Molecular Belt Models for the Apolipoprotein A-I Paris and Milano Mutations

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ABSTRACT Models for the binding of the 200-residue carboxy-terminal domain of two mutants of apolipoprotein A-I (apo A-I), apo A-I(R173C)$_{Milano}$ and apo A-I(R151C)$_{Paris}$, to lipid in discoidal high-density lipoprotein (HDL) particles are presented. In both models, two monomers of the mutant apo A-I molecule bind to lipid in an antiparallel manner, with the long axes of their helical repeats running perpendicular to the normal of the lipid bilayer to form a single disulfide-linked homodimer. The overall structures of the models of these two mutants are very similar, differing only in helix-helix registration. Thus these models are consistent with experimental observations that reconstituted HDL particles containing apo A-I$_{Milano}$ and apo A-I$_{Paris}$ are very similar in diameter to reconstituted HDL particles containing wild-type apo A-I, and they support the belief that apo A-I binds to lipid in discoidal HDL particles via the belt conformation.

INTRODUCTION

Apolipoprotein A-I (apo A-I) is the primary protein component of high-density lipoprotein (HDL) particles. The structure of apo A-I has been a subject of intense study because elevated levels of HDL correlate strongly with a reduced risk for atherosclerosis (Miller and Miller, 1975; Miller et al., 1977; Castelli et al., 1986). In addition to its ability to solubilize lipid, apo A-I serves as the major activator of lecithin cholesterol acyltransferase (LCAT) and promotes cellular cholesterol efflux (Rothblat et al., 1992).

Apo A-I is a major constituent of both spherical and nascent discoidal HDL particles. The size of both spherical and discoidal HDL particles is heterogeneous, with diameters ranging from 7 to 13 nm (Atkinson et al., 1974; Brouillette and Anantharamaiah, 1995). The smallest discoidal particles contain two monomers of apo A-I per HDL particle (Jonas et al., 1990). The 200-residue carboxy-terminal lipid-binding domain of apo A-I is characterized by eight 22-mer and two 11-mer repeats defined by the presence of proline residues at the beginning of most repeats. The sequence of these repeats is characteristic of that of an amphipathic α-helix (Segrest et al., 1974, 1992).

There are two prevailing models of the structure of apo A-I in discoidal HDL particles. In the picket fence model, the lipid-binding domain, made up of 8–10 short antiparallel helices, runs along the circumference of the disc with its hydrophobic face oriented toward the interior of the disc and the long axes of the helices parallel to the normal of the lipid bilayer (Tall et al., 1977; Nolte and Atkinson, 1992; Phillips et al., 1997). In the belt model, long continuous antiparallel amphipathic helices of the lipid-binding domain are arranged on the exterior of the disc with their hydrophobic faces curved toward the center of the disk and the long axes of the helices perpendicular to the normal of the lipid bilayer (Segrest, 1977, 1999; Borhani et al., 1997; Rogers et al., 1998; Koppaka et al., 1999).

An amino-terminal mutant of human apo A-I, apo Δ(1–43) A-I, in which the first 43 residues were deleted, has been reported previously (Rogers et al., 1997). Studies of apo Δ(1–43) A-I have shown this mutant to be very similar to wild-type apo A-I in lipid association with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) vesicles and activation of LCAT activity. Wild-type apo A-I and apo Δ(1–43) A-I have similar α helical composition when bound to lipid (~80%). Interestingly, unlike wild-type apo A-I, there is no statistically significant difference in the α-helicity for apo Δ(1–43) A-I between its lipid-free and lipid-bound conformations (Rogers et al., 1997). These results suggested that the structure of lipid-free apo Δ(1–43) A-I was similar to that of lipid-bound wild-type apo A-I. In the crystal structure of human apo Δ(1–43) A-I, residues 44–243 formed a continuous amphipathic α-helix. Two monomers of apo A-I associated with each other in an antiparallel manner in the crystal structure to form a dimer. The authors explained why the crystal structure supports the belief that apo A-I binds to lipid via the belt model (Borhani et al., 1997).

Earlier we proposed a detailed model for the binding of apo A-I to lipid in discoidal HDL particles in the belt conformation (Segrest et al., 1999). In this model, two monomers of apo A-I run around the edges of the lipid bilayer in an antiparallel fashion. Although the binding of apo A-I to lipid is driven by hydrophobic interactions, these interactions are nonspecific. In the belt model interhelical salt bridging plays a role in determining the specificity of helix-helix registration. The salt bridging hypothesis of the belt model is supported by evidence from a molecular dynamics simulation on a model HDL particle containing POPC and a model peptide (Sheldahl and Harvey, 1999).

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This molecular dynamics simulation showed that interhelical energetics were dominated by the formation of salt bridges, and the authors predicted that interhelical salt bridging was likely to play a role in determining helix-helix registration in belt models.

