence of lipid. At the center of the protein lies the central loop, which is a sustained conformational feature present also on discoidal HDL. On the left is the cartoon structure of lipid-free apoA-I, in the unit cell conformation it would adopt within a dimer. On the right is a schematic representation of the apoA-I unit cell illustrating the relative position of functional elements. The residues of apoA-I that exhibit spin coupling are colored in red. (B) The unit cell monomers of apoA-I (as depicted in panel A) colored in orange and blue, respectively are aligned based on the residues that exhibit dipolar coupling (red). In the presence of lipid, the β -strands transition to α -helix and the protein adopts an extended helical structure. ApoA-I is a highly dynamic molecule so the lipid-free model represents the average state of the protein in solution and not necessarily the precise conformation of apoA-I.