Metastable Alpha-rich and Beta-rich Conformations of Small Aβ42 Peptide Oligomers

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December 15, 2022

Abstract

Probing the structures of amyloid-beta (Aβ) peptides in the early steps of aggregation is extremely difficult experimentally and computationally. Yet, this knowledge is extremely important as small oligomers are the most toxic species. Experiments and simulations on Aβ42 monomer point to random coil conformations with either transient helical or β-strand content. Our current conformational description of small Aβ42 oligomers is funneled toward amorphous aggregates with some β-sheet content and rare excited states with well-ordered assemblies of β-sheets. In this study, we emphasize another view based on metastable α-helix bundle oligomers spanning the C-terminus residues which are predicted by the machine-learning AlphaFold2 method and supported indirectly by low-resolution experimental data on many amyloid polypeptides. This finding has consequences in designing drugs to reduce aggregation and toxicity.

Μεταστaβλε Αλπηα-ριζη ανδ Βετα-ριζη ὀνφορματιονς οφ Σμαλλ Αβ42 Πεπτιδε Ολιγομερς

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Abstract: Probing the structures of amyloid-beta (Aβ) peptides in the early steps of aggregation is extremely difficult experimentally and computationally. Yet, this knowledge is extremely important as small oligomers are the most toxic species. Experiments and simulations on Aβ42 monomer point to random coil conformations with either transient helical or β-strand content. Our current conformational description of small Aβ42 oligomers is funneled toward amorphous aggregates with some β-sheet content and rare excited states with well-ordered assemblies of β-sheets. In this study, we emphasize another view based on metastable α-helix bundle oligomers spanning the C-terminus residues which are predicted by the machine-learning AlphaFold2 method and supported indirectly by low-resolution experimental data on many amyloid polypeptides. This finding has consequences in designing drugs to reduce aggregation and toxicity.

Keywords: amyloid-beta, aggregation, simulations, atomistic, coarse-grained, small oligomers

1.Introduction

Amyloid-beta (Aβ) peptides of 40 and 42 amino acids are proteolytically cleaved form amyloid precursor protein by β- and γ-secretases. Soluble Aβ dimers isolated from Alzheimer's cortex directly induce tau hyperphosphorylation and neuritic degeneration, and small Aβ oligomers are believed to the most toxic species.[1,2] The aggregation kinetics of Aβ follows a sigmoidal curve with three phases: a lag phase free of any Thioflavin T fluorescence signal, a growth phase or fibril elongation followed by a saturation phase.[3,4]

Aβ42, less abundant but more toxic than in its Aβ40 counterpart, is very sensitive to protein concentration upon aggregation in the bulk solution, and also pH, temperature, the presence of membrane and the addition of seeds. Characterizing the early Aβ oligomers is challenging experimentally and computationally.[5-7]

This is an experimental challenge due to the transient and heterogeneous ensemble of oligomer structures, the fact that most experimental observables provide time- and space-averaged properties,[6] and the μs time resolution cannot be achieved yet.[8] This is a challenge for computer simulations due to the accuracy of the protein and water force fields,[9,10] and the time scale of primary nucleation in pure buffer which is on the order of several hours for Aβ42 at μM concentrations.[4] By using atomistic molecular dynamics (MD) simulations, the current time lengths vary from 2.5 to 30 μs for 20 Aβ42 peptides with an implicit solvent model,[11] and Aβ40 monomer in explicit water,[12] respectively. Simulations of Aβ40/42 species with free lipids and calcium ions in aqueous solution are also currently limited to the μs time scale.[13-15] Going beyond this time scale or sampling rare events has been made possible by the use of coarse-grained or mesoscopic models and enhanced sampling techniques such as path or umbrella sampling and metadynamics, among others. Our current structural view of Aβ40/42 monomers and small oligomers is random coil, with increasing β-sheet content as the oligomer size increases.[16] In this study, we emphasize other metastable oligomers based on α-helix bundles that are predicted by the AlphaFold2 machine learning and are indirectly supported by low-resolution experimental data on many amyloid polypeptides.

