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A DFT-assisted topological analysis of four polymorphic, S-shaped A β 42 fibril structures

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Abstract

Amyloid β 42 (A β 42) is an inherently disordered peptide, whose toxic actions are believed to play important roles for the etiology of Alzheimer's Disease (AD). Four fibril structures of the peptide that display broadly similar characteristics were recently published, but a systematic comparison of these structures is lacking. In this paper, a topological framework was created to enable such understanding and produced new insights into subtle structural elements, which underlie the overall structural diversity, and a DFT-based analysis illuminated some of the energetic differences that arise as their consequence.

Graphical Abstract

Similar but different: Four S-shaped Amyloid β 42 structures have been reported. The structures are qualitatively similar, but distinct in ways that are hard to capture. This paper proposes a topological framework to enable a similarity analysis. DFT calculations illuminate some energetic differences that arise as a consequence of the tightly circumscribed shape diversity.

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A β 42 is an aggregation-prone peptide, and its toxic actions are believed to play an important role for the etiology of AD.^[1] The A β framework is inherently disordered and, as such, capable of sampling a vast conformational space, which allows it to yield diverse structures in response to minute modifications of aggregation conditions.^[2]

Four A β 42 fibrillary structures were recently published that share a common S-shaped feature, which extends from the central hydrophobic cluster (CHC) LVFF (amino acids 17–20) through to the C-terminus, thus spanning amino acids 17 through 42.^[3] Whereas the N-terminal domain of A β 42 (amino acids 1–16) is flexible, prone to disorder and was only resolved in two of four structures, the S-shaped fibril core element was observed in all four structures. Whilst clearly displaying strong structural similarities at the qualitative level, the S-shaped folding elements are different in subtle but significant ways between the four structures (Figure 1A). The purpose of this paper is to provide a topological framework to enable a more systematic comparison between the four structures and to use Density Functional Theory (DFT)-based approaches to better understand the stabilities of the hydrophobic C-terminal region of the peptide, which is believed to be responsible for initial steps of A β 42 folding that leads to its aggregation into fibrils.

The significance of the C-terminal hydrophobic loop formation (loop 2) and its subsequent folding onto the CHC leading to the formation of loop 1 (Figure 1B,C) has been previously recognized as a key structural event that is needed for the formation of those folded scaffolds.^[4] However, an adequate framework within which these assemblies can be compared in quantitative fashion is lacking.

A straightforward topological framework was created to enable the systematic comparison of the four S-shaped motifs (extending from L17 to A42) associated with the A β 42 fibril structures (Figure 2). The hydrophobic sidechains of the residues L17–V18–F19–F20 of CHC form hydrophobic segment LVFF (A) and can be projected into loop 1 or away from loop 1, respectively. This can be represented through a simple 4×2 “chessboard” motif, in which direction of residue projection is represented by a filled square and the absence of the residue is represented by an open square. Analogously, the I31–I32 segment (B in Figure 2) can project its sidechains into loop 1 or loop 2 (a 2×2 chessboard motif), and the segments L34–M35–V36 and V39–V40–I41 (C and D in Figure 2) can project their sidechains either into loop 2 or away from it (2×3 chessboard motifs).

The four structures were subjected to this topological analysis, as displayed in Figure 2. The analysis of the chessboard patterns reveals that the most recently obtained structure 5oqv is the most distinct one of the four. It shows topological equivalence with 2mxu in segment 3, whereas segments 1, 2 and 4 are different in orientation of at least one of the four residues. There is no topological equivalence, defined in this paper as qualitative identity at the level of the simplified chessboard patterns shown in Figure 2, in any of the four segments when compared with 2nao and 5kk3, and segment 4 of 5oqv (out-in-out) is fully inverted as compared with the other three structures (in-out-in). The structure 2mxu is topologically equivalent with structures 2nao and 5kk3 in segments 1, 2 and 4, but different in segment 3 (out-in-out vs out-out-in), and the structures 2nao and 5kk3 are topologically identical. Topological identity is not to be mistaken for more subtle local conformational differences,

which clearly still exist between 5kk3 and 2nao. None of the four segments shows topological equivalence across all four structures. This is noteworthy, since there is evidence that different AD patients have fibrillary deposits that can differ structurally between people.^[5] We believe that our topological framework may provide a helpful tool to facilitate systematic comparison and deeper molecular level analysis of such structures.

Formation of loop 2 is believed to be one of the early A β 42 folding events, which is readily rationalized through its highly hydrophobic nature.^[4a, 6] The experimentally well-documented, increased A β 42 fibril forming propensity at elevated temperatures is likely to arise, at least in part, as a consequence of the loss of hydrophobic hydration of loop 2 upon heating,^[2a] although other factors, such as enhanced sampling of fibril-seeding conformers is also likely to contribute.^[4c, 7]

In an effort to better understand the local conformational differences of loop 2, the Ramachandran angles associated with the three segments were systematically analyzed (Figure 3 and Table 1). A high degree of variance was noted, particularly so for the LMV fragment, in which some of the angles covered a range significantly larger than 90° (e.g., $\Psi_{L34} = 173.3^\circ$ and $\Phi_{M35} = 161.0^\circ$ for Φ_{M35}). The values associated with I41, on the other hand, were amongst the most tightly circumscribed ones (Figure 3, see also Figure S1), ranging from $\Phi_{I41} = -117.6^\circ$ (5kk3) to -128.9° (2nao) and $\Psi_{I41} = +116.0^\circ$ (5oqv) to $+143.1^\circ$ (5kk3), which is noteworthy because I41 is the key residue that is responsible for structural and functional differences between A β 42 and its more soluble and less toxic A β 40 counterpart.^[8]

