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Inhibition of peptide aggregation by lipids: Insights from coarse-grained molecular simulations

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ABSTRACT

The amyloidogenic peptide apolipoprotein C-II(60–70) is known to exhibit lipid-dependent aggregation behaviour. While the peptide rapidly forms amyloid fibrils in solution, fibrillisation is completely inhibited in the presence of lipids. In order to obtain molecular-level insights into the mechanism of lipid-dependent fibril inhibition, we have employed molecular dynamics simulations in conjunction with a coarse-grained model to study the aggregation of an amyloidogenic peptide, apoC-II(60–70), in the absence and presence of a short-chained lipid, dihexanoylphosphatidylcholine (DHPC). Simulation of a solution of initially dispersed peptides predicts the rapid formation of an elongated aggregate with an internal hydrophobic core, while charged sidechains and termini are solvent-exposed. Inter-peptide interactions between aromatic residues serve as the principal driving force for aggregation. In contrast, simulation of a mixed peptide–DHPC solution predicts markedly reduced peptide aggregation kinetics, with subsequent formation of a suspension of aggregates composed of smaller peptide oligomers partially inserted into lipid micelles. Both effects are caused by strong interactions between the aromatic residues of the peptide with the lipid hydrophobic tails. This suggests that lipid-induced aggregate inhibition is partly due to the preferential binding of peptide aromatic sidechains with lipid hydrophobic tails, reducing inter-peptide hydrophobic interactions. Furthermore, our simulations suggest that the morphology of peptide aggregates is strongly dependent on their local lipid environment, with greater contacts with lipids resulting in the formation of more elongated aggregates. Finally, we find that peptides disrupt lipid self-assembly, which has possible implications for explaining the cytotoxicity of peptide oligomers.

1. Introduction

A number of debilitating human disorders are characterised by the extracellular deposition of insoluble aggregates composed of proteins and other biomolecules in the body, including Alzheimer's, Parkinson's, Huntington's and the transmissible prion diseases [1]. Numerous experimental evidence now indicates that many sequentially and structurally non-homologous proteins and peptides, as yet un-associated with any known physiological disorders, are capable of fibril-formation given certain environmental conditions, such as pH, temperature, pressure and ionic strength [2,3]. In addition to full-length proteins, "core fibril regions" within intact proteins have been identified using computational techniques [4]. These derivative peptides, when cleaved from the full-length chain, have been shown to be capable of independent fibril formation. Examples include short peptides derived from islet amyloid polypeptide (IAPP) [5], the central motif of human calcitonin [6], fibril-forming segments of yeast prion protein sup35 [7], 21-residue [8] and 11-residue [9] fragments of human apolipoprotein C-II (apo-CII). Understanding the aggregation mechanism of amyloidogenic derivative peptides is important due to the possible insights that may be obtained into the fundamental fibrillisation principles of larger proteins.

Conversely, conditions may be contrived which inhibit the formation of insoluble aggregates and, in some cases, result in the dissociation of mature fibrils. Examples include oxidation [10,11] and binding to cyclic organic molecules (cyclodextrin) [12,13] and peptides [14]. More recent work have also indicated the role of surfactants and lipids in regulating the morphology of mature fibrils formed from human apolipoprotein C-II [8], as well as their formation kinetics, in some cases fully inhibiting their formation [8]. Phosphocholine (PC) lipids is known to inhibit fibrillisation of apoC-II protein and its fibrillogenic tryptic peptides, apoC-II(56–76) [15,16] and apoC-II(60–70) [9,17], in a concentration-dependant manner.

Theoretical molecular modelling is long-recognised as a powerful complement to experimental studies in the biological sciences, and has been employed to study protein aggregation. We have previously applied all-atom molecular dynamics (MD) simulations to study the monomeric structure of the apoC-II(60–70) peptide

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under experimentally known fibril-enhancing and fibril-inhibiting conditions [9]. To enhance sampling of all-atom MD, we have applied umbrella sampling methods to calculate the free energy of dimerisation of this peptide under lipid-rich and lipid-depleted conditions [17]. Based on these simulations, it was hypothesised that lipids inhibit fibrillisation by stabilising oligomers which are incapable of propagating fibril formation. This hypothesis was consistent with results from subsequent ultracentrifugation experiments [17], which confirmed the presence of oligomers in mixed lipid-peptide solutions. Other advanced methods, such as replica-exchange MD [18], have also been applied to study aggregation of amyloidogenic peptides [19,20]. A more recent method of enhanced sampling for studying protein dynamics and aggregation is metadynamics, which enables exploration of conformational space along pre-defined reaction coordinates [21,22].

Although advanced methods in conformational sampling have enabled the study of the formation of oligomers at atomistic resolution, aggregation phenomena at biologically relevant length and time-scales are still computationally intractable. To bridge the gap between the short time and length-scales of atomistic molecular simulations and experimental-scale phenomena, mesoscale coarse-grained (CG) molecular models have been developed, enabling faster computation albeit at the cost of atomistic detail. CG models have been applied specifically for the study of amyloid fibril formation. A CG forcefield, together with the "activation–relaxation technique" sampling method was applied by Wei et al. [23] to study fibrillation of KFFE. Similarly, Urbanc et al. [24] used a 4-colour bead model, in conjunction with discrete MD (DMD), to examine Aβ oligomerisation. Models capable of describing mature fibril formation include those of Pellarin and Caflisch [25], who recently developed a simple, two-state model capable of imitating fibril-like peptide ordering from an initial random peptide dispersion; and Bellesia and Shea [26], whose chiral model reproduces fibril ribbon twist.