2. Results and discussion

2.1 Monomer

The current experimental view we have for Aβ40 and Aβ42 monomers is that they lack stable secondary and tertiary structures and have flat free energy surfaces.[17] The monomers consist of a heterogeneous ensemble of random coil states with little α-helix and β-strand character. Both extended and compact conformations were obtained by SOP-IDP coarse-grained Langevin dynamics simulations at 300 K,[18] all-atom metadynamics simulations at 350 K using CHARMM22-TIP3P force field,[19] and atomistic MD simulations at 300 K using the AMBER99SB-disp[12] and CHARMM36m-TIP3P modified[20] force fields.

Small helical contents in monomer were evidenced by many theoretical studies. Metadynamics at 350 K applied to Aβ40 monomer predicted high energy states with α-helix at residues 21-26 and 30-37.[19] Aβ42 conformations with α-helix content spanning residues 10-20 were predicted by the Folding@home approach using thousands of MD simulations with the AMBER99sb-TIP3P force field,[21] and by multiple-reservoir replica exchange simulations with the AMBER99sb/TIP4P-Ew force field.[22]Partially folded α-helical structures spanning the CHC (central hydrophobic core, residues 17-21) and residues 30-38 of Aβ42 were reported by MD simulations and Hamiltonian replica exchange with solute scaling.[23] A short helix covering residues 17-23 was also reported for Aβ40 monomer using a predictive coarse-grained force field.[24]

Transient helical conformations were also evidenced experimentally. They were reported by a SERS (surface enhanced Raman spectroscopy) study on Aβ40 monomer between pH 5.5 and 10.5.[25] A nuclear magnetic resonance (NMR) structure of Aβ40 monomer reported on the formation of a 3-10 helix spanning residues 13-23 at pH 7.3 at 50mM NaCl.[26] Aβ42 monomer was found essentially disordered but displays α-helix spanning residues 15-24 and 29-35 in the presence of micelles.[7]

Small β-strand contents were evidenced by circular dichroism (CD) experiments[17] and many simulations using atomistic or coarse-grained models, suggesting notably the existence of multiple transient β-hairpin conformations covering the CHC and the C-terminus (30-42),[7,27,28] and revealing the very low probabilities of the aggregation-prone N* states with U-shaped or S-shaped fibrillar conformations.[18]

2.2 Ρανδομ ϖιλ Ολιγομερς ωιτη β-σηεετ ςοντεντ

While small α-helix and β-strand contents are present in the spectrum of conformations of Aβ42, our current conformational view for small Aβ42 oligomers is funneled toward β-sheet conformations for several reasons.

The first reason comes from the high propensity of β-sheets revealed by oligomer simulations at a very high

concentration of small fragments of Aβ (Aβ16-22, Aβ37-42, Aβ25-35, Aβ10-24 and Aβ35-40), tau (PHF6 motif, repeats R1-R4), transthyretin (105-115) and β2-microglobulin (83-89) peptides which also form fibrils.[29-36] It is notable, however, that two simulations on Aβ16-22 oligomers proposed helical intermediates.[37-39]

Second, the preference for β-sheet formation comes from the fact that many computational methods do not explore the full conformational ensemble. On-lattice Monte Carlo simulations do not allow the formation of α-helix oligomers[40-42] and atomistic metadynamics simulations do not include collective variables associated with side chain packings of α-rich oligomers. It is important to note that the introduction of the steric zipper interface between the side chains as a collective variable was found critical in metadynamics simulations to understand the primary nucleation of 18 Aβ37-42 peptides.[35]

Additionally, off-lattice simplified models aimed at understanding primary and second nucleation mechanisms either tune the probability of the β-strand monomer,[43] or consider three states for Aβ dimers with coil-coil, coil-β, and β-β character to explain the transition from amorphous to fibrils.[44] These models suggest that fibril formation at a concentration of mM can occur through the assembly of early ordered oligomers, the assembly of nonfibrillar aggregates rich in β-sheet content, or the formation of amorphous aggregates which reorganize to β-sheet aggregates and to fibrils.

