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Son Tung Ngo, Phuong H. Nguyen, Philippe Derreumaux. Impact of A2T and D23N Mutations on Tetrameric A β 42 Barrel within a Dipalmitoylphosphatidylcholine Lipid Bilayer Membrane by Replica Exchange Molecular Dynamics. Journal of Physical Chemistry B, 2020, 124 (7), pp.1175-1182. 10.1021/acs.jpcb.9b11881 . hal-03087700

HAL Id: hal-03087700

<https://hal.science/hal-03087700>

Submitted on 29 Dec 2020

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Impact of A2T and D23N Mutations on Tetrameric A β 42 Barrel within a DPPC Lipid Bilayer Membrane by Replica Exchange Molecular Dynamics

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Abstract: In Alzheimer's disease (AD), many experimental and computational studies support the amyloid pore hypothesis of the A β 42 peptide. We recently designed a β -barrel tetramer in a membrane-mimicking environment consistent with some low-resolution experimental data. In this earlier study by using extensive replica exchange molecular dynamics simulations we found that the wild-type (WT) A β 42 peptides have a high propensity to form β -barrels, while the WT A β 40 peptides do not. In this work, we have investigated the effect of the mutations D23N and A2T on the A β 42 barrel tetramer by using the same enhanced conformational sampling technique. It is known that the D23N mutation leads to early-onset AD, while the A2T mutation protects from AD. This computational study in a DPPC lipid bilayer membrane shows that the WT sequence and its A2T variant have similar β -barrel populations, and the 3D model is slightly destabilized for D23N compared to its WT sequence. These extensive modelling calculations indicate the lower and higher induced-toxicity of these two mutations in AD cannot be correlated to their β -barrel tetramer stabilities in a DPPC lipid bilayer membrane.

Introduction

In Alzheimer's disease (AD), the amyloid cascade, based on the deleterious effect of A β oligomers, is the most accepted hypothesis.^{1,2} Although all clinical attempts targeting these oligomers have failed to deliver drugs,^{3,4} negative Alzheimer's trials have not killed the leading theory of disease.⁵ It is known that A β oligomers are formed during the lag-phase of the aggregation kinetics (primary nucleation) or from the fragmentation of fibrils (secondary nucleation).⁶ *In vitro* interactions of these A β oligomers with the prion protein,⁷ the tau protein,⁸ the NMDA receptor,⁹ and many other cellular proteins have been characterized.

Amyloid oligomers interactions with model and cellular membranes are documented by a large number of experimental techniques, and A β oligomers, but not monomers, induce toxicity.¹⁰⁻¹⁵ Among the different types of amyloid protein-membrane interactions, there is increasing evidence that A β 42 can form β -barrels in various membrane-mimicking environments; micelles composed of dodecylmaltoside (DDM, 2 $\times$ CMC), 200 mM ammonium acetate and PC12 lipids,¹⁶ micelles composed of dodecylphosphocholine (DPC) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) lipids,¹⁷ and cellular membranes,¹⁸ based on electrical currents using patch-clamp^{18,19} or biochemical and biophysical experiments.^{16,17} Overall these annular pores can span various oligomer sizes as small as tetramers and hexamers and up to 24-mers.^{16,17,20}

AD is sporadic in 95% of the cases, but several familial AD (FAD) mutations provoke early-onset disease: H6R (English), D7H (Taiwanese), D7N (Tottori), A21G (Flemish), E22Q (Dutch), E22G (Arctic), E22 Δ (Osaka), and D23N (Iowa).⁸ These mutations are located in the N-terminus of A β (residues 1-11), the central hydrophobic core (residues 17-21) and in the loop region (residues 23-28). All these FAD mutations impact the aggregation kinetics in aqueous solution,^{6,8,21} change the ratio of A β 40 and A β 42 peptides released by the β - and γ -secretases, and notably augment the production of the most toxic A β 42 peptide.²² The D23N mutation also led to the discovery to a new amyloid fibril structure with antiparallel β -strands rather than parallel β -strands within

the sheets as observed for the WT A β 40 and A β 42 peptides under various experimental conditions, and for the α -synuclein and tau fibrils.²³ This transition from antiparallel to parallel β -strands in the sheets is not unique in the amyloid world, as it was observed for the A β 16-22 variant with E22Q mutation.²⁴

Due to the intrinsic disorder of all amyloid proteins and the transient character of all oligomers, experimentally-derived atomic structures are lacking, and computer simulations have been employed to determine the dominant microstates of the system in aqueous solution,^{8,25,26} on material surface,²⁷ and interacting with membrane models.^{28,29} The influence of D23N has motivated computational studies aimed at determining the free energy landscapes of A β 21-30,^{30,31} and A β 40/A β 42 monomers,^{32,33} A β 40/42 dimers,³⁴ A β 16-35 6-mers,³⁵ and the thermodynamics of fibril elongation,³⁶ all simulations performed in aqueous solution with various force fields and protein representations.

