Molecular Dynamics Simulations on Discoidal HDL
Particles Suggest a Mechanism for Rotation in the
Apo A-I Belt Model

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Apolipoprotein A-I (apo A-I) is the major protein component of high-density lipoprotein (HDL) particles. Elevated levels of HDL in the bloodstream have been shown to correlate strongly with a reduced risk factor for atherosclerosis. Molecular dynamics simulations have been carried out on three separate model discoidal high-density lipoprotein particles (HDL) containing two monomers of apo A-I and 160 molecules of palmitoyloleoylphosphatidylcholine (POPC), to a time-scale of 1 ns. The starting structures were on the basis of previously published molecular belt models of HDL consisting of the lipid-binding C-terminal domain (residues 44–243) wrapped around the circumference of a discoidal HDL particle. Subtle changes between two of the starting structures resulted in significantly different behavior during the course of the simulation. The results provide support for the hypothesis of Segrest et al. that helical registration in the molecular belt model of apo A-I is modulated by intermolecular salt bridges. In addition, we propose an explanation for the presence of proline punctuation in the molecular belt model, and for the presence of two 11-mer helical repeats interrupting the otherwise regular pattern of 22-mer helical repeats in the lipid-binding domain of apo A-I.

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Introduction

Apolipoprotein A-I (apo A-I) is the primary protein component of nascent (discoidal) and circulating (spherical) high-density lipoprotein (HDL) particles. It has been well established that levels of circulating HDL particles in the bloodstream correlate with a reduced risk of atherosclerosis. In addition to the structural role apo A-I plays in HDL particles, it has been shown to exhibit a range of biological activities believed to be responsible for HDL’s protective effects against atherosclerosis. Apo A-I is capable of solvating lipids and cholesterol, spontaneously forming discoidal reconstituted high-density lipoprotein (rHDL) complexes in vitro. Apo A-I has also been shown to promote cholesterol efflux from peripheral tissues, activate lecithin:cholesteryl acyltransferase (LCAT), an enzyme which catalyzes the esterification of cholesterol taken up by apo A-I, and deliver the cholesteryl ester to the liver through receptor-mediated uptake.

The C-terminal lipid-binding domain of apo A-I (residues 44–243) consists of ten class A amphipathic α-helical repeats punctuated at regular intervals by proline residues. Eight of these repeats are 22 residues in length, while two of them are 11 residues in length. There have been two competing models for the binding of apo A-I to the circumference of discoidal HDL particles. The first of these is commonly referred to as the picket-fence model, in which the long axes of the helical repeats are perpendicular to the plane of the lipid bilayer. In this model, the regularly spaced proline residues function as helix breakers and adjacent helical repeats are connected by β-turns. This model was a logical starting point for the investigation of the structure of apo A-I in discoidal HDL particles because a 22-mer amphipathic α-helix is long enough to span the hydrophobic distance across the lipid bilayer if a standard rise/residue of 1.5 Å is assumed.
Previously, Phillips et al. have performed a short molecular dynamics (MD) simulation for the picket-fence model.8 The alternative model for the binding of apo A-I to discoidal HDL particles is commonly known as the molecular belt model. In this model, the molecules of apo A-I form a continuous amphipathic α-helix with the long axes of the helical repeats parallel to the plane of the lipid bilayer. The overall structure of a single monomer of apo A-I in this model is both planar and circular, with the continuous hydrophobic face of the lipid-binding domain oriented towards the lipid.4,10,11–13 We have previously proposed an all-atom model for the molecular belt model consisting of two monomers of apo A-I and 160 molecules of palmitoyloleoylphosphatidylcholine (POPC).13

Figure 1 is a wheel diagram showing helical repeats of 4 and 6 as they interact in the molecular belt model. Helix 4 is viewed from the carboxy to the amino terminus, while helix 6 is viewed from the amino to the carboxy terminus. Both helices fit the pattern of the class A amphipathic α-helix. In this view, the hydrophobic face of the helices are oriented upwards, and the hydrophilic face of the helices are oriented downwards. The negatively charged side-chains are mainly concentrated in the hydrophilic phase, and the positively charged residues are localized at the interface between the hydrophobic and hydrophilic faces of the helices. Figure 1 shows how in the molecular belt model, interhelical salt bridges are predicted to form between residues at position 9 of one helix and 5 of the other, as well as between residues at position 2 of both helices. In this example, there are three possible interhelical salt bridges.

Using our molecular belt model as a starting point, we were able to propose additional models for two naturally occurring mutations of human apo A-I, apo A-I Milano in which Arg173 is mutated to a cysteine, and apo A-I Paris, in which Arg151 is mutated to a cysteine.14 In individuals heterozygous for either of these mutations, the mutant apo A-I molecules are found on HDL particles as disulfide-linked homodimers or as heterodimers with apo A-II.15,16 Models for these mutant HDLs were constructed from the molecular belt of Segrest et al. by the rotation of the monomers around the circumference of the lipid bilayer until the two Cys173 residues (apo A-I Milano) or until the two Cys151 residues (apo A-I Paris) were juxtaposed, bringing them close enough to form intermolecular disulfide bonds. As a result, both of these models retained the overall structural features of the double belt model, but differed in their interhelical interactions.

For the 1 ns MD simulations reported here, the molecular belt model of Segrest et al. (LL5/5) and the model for apo A-I Milano (C173/C173) were selected to be the first two systems studied. A third model discoidal HDL particle was chosen with a helix–helix registration differing from the LL5/5 and Milano models on the basis of the heuristic salt bridge scoring function implemented in ALIGN.13 In order to test the hypothesis that interhelical salt bridges are critical in deciding the specificity of the helix–helix registration in molecular belt models,13,17 we built a model with a registration close to that of the wild-type LL5/5 registration, but that was predicted by the ALIGN algorithm to have unfavorable interhelical salt-bridging interactions. The resulting model consists...
of two wild-type monomers of apo A-I rotated by four residues, or approximately a single turn of an 11/3 helix relative to the LL5/5 registration, juxtaposing Lys133 from each monomer with the same residue on the opposite monomer. We will refer to the model with this registration as the K133/K133 model. Because the K133/K133 model represents essentially the same overall structure as the LL5/5 alignment with a small perturbation, we shall refer to the LL5/5 rotamer of Segrest et al. as the G129/G129 rotamer due to the fact that Gly129 from each monomer is juxtaposed in this model. The rotational alignments used in these simulations are shown in Figure 2. According to ALIGN, the K133/K133 rotamer should have a greatly reduced number of interhelical salt bridges and more charge–charge appositions compared to the G129/G129 rotamer, resulting in a less energetically favorable structure.