Two antiparallel amphipathic α-helices can interact with one another through either of two interfaces. Interhelical salt bridges could determine the preference for one antiparallel interface and could dictate the registration of the helices. This hypothesis is supported by our analysis of all possible helix-helix alignments in alternative belt models, in which we found that the model with maximum salt bridging corresponded to the alignment found in the apo $\Delta(1-43)$ A-I crystal structure (Segrest et al., 1999).

Two naturally occurring human mutations of apolipoprotein A-I have been isolated in which an arginine residue is mutated to cysteine. In apo A-I_{Milano} this substitution occurs at residue 173, whereas in apo A-I_{Paris} this substitution occurs at residue 151 (Weisgraber et al., 1983; Bruckert et al., 1997). These mutations are interesting because wild-type apo A-I does not contain any cysteine residues. Reconstituted HDL particles containing disulfide-linked homodimers of either apo A-I_{Milano} or apo A-I_{Paris} were similar to reconstituted HDL particles containing wild-type apo A-I in their ability to clear dimyristoylphosphatidylcholine (DMPC) emulsions and their ability to promote cholesterol efflux (Calabresi et al., 1997b; Franceschini et al., 1999; Daum et al., 1999). In both mutations, heterozygous individuals have decreased levels of HDL but are paradoxically at a reduced risk for atherosclerosis (Franceschini et al., 1980; Weisgraber et al., 1983; Bruckert et al., 1997). In addition, it has been shown that reconstituted HDL particles containing apo A-I_{Milano} or apo A-I_{Paris} are capable of LCAT activation, although at a decreased efficiency compared to reconstituted HDL particles containing wild-type apo A-I (Calabresi et al., 1997a; Daum et al., 1999). In individuals heterozygous for either mutation, HDL particles containing apo A-I_{Milano} or apo A-I_{Paris} form either disulfide-linked homodimers of apo A-I or heterodimers of apo A-I and apo A-II (Weisgraber et al., 1983; Bruckert et al., 1997). Sirtori et al. (1999) have previously presented a picket fence model for the binding of apo A-I_{Milano} to discoidal HDL particles.

Apo A-I_{Milano} forms reconstituted HDL particles with two distinct diameters containing either two or four molecules of apo A-I present as disulfide linked homodimers. These reconstituted HDL particles are of a size comparable to that of reconstituted HDL particles containing wild-type apo A-I (Calabresi et al., 1997a). Apo A-I_{Paris} forms reconstituted HDL particles with three distinct diameters. The majority of the reconstituted HDL particles containing apo A-I_{Paris} were found to have a mean diameter equal to that of reconstituted HDL particles containing wild-type apo A-I (Daum et al., 1999). The models for apo A-I_{Milano} and apo A-I_{Paris} presented in this paper represent the smaller sized particles; they contain two monomers of mutant apo A-I and present a single disulfide-linked homodimer per reconstituted HDL particle.

Based upon the belt model for apo A-I published previously, we have constructed similar models for disulfide-linked homodimers of apo A-I_{Milano} and apo A-I_{Paris} bound to discoidal HDL particles. We argue that the models presented for apo A-I_{Milano} and apo A-I_{Paris} agree with the experimental data for the size and shape of reconstituted HDL particles containing the Paris and Milano mutations.

METHODS

The belt model of apo A-I published previously (Segrest et al., 1999) was used as a starting structure. Modeling was carried out with the Quanta/Charmm package (Molecular Simulations, San Diego, CA) on an SGI Octane workstation. For the apo A-I_{Milano} mutant, Arg^{173} on both monomers was mutated to a cysteine. One of the monomers was then rotated with respect to the other one until Cys^{173} was directly across from the corresponding cysteine residue in the other monomer. A disulfide bridge was then added between the two gamma sulfurs, which were $\sim$2.0 Å apart. One hundred cycles of steepest descent energy minimization were then carried out to eliminate unacceptable steric interactions. The same procedure was then repeated for apo A-I_{Paris}, with Arg^{151} mutated to a cysteine.

RESULTS

Models for Apo A-I Milano and Paris mutations

Shown in Fig. 1 is a helical wheel diagram of helical repeats 2–9 from the carboxy-terminal lipid-binding domain of apo A-I. When these amphipathic α-helical repeats of apo A-I are plotted on a helical wheel as an α11/3-helix, the hydrophobic face of the resulting 198-residue amphipathic α-helix is clearly continuous along one face of the helix (Segrest et al., 1999). We assume that the transformation of an idealized α-helix to an α11/3-helix would involve some small energy costs that would be offset by lipid association. Analysis of the individual 22mer helical domains in apo A-I, using the 76 algorithm (Palgunachari et al., 1996; Mishra and Palgunachari, 1996) to calculate affinity, shows that helices 2–9, individually or together, have a significantly greater lipid affinity as α11/3-helices than as idealized α-helices. We hypothesize that this increased affinity provides more free energy than is required to convert a standard α-helix into the α11/3-helix conformation. In addition, 76 affinity calculations indicate that the lipid affinity of helix 10 is largely insensitive to the idealized α-helix–to–α11/3-helix transition, and helix 1 prefers the idealized α-helical conformation.