Along with disease-causing mutations, the A2T (homozygous) and A2V (heterozygous) mutations protect from AD and increase the lag-phase of aggregation.^{8,37} These mutations are particularly intriguing as the amino acid Ala is not located in the core of A β 40/42 amyloid fibril structures.²³ Atomistic simulations have investigated the impact of these substitutions on the equilibrium ensemble of A β 28, A β 40 and A β 42 in aqueous solution, revealing a significant change in the intrinsic disorder of the monomers,^{37,38} and the higher content of intrapeptide-stabilized conformations in the mutant dimers compared to its WT counterpart dimer.^{39,40}

While the impact of mutations (E22 Δ , F19P, F20C) and N-terminal truncations (A β 17-42, A β p3-42) has been investigated on the pore ability of double layers of β -sheets in a lipid bilayer membrane by MD simulations,²⁹ the influence of D23N and A2T on A β 42 pore ability has not been studied by experimental and computational means. Recently, we proposed a 3D model for A β 42 β -barrel tetramer that remains formed for 2 microseconds molecular dynamics (MD) simulation in a bilayer consisting of POPC/POPS/cholesterol/sphingomyelin with a 40/10/25/25 molar ratio.⁴¹ Extensive

replica exchange molecular dynamics (REMD) simulations of this 3D model in a DPPC lipid bilayer membrane showed that A β 42 forms barrels with a very high probability, while A β 40 does not.⁴¹ In this study, our aim is to determine whether the A2T and D23N mutations change the probability of β -barrel formation of the A β 42 peptides in the DPPC lipid bilayer membrane and if the predicted barrel stabilities can be correlated to the reduced and increased toxicity of these two variants in AD.

Materials and Methods

A2T and D23N barrels were centred in rectangular box of 7.12×7.15×6.60 and 7.12×7.15×7.60 nm, respectively, with (x, y, z) lipid bilayer dimensions of 7.12×7.15×4.00 nm solvated by the SPC water model.⁴² We used the united-atom force field GROMOS 53a6⁴³ for the peptides and the Berger force field⁴⁴ for the DPPC lipid bilayer. The peptides at pH 7 have NH₃⁺ and CO₂⁻ termini, deprotonated Glu and Asp, protonated Arg and Lys, and neutral His with a protonated N ϵ atom. In total, the transmembrane D23N A β 42 system consists of 123 DPPC lipid molecules, 4908 water molecules, 8 Na⁺ atoms (22,526 atoms), while the transmembrane A2T A β 42 system consists of 123 DPPC lipid molecules, 3282 water molecules, 12 Na⁺ atoms (17,656 atoms).

The WT MD-refined A β 42 structure obtained in Ref. 41 was mutated by using PyMOL⁴⁵ and further refined by a 5 ns MD simulation with decreasing restraints on the backbone and all side-chain atoms of all A β residues, except at positions 2 and 23.

REMD simulations were performed using GROMACS version 5.1.3⁴⁶ and all parameters described in Ref. 41. As for the WT A β 42 barrel, the D23N and A2T barrels were sampled by REMD with 28 replicas at 310.0, 312.8, 315.6, 318.4, 321.3, 324.1, 327.0, 329.9, 332.8, 335.8, 338.8, 341.7, 344.7, 347.8, 350.8, 353.9, 357.0, 360.1, 363.2, 366.4, 369.5, 372.7, 375.9, 379.2, 382.4, 385.7, 389.0, and 392.3 K. The D23N and A2T barrels were simulated by 500 ns of REMD, the first 50 ns were removed, and the comparison between the three sequences was monitored over the time interval 50-500 ns at 310 K. The data were recorded every 10 ps and 45,000 snapshots per system were used to determine the physical impact of the two mutations.