A number of biophysical properties were calculated from the resulting coordinate trajectories and compared with experimental data to evaluate the stability as well as the dynamic aspects of the model systems over time. We find that the values calculated from all three simulations are in excellent agreement with existing experimental data. We also observe significant differences among the three simulations, which may have critical implications for the structure and function of apo A-I when bound to discoidal HDL particles.

Results

Overall structure of the three systems

Figure 3(a) is a Ribbons diagram of the fully solvated, annealed structure of the model rHDL particle with the two monomers of apo A-I in the G129/G129 rotamer. The view is of the helix-5/helix-5 interface as seen from the outside of the HDL particle, looking perpendicular to the bilayer.
Table 1. The solvent-accessible surface area for the methylene carbon atoms and the total SASA for each of the three model systems used in the simulations. Values were averaged from the SASA every 10 ps during the course of the simulation.

<table>
<thead>
<tr>
<th>Rotamer</th>
<th>Methylene carbon atoms (Å²)</th>
<th>Total (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G129/G129</td>
<td>2664.2(± 100.3)</td>
<td>56818.1(± 853.9)</td>
</tr>
<tr>
<td>K133/K133</td>
<td>2234.5(± 98.4)</td>
<td>55124.6(± 836.6)</td>
</tr>
<tr>
<td>C173/C173</td>
<td>2984.9(± 120.3)</td>
<td>62228.5(± 967.9)</td>
</tr>
</tbody>
</table>

normal. Overall, the packing of the lipid is good, with no gaps between the chains of the POPC molecules and the hydrophobic face of the amphipathic α-helices of the two apo A-I monomers.

The solvent accessible surface area (SASA) for the methylene carbon atoms in the POPC molecules and the total SASA for the entire HDL particle for all three simulations are shown in Table 1. The total SASA for the G129/G129 and K133/K133 simulations is comparable, while in the K133/K133 model there is clearly less methylene SASA, indicating that the protein in this model does a better job of protecting the methylene groups from solvation. Very little of the surface area of the methylene groups is exposed to solvent, and contributes little to the overall SASA for all three simulations. This indicates that the 13 Å interhelical distance observed in Figure 3(a) before the 1 ns MD simulation is sufficient to protect the hydrophobic phase of the lipid bilayer from solvation. This is not intuitively obvious because Wiener & White have previously reported that the experimentally observed effective hydrophobic thickness of a DOPC bilayer is 28.6 Å.18 The total hydrophobic thickness of the POPC bilayer that can be effectively covered by the two monomers of apo A-I in our simulation, however, is approximately 25 Å.

Figure 4 shows the distribution of atom types along the normal of the lipid bilayer for each of the three simulations. Coordinates for the atomic positions are averaged over all 500 coordinate sets, which were taken at 2 ps intervals. The two peaks in the plot representing the distribution of the protein atoms indicate the presence of the two monomers of apo A-I in the molecular belt model and have mean positions of −4.7 Å and −6.6 Å for the G129/G129 model, −6.0 Å and 5.2 Å for the K133/K133 model, and −7.7 Å and 6.2 Å for the C173/C173 model. The distance between these two peaks is 11.3 Å and 11.2 Å for the G129/G129 and K133/K133 rotamers, about that of the interhelical separation between the two monomers observed in the crystal structure of apo A-I,10 and 13.9 Å in the C173/C173 model. As can be seen in the figure, the trailing portions of the phosphate, choline, and protein peaks all overlap between 10 Å and 20 Å along the normal of the lipid bilayer. A close examination of the structure of the model HDL particles reveals that at the edges of the discloidal particle, the POPC headgroups bend down towards the protein side-chains, thus protecting the lipid hydrocarbon chains from solvation.

Also clearly visible in the G129/G129 simulation is the methylene trough defining the separation between the two leaflets. Clearly, the hydrocarbon chains of the lipid molecules are not extensively interdigitated as observed in previous simulations of POPC in the liquid-crystal phase,19 but rather there is a clear delineation between the two leaflets as observed experimentally.18 In the G129/G129 simulation, the phosphate groups are located at a mean distance of +17.8 Å and −16.8 Å along the bilayer normal, and the choline groups are located at +17.6 Å and −18.3 Å. The distribution of lipid atoms along the bilayer normal agree well with data determined from small angle X-ray scattering experiments.18

Figure 5 is a plot of the fraction of amino acid residues in an α-helical conformation in each simulation over 1 ns at 10 ps intervals. The average α-helical content over the entire simulation for apo A-I in each rHDL particle was 75.9% (G129/G129), 77.4% (C173/C173), and 80.4% (K133/K133). These results are in excellent agreement with previously published circular dichroism data reporting that the percentage of α-helical content for lipid-bound wild-type apo A-I was 82 (±6%), and 78 (±1)% for apo A-I (1–43) A-I.20 The G129/G129 and C173/C173 rotamers had similar α-helical content over the time-course of the simulation despite significant differences in their rotational alignment and the presence of a disulfide bond linking the two molecules of apo A-I in the C173/C173 rotamer. Interestingly, the K133/K133 rotamer, which was rotated from the G129/G129 alignment by a single turn of an α-helix, was slightly more stable than the other two models, with a higher helicity throughout most of the simulation than either the G129/G129 or the C173/C173 rotamers.