The locations of the arginine-to-cysteine substitutions in apo A-I_{Milano} and apo A-I_{Paris} are particularly striking because they occur exactly 22 residues from one another in the amino acid sequence. When the carboxy-terminal domains of apo A-I_{Milano} and apo A-I_{Paris} are plotted on the α11/3-helix, residues 151 and 173 both fall on position 9 of the helical wheel. This, combined with the phenotypic similar-

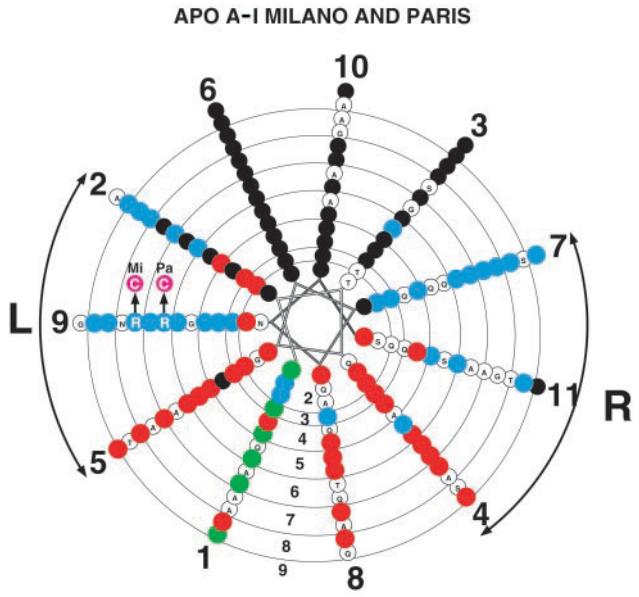

FIGURE 1 Helical wheel of helices 2–9 (residues 66–219) of human apo A-I. This view is down the long axis of the helices from the N-terminal toward the C-terminal end, with a pitch of 11/3 residues per turn. Helices are numbered on the concentric circles. Hydrophobic residues are indicated by solid black circles, acidic residues by solid red circles, basic residues by solid blue circles, and proline residues by solid green circles. The locations of the Milano and Paris mutations are indicated by purple circles. This figure was generated by WHEEL (Jones et al., 1992).

ities of the two mutants, suggests that apo A-I_{Milano} and apo A-I_{Paris} are structurally very similar.

There are two possible orientations in which two molecules of apo A-I can interact with one another in an antiparallel manner to form a dimeric belt. We have previously referred to these two orientations as the LL and RR orientations (Segrest et al., 1999). The LL interface brings the side chains at positions 2, 5, and 9 of the helical wheel into position to participate in interhelical salt bridging, and this is the orientation that is observed in the crystal structure of human apo Δ(1–43) A-I (Borhani et al., 1997). The LL alignment is the orientation in which cysteines at position 9 of the helical wheel can be opposed to form a disulfide bridge. In this orientation, residues at position 9 of the helical wheel are in the middle of the docking interface, whereas in the RR orientation, these residues would lie on opposite sides of the helices and would be unable to interact with each other. As a result, only the LL interface is favorable for the formation of disulfide linked homodimers of apo A-I_{Milano} or apo A-I_{Paris}.

As a consequence of the LL orientation of apo A-I in the belt model, cysteine residues located at position 9 of the helical wheel, as they are in both the apo A-I_{Milano} and apo A-I_{Paris} mutations, would be in a better position to form interhelical disulfide bonds than mutations at positions 2 or 5 on the helical wheel. To make disulfide bridges between residues at positions 2 or 5 of the helical wheel, the structure of the belt model would have to be distorted. However, cysteine can be substituted for arginine at position 9 on the helical wheel, preserving the 10-Å distance between the centers of the neighboring helices and providing good coverage of the hydrophobic surface area of the lipid in the HDL particle.