The time-averaged secondary structure compositions were predicted using DSSP tool,⁴⁷ and the REMD-averaged collision cross sections (CCS) were determined using the trajectory method of the IMPACT software.⁴⁸ The free energy landscape of each system was projected on the RMSD using all heavy backbone atoms of residues 9-40 with respected to its MD-refined structure, and the mean number of intermolecular side chain contacts using residues 9-40. Residues 1-8 are not included because they are disordered, and residues 41-42 are excluded to allow comparison with our earlier study on A β 40 peptide.⁴¹ An intermolecular side-chain (SC) contact n_{ij}^{kl} between residue i of chain l and j of chain k is counted when the minimum distance r_{ij}^{lk} between two residues is smaller than 0.45 nm, the indexes i, j are in the range of [9,40] and the indexes l, k are in the range of [1,4] with $l \neq k$. The total SC contact between two chains is the sum of all available SC between residues of two chains $N_{SC}^{kl} = \sum_{ij} n_{ij}^{kl}$, and the mean value of intermolecular SC contacts per pair is computed by $\langle N_{SC} \rangle = \frac{\sum_{k \neq l} N_{SC}^{kl}}{6}$.

Because of the high dimensionality of the conformational space, the trajectories were also analysed by computing the distribution of the tilt angle of the β -strands relative to the axis of the barrel, the distribution of the inner pore diameter, and the distributions of intra-molecular and intermolecular H-bonds. A H-bond was considered formed if the acceptor-donor-H angle is less than 30 degrees and the acceptor – donor distance is less than 0.35 nm.⁴¹

Results and Discussion

In our earlier study, the convergence of the WT A β 42 REMD simulation was assessed by the average of exchange rates between consecutive replicas (18%), the propagation of the 1st and 26th replicas over the temperature index during the simulation time, and the high similarity of the free energy landscapes projected on the RMSD and number of intermolecular contacts using the two time interval 50-500 ns and 150-500 ns.⁴¹ The same mean exchange ratio between consecutive replicas was obtained for the two variants (18-20%) and the other two controls for convergence are also satisfied for A2T and D23N mutants (data not shown).

The secondary structure remains constant upon mutation within statistical errors (2%): 48% of β -strand, 48% of coil, 2% of turn and almost 0% of α -helix (Table 1). The β -strand content along the sequence, see Fig. 1, remains identical between the WT and A2T sequences with the residues 12-22 and the residues 29-40 having each more than 50% of β -character. There is an averaged β -strand increase of 10% for residues 13-16, 21-24, 28-29 and 35-40 from WT to D23N. Upon D23N mutation, the turn content of residues 23-26 increases from 8 to 16% and the coil content augments moderately in the N-terminus. The coil and turn profiles of A2T and WT are nearly identical. In all species, the α -helix signal is less than 5% except in the region 2-12 for WT and D23N and the region 23-27 in A2T. The averaged CCS for the WT system ($22.6 \pm 1.4 \text{ nm}^2$) is in between that for D23N ($23.1 \pm 1.0 \text{ nm}^2$) and A2T ($20.6 \pm 0.8 \text{ nm}^2$).

Figure 2 shows the free energy landscapes (FEL) of the D23N and A2T A β 42 tetramers projected on the backbone RMSD with respect to the MD-refined structure and the mean value of intermolecular SC contacts per pair (N_c). The six free energy minima on the A2T FEL (Fig. 2A) are denoted by A0, B0, C0, D0, E0, and F0 with (N_c , population) values of (27.0, 34%), (32.6, 14%), (29.0, 17%), (25.0, 4%), (26.2, 5%), and (20.2, 7%), respectively. The seven free energy minima on the D23N FEL (Fig. 2B) are designated by A1, B1, C1, D1, E1, F1, and G1 with (N_c , population) values of (33.0, 29%), (36.2, 17%), (31.0, 9%), (28.2, 12%), (28.2, 13%), (28.2, 7%) and (29.0, 5%), respectively.

Our earlier study on the WT FEL revealed six free energy minima A-F (see Fig. 4A in Ref. 41) characterized by (N_c , population) values of (A: 24.8, 48%), (B: 22.4, 30%), (C: 25.4, 4%), (D: 28.6, 5%), (E: 27.0, 2%), and (F: 36.5, 5%). The minimum A is a perfect close barrel with an inner pore diameter of 0.75 nm and a tilt angle of 37.5 degrees, the minima B-D and F representing 48% of the ensemble are barrel-like and the minimum E is non-barrel.

