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Biophysical Chemistry 130 (2007) 102–113

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Molecular dynamics simulations of a fibrillogenic peptide derived from apolipoprotein C-II

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Received 6 June 2007; received in revised form 3 August 2007; accepted 3 August 2007
Available online 22 August 2007

Abstract

The pathway to amyloid fibril formation in proteins involves specific structural changes leading to the combination of misfolded intermediates into oligomeric assemblies. Recent NMR studies showed the presence of "turns" in amyloid peptides, indicating that turn formation may play an important role in the nucleation of the intramolecular folding and possible assembly of amyloid. Fully solvated all-atom molecular dynamics simulations were used to study the structure and dynamics of the apolipoprotein C-II peptide 56 to 76, associated with the formation of amyloid fibrils. The peptide populated an ensemble of turn structures, stabilized by hydrogen bonds and hydrophobic interactions enabling the formation of a strong hydrophobic core which may provide the conditions required to initiate aggregation. Two competing mechanisms discussed in the literature were observed. This has implications in understanding the mechanism of amyloid formation in not only apoC-II and its fragments, but also in other amyloidogenic peptides.

Keywords: Apolipoprotein; Molecular dynamics; Amyloid; Turn; Fibril; Simulation

1. Introduction

The misfolding of protein and peptides is subject to regulatory control mechanisms that lead to either refolding or degradation. However, sometimes misfolded proteins will aggregate and form insoluble amyloid fibrils. These amyloid assemblies form the basis of degenerative amyloid based diseases, such as Alzheimer's disease, Parkinson's disease and variant Creutzfeldt–Jakob disease. It is widely believed that the amyloidoses share common pathogenic mechanisms which lead to protein fibril formation and deposition. Consequently, an understanding of the folding mechanism of proteins is critical in understanding the structural changes which cause amyloid diseases. There are a family of proteins that appear to be structurally and functionally unrelated, but nevertheless, undergo the same biochemical and biophysical processes to form insoluble fibril aggregates [1]. One such protein is the human plasma apolipoprotein protein C-II (apoC-II), a component of lipoproteins and a cofactor for lipoprotein lipase [2]. ApoC-II is a 79 amino acid protein, which in lipid-free conditions folds into cross-β sheet structure to form amyloid fibrils [3]. Hydrogen–deuterium exchange and NMR spectroscopy of apoC-II fibrils revealed core regions between residues 19–37 and 57–74 [4]. This was confirmed by tryptic hydrolysis of the apoC-II fibrils yielding apoC-II(56–76), which readily formed fibrils. The ability of apoC-II to form amyloid is typical of other apolipoproteins (see Hatters et al., 2002 [5] and references therein). ApoC-II is of particular interest because aggregates of apoC-II are known to be associated with human atherosclerotic plaques and the macrophage inflammatory response [6].

Traditional experimental methods have contributed to the understanding of amyloid formation, however experimental analysis has been limited by the insolubility of the amyloid assemblies. X-ray fibre diffraction and solid state NMR studies have shown that amyloid fibrils are typically composed of cross

Abbreviations: apo, apolipoprotein; NMR, nuclear magnetic resonance; Aβ, β-amyloid; MD, molecular dynamics; PME, particle mesh Ewald; VMD, visual molecular dynamics; PEPCAT, peptide conformational analysis tool; SASA, solvent accessible surface area; ROG_{hyd}, radius of gyration of the hydrophobic residues.

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0301-4622/$ - see front matter.
doi:10.1016/j.bpc.2007.08.002

β-structure. This structure consists of β-strands running perpendicular to the fibril axis and hydrogen bonded to form β-sheets which run parallel to the fibril axis [7,8]. Advances recently have provided insights into understanding the structure, mechanisms and disease association of amyloidogenic peptides, including atomic structures of the spine of amyloid-like fibrils [9–12]. Despite the progress which has been made in experimental work, at the atomistic level there is limited information on the insoluble aggregates and of the mechanism relating to the structural change.

Protein fibrillation involves the formation of specific partially folded intermediates, which combine until a stable oligomeric assembly is formed. The partially folded intermediates are a result of specific structural transitions. Experimental work has shown the consistent occurrence of helix-to-β-strand transitions [13–15]. This is clearly shown in the prion protein where the transition from α-helix-to-β-sheet involves a large part of the protein [13]. Other conformational changes believed to occur are random coil→α-helix and random coil→β-strand transitions [16–21]. Studies have indicated the presence of turns in amyloid peptides which are stabilized by hydrophobic and electrostatic interactions [22]. Various studies, including solid state NMR studies, also suggest the presence of turn or bend structure in amyloid fibrils [11,23,24]. It has been postulated that "turn" formation may nucleate the intramolecular folding and subsequent assembly of amyloid [25]. In fact, one recent study showed that the amount of β-turn present in the Alzheimer's associated amyloid-β peptides correlates with the potential to aggregate into fibrils [26].