Fig. 2 is a Ribbons diagram of the belt model for a disulfide-linked homodimer of apo A-I_{Milano} bound to discoidal HDL particles. The overall structure of the resulting model is very similar to our previously published belt model for wild-type apo A-I (Segrest et al., 1999). The hydrophobic faces of the helices are oriented toward the center of the disk to bind lipid, and interhelical interactions in the model are dominated by salt bridges that form between positions 2 and 2′, as well as between positions 5 and 9′, and 9 and 5′ on the helical wheel (Fig. 1). Also shown in Fig. 2 is the location of the disulfide bond in the apo A-I_{Milano} homodimer. To form the disulfide bond between the two molecules of apo A-I_{Milano} in the helix-helix registration published by Segrest et al., the two monomers simply need to rotate about the discoidal core until the two cysteine residues are brought into position to form a covalent bond. This rotation forces a helix-helix registration that is different from the one predicted for wild-type apo A-I in our previous paper. A similar model has been built for the structure of a disulfide-linked homodimer of apo A-I_{Paris} (data not shown). Although the overall structure of the belt model would remain the same, different salt bridges would be predicted to form between helices in the dimer.

Implications for HDL structure

Fig. 3 depicts an analysis of salt bridging as a function of helix-helix registration in the LL interface, using a previously published empirical salt bridge scoring function (Segrest et al., 1999). The wild-type orientation corresponds to the helix-helix registration observed in the crystal structure (Borhani et al., 1997). Using an α11/3-helix results in a

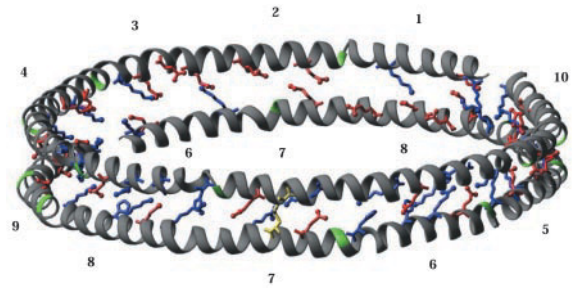

FIGURE 2 Ribbon diagram of a model for a disulfide-linked homodimer of apo A-I_{Milano} for helices 1–10 (residues 44–241). The location of proline residues is indicated in green. Only acidic and basic side chains at positions 2, 5, and 9 in the helical wheel are shown. Acidic side chains are in red, basic side chains in blue, and the location of the cystine disulfide bridge in yellow. This figure was generated by RIBBONS (Carson, 1997).

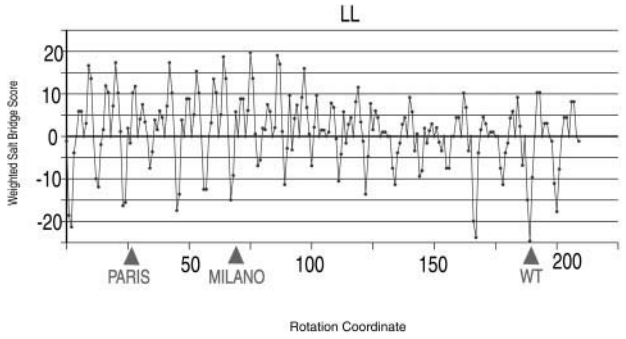

FIGURE 3 Weighted salt bridge scores of the LL docking interface. This figure was generated using ALIGN, a modification of the HELNET program (Jones et al., 1992; Segrest et al., 1999). The rotation coordinate (*x* axis) represents the rotation of one monomer of apo A-I in the belt model relative to the other in one-residue increments. Points along the *x* axis corresponding to the helix-helix registrations for apo A-I_{Milano} (MILANO), apo A-I_{Paris} (PARIS), and wild-type apo A-I (WT) are labeled. The weighted salt bridge score for each rotational orientation is indicated on the *y* axis.

favorable helix-helix interaction as one monomer is rotated relative to the other at intervals of 11 residues (Segrest et al., 1999). This periodicity in the weighted salt bridge score is particularly striking, considering the fact that the cysteine substitutions in apo A-I_{Milano} and 151 in apo A-I_{Paris} are exactly 22 residues apart. The orientations that bring residues 173 into position to form a disulfide bond in apo A-I_{Milano} (and residues 151 in apo A-I_{Paris}) are labeled in Fig. 3. These two orientations also have favorable weighted salt-bridge scores. Thus the belt model is consistent with experimental observations that reconstituted HDL particles containing disulfide-linked homodimers of apo A-I_{Milano} and apo A-I_{Paris} would be structurally very similar to each other and predicts that these mutants would have favorable interhelical interactions, based upon their potential to form salt bridges.

DISCUSSION

If the belt model proposed by Segrest et al. is correct, the function of interhelical salt bridges in HDL particles is to provide specificity for helix-helix interactions while adding little to the overall stability of the protein-lipid complex. When we use the weighted salt bridge scoring function in the ALIGN program, the helix-helix registration observed in the crystal structure of apo $\Delta(1-43)$ A-I is the most favored orientation. Because of the presence of a covalent bond between two monomers of apo A-I in disulfide-linked homodimers of apo A-I_{Milano} and apo A-I_{Paris}, the helix-helix registration in the belt model would be locked into rotational orientations different from that seen in the crystal structure, effectively forcing the dimer into an orientation with different salt bridging interactions. However, the helix-helix registrations corresponding to the apo A-I_{Milano} and apo A-I_{Paris} models presented in this paper are also predicted by ALIGN to be favorable interactions (Fig. 3).