Structure of the POPC bilayer

The average calculated areas per POPC headgroup for each simulation were 65.0(± 1.0) Å², 67.0(± 1.0) Å², and 65(± 1.0) Å² for the G129/G129, C173/C173, and K133/K133 simulations, respectively. The observed area per headgroup was stable over the simulation for the G129/G129 and K133/K133 rotamers, and these values compare favorably to the values of 64(± 1) Å² calculated in a recent MD simulation of fully hydrated POPC bilayers,21 as well as 65.5(± 0.04) Å² reported in an earlier simulation.19 In the case of the simulation of the C173/C173 rotamer, the area per headgroup began with a value of 68 Å² before decreasing to a value of 66 Å² after 700 ps. The area per lipid headgroup was then stable for the remainder of the simulation, suggesting that the lipid packing in the initial model was poor and that translational diffusion of the POPC molecules within the lipid bilayer redistributed the lipids, resulting in values
Figure 4. Distribution of atomic positions along the bilayer normal for the G129/G129 (a), K133/K133 (b), and C173/C173 (c) models. The model rHDL particle in each simulation was placed with its center-of-mass at the origin and its principal axes parallel with the x, y, z axes of the coordinate system.
which are comparable to that of the other two rotamers.

Figure 6 is a plot of the deuterium order parameter profile for the sn-1 and sn-2 chains of POPC from the three simulations. The resulting $S_{CD}$ profile agrees well with previously reported experimental values, as well as other dynamics simulations on POPC bilayers. As with other simulations, we do not observe the increased order parameter for carbon number 2 of the sn-1 chain that is observed experimentally. This behavior has been observed in a number of simulations of lipid bilayers to date, and it has been proposed that this is due to the brevity of simulation time. Although our values for the $S_{CD}$ agree well with other simulations, it is apparent that the order parameters are still lower than those measured experimentally.

We calculated values of $3.5 \times 10^{-7}$ cm$^2$ s$^{-1}$, $2.65 \times 10^{-7}$ cm$^2$ s$^{-1}$, and $2.74 \times 10^{-7}$ cm$^2$ s$^{-1}$ for the lateral diffusion constant, $D$, of G129/G129, C173/C173, and K133/K133 rotamers, respectively (Figure 7). Heller et al. have previously shown that the experimentally reported value of $7.0 \times 10^{-5}$ cm$^2$ s$^{-1}$ for $D$ for pyrene in the liquid-crystal phase of DOPC translates to $3.6 \times 10^{-5}$ cm$^2$ s$^{-1}$ for the self-diffusion coefficient of DOPC, through the application of the free volume model to lipid molecules in the liquid-crystal phase.

**Interhelical distance at the helix-5/helix-5 interface is greater for the G129/G129 rotamer than the K133/K133 rotamer**

We have previously reported that interhelical interactions between helix 5 and helix 5 of the two monomers of apo A-I in the G129/G129 rotamer were predicted to be slightly unfavorable on the basis of the results of analysis using the program ALIGN. There were two predicted interhelical salt bridges between the two monomers in the G129/G129 alignment, and a single charge–charge apposition was predicted because the model brought Lys133 from each monomer into close proximity with Lys133 on the opposite chain. On the basis of these observations, we postulated that the helix-5/helix-5 interaction was the weakest interhelical interaction in the molecular belt model. Indeed, in the crystal structure of apo A-I, it was observed that the interhelical distance between helix-5 and helix-5 was slightly larger than the interhelical distances along the rest of the dimer.

In order to examine the opening and closing of the “mouth” formed by the helix-5/helix-5 interaction, we calculated the interhelical center-of-mass distance in the $z$-direction as a function of time. This is an accurate measure of the interhelix separation, because both copies of helix 5 lie nearly parallel with the $xy$ plane. Figure 8 is a plot of this...
Figure 6. Deuterium order parameters for the G129/G129 (a), K133/K133 (b), and C173/C173 (c) rotamers averaged over 1 ns of MD simulation time. The values for the $S_{CD}$ are averages over both hydrogen atoms on each methylene carbon and are shown for the sn-1 (palmitoyl) chain, and the sn-2 (oleoyl) chain.
Figure 7. Calculation of translational diffusion coefficient for the G129/G129 (blue), K133/K133 (green), and C173/C173 (pink) models. A plot of $<\Delta r^2(t)>$ versus $t$ has a slope equal to $4D$ in the linear regime (black lines), where $D$ is the two-dimensional translational diffusion coefficient.

Figure 8. Helix-5/helix-5 center-of-mass distances for the G129/G129 and K133/K133 rotamers over 1 ns of MD simulation, illustrating the differences in interhelical distances between the two monomers as a function of time. This distance was measured every 2 ps during the course of the simulations.
interhelical distance over 1 ns of MD simulation for the G129/G129 and K133/K133 rotamers, at intervals of 2 ps. It is readily apparent that the interhelical distance in the G129/G129 rotamer varies quite substantially over time, with a maximal value of almost 17.6 Å at 594 ps into the simulation. Figure 3(b) shows the structure of the G129/G129 model after 600 ps of MD. The separation between the two helices is quite evident, and has resulted in the exposure of hydrocarbon surface area to the solvent.

In the K133/K133 simulation, a strikingly different result is obtained: the helix-5/helix-5 interhelical distance remained stable over the simulation, with small fluctuations about a mean value of 13.4 Å, and a standard deviation of 0.5 Å. It was unexpected that rotating one monomer relative to the other by a single turn of an α-helix would result in such significant structural changes over the course of the simulation between the G129/G129 and K133/K133 rotamers. The significance of this observation will be discussed below.

**Interhelical salt bridges provide specificity for a particular rotamer**

We have postulated that the primary purpose of interhelical salt bridges in the molecular belt model is to provide specificity for favorable rotational alignments of two apo A-I monomers bound to discoidal HDL particles. In order to examine the role of interhelical salt bridges in HDL structure, we monitored their association and disassociation over 1 ns for all three systems using a radial distribution function (rdf) following the method of Sheldahl & Harvey. ALIGN predicted that there would be no interhelical salt bridges in the K133/K133 rotamer, and several charge apportionings, so it was somewhat surprising to see that this rotamer actually formed approximately the same total number of interhelical salt bridges as the KG129/G129 rotamer. Even before the MD simulation, but after the 100 ps of simulated annealing, many of the salt bridges were in fact identical with those in the G129/G129 rotamer. Other residues were observed to form salt bridges with different partners on the opposite monomer in the K133/K133 rotamer and the G129/G129 rotamer. This result indicates that intermolecular salt bridging is more dynamic than would be predicted by a strict interpretation of the ALIGN analysis.