Beta-rich Aβ42 oligomers ranging from elongated to compact shapes were described by ss-NMR spectroscopy, ion mobility separation coupled to mass spectrometry, and simulations, featuring multiple interfaces, mixed parallel/antiparallel strands, perpendicular β-sheets and β-barrels.[6,7,11,28,45-49] For instance, atomistic simulations in explicit solvent revealed β-barrel motifs in Aβ42 trimer and tetramer.[48,50,51] An hexamer peptide barrel was found experimentally to be the building block of Aβ protofibrils.[52]

Finally, using the multimer version of AlphaFold2,[53]we found that Aβ42 dimers up to hexamers have a non-negligible probability to display intramolecular β-hairpin conformations spanning the CHC and the C-terminus (residues 30-42), and in some cases to form β-barrels.[54]

2.3 Random coil Oligomers with alpha-helical content

The AlphaFold2 machine-learning approach is based on protein data bank (PDB) templates, sequence alignments, co-evolution rules and multiple algorithms to design a protein-specific potential of mean force. AlphaFold2 success stories include the prediction of single domain protein structures,[55] and most transmembrane protein structures.[56] AlphaFold2 limitations to predict very accurately the structures of protein – protein (peptide) complexes[57,58] and generate conformational heterogeneity[59] were reported.

At the date of the present study, the PDB contained about 200,000 structures.[60] The most striking AlphaFold2 result for the structures of Aβ42 dimers up to hexamers is the prediction of α-helix topologies for all species in addition to β-rich topologies.[54] The AlphaFold2 structures are shown in Figure 1. While the dimer displays an antiparallel helix bundle spanning the C-terminus (Figure 1A), all higher aggregates display parallel helix bundles spanning the C-terminal residues 29-39. (Figures 1B-E). These α-rich oligomers are supported indirectly by numerous experiments on Aβ and many other amyloid polypeptides.

CD experiments on Aβ42 and Aβ40 peptides in pure buffer give 19% and 32% of α-helix structure after 4 days of incubation.[61] Addition of trifluoroethanol suggested α-helical intermediates during Aβ assembly,[62] and addition of low solvent polarity stabilized partial α-helical structures and accelerated Aβ40 amyloid fibrillation.[63]Pyroglutamate-modified pEAβ(3-42) aggregation also pointed to α-helical intermediates, stabilized by parallel C-terminus interactions, each monomer forming a helix-turn-helix spanning residues 10-23 and 30-36.[64]

Slow nucleation of short polyglutamine-containing Huntingtin fragments via α-helix-rich oligomers and inhibition of amyloid structure in a Huntingtin fragment by targeting α-helix-rich oligomers were also reported experimentally.[65,66] Using computational and experimental approaches, human islet amyloid polypeptide (hIAPP) fragment 8-20 fibril formation starts from isolated helical monomers, helical dimers to hexamers, followed by the conversion to β at the hexamer level.[67] PolyQ-Aβ30-42 peptides at μM concentration suggested an aggregation triggered by a rapid formation of α-helical oligomers mediated by the C-terminal

residues, as assessed by CD and FTIR (Fourier Transformed Infrared) spectroscopies.[68] Infrared nanospectrometry monitored a α-to-β transition during the self-assembly of the N-terminal Josephin domain of ataxin 3.[69] The conversion of rationally designed α-helical peptides to amyloid fibrils and the oligomerization of natural hexapeptides into amyloid fibrils through α-helical oligomers are also well established.[70,71] Overall, there are many experiments reporting a minor population of partially folded helical oligomers during amyloid fibril formation.[72,73]

Additionally, a rational design of α-helical peptide inhibitors targeting Aβ40 surface reduces the generation of toxic Aβ toxic oligomers.[74] Helical peptide foldamers and peptidometics were found dual inhibitors of Aβ and hIAPP fibrillization.[75] Alpha-helix mimetics, which induce α-helicity in Aβ using NMR and CD, inhibit the seed-catalyzed aggregation of Aβ.[76] Based on ion mobility spectrometry – mass spectrometry combined to MD simulations, it was suggested that Aβ C-terminal interactions play a key role in their inhibitory activity.[77] Finally, it was found that Aβ25-35 peptide forms early stage helical conformations by CD and Raman spectroscopic techniques, and carvedilol inhibits Aβ25-35 fibrillation.[78]