Analysis of the three FELs reveals that both A2T and D23N mutations explore a larger conformational space than the WT sequence, RMSD varying between 0.57 and 0.78 nm (Fig. 2) vs. 0.42 and 0.67 nm for WT. The mean N_c value is 27 for both WT and

A2T and increases to 31 for D23N, indicating an additional difference upon D23N mutation compared to A2T.

Figure 3 shows the distributions of the numbers of intra-molecular H-bonds formed between the residues 12-22 and the residues 29-40 in the four consecutive chains (hairpins). These selections of amino acids is consistent with the β -strand secondary profile, see Fig. 1. It is not surprising that the distributions of the chains 1 and 4, that close the barrel, have similar profiles (many states with 5, 6, 7 and 8 H-bonds, see panels 3A and 3D) and differ from the two similar profiles of chains 2 and 3 (many states with 7, 8, 9 and 10 H-bonds, see panels 3B and 3C). Looking at the effect of the mutations on each profile, they both reduce the population of 8 H-bonds in chain 1 (panel 3A), the population of 7 and 9 H-bonds in chain 2 (panel 3B), and increase the populations of 7 and 8 H-bonds in chain 4 (panel 3D). Interestingly, D23N is able to destabilize the first hairpin (0 H-bonds with a population of 2%, see panel 3A).

Figure 4 shows the distributions of the numbers of intermolecular H-bonds formed between the residues 12-22 and 12-22 and between the residues 29-40 and 29-40 of the four interfaces. The four distributions differ substantially from each other, the profile of the first interface varying between 0 and 9 H-bonds (panel 4A), for the third interface it is from 0 to 12 H-bonds (panel 4C) and for the fourth interface it is between 1 and 10 H-bonds (panel 4D). Looking at the effect of the mutations on each profile, we see that D23N either increases the population of the maximum number of H-bonds (e.g., peaks for 7 and 8 H-bonds in interface 1 or peaks for 7, 8 and 9 H-bonds in interface 2) or decreases the populations of the same interface at other locations (e.g., peaks for 4, 5 and 6 H-bonds in interface 2). For A2T, its long-range effect is reflected for instance by the population of the maximum number of H-bonds stabilizing the interface 4 (almost 2% for 9 and 10 H-bonds) or destabilizing the same interface 4 (peaks for 2 and 3 H-bonds, panel 4D).

Upon mutation, there are some variations in the intermolecular side-chain--side-chain contact map of each individual interface, indicating structural and packing

reorganization (data not shown). Averaged over the four interfaces, however, the intermolecular contact maps of the WT and A2T sequences are very similar and have identical probabilities for the region 12-22, but the A2T map shows more intermolecular contacts in the C-terminus region (Figures 5A-B). The intermolecular pattern changes for D23N where both the hydrophobic core region and the C-terminus region display different and more contacts than their WT and A2T counterparts (Fig. 5C).

Figure 6 shows the distribution of the tilt angle and the inner pore diameter for the three species using all frames between 50 and 500 ns. The tilt angle profiles for D23N and A2T are very similar with two peaks of identical probabilities at 37-38 and 35.5 degrees, respectively. In contrast, the tilt angle profile for WT displays a dominant peak at 36.2 degrees with a shoulder at 37.2 degrees, see Fig. 6A. The distribution of the inner pore diameter is rather uniform for the WT sequence varying between 0.42 and 0.8 nm; those for A2T and D23 display a more pronounced peak at 0.5 nm and smaller fluctuations than the WT sequence (Fig. 6B).

Based on a minimal number of 5 residues with a β Ramachandran state in each β -strand and a minimal number of x H-bonds within all hairpins and connecting the four consecutive hairpins, we can quantify the population of perfect close eight-stranded β -sheets or β -barrels for the two variants. Using all frames between 50 and 500 ns, i.e., 45000 for each species, we find 75% for WT, 71% for A2T and 44% of barrels for D23N if $x=2$ (loose condition); and 48% for WT (identical to previous estimations),⁴¹ 45% for A2T and 32% of barrels for D23N if $x=3$ (more stringent condition).