Upon analysis of the rdfs for the interhelical salt bridges observed in each simulation, we noticed that several amino acid residues spent a substantial portion of their time with multiple salt bridging partners. The observed promiscuous side-chains are summarized in Table 2. The number of promiscuous salt bridges is larger in the K133/K133 rotamer than in the G129/G129 rotamer. If one considers salt bridges involving the amino acid residues in the last three columns of Table 2 and considers the residue in the next to last column (the first partner) as position i, then the second salt bridging partner appears primarily at positions i + 4, i + 7, or i + 11 in the sequence. These correspond to salt bridging interactions with one, two, or three turns of an α(11/3)-helix separating the two salt bridging partners on the opposite monomer. It is striking that there are very few deviations to this salt bridging pattern, and those that are present only occur at positions i + 3 or i + 5.

An example of one such promiscuous salt bridge in the K133/K133 rotamer is shown in Figure 9. This structure was taken from 872 ps into the MD simulation, and clearly shows the geometry of the simultaneous interaction between Asp89 on one apo A-I monomer with both Arg177 and Arg188 of the opposite monomer. At the start of our simulation, Asp89 is salt bridged to Arg177. Asp89 remains paired with Arg177 until the formation of the simultaneous salt bridge with Arg188. The Asp89-Arg177 salt bridge soon breaks, and Asp89 is salt bridged exclusively to Arg188 during the final 100 ps of the simulation.

This result suggests a structural and dynamic role for the α(11/3)-helix. In the α(11/3)-helix, two amino acid residues 11 residues apart in the sequence fall on the same position of the helical wheel. A promiscuous side-chain like Asp89 is positioned so that it can pair with either or both of the two side-chains spaced exactly 11 residues (16.5 Å) apart on the other monomer. The length of the side-chains is sufficient to allow the simultaneous formation of these two salt bridges. If the α helix had the canonical α 18/5 form, however, two residues would have to be 18 residues apart in order to fall on the same position of the helical.

### Table 2. Table of promiscuous salt bridges observed over the course of 1 ns MD simulations for model HDL particles corresponding to each of the three rotational alignments illustrated in Figure 2.

<table>
<thead>
<tr>
<th>Rotamer</th>
<th>Promiscuous side-chains</th>
<th>Salt bridge partners</th>
</tr>
</thead>
<tbody>
<tr>
<td>G129/G129</td>
<td>Glu111</td>
<td>Arg151</td>
</tr>
<tr>
<td></td>
<td>Glu125</td>
<td>His155</td>
</tr>
<tr>
<td>K133/K133</td>
<td>Glu70</td>
<td>His199</td>
</tr>
<tr>
<td></td>
<td>Lys77</td>
<td>Lys206</td>
</tr>
<tr>
<td></td>
<td>Glu78</td>
<td>Arg188</td>
</tr>
<tr>
<td></td>
<td>Asp85</td>
<td>Arg177</td>
</tr>
<tr>
<td></td>
<td>Asp89</td>
<td>Arg188</td>
</tr>
<tr>
<td></td>
<td>Glu92</td>
<td>Arg173</td>
</tr>
<tr>
<td></td>
<td>Glu125</td>
<td>Lys140</td>
</tr>
<tr>
<td>C173/C173</td>
<td>Lys59</td>
<td>Asp73</td>
</tr>
<tr>
<td></td>
<td>Glu92</td>
<td>Lys40</td>
</tr>
<tr>
<td></td>
<td>Arg151</td>
<td>Lys191</td>
</tr>
<tr>
<td></td>
<td>Glu191</td>
<td>Glu198</td>
</tr>
<tr>
<td></td>
<td>Arg151</td>
<td>His155</td>
</tr>
<tr>
<td></td>
<td>Arg215</td>
<td>Glu125</td>
</tr>
</tbody>
</table>

Promiscuous side-chains are defined as those that either (a) spent part of their time salt bridged to multiple partners on the opposite apo A-I monomer simultaneously, or (b) alternated between two or more salt bridging partners over the course of the simulation.
wheel. The resulting distance between two such amino acid residues would then increase to 27 Å, which is too great to be spanned by the side-chains, precluding the formation of a salt bridging network such as the one seen in Figure 9.

This suggests a mechanism to facilitate the sliding of the two helices past one another, allowing the two ring-shaped monomers to rotate relative to one another. In the α11/3-helix of apo A-I, positively charged residues fall predominantly at position 9 of the helical wheel, and negatively charged residues at position 5. Position 2 of the helical wheel is evenly split, with negatively charged amino acid residues at the amino-terminal portion of the sequence and positively charged amino acid residues towards the carboxy-terminal region of the protein. Interhelical salt bridges occur predominantly between residues at positions 9 and 5 of opposite chains, or between amino acid residues at position 2 of opposite chains.13 Since there are groups with the same charge spaced roughly every 11 residues along the helical wheel at positions 9 and 5, an amino acid such as Asp89, which forms a salt bridge with a basic residue on the other monomer would be able to form a second salt bridge with another basic residue 11 residues down in the sequence, and would then be free to disassociate from its initial salt bridging partner. A large number of such interactions can occur, so the monomers are able to rotate past one another relatively easily, without the free energy cost of first disassociating large numbers of interhelical salt bridges.

This observation is particularly important in the case of salt bridges formed between two residues at position 2 of the helical wheel. While residues at positions 5 and 9 are exposed to solvent and would therefore be solvated by water molecules when their salt bridges disassociate, the charged groups at position 2 of the helical wheel are buried in a hydrophobic environment, which precludes this compensatory solvation. Salt bridges that do form in a hydrophobic environment have a much higher energy barrier to disassociation than those exposed to solvent.26 In order to form a more stable structure therefore, a preferred rotamer would be one which maximizes the number of interhelical salt bridges in the hydrophobic environment. As described above, there would be very little free energy change associated with the salt bridge association/disassociation between side-chains at positions 5 and 9 of the helical wheel. However, this is not the case for interhelical salt bridges between residues at position 2. As the two monomers of apo A-I rotate relative to one another, there would be no free energy penalty associated with the buried side-chains provided that salt bridges were not broken. Rotation in a direction that would necessitate the disassociation of buried salt bridges without first forming new ones would be unfavorable. The most favorable direction for rotation would therefore rotate the carboxy-terminal domain of one apo A-I molecule towards the amino-terminal domain of the other. This would allow for the formation of additional salt bridges, while allowing existing salt bridges to change partners through the mechanism described above. Rotational rearrangement is therefore unidirectional. By the rotation of one apo A-I monomer relative to the other, however, a more favorable orientation could be obtained which would maximize the number of these buried salt bridges. Once formed, these rotamers would be more stable due to the much higher free energy barriers associated with the disassociation of the salt bridges in a hydrophobic environment.