The study of β-turns and β-hairpin formation provide a good system for investigating the fundamental issues in protein folding because they are often initiation sites in early protein folding events [27,28]. The β-turn is stabilized by hydrophobic interactions, and in the case of the β-hairpin, by hydrogen bonding. The side chains move to a position to promote the formation of a hydrophobic cluster essential for the folding of the hairpin. There are different theories in the relative timing of the formation of the interstrand hydrogen bonds near the turn and the hydrophobic core. The "kinetic zipping mechanism" of Munoz hypothesized that in the folding of the hairpin [29], the β-turn forms initially thus placing the strands in a position such that the remaining hydrogen bonds are formed between the strands towards the termini regions. The turn is then further stabilized by the hydrophobic cluster formed by the aromatic residues. Alternatively, Pande [30] suggested the β-hairpin is initiated by the "hydrophobic collapse" mechanism [31], involving clustering of the hydrophobic sidechains and the subsequent formation of interstrand hydrogen bonds. The early development of the hydrophobic assembly is also discussed in a study of β-hairpin formation by Karplus [32]. This study suggests the hydrogen bonds are formed between the strands in both directions from the hydrophobic cluster. Klimov suggested the relevance of both mechanisms depending on the position of the hydrophobic cluster which in turn defines the rigidity of the hairpin [33]. The turn is likely to form first if the hydrophobic residues are located near the turn region, as in the former kinetic zipping mechanism. The latter hydrophobic collapse mechanism may be more likely when the hydrophobic residues are located in the middle of the strands such that the hydrophobic core is readily formed. On the other hand, if the hydrophobic residues are not in either position, it may be a combination of the two mechanisms.

Previous molecular dynamics (MD) studies have provided insights into the mechanism of the formation of β-turns. Bonvin simulated several starting conformations of the α-amylase inhibitor (native β-hairpin, α-left hand helical and extended conformation) over 10–30 ns at 300 K, 360 K, 400 K and observed consistent β-hairpin formation [34]. Roccatano performed simulations of protein G [35], which showed that the dynamical behaviour of the hairpin at different temperatures is very similar and large motions of the turn and end residues were observed (in agreement with experimental observations). MD has also been widely used to study the molecular properties of specific fragments of amyloidogenic proteins. The simulations have explored the mechanistic details of the fibrillation pathway and reproduced the structural transitions, including the presence of bend-motifs. These studies include simulations of single peptides [36–47] and of oligomers in various sizes and arrangements [40,48–56]. Many of these MD studies have been performed on the various peptides of the amyloid β-protein which is linked to Alzheimer's disease [41,42,44,49,57]. Conformational analysis of the Aβ(21–30) fragment, believed to be the nucleation site, have reproduced native conformations, including a bend-motif [38,42]. β-hairpins were also observed in simulations of the Aβ(25–35) fragment in water [41]. Simulations of the Aβ(1–42) fragment suggests the location of β-conformation seeding residues [58]. A conformational template identifying alternating β-bend/α-helix structure has shown the presence of a reoccurring bend-like motif in the amyloid-β peptide which would enable efficient folding of the strands of the β-sheet forming the fibrils [43]. The role of different structural elements of the Aβ(9–40) fragment, including the turn regions and the importance of hydrophobic packing in stabilizing the amyloid structure, has been investigated by Hummer [55,56]. The stability and dynamics of the prion protein has also been widely studied by MD. Conformational changes between α-helices and β-strand conformation including β-hairpin have been observed [46,54,59–61]. The presence of β-hairpins in the fibrillogenic process strongly suggests a role in the protein folding nucleation sites [62].

It is believed that small fragments of amyloidogenic proteins are integral in forming the partially folded intermediates of the fibrillation process [63,64] and that these fragments contain core regions which play a key role in aggregation [40,63,64] In our current study all-atom MD simulations have been used to investigate the dynamics of a tryptic peptide, apoC-II (56–76), derived from apoC-II for a total of 400 ns. The 56–76 peptide was chosen for study because it also has been shown experimentally to form fibrils and is believed to contain the core residues initiating the conformational change [4]. Several initial peptide conformations containing secondary structure elements associated with the structural transitions observed in fibrils were used for the MD study. Initial structures forming an α-helix, an extended strand and a β-strand as well as the experimental NMR structure of apoC-II in lipids were used. As a result 4 trajectories with the simulation time of 100 ns for each system have been generated thereby enabling us to observe the dynamical behaviour of the apoC-II peptide over a longer time period sampling different