Given the periodicity of the 22-mer repeats seen in the sequence of the carboxy-terminal lipid-binding domain of apo A-I, the existence of two cysteine mutations occurring exactly 22 residues apart in the sequence is probably not coincidental. As shown in Fig. 3, ALIGN predicts that a rotation of one monomer relative to the other would produce a favorable interhelical salt bridging pattern every 11 residues. These belt models for wild-type apo A-I, apo A-I_{Milano}, and apo A-I_{Paris} are dependent upon the presence of the α11/3-helix discussed previously. In addition to these observations, there are experimental data showing that both apo A-I_{Milano} and apo A-I_{Paris} form reconstituted HDL particles very similar in size and shape to reconstituted HDL particles containing wild-type apo A-I (Calabresi et al., 1997a; Daum et al., 1999). Calabresi et al. have shown that reconstituted HDL particles containing a single disulfide-linked homodimer of apo A-I_{Milano} have diameters very similar to those of reconstituted HDL particles containing two monomers of wild-type apo A-I (Calabresi et al., 1997a,b). Daum et al. (1999) have shown similar results for apo A-I_{Paris}. The authors of both papers have also shown that the apo A-I_{Milano} and apo A-I_{Paris} mutations do not affect lipid affinity or the promotion of cholesterol efflux when compared to wild-type apo A-I. In addition, both apo A-I_{Milano} and apo A-I_{Paris} are capable of activating LCAT activity, although at decreased efficiency compared to wild-type apo A-I. Our models for wild-type apo A-I, apo A-I_{Milano}, and apo A-I_{Paris} are similar in their overall structure, an observation consistent with experimental data that reconstituted HDL particles containing apo A-I_{Milano} or apo A-I_{Paris} are very similar in size and shape to reconstituted HDL particles containing wild-type apo A-I (Calabresi et al., 1997b; Daum et al., 1999).

Simple packing arguments favor belt models over picket fence models. Fig. 4 shows the relationship between the

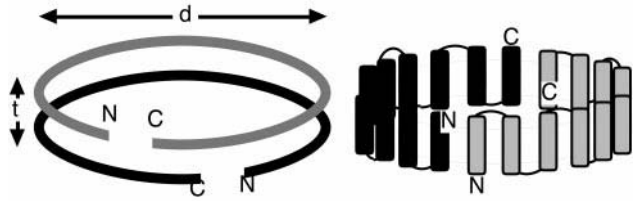

FIGURE 4 Alternative geometries for two monomers of the apo A-I lipid-binding domain (residues 44–243) bound to discoidal HDLs. The protein must cover a discoidal particle of diameter *d* and thickness *t*. A typical small HDL has a diameter of ~90–100 Å (Jonas et al., 1990) and a hydrocarbon thickness of ~30 Å (Wiener and White, 1992). The circumference is thus on the order of 300 Å, which is easily covered in the belt model (*left*), because this perfectly matches an α-helix containing 200 residues with a standard rise per residue of 1.5 Å. Picket fence models contain 8–10 α-helices, for which the interhelical spacing would have to be 15 Å or more to cover the circumference.

dimensions of a discoidal HDL particle and the alternative arrangements of apo A-I around the disk. A typical small discoidal HDL has a diameter of ~90–100 Å (Jonas et al., 1990), with a hydrocarbon thickness of ~30 Å (Wiener and White, 1992).

Picket fence models (Tall et al., 1977; Brasseur et al., 1990, 1992; Sparks et al., 1992; Phillips et al., 1997) with two molecules of apo A-I and 160 molecules of POPC (Jonas et al., 1990) are based on the observation that apo A-I is punctuated by prolines at regular intervals, generally 22 residues, and on the assumption that prolines mark the beginnings of α-helices. With roughly 20 residues per α-helix and a standard rise per residue of 1.5 Å, such helices would span the thickness of a typical bilayer quite nicely. Unfortunately, picket fence models have only 8–10 α-helices, and with a helix-helix packing distance of ~10 Å observed in crystal structures, the maximum circumference that can be covered in the picket fence geometry is only ~200 Å. Even if the water-soluble amino-terminal domain of 43 residues is used to plug the gap, it could provide at most two more α-helices per monomer. The dimer would then have a maximum of 24 α-helices, for a circumference of 240 Å, giving a diameter of 76 Å, which is considerably smaller than that observed in reconstituted HDL particles containing two monomers of wild-type apo A-I (Jonas et al., 1990). The packing problem with picket fence models is clearly demonstrated by figures in previously published models (Brasseur et al., 1990, 1992; Phillips et al., 1997), where an interhelix distance of ~15 Å is required to cover the perimeter of the disk, in at least one case leaving gaps through which the hydrophobic lipid tails are clearly visible (Phillips et al., 1997).