Figure 9. A salt bridging triad observed in the K133/K133 rotamer at t = 872 ps in the MD simulation. Asp89 begins the simulation salt bridged to Arg177 of the opposite monomer before forming the structure shown here, where it is salt bridged simultaneously to Arg177 and Arg188. The original salt bridge then breaks, and Asp89 forms an interhelical salt bridge exclusively with Arg188 during the last 100 ps of the simulation. This figure was generated with Ribbons.50
Proline stacking in the K133/K133 rotamer gives rise to kinks in the structure of discoidal HDL particles

While all of the models were constructed as discoidal HDL particles, the K133/K133 rotamer adopted a distinctly hexagonal conformation during the course of the simulation (Figure 10). It was initially surprising that such small changes in the rotational alignment of the apo A-I monomers relative to each other would give rise to such gross changes in HDL structure. However, a retrospective examination of the alignment of the two sequences suggests an explanation. Table 3 compares the observed kink angles from the K133/K133 simulation with those of Borhani et al. for the crystal structure of apo Δ(1–43) A-I. The

Table 3. Interhelical angles measured for the K133/K133 simulation after 1 ns of MD, compared with the values for the apo Δ(1–43) A-I

<table>
<thead>
<tr>
<th>Helix junction</th>
<th>K133/K133 MD simulation</th>
<th>Apo Δ(1–43) A-I crystal structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–2</td>
<td>58 59</td>
<td>15 14</td>
</tr>
<tr>
<td>2–3</td>
<td>34 29</td>
<td>19 8</td>
</tr>
<tr>
<td>3–4</td>
<td>18 62</td>
<td>47 57</td>
</tr>
<tr>
<td>4–5</td>
<td>62 18</td>
<td>47 57</td>
</tr>
<tr>
<td>5–6</td>
<td>14 69</td>
<td>58 42</td>
</tr>
<tr>
<td>6–7</td>
<td>53 36</td>
<td>48 54</td>
</tr>
<tr>
<td>7–8</td>
<td>51 17</td>
<td>12 18</td>
</tr>
<tr>
<td>8–9</td>
<td>44 43</td>
<td>17 29</td>
</tr>
</tbody>
</table>

Figure 10. Proline-induced kinks observed in the K133/K133 rotamer after 1 ns of MD simulation. The sites of the proline residues are shown in green, and the interhelical kink angles for the monomer closest to the viewer are displayed. The pronounced kinking observed between helical junctions 3–4, 4–5, 5–6, and 6–7 is comparable to those observed in the apo Δ(1–43) A-I crystal structure. This figure was generated by Ribbons.
interhelical kink angles, particularly between helical repeats 4–7 are very close to those observed in the X-ray crystal structure, with two exceptions; the 4–5 junction in molecule B, and the 5–6 junction in molecule A. This corresponds to the region where helix 4 of molecule B and helix 6 of molecule A are antiparallel. These two helix junctions are straight rather than kinked in the MD simulation. However, these same helix junctions are clearly kinked in the opposite monomers, where helix 6 of molecule B is antiparallel with helix 4 of molecule A. In the G129/G129 alignment, the proline residues that punctuate helical repeats 4–6 are displaced from the same proline residues on the opposite monomer by four residues, or a single turn of an α11/3-helix. As discussed previously, the K133/K133 rotamer corresponds to the rotation of one apo A-I monomer in the G129/G129 model relative to the other by four residues. It became apparent in retrospect that the direction in which the two rotamers were slid relative to the G129/G129 model resulted in the juxtaposition of the proline residues in helical repeats 4–6 with those from the opposite chain. The kinks that were observed in the K133/K133 simulation occurred unequivocally at the sites where this proline stacking occurred, implicating the proline stacking in the formation of this kinked structure (Figure 11).

The kinetic aspects of the evolution of the model from circular to hexagonal are interesting. During the course of the simulation, the rearrangement of the protein/lipid complex occurred in two distinct stages. The fastest step involves the kinking of the α-helices at the proline residues. This event occurs on a time-scale of 100 ps. During this phase, the kinking pulls the ends of the helices away from the lipid, creating a small gap between the lipid and the protein, exposing hydrophobic surface area to the solvent. The largest gap between the protein and the lipid occurs at approximately 500 ps into the simulation. The second step in the restructuring process occurs on the nanosecond time-scale and involves the translational diffusion of the POPC molecules to close the gaps formed by the proline-induced kinking. The gaps between lipid and protein have disappeared almost entirely after 1 ns.

Also unexpected in the K133/K133 simulation was the observation that the amphipathic helical repeats of the lipid-binding domain of apo A-I straightened out during the simulation. This was contrary to their predicted behavior on the basis of the analysis of protein structures from the Brookhaven Protein Data Bank which showed that amphipathic α-helices tend to curve towards the hydrophobic environment, and away from proline-induced kinks in the hydrophilic environment.

We hypothesize that the proline residues that anchor either end of the amphipathic helical repeats add another geometric constraint on the structure of apo A-I bound to lipid. While a single proline residue on the hydrophilic side of an amphipathic helix would induce curvature towards the hydrophobic face, the presence of two proline residues at either end of an already curved helix would straighten out the helix, resulting in the polygon-like structure observed in the simulation of the K133/K133 rotamer when viewed down the normal of the lipid bilayer. This effect appears to be more pronounced when the proline residues are stacked in the belt model. Once locked into this hexagonal structure, it would become difficult for the monomers to rotate relative to one another. Because the lipid-binding domain is no longer a single, continuous, smoothly curving amphipathic α-helix, but rather an object with fairly sharp angles, rotation of one monomer relative to the other would expose large hydrophobic surface area at the corners between the lipid and the protein.