CG methods have also been applied to the study of biological lipids and their interactions with proteins and peptides. One of the most demonstrably successful CG forcefields is MARTINI [27,28], which has been employed to study peptide–micelle interactions, peptide toxin self-assembly and membrane insertion [29,30], and membrane protein–lipid self-assembly [31,32]; more examples are described in [33]. In this model, significant computational speed-up is possible due to a number of factors. Firstly, the coarse-graining scheme produces a reduction in the total number of particles, and therefore a significant reduction in the number of non-bonded interactions, in the simulation system. Secondly, larger simulation timesteps may be used as a result of the removal of fast degrees of freedom such as individual (i.e. atomic-level) bond length/angle vibrations and torsion angle rotations, the latter in cases where four or more atoms are combined and treated as a single interaction site. Furthermore, there is a "smoothing" of the potential as a result of combining several atoms into single sites, which in turn produces smoother free energy landscapes which are more easily explored by unbiased simulation dynamics (in contrast to rugged, atomistic landscapes, which contain multitudes of local energy minima). Since the MARTINI forcefield was parameterised specifically for interactions between amino acids and lipid bilayers, it is a potentially powerful approach to study the interactions between lipids and peptides, such as apoC-II(60–70), which may lend further important insights into the mechanisms by which lipids inhibit fibrillisation.

In the present work, we have applied the MARTINI forcefield to study the aggregation of multiple copies of the apoC-II(60–70) peptide, the self-assembly of short-chain phosphatidylcholine (PC) lipids (experimentally demonstrated to inhibit fibrillisation), as well as the self-assembly of a combined peptide–lipid mixture, in order to obtain qualitative insights into the fibril-inhibition mechanisms of the lipid. These CG simulation results provide experimentally verifiable hypotheses regarding the structures of the non-fibrillar peptide–micelle complexes. We compare the results obtained by the above-mentioned CG methods with our previous atomistic simulations and experimental studies [8,9,17].

2. Methods

2.1. MARTINI molecular models and parameters

We have employed the MARTINI forcefield [27,28] to study the aggregation behaviour of the apoC-II(60–70) peptide in pure water and in the presence of short-chain PC-like lipids. This forcefield has been successfully applied in numerous studies of peptide and protein–lipid interactions [33], C60–lipid interactions [34], and lipid phase separation [35]. It uses a four-to-one (4–1) atom-site mapping, in which typically four (4) heavy atoms (i.e. non-hydrogen atoms) are represented by a single interaction site, reducing the granularity of representation of the molecule. The model takes into account four main types of interaction sites: polar (P), nonpolar (N), apolar (C), and charged (Q). For particles of type N and Q, four subtypes (0, d, a, and da) are further distinguished. Subtype 0 applies to groups in which no hydrogen-bonding capabilities exist, d and a are for groups that could act as a hydrogen-bond donor or acceptor, respectively, and da is for groups with both donor and acceptor options. All non-bonded particles interact via a Lennard–Jones (LJ) potential. In addition to the LJ interaction, charged groups (type Q) interact via the electrostatic Coulombic potential. The particle types for most amino acids were determined by comparison between simulation results and experimental measurements of the water-oil partition coefficients of the amino acid analogues. Details of the assignment of particle types and coarse-grained force field parameters for amino acids can be found in Monticelli et al. [28]

We have constructed models of the apoC-II(60–70) peptide and PC lipid molecules following the methodology prescribed by Monticelli et al. [28]. The amino acid sequence for the peptide is MSTYTGIFTDQ. For the peptide, each amino acid is represented by a single backbone bead (shown as green spheres in Fig. 1A) harmonically bonded to sidechain beads (yellow spheres, Fig. 1A). Except for glycine, all residues consist of at least one sidechain bead. Tyrosine and phenylalanine sidechains are composed of three beads to model their molecular planarity. We assign all bonded and non-bonded interaction parameters for the amino acids according to Monticelli et al. [28]. The peptide is composed of a series of CG residues harmonically linked via the backbone beads. Backbone bonded parameters are dependent on the pre-defined secondary structure of the residues (helix, strand or coil). Thus, in the current implementation of MARTINI, significant backbone structural transitions generally do not take place. In the current work, we assign backbone bonded parameters consistent with random coil for all residues of the peptide. This choice is justified, given that the peptide exhibits a significant bias (>60% of the time from a 600 ns trajectory) towards random coil conformations in atomistic MD simulations [9].

For the DHPC lipid, we have used all bonded and non-bonded parameters as provided in the MARTINI forcefield [27,28]. Specifically, we model each of the two hydrophobic tails with two C-type beads; the choline group modelled as a positively charged Q_0-type bead; the phosphate group as a negatively-charged Q_a bead; and the glycerol backbone as two polar P-type beads. The model is shown in Fig. 1B. This model represents lipids of aliphatic carbon chain lengths between 8 and 11. Although lipid chain length is ambiguous in a CG model, this is not a significant problem in our current study, as PC lipids of lengths from C4 to C12 have been