In contrast, the 200 residues of the lipid-binding domain of apo A-I (residues 44–243) would cover the circumference of a disk with the same stoichiometry almost perfectly in the belt model (Segrest et al., 1999), assuming a rise per residue of 1.5 Å for an α-helix. Slightly larger diameters can be accommodated by using the globular amino-terminal domain to plug any gap (Segrest et al., 1999). Because each α-helix has a diameter of 10 Å, the belt is only ~20 Å across, so the bilayer is somewhat thinner at the periphery than at the center.

Sirtori et al. have argued that the picket fence model of apo A-I is compatible with apo A-I_{Milano} (Sirtori et al., 1999). The all-atom picket fence model they have published has serious problems. First, it requires that the last ~55 residues of apo A-I not associate with the lipid core, to promote the formation of the intermolecular disulfide bridge between residues 173 on each monomer. This appears highly unlikely, given the fact that that the carboxy-terminal region of apo A-I is one of the strongest lipid-binding domains. Second, it requires an interhelical distance of 15 Å to cover the circumference of the disk, which we believe to be unfavorable for reasons discussed previously.

The picket fence models do not appear to explain how a single disulfide-linked homodimer of apo A-I_{Milano} or apo A-I_{Paris} could form reconstituted HDL particles while still retaining the same overall structure as reconstituted HDL particles containing two monomers of wild-type apo A-I. As described above, a picket fence model for apo A-I_{Milano} would require that a substantial portion of the lipid-binding domain be removed from the surface of the HDL particle for the cysteine residues to be brought into position to form a disulfide bond. Using similar logic, we conclude that ~80 residues would have to be excluded from lipid binding in reconstituted HDL particles containing apo A-I_{Paris} to form a disulfide-linked homodimer. This would result in reconstituted HDL particles containing two monomers of apo A-I_{Milano} with much smaller diameters than HDL particles containing wild-type apo A-I, and reconstituted HDL particles containing two monomers of apo A-I_{Paris} would be predicted to have even smaller diameters. Each apo A-I mutant containing a disulfide-linked homodimer would therefore differ dramatically in its overall structure from wild-type apo A-I and other cysteine-containing mutants.

The belt model, however, predicts that relatively small-scale structural rearrangements involving rotation of the mutant apo A-I proteins, when bound to lipid, would be sufficient to change the helix-helix registration observed in the crystal structure to one accommodating the presence of disulfide bonds linking the two monomers. The observation that the apo A-I_{Milano} and apo A-I_{Paris} mutations occur exactly 22 residues apart in the sequence, combined with the observation that the overall structures of reconstituted HDL particles containing these mutants are very similar in size and shape to that of wild-type apo A-I, provides strong evidence in support of the belt model. The structural similarity between the belt models could explain the experimental observations that the Milano and Paris mutants exhibit rates of clearance for DMPC emulsions in vitro and cholesterol efflux similar to those of wild-type apo A-I. This, too, argues that there cannot be major structural differences between the wild-type and mutant apo A-I molecules in HDL particles.

Although the structure and activities of apo A-I_{Milano} and apo A-I_{Paris} are similar to those of wild-type apo A-I, their ability to activate LCAT is decreased. It is known that the central region of apo A-I, particularly repeat 6 (residues 143–164), is important in LCAT activation (Fielding and Fielding, 1995). The major hypothesis of the belt model presented by Segrest et al. argues that salt-bridging interactions between helices favor the helix-helix registration observed in the crystal structure over other possible registrations, as shown in Fig. 3 (Segrest et al., 1999; Borhani et al., 1997). That the disulfide-linked homodimers observed in apo A-I_{Milano} and apo A-I_{Paris} are effectively locked into different helix-helix registrations suggests that the helix-helix registration of the central domain may be crucial to the regulation of LCAT activity.

These models can be tested experimentally. Based upon the structural and functional similarities between apo A-I_{Milano} and apo A-I_{Paris}, the 11mer periodicity observed in the weighted salt bridge score shown in Fig. 3, and the fact that the Milano and Paris mutations occur at the same position on the helical wheel shown in Fig. 1, the belt model predicts that similar mutants of apo A-I could be generated. The substitution of a cysteine residue for any residue in the wild-type sequence of apo A-I occurring at position 9 of the helical wheel should generate mutants with structural and functional properties similar to those of apo A-I_{Milano} and apo A-I_{Paris}. The belt model predicts that, like the Paris and Milano mutations, these mutants would be able to form reconstituted HDL particles containing disulfide-linked homodimers of mutant apo A-I molecules with sizes and shapes similar to those of reconstituted HDL particles containing wild-type apo A-I and should show a similar ability to clear DMPC emulsions and promote cholesterol efflux. To form reconstituted HDL particles, the picket fence model predicts that large-scale structural differences would be required for each mutation and that these reconstituted HDL particles would differ dramatically in structure and function from reconstituted HDL particles containing wild-type apo A-I.