**Solvation free energies for all three model systems over time are similar**

The solvation free energies for the three model systems were evaluated as a function of time during the simulations as discussed in Materials and Methods (Figure 12). The average free energies of the G129/G129, C173/C173, and K133/K133 model systems over the final 500 ps of MD simulation were $-81.0$ kcal mol$^{-1}$, $-76.7$ kcal mol$^{-1}$, and $-86.1$ kcal mol$^{-1}$, with standard deviations of $7.7$ kcal mol$^{-1}$, $9.0$ kcal mol$^{-1}$, and $9.5$ kcal mol$^{-1}$, respectively. All three systems exhibit comparable values for the solvation free energies within a similar range of oscillatory behavior, suggesting that there is no strong preference for one alignment over the other. This indicates that even in the case of the G129/G129 rotamer where the helix-5/helix-5 interhelical distance varies over time, or
Continuum electrostatics models of model HDL particles suggest an explanation for apo A-I lipid preference

Continuum electrostatics calculations were carried out on the G129/G129 rotamer after the initial construction of the model, after simulated annealing but before MD, and after 1 ns of MD simulation. Figure 13 shows two views of the electrostatic potential map for the G129/G129 rotamer after 1 ns of simulation time. The positive (blue) and negative (red) potentials are shown in the Figure at $\pm 3 \, kT/e$, respectively. The fact that the positive potential extending across the hydrocarbon phase persists after a nanosecond suggests that it is not an artifact of construction of the model system due to orientations of the amino acid side-chains. In all three cases, a positive electrostatic potential was observed extending across the inner diameter of the HDL particle in the hydrocarbon portion of the lipid bilayer. In coordinate trajectories taken from the final stages of the simulation, there is very little potential at the surface of the lipid bilayer. This is due to the fact that because POPC is a zwitterionic lipid, the positively charged choline groups interact with the negatively charged phosphate groups to cancel out their electrostatic potential. The source of the positive electrostatic potential is the dominance of class A amphipathic helices in apo A-I. The class A amphipathic $\alpha$-helix is characterized by the localization of negatively charged residues at the middle of the polar face of the helix and the clustering of positively charged residues at the interface between the hydrophobic and hydrophilic faces, particularly at position 9 of the $\alpha$11/3-helix.$^{29,30}$ The binding of two monomers of apo A-I to lipid in an antiparallel manner concentrates the positive charges at the interhelical region between the two helices (Figure 1), leading to the positive potential observed in Figure 13. Even when a basic side-chain at position 9 is associated with an acidic side-chain at position 5, the latter will be closer, on average, to the solvent; salt bridges are transient and frequently broken,$^{17}$ and the unpaired acidic side-chain at position 5 will be better solvated than the basic partner at position 9. This concentration of relatively buried positive charge is probably the explanation for the observation that apo A-I binds preferentially to anionic lipids.$^{31}$

Figure 12. Eisenberg–McLachlan solvation free energies for all three rotamers as a function of time. The values for the SFEs are comparable for all three model HDL particles over 1 ns and are stable over time, indicating that all three structures are equally well solvated.
Discussion

Here, we have presented the results of MD simulations for three separate HDL particles, each one a distinct rotational alignment of the molecular belt model. The properties of the POPC molecules in the lipid bilayer over the course of 1 ns of simulation time for all three rotamers are in good agreement with previously reported values from experimental studies on POPC bilayers. The behavior of POPC in these simulations also agrees well with previously reported simulations of lipid bilayers in general, and POPC bilayers in particular. In addition, the percent of amino acid residues in apo A-I monomers that are in an α helical conformation in all three systems agrees well with reported values for the percentage of α helicity for apo Δ(1–43) A-I bound to lipid.

On the basis of a comparison between the G129/G129 and the K133/K133 MD simulations, we believe that both interhelical salt bridges as well as proline punctuation provide the specificity for the rotational alignment of apo A-I monomers in discoidal HDL particles. The periodicity of an α11/3-helix allows for side-chains participating in interhelical salt bridging interactions to have multiple partners. Salt bridges at positions 5 and 9 of the helical wheel are exposed to solvent and spend a substantial amount of their time solvated by water. We have previously shown that these partially solvated salt bridges are capable of significant contributions to interhelical energetics. The α11/3-helix allows charged side-chains to form interhelical salt bridges with oppositely charged side-chains three, four, seven, or 11 residues downstream in the other monomer. These salt bridges are free to disassociate from one partner and form a different salt bridge with another. Salt bridges at position 2 of the helical wheel are buried in the hydrophobic phase of the HDL particle, but again the periodicity of the α11/3-helix allows amino acid residues participating in these salt bridges to simultaneously form additional salt bridges with oppositely charged side-chains as much as 11 residues downstream from their original partner. This would imply that a residue at position 2 on one monomer can salt bridge to residues at positions 5, 6, 9, or 2 on the second monomer; a residue at position 5 on one monomer can salt bridge to residues at positions 8, 9, 1, or 5 of the other monomer; and a residue at position 9 on one monomer can salt bridge to residues at positions 2, 5, 8, and 9 of the other monomer. Thus, the transient formation of two salt bridges facilitates rotation/sliding of one monomer relative to the other.

With two monomers of apo A-I binding to a discoidal HDL particle in an antiparallel orientation with a random rotational alignment, the interhelical salt bridges would provide enough mobility...
for the rotational rearrangement of one monomer relative to the other. From the results of the K133/K133 simulation, it seems clear that proline-induced kinking of the \( \alpha \) helices occurs on a time-scale much shorter (\( \sim 100 \) ps) than rotation (\( \sim 1-10 \) ns). Once the proline residues become stacked due to rotation rearrangements on the nanosecond time-scale, they would quickly introduce kinks in the structure, particularly at interhelical junctions 4–5, 5–6, and 6–7, more rapidly than monomer rotation would unstack them. Once these kinks form, there would be a large positive free energy associated with further rotation as additional movements would expose large hydrophobic surface area on both the protein and lipid phase at each kink for each monomer. In order for further rotation to occur, these proline-induced kinks must straighten out and the two apo A-I monomers must rotate enough to prevent the proline residues from returning the helices to their kinked state. Such events would be rarer because the proline kinking event occurs on the 100 ps time-scale, while it would take a nanosecond or more for the two monomers to rotate the minimum four residues (a single turn of the \( \alpha \)11/3-helix) required to prevent kinking. The proline-induced kinking therefore introduces a kinetic barrier to the rotation of two apo A-I monomers on an HDL disk. This model is consistent with experimental data showing that rotational realignment does not occur even on the scale of several days (L. Li & J.P.S., unpublished results).

