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Structural and dynamic features of apolipoprotein A-I cysteine mutants, Milano and Paris, in synthetic HDL

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ABSTRACT

Pursuing an established research interest in our group, we built two models for synthetic HDL containing the natural cysteine mutants of apolipoprotein A-I, apolipoprotein A-I Milano (apoA-IM) and apolipoprotein A-I Paris (apoA-IP), both in their homodimeric form. Data on the structural and dynamic properties of such s-HDL are an essential preliminary step for the understanding of the biological activity of the two mutants. Furthermore, comparison between apoA-IM and apoA-IP allows evaluating the effects of the same mutation in a different position in the primary structure and to directly compare our findings with previously published models. We computed for 50 ns in explicit solvent the molecular dynamics of the two complexes and analyzed different properties as a function of time. The proposed s-HDL structures differ significantly from one another and from wild type apolipoprotein A-I. All features of the apoA-IM model are consistent with experimental data. The higher RMSF of apoA-IM has a counterpart in the finding that trypsin, matrix metalloproteases, and chymase degrade apoA-IM much faster than wild type apoA-I; the primary cutting site is correctly identified by molecular dynamics data on our model of apoA-IM-containing s-HDL. The few experimental data for apoA-IP prevent direct comparison with our findings.

1. Introduction

Experience with synthetic high-density lipoproteins (s-HDL) has proven that the anti-atherogenic, anti-thrombotic and anti-inflammatory properties of natural HDL (reviewed in [1,2]) rest on their main protein component, apolipoprotein A-I (apoA-I). A number of apoA-I mutants have been characterised [3–5]. While most of them are linked with pathological phenotypes (e.g. deletion [6], non-conservative [7], nonsense [8], frameshift [9]), two variants, apoA-I Milano (apoA-IM) [10] and apoA-I Paris (apoA-IP) [11], appear to exhibit some unique, favourable features [10,12]. A common structural feature of the two variants is the presence of an arginine-to-cysteine mutation, at amino acid 173 in Milano and 151 in Paris. This leads to the formation of homodimers (apoA-IM–apoA-IM; apoA-IP–apoA-IP) and heterodimers with apoA-II (apoA-IM–apoA-II; apoA-IP–apoA-II).

Carriers of both mutants (all of them heterozygous) have low levels of circulating HDL [13]; in contrast to their hypo-α-cholesterolemic phenotype, however, apoA-IM carriers do not differ to any extent in the entity of carotid intima-media thickness with respect to close relatives living in the same environment and with HDL levels in the normal range [14]. For both apoA-I variants a gain-of-function has been reported in their being able to prevent lipoxygenase-mediated phospholipids oxidation (apoA-IM > apoA-IP), whereas wild type apoA-I is poorly active in this respect [15]. Cholesterol-effluxing capacity has been investigated only for apoA-IM: small r-HDL (7.8 nm in diameter) containing apoA-IM–apoA-IM are more efficient than the corresponding wild type apoA-I particles as acceptors of cell membrane cholesterol. Large particles (12.5 nm in diameter), either with wild type apoA-I or apoA-IM, are instead equivalent in their cholesterol-effluxing capacity [16]. Previous data on apoA-IM published by our research group [17,18] suggested that the sensitivity to proteases, which is higher for the apoAIM–apoA-II heterodimer and the apoA-IM–apoA-IM homodimer than for wild type apoA-I, could be a further protective mechanism of this natural mutant. ApoA-IM could enter the artery wall from circulation, acting as a decoy substrate for plaque proteases, thus exerting a protective effect against plaque rupture.

To make sense of the specific features of the mutants, structure–function relationships need to be investigated on a molecular model of the proteins at atomic resolution. With their

Abbreviations: apoA-I, apolipoprotein A-I; apoA-IM, apolipoprotein A-I Milano; apoA-IP, apolipoprotein A-I Paris; DMPC, dimyristoylphosphatidylcholine; EM, energy minimisation; FFT, fast Fourier transform; HDL, high-density lipoproteins; LCAT, lecithin-cholesterol acyltransferase; MD, molecular dynamics; PME, particle-mesh Ewald; POPC, palmitoyloleoyl phosphatidylcholine; RMS, root mean-square; SA, simulated annealing; SAS, solvent accessible area.

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amphipathic nature, apolipoproteins are natively folded only in a hydrophobic environment, i.e. when bound to lipids inside the lipoprotein particles [19]. Between amino acid 44 and 243 the secondary structure of apoA-I is composed of ten amphipathic α-helices, punctuated at regular intervals by proline residues [20,21]. The tertiary structure is known at atomic resolution only for lipid-free apoA-I based on a crystal structure published in 1997 [22]. Because of the lack of structural data at atomic resolution on apoA-I in HDL, several models for the three-dimensional structure of such an assembly have been proposed (see a recent review in [23]). The published models have been obtained through computational [24–26], experimental [27,28], or combined approaches [29,30]. Often the proposed structures differ in several respects, and it is quite difficult to sum-up all features in a single model to envision a 'global prophesy'. Even methods based on the direct observations of apoA-I in synthetic discoidal HDL gave conflicting results: Wu et al. [30] proposed a double superhelix structure from small angle neutron scattering data supported by mass spectrometry, whereas Culot et al. [31] observed a picket-fence arrangement by scanning tunnelling microscopy. However none of these approaches has a resolution adequate to solve apoA-I structure at atomic level, which makes the proposed models inadequate for molecular mechanics studies. Moreover, in both natural mutants in this investigation the formation of a covalent cystine bond between the mutated amino acids induces relevant structural constraints, which are not compatible with some of the models proposed for wild type apoA-I. Conversely, the structural restraints present in the homodimeric forms of apoA-IM and apoA-IP have been used to validate the most credited of these models, the double belt, and to extend its applicability [32].