Coordinates of the models are available at http://uracil.cmc.uab.edu/Publications.

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REFERENCES

Atkinson, D., M. A. F. Davis, and R. B. Leslie. 1974. The structure of a high density lipoprotein (HDL_3) from porcine plasma. *Proc. R. Soc. Lond. B.* 186:165–180.

Borhani, D. W., D. P. Rogers, J. A. Engler, and C. G. Brouillette. 1997. Crystal structure of truncated human apolipoprotein A-I suggests a lipid-bound conformation. *Proc. Natl. Acad. Sci. USA.* 94:12291–12296.

Brasseur, R., J. De Meutter, B. Vanloo, E. Goormaghtigh, J. M. Ruysschaert, and M. Rosseneu. 1990. Mode of assembly of amphipathic helical segments in model high-density lipoproteins. *Biochim. Biophys. Acta.* 1043:245–252.

Brasseur, R., L. Lins, B. Vanloo, and J. M. Ruysschaert. 1992. Molecular modeling of the amphipathic helices of the plasma apolipoproteins. *Proteins Struct. Funct. Genet.* 13:246–257.

Brouillette, C. G., and G. M. Anantharamaiah. 1995. Structural models of human apolipoprotein A-I. *Biochim. Biophys. Acta.* 1256:103–129.

Bruckert, E., A. von Eckardstein, H. Funke, I. Beucler, H. Wiebusch, G. Turpin, and G. Assmann. 1997. The replacement of arginine by cysteine at residue 151 in apolipoprotein A-1 Milano produces a phenotype similar to that of apolipoprotein A-1. *Atherosclerosis.* 128:121–128.

Calabresi, L., G. Franceschini, A. Burkybile, and A. Jonas. 1997a. Activation of lecithin cholesterol acyltransferase by a disulfide-linked apolipoprotein A-I dimer. *Biochem. Biophys. Res. Commun.* 232:345–349.

Calabresi, L., G. Vecchio, F. Frigerio, L. Vavassori, C. R. Sirtori, and G. Franceschini. 1997b. Reconstituted high-density lipoproteins with a disulfide-linked apolipoprotein A-1 dimer: evidence for restricted particle size heterogeneity. *Biochemistry.* 36:12428–12433.

Carson, M. 1997. Ribbons. *Methods Enzymol.* 277:493–505.

Castelli, W. P., R. J. Garrison, P. W. F. Wilson, R. D. Abbott, S. Kalousdian, and W. B. Kannel. 1986. Incidence of coronary heart disease and lipoprotein cholesterol levels. *JAMA.* 256:2835–2838.

Daum, U., C. Lanager, N. Duverger, F. Emmanuel, P. Benoit, P. Denefle, A. Chirazi, P. Cullen, P. H. Pritchard, E. Bruckert, G. Assmann, and A. von Eckardstein. 1999. Apolipoprotein A-1($R151C)_{Paris}$ is defective in activation of lecithin: cholesterol acyltransferase but not in initial lipid binding, formation of reconstituted lipoproteins, or promotion of cholesterol efflux. *J. Mol. Med.* 77:614–622.

Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* 36:211–228.

Franceschini, G., L. Calabresi, G. Chiesa, C. Parolini, C. R. Sirtori, M. Canavesi, and F. Bernini. 1999. Increased cholesterol efflux potential of sera from ApoA-I_{Milano} carriers and transgenic mice. *Arterioscler. Thromb. Vasc. Biol.* 19:1257–1262.

Franceschini, G., C. R. Sirtori, A. Capurso, K. H. Weisgraber, and R. W. Mahley. 1980. A-I_{Milano} Apoprotein-decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and without clinical atherosclerosis in an Italian family. *J. Clin. Invest.* 66:892–900.

Jonas, A., J. H. Wald, K. L. H. Toohill, E. S. Krul, and K. E. Kézdy. 1990. Apolipoprotein A-I structure and lipid properties in homogeneous, reconstituted spherical and discoidal high density lipoproteins. *J. Biol. Chem.* 265:22123–22129.

Jones, M. K., G. M. Anantharamaiah, and J. P. Segrest. 1992. Computer programs to identify and classify amphipathic α helical domains. *J. Lipid Res.* 33:287–296.