It has been shown experimentally through a combination of deletion analysis and LCAT inhibition by monoclonal antibodies to helical repeats 6 and 7 and \( \alpha \) helical repeats 6 and 7 of the lipid-binding domain of apo A-I are critical for LCAT activation. Because helical repeats 4–7 each are 22 amino acid residues in length and are each punctuated by a proline at position 1 of the helical repeats, the proline kinking that we propose will be strongly favored when these repeats are aligned. This suggests that the role of proline punctuation in apo A-I structure is to properly orient the LCAT activation domain of apo A-I for its interaction with the enzyme. Figure 14 is a schematic showing the rotational alignments of the LL5/6, LL5/5, and LL5/4 rotamers. The LL5/6 rotamer aligns six 22-mer helical repeats in an antiparallel fashion, and juxtaposes five pairs of proline residues. The LL5/5 and LL5/4 rotamers align three and two helical repeats, stacking four and three pairs of proline residues, respectively. This suggests that the LL5/6 rotamer might be the most stable, since it maximizes the alignment of the 22-residue helices.

This hypothesis agrees well with recent interhelical cross-linking experiments showing that the LL5/6 rotamer is substantially preferred over the LL5/4 and LL5/5 rotamers (L. Li & J.P.S., unpublished results). It is interesting to note that there would be no preference for any of these rotamers over the other if it were not for the presence of the two 11-mer helical repeats 3 and 9. These helical repeats introduce a disruption into the periodic 22-mer motif observed in the lipid-binding domain of apo A-I. If the lipid-binding domain of apo A-I consisted entirely of 22-mer helices, nine in all, there would be nine separate rotamers that would each align nine 22-mer helical repeats. The presence of the two 11-mer helices guarantees that there is one rotational alignment which is preferred over the others.

We have presented the results of three MD simulations and propose a role for interhelical salt bridging and proline-induced kinking in the molecular belt model for apo A-I. These two structural aspects of the belt model dominate the dynamics of apo A-I when bound to discoidal HDL particles on two different time-scales. The
salt-bridging hypothesis first proposed by Segrest et al. argued that interhelical salt bridges provided specificity for preferred rotational orientations of two apo A-I monomers when bound to discoidal HDL particles. We believe on the basis of these simulations that this is not entirely correct, and that interhelical salt bridging provides a mechanism for rotational rearrangements on the 10 ns time-scale. On the basis of the results from the simulations presented here, we believe instead that rotational orientations are selected for by the stacking of proline residues that maximizes the alignment of the 22-mer helices in the lipid-binding domain of apo A-I. The proline-induced kinking observed in the K133/K133 simulation occurs on the 100 ps time-scale, and provides a kinetic barrier to rotational reorientation once preferred rotational alignments form. Thus, interhelical salt bridges facilitate the rotation of two monomers of apo A-I when bound to discoidal HDL particles, while proline kinking and the presence of the two 11-mer helices select for preferred rotational alignments of apo A-I bound to discoidal HDL.

Materials and Methods

Construction of the model discoidal HDL particles

The construction of model HDL particles containing two monomers in the G129/G129 rotamer wrapped around a core discoidal lipid bilayer containing 160 molecules of POPC has been described, as has the model for a disulfide-linked homodimer of apo A-I/apolipoprotein A-I. In the original models, there was a sizable gap between the amino and carboxy-terminal ends of the two monomers, exposing a large hydrophobic surface area of the POPC hydrocarbon chains to solvent. The LOCATE program identified another potential amphipathic α-helical domain in residues 33–43 of the amino-terminal globular domain of apo A-I. We therefore inserted an additional 11 α-helical residues from this region into the molecular belt models in order to close the gap. Construction of the K133/K133 rotamer was achieved through the rotation of one of the monomers from the G129/G129 model until residues Lys133 from both chains were juxtaposed with one another, using the program O. This rotation corresponds to a single turn of an 11/3 α-helix (four residues). The starting coordinates for the lipid portion of the model HDL particles were taken from an earlier simulation of the liquid-crystal phase of a patch of 200 molecules of POPC. A circular patch of 160 lipids was docked into the center of the disc whose circumference was defined by the atomic coordinates of the apo A-I molecules. The final structures had a total diameter of 105 Å and the thickness across the lipid bilayer was 50 Å. The resulting rHDL models had a total of 28,340 atoms (including hydrogen atoms). All three rHDL particles were subjected to 3000 steps of conjugate gradient energy minimization using XPLOR in order to eliminate steric conflicts.

Solvation of the model HDL particles

The model HDL particles were solvated in a sphere of water 75 Å in radius. The sphere was generated by the method as described. A lattice of cubes 15.5516 Å on a side was constructed, each containing 125 water molecules equilibrated by Monte Carlo, and all water molecules with atoms greater than 75 Å from the origin or containing any atoms within 1.0 Å of a POPC or protein atom were deleted. The density of the water molecules in the cubes was 1 g ml⁻¹. The TIP3P model was used to simulate the water molecules. The solvated rHDL particles were then subjected to another 1000 steps of conjugate gradient energy minimization using XPLOR in order to remove unacceptable steric clashes between water molecules and the protein/lipid complexes. The final model systems with protein, lipid, and water molecules had a total of approximately 175,000 atoms each (including hydrogen atoms).

Annealing of the solvated model HDL particles

Annealing for all three model systems was carried out using NAMD on a 4-processor ORIGIN 200 (Silicon Graphics, Inc., Mountain View, CA). The fully solvated energy minimized rHDL models were each heated from an initial temperature of 30 K to a final temperature of 300 K over 27 ps in increments of 10 K ps⁻¹. The structures remained at 300 K for another 73 ps to allow for equilibration. The annealed structures were examined for stability prior to beginning the MD simulations. Stability after 100 ps of the simulated annealing protocol was evaluated by the extent of water penetration into the lipid bilayer as well as lipid packing, to ensure that all 80 POPC molecules in each leaflet remained in the lipid bilayer and that there were no regions with unacceptable steric clashes. Analysis of the solvent-accessible hydrophobic surface area of the particle at the lipid–protein interface ensured that there were no regions with gaps between the hydrophobic face of the amphipathic α-helices and the methylene groups of the lipid acyl chains. An example of fully solvated, annealed model for the G129/G129 alignment is shown in Figure 3(a).