With an approach similar to our modelling of s-HDL containing wild type apoA-I [26], in the present investigation we made direct reference to the published crystal structure of apoA-I, and applied computational procedures to model the homodimeric forms of apoA-IM and apoA-IP. The available crystal could only be solved at low resolution (4.0 Å) [22]. We already discussed thoroughly [26] why we maintain that the use of such structure implies some obvious approximations, but no real limitation to our computational work.

We next built the models of two s-HDL, containing one molecule each of either apoA-IM–apoA-IM or of apoA-IP–apoA-IP, together with a homogeneous phospholipid bilayer of L-α-palmitoyloleoyl phosphatidylcholine (POPC). We then computed for 50 ns in explicit solvent the MD simulations of the two macromolecular complexes to obtain data on their structural and dynamic properties. Such MD results are an essential preliminary step for the understanding of the biological activity of the two mutants. Furthermore, comparison between apoA-IM and apoA-IP allows evaluating the effects of the same mutation in a different position in the primary structure and to directly compare our findings with previously published models.

2. Materials and methods

2.1. Preparation of the apoA-IM and apoA-IP molecules

ApoA-IM and apoA-IP were obtained introducing, respectively, the R173C and the R151C mutations in both A and B chains of wild type apoA-I (PDB ID code: 1AV1, chains A and B, both corresponding to amino acids 44–243 of the protein primary structure) with the Biopolymer module of the Insight II suite (Accelrys, San Diego, CA). The rotamer library for cysteine was then manually explored and the most favourable energetic arrangements were selected. In agreement with previous data [33,34], the lack of the N-terminal domain of apoA-I is not expected to influence the formation of small s-HDL.

2.2. Preparation of the apoA-IM–apoA-IM and apoA-IP–apoA-IP homodimers

ApoA-IM and apoA-IP were docked to another copy of themselves by using the rigid docking approach implemented in ZDOCK with default parameters, generating 2000 solutions for each A–A′, A–B and B–B′ chain pairs. The 20 solutions with lowest energy were visually inspected. Only the complexes with a cysteine sulphur–sulphur distance compatible with cystine formation after EM were accepted. The selected complexes were subjected to energy minimisation (EM) with MOE (Chemical Computing Group, Quebec, Canada), using the CHARMM27 force field, down to a root mean-square (RMS) gradient of 0.001 kcal mol^{-1} Å^{-1};a distance-dependent dielectric term ($e = 4r$) was applied, as in Vitale et al. [35]. The structures of apoA-IM–apoA-IM and of apoA-IP–apoA-IP with the lowest energy were used to set up the system for the two main MD simulations.

2.3. Preparation of the s-HDL particles and MD simulations

The preparation of the s-HDL particles containing the homodimeric forms of apoA-IM or apoA-IP required a large and equilibrated double layer of POPC: an equilibrated 32×32 lipid-molecule bilayer was obtained from the free-access database of bilayer structures of Peter Tieleman (http://moose.bio.ucalgary.ca/) and used to manually create a larger bilayer of 64×64 POPC molecules. This novel membrane structure was energy-minimised, solvated with water in a $16 \times 16 \times 16$ nm^3 box, and minimised again; then its dynamics was simulated for 2 ns to get an equilibrated structure and to avoid any boundary artefact. The apoA-IM–apoA-IM and the apoA-IP–apoA-IP molecules were placed into the double layer while getting as many lipid molecules as possible trapped into the hole formed by the ringlike dimer structure, in agreement with relevant experimental data on the s-HDL composition [36]. The lipid molecules remaining outside the hole were manually removed using the Insight II suite, running on SGI Fuel, with the aid of Stereo Graphics Crystal Eyes stereo view. The resulting s-HDL particle with the apoA-IM–apoA-IM homodimer contained 98 POPC molecules; it was inserted in a $16 \times 16 \times 16$ nm^3 box, and neutralised with 14 Na^+ ions. The s-HDL particle with the apoA-IP–apoA-IP homodimer contained 94 POPC molecules; it was inserted in a $15 \times 15 \times 15$ nm^3 box, and neutralised with 14 Na^+ ions. Both systems were solvated with the simple point charge model of water. After an initial energy minimisation, they were simulated in two different steps: (1) 1 ns of position-restrained MD with an isotropic force (1000 kJ mol^{-1} nm^{-2}) applied to all protein atoms to allow for both water exit from the hydrophobic bilayer core and solvent relaxation; and (2) 50 ns of unrestrained MD. The GROMOS96 force field, modified according to Berger et al. [37] to improve lipid properties, was applied. All the simulations were performed at 300 K and 1 bar with a coupling constant of 0.1 ps for temperature and 1.0 ps for pressure, in both cases applying the Berendsen weak coupling algorithms [38]. The time step for integration was set at 2 fs. Fast particle-mesh Ewald (PME) electrostatics [39,40] was applied with the following parameters: distance for the Lennard–Jones cutoff = 0.9 nm; distance for the Coulomb cutoff = 0.9 nm; maximum spacing for the fast Fourier transform (FFT) grid = 0.12 nm and a cubic interpolation order, with neighbour list searching (updated every 10 steps). The main simulations were carried out on the Vital-IT Linux cluster at Lausanne.

2.4. Simulation of apoA-IP–apoA-IP with wall restraints

Simulated annealing (SA) of two models of s-HDL containing apoA-IP–apoA-IP and POPC with either parallel or antiparallel arrangements were carried out. A s-HDL model containing two