Koppaka, V., L. Silvestro, J. Engler, C. Brouillette, and P. H. Axelsen. 1999. The structure of human lipoprotein A-I. *J. Biol. Chem.* 274: 14541–14544.

Miller, G. J., and N. E. Miller. 1975. Plasma-high-density-lipoprotein concentration and development of ischaemic heart disease. *Lancet.* i:16–19.

Miller, N. E., D. S. Thelle, O. H. Førde, and O. D. Mjøs. 1977. The Tromsø Heart Study: high-density lipoprotein and coronary heart disease: a prospective case-control study. *Lancet.* i:965–968.

Mishra, V. K., and M. N. Palgunachari. 1996. Interaction of model class A_1, class A_2, and class Y amphipathic helical peptides with membranes. *Biochemistry.* 35:11210–11220.

Nolte, R. T., and D. Atkinson. 1992. Conformational analysis of apolipoprotein A-I and E-3 based on primary sequence and circular dichroism. *Biophys. J.* 63:1221–1239.

Palgunachari, M. N., V. K. Mishra, S. Lund-Katz, M. C. Phillips, S. O. Adeyeye, S. Alluri, G. M. Anantharamaiah, and J. P. Segrest. 1996. Only the two end helixes of eight tandem amphipathic helical domains of human Apo A-I have significant lipid affinity: implications for HDL assembly. *Arterioscler. Thromb. Vasc. Biol.* 16:328–338.

Phillips, J. C., W. Wriggers, Z. Li, A. Jonas, and K. Schulten. 1997. Predicting the structure of apolipoprotein A-1 in reconstituted high-density lipoprotein disks. *Biophys. J.* 73:2337–2346.

Rogers, D. P., C. G. Brouillette, J. A. Engler, S. W. Tendian, L. Roberts, V. K. Mishra, G. M. Anantharamaiah, S. Lund-Katz, M. C. Phillips, and M. J. Ray. 1997. Truncation of the amino terminus of human apolipoprotein A-I substantially alters only the lipid-free conformation. *Biochemistry.* 36:288–300.

Rogers, D. P., L. M. Roberts, J. Lebowitz, G. Datta, G. M. Anantharamaiah, J. A. Engler, and C. G. Brouillette. 1998. The lipid-free structure of apolipoprotein A-I: effects of amino-terminal deletions. *Biochemistry.* 37:11714–11725.

Rothblat, G. H., F. H. Mahlberg, W. J. Johnson, and M. C. Phillips. 1992. Apolipoproteins, membrane cholesterol domains, and the regulation of cholesterol efflux. *J. Lipid Res.* 33:1091–1097.

Segrest, J. P. 1977. Amphipathic helixes and plasma lipoproteins: thermodynamic and geometric considerations. *Chem. Phys. Lipids.* 18:7–22.

Segrest, J. P., R. L. Jackson, J. D. Morrisett, and A. M. Gotto, Jr. 1974. A molecular theory of lipid-protein interactions in the plasma lipoproteins. *FEBS Lett.* 38:247–253.

Segrest, J. P., M. K. Jones, H. De Loof, C. G. Brouillette, Y. V. Venkatachalapathi, and G. M. Anantharamaiah. 1992. The amphipathic helix in the exchangeable apolipoproteins: a review of secondary structure and function. *J. Lipid Res.* 33:141–166.

Segrest, J. P., M. K. Jones, A. E. Klon, C. J. Sheldahl, M. Hellinger, H. De Loof, and S. C. Harvey. 1999. A detailed molecular belt model for apolipoprotein A-I in discoidal high density lipoprotein. *J. Biol. Chem.* 274:31755–31758.

Sheldahl, C., and S. C. Harvey. 1999. Molecular dynamics on a model for nascent high-density lipoprotein: role of salt bridges. *Biophys. J.* 76: 1190–1198.

Sirtori, C. R., L. Calabresi, and G. Franceschini. 1999. Recombinant apolipoproteins for the treatment of vascular diseases. *Atherosclerosis.* 142:29–40.

Sparks, D. L., M. C. Phillips, and S. Lund-Katz. 1992. The conformation of apolipoprotein A-I in discoidal and spherical recombinant high density lipoprotein particles. *J. Biol. Chem.* 267:25830–25838.

Tall, A. R., D. M. Small, R. J. Deckelbaum, and G. Shipley. 1977. Structure and thermodynamic properties of high density lipoprotein recombinants. *J. Biol. Chem.* 252:4701–4711.

Weisgraber, K. H., S. C. Rall, Jr., T. P. Bersot, R. W. Mahley, G. Franceschini, and C. R. Sirtori. 1983. Apolipoprotein A-I_{Milano}. *J. Biol. Chem.* 258:2508–2513.

Wiener, M. C., and S. H. White. 1992. Structure of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of x-ray and neutron diffraction data. *Biophys. J.* 61:428–433.