Molecular dynamics

MD simulations for all three model systems were carried out on a 32-processor beowulf cluster at the Theoretical Biophysics Group at the University of Illinois using NAMD. Each MD simulation covered 1 ns. The CHARMM22 force field was used with modifications to allow for the introduction of parameters for POPC molecules. The necessary unsaturated group parameters for POPC were taken from the parameters for 2-hexene in the CHARMM24 lipid parameter set. Velocity reassignment was carried out every 2 ps during the simulation. Non-bonded interactions (electrostatic and Lennard–Jones potentials) were truncated at a distance of 75 Å taking effect at a distance of 75 Å from the center of the system. The total energies of all three model systems remained constant throughout the time-course of the simulation. The temperature for all three systems was also stable at 300 K during the simulations. Coordinate trajectories were written to output files every 2 ps throughout the simulation, and all 500 structures were used for analysis. Analysis scripts were written in XPLOR.
Calculation of percentage of \( \alpha \)-helicity

The percentage of \( \alpha \)-helicity over time for each of the three simulations was calculated by determining fraction of amino acid residues that lie within \(-100^\circ < \phi < -30^\circ\) and \(-80^\circ < \psi < 5^\circ\) in torsion space.\(^46\) This value was calculated every 10 ps during the MD simulation.

Deuterium order parameters

The deuterium order parameter (\( S_{CD} \)), is an important piece of structural and dynamical information for lipid bilayers.\(^{23}\) It measures the motional anisotropy of a given C–H bond in a methylene group. The deuterium order parameter is given by:

\[
S_{CD} = \frac{1}{2}(3 \cos^2 \theta - 1)
\]

where \( \theta \) is the angle between the C–H bond and the bilayer normal. The \( S_{CD} \) varies between a value of \(-0.5\) for a fully ordered, all-trans chain that is oriented parallel with the normal of the bilayer, and \(0.0\) for a completely randomly oriented hydrocarbon chain. \( S_{CD} \) was calculated by averaging the term in brackets for both C–H bonds and all 160 lipids for a given methylene group. This parameter was calculated every 10 ps during the simulation, and the reported results represent averages over the resulting 100 structures.

Area per lipid headgroup

The total area per leaflet is a product of the number of lipid molecules, \( N \), and the average area per headgroup, \( a \). If the \( N \) lipids form a disc whose face is a circle with a radius of \( r \), then:

\[
\pi r^2 = Na
\]

For a given structure, the average area per headgroup can be calculated as follows. The structure is oriented so that the z-axis is perpendicular to the mean plane of the phosphate groups, and the radial position of each phosphorous atom \( i \) is calculated from the atomic coordinates \( x \) and \( y \) using the relationship:

\[
r_i = \left(\left(x_i - \bar{x}\right)^2 + \left(y_i - \bar{y}\right)^2\right)^{1/2}
\]

where \( \bar{x} \) and \( \bar{y} \) are the mean \( x \) and \( y \) coordinates of the full set of phosphorous atoms. If the atoms are then ordered according to their radii, \( n = 1, 2, ..., N \), a plot of \( r_i^2 \) versus \( n \) will have a slope equal to \( a/\pi \). Figure 15 shows a series of such plots, for structures captured at 100 ps intervals.

Continuum electrostatics calculations

Continuum electrostatics calculations were carried out using the DelPhi\(^\text{18}\) module in INSIGHT II (Accelrys, San Diego, CA, 2000). Graphical representations of the results of electrostatics calculations obtained from DelPhi were generated in Insight II. A solute dielectric constant of 4.0 was used, with the solute extending across 20% of the grid, with 65 grid points/axis. The ionic strength was set to 0.145 M, and an ionic radius of 2.0 Å was used with a solvent radius of 1.4 Å and solvent dielectric constant of 80.0. In order to obtain more accurate results for the electrostatic potential of the system, the resulting grid was treated as a coarse potential map and was used in a focusing calculation in which the solute

\[
\text{Figure 15. Calculation of area per headgroup. A plot of } r_i^2 \text{ versus } n \text{ has a slope of } a/\pi, \text{ giving } a = 65.0(\pm1.0) \text{ Å}^2, 67.0(\pm1.0) \text{ Å}^2, \text{ and 65.0(\pm1.0) Å}^2 \text{ for the average area per lipid headgroup for the G129/G129, C173/C173, and K133/K133 simulations, respectively.}
\]
extended across 80% of the grid. Charges from the modified CHARMM22 force field used for the MD simulations were also used for the electrostatics calculations.

A number of structures were examined, but results are reported only for the coordinate sets at the end of each of the MD simulations.

**Translational diffusion of lipid headgroups**

The diffusion constant can be calculated from the Einstein relationship:

$$\langle R^2(t) \rangle = 2dDt$$

where $D$ is the diffusion constant, $d$ is the number of dimensions, and $\langle R^2(t) \rangle$ is the mean square distance traveled in time $t$. In order to monitor the lateral diffusion of lipid headgroups in the bilayer, we calculate the displacement of each phosphorous atom in the $X,Y$ plane as a function of time. The slope of a plot of $\langle R^2(t) \rangle$ versus time through the linear portion of the graph is equal to $4D$ for the case of diffusion in two dimensions (Figure 7). The translational diffusion coefficient for each lipid headgroup in a bilayer can therefore be calculated directly.

**Solvation free energies**

The solvation free energies, $\Delta G_s^i$ for all three model rHDL particles were calculated through the method outlined by Eisenberg & McLachlan:\[48, 49\]

$$\Delta G_s^i = \sum_i \sigma_i A_i$$

where $A_i$ represents the SASA for atom $i$ and $\sigma_i$ is the atomic solvation parameter for atom $i$.

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