To better understand the energetics of loop 2 of A β 42 in those four structures, DFT calculations were conducted. For that purpose, A β 42 was truncated after residue 28 and residue 29 (glycine) replaced by an acetyl cap, yielding the four singly negatively charged, folded hydrophobic peptides Ac-AIIGLMVGGVVIA, referred to as 5oqv-L2, 2mxu-L2, 2nao-L2 and 5kk3-L2 (Figure S2). The lack of charge separation in the loop 2 region makes it a system that is particularly well suited for a DFT computational study, where solvent contribution is modeled through a dielectric continuum, which can lead to issues with multiply charged frameworks, especially if opposite charges are present.^[9] The backbone dihedral angles of the four peptides were frozen to remain in the conformations found in fibrils and rest optimized. The most stable of the four conformers was found to be 2mxu-L2 ($E_{\text{rel};2\text{mxu-L2}} = 0.0$ kcal/mol), followed by 2nao-L2 ($E_{\text{rel};2\text{nao-L2}} = +3.1$ kcal/mol), 5kk3-L2 ($E_{\text{rel};5\text{kk3-L2}} = +4.9$ kcal/mol) and, finally, 5oqv-L2 ($E_{\text{rel};5\text{oqv-L2}} = +9.5$ kcal/mol), thus establishing that the structural flexibility of the loop 2 region of A β 42 endows it with the ability to adopt shapes that are structurally sufficiently distinct to cover a rather wide energetic window of ~10 kcal/mol. The lower stability of 5oqv-L2 in our DFT calculations may be reflective of the fact that organic co-solvent was employed in the protocol that was used to grow A β 42 fibrils for structural determination in that study.^[3d]

The conformational flexibility of the hydrophobic loop 2 region is likely to be one of the key aspects of A β 42 that underlie the remarkable structural diversity of this peptide. Although the 2mxu-L2 motif has emerged as the most stable one of the four from this computational study, it has to be kept in mind that other, more thermodynamically stable conformational

states of loop 2 may exist and have not been reached in any of those structures due to kinetic reasons.

Computational Details

All DFT computations were performed with the Gaussian 09 program package,^[10] using the M062X functional, which has been recommended for computations of compounds composed of main group elements (such as peptides).^[11] All input structures were modified from the deposited pdb datafiles, using the programs Molden and Chemcraft. Solvent (water) was modeled using the polarizable continuum model (PCM), as implemented in Gaussian 09 ($\epsilon_r = 78.3553$). Backbone torsions were frozen at values found in fibrils and the rest optimized at the M062X/6–31G* level of theory. The optimized structures were subsequently subjected to single point calculations at the M062X/6–311++G** level of theory to then rank-order their relative energies.^[12] Coordinate superpositions to generate Figure 1A was performed with the Schroedinger Maestro 2009 program package by simultaneously minimizing distances of the C $_{\alpha}$ atoms of amino acids 17–42 in all structures. All geometry-optimized coordinate sets have been deposited as part of the Supporting Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A) Superposition of the four Aβ42 structural motifs found in the structures 5oqv, 5kk3, 2mxu and 2nao; B,C) location of the hydrophobic regions of Aβ42 (5oqv): central hydrophobic cluster (CHC) LVFF; C-terminal loop 2 IIGLMVGGVVIA, with the three hydrophobic segments shown.

Figure 2.

A topological analysis of the four S-shaped fibril core regions of A β 42 to classify the structures with regard to their similarity. Residues 17–42 are shown with the four hydrophobic segments highlighted by space filling.

Figure 3. Ramachandran angles for the segments AII, LMV and VVIA from the four structures.

Table 1.

Ramachandran angles for the hydrophobic fragments AII, LMV and GVVIA of the loop 2 region of A β 42.

Residue	Angle	5oqv	2nao	5kk3	2mxu
A30	ϕ	-135.7	-79.1	-150.9	-131.05
	ψ	+143.3	+107.3	+137.3	+114.9
I31	ϕ	-149.2	-135.6	-160.0	-140.85
	ψ	+108.0	+137.7	+149.4	+143.6
I32	ϕ	-148.1	-142.4	-135.3	-119.6
	ψ	+170.2	+86.7	+125.4	+129.3
L34	ϕ	+59.8	-78.8	-68.5	-85.6
	ψ	+74.5	+137.8	-35.5	-35.0
M35	ϕ	-106.4	+63.7	-135.3	+53.6
	ψ	+134.2	+41.4	+93.3	+90.1
V36	ϕ	-103.2	-101.2	-159.0	-132.0
	ψ	+91.2	+88.1	+141.75	+120.1
V39	ϕ	-148.3	-177.5	-139.3	-137.5
	ψ	+168.7	+123.7	+126.1	+146.4
V40	ϕ	-136.3	-127.5	-128.85	-123.2
	ψ	+139.9	+126.4	+132.1	+137.8
I41	ϕ	-119.65	-128.9	-117.6	-128.8
	ψ	+116.0	+130.2	+143.1	+128.7
A42	ϕ	-114.0	-81.3	-100.0	-134.5
	ψ	N/A	N/A	N/A	N/A