Computationally, AlphaFold2 α-helical tetramer and hexamer structures are very stable using CHARMM36m-TIP3P modified and AMBER99SB-DISP for 0.3 μs MD simulations at 310 K.[54] Transient formation of helical conformations differing from helix bundles was reported by numerous simulations of Aβ40 and Aβ42 oligomers,[7,28,46,79] but a recent simulation proposed that conformations with α-helical structure have a high propensity to initiate Aβ42 aggregation.[80] Finally, it should be noted that the helix propensity of amyloid peptides is a fundamental requirement to fulfill the lipid-chaperon model,[81] and helical intermediates during amyloid formation are catalysed by membranes.[36,72]

3. Conclusions

The Aβ42 monomer and oligomer structures in aqueous solution are of high importance as they initiate fibril formation and are believed to be the most toxic species. While the community believes on random coil – β-sheet oligomers and the role of β-hairpin[82] in the early steps of aggregation, the existence of α-helical bundle metastable intermediates of Aβ42 oligomers is rarely cited, while it is predicted by AlphaFold2 and is, more importantly, supported indirectly by a large number of experimental studies on Aβ and many amyloid polypeptides under various conditions. It is important to note that there is a general resistance of the field to believing CD in detecting α-helix in aggregates, because of light-scattering interference and skewing of the CD spectrum. But the α-helix signal in oligomers was further evidenced by FTIR and Raman spectroscopies in addition to CD. Clearly, the coexistence of α-rich oligomers and β-rich oligomers en route to fibril formation has to be considered when designing drugs targeting Aβ monomers and oligomers.[76,81,83,84]

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Notes. The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the "Initiative d'Excellence" program from the French State, Grant "DYNAMO", ANR-11-LABX-0011. PD thanks Ronald Wetzel for useful discussions and Geoffrey Letessier of the Laboratoire de Biochimie Théorique for technical support.

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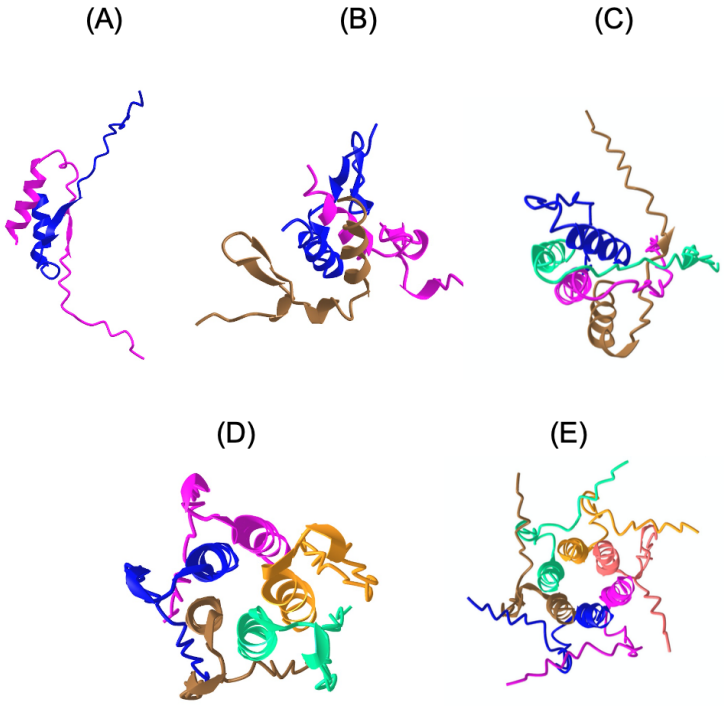

Figure 1. Representative structures of AlphaFold2 structures of Aβ42 aggregates. (A) dimer, (B) trimer, (C) tetramer, (D) pentamer and (E) hexamer showing the interface made by the C-terminus in helical conformations.[54]