Irrespective of the values of minimal number of H-bonds, the β -barrel tetramer has the same probability for WT and A2T and is slightly destabilized for D23N. There is therefore no correlation between the predicted β -barrel populations and the experimentally increased toxicity upon D23N and decreased toxicity upon A2T. The predicted stability of D23N barrel is consistent with MD simulations demonstrating that the Osaka (E22 Δ) A β 42 barrel, i.e. with a deletion of E22 before the D23 amino acid, and the WT A β 42 barrel made of annular double layers of β -sheets have similar morphologies

and dimensions.²⁹ Its lower stability compared to WT results from the absence of the D23-K28 salt-bridge that leads to subtle change of short-range and long-range interactions between the different hairpins and interfaces (see Figures 4 and 5) leading to more flexibility, although the loop region (residues 23 to 28) remains exposed to solvent as for WT, see Figure 7 reporting the time-averaged number of water molecules interacting with specific A β residues.

It is not surprising that A2T does not impact the global barrel population compared to its WT counterpart, as this mutation does not change significantly the conformational space of the N-terminal region mainly coil (98%) compared to the WT sequence, and this N-terminus resides in the bulk water region (see Fig.7), with transient interactions with the phosphate atoms of the lipid membrane bilayer. Our finding for A2T is also in agreement with the small variation in the experimental pore conductance of the A β 42 and A β p3-42 peptides, where in the latter peptide, the two first residues are cleaved and the glutamate is replaced by a pyroglutamate, although MD simulations revealed that the N-termini of A β p3-42 peptides tend to be located in the hydrophobic lipid core.⁴⁹

We find that both mutations do not change the lipid order parameters of the bilayer membrane within our simulation time, and much longer timescales might be necessary to see distinct detergent-like effects on lipid membrane bilayers upon mutation.¹³ It is possible that toxicity decrease upon A2T mutation may result from distinct metal site structure and metal exchange of the Cu²⁺-A β complex, as it has been found for the A2V mutant.⁵⁰ Toxicity variation upon specific mutations might also result from distinct early oligomer-membrane interactions,^{51,52} as the N-terminal residues modulate these interactions,⁵³ from larger amyloid β -barrel pores,^{16,20} or from variation in the lipid composition of membranes upon aging.⁵⁴

Conclusion

In summary, based on REMD simulations of the WT, A2T and D23N A β 42 β -barrel tetramers, we have shown that the probability of a perfect close β -barrel remains the

same between WT and A2T, approximately of 45%, and only slightly decreases to 32% in a DPPC lipid membrane bilayer. The A β 42 barrel conformation is dynamic in all species and its heterogeneity varies with the amino acid sequence.

Overall we do not find an enhancement of barrel stability for D23N known to be more toxic, and a reduction of barrel stability for A2T known to be protective. Whether our results on D23N mutation may be extrapolated to the FAD mutations located at positions 21 and 22 remain to be explored.⁵⁵ At the present time, we go beyond the DPPC membrane model and explore other compositions of cellular membranes, as they change in aging and AD.^{54,56}

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the support of the Vietnam National Foundation for Science & Technology Development (NAFOSTED) under the grant number 104.99-2019.57, University of Paris, and the French State (Grant “DYNAMO”, ANR-11-LABX-0011-01, and “CACSICE”, ANR-11-EQPX-0008). PhD also thanks the support of CNRS.

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Table 1. Secondary structures and cross-collision sections of A2T, D23N and WT systems at 310 K using the time interval from 50 to 500 ns of REMD simulations.

	β -strand	α -helix	turn	coil	CCS (nm ²)
A2T	47.9 $\pm$ 1.7	0.1 $\pm$ 0.3	2.7 $\pm$ 1.5	49.4 $\pm$ 2.2	20.6 $\pm$ 0.1
D23N	49.7 $\pm$ 2.0	0.1 $\pm$ 0.4	2.6 $\pm$ 1.7	47.6 $\pm$ 2.1	23.1 $\pm$ 0.1
WT	46.6 $\pm$ 2.1	0.1 $\pm$ 0.4	2.0 $\pm$ 1.4	51.1 $\pm$ 2.4	22.6 $\pm$ 0.1

Figure 1. Secondary structure compositions along the sequence. The results are obtained using the interval 50-500 ns of REMD at 310 K

Figure 2. Free energy landscapes of the transmembrane full-length A2T (A) and D23N (B) systems projected on the backbone RMSD (in nm) and the mean number of inter-molecular side-chain contacts per pair (N_c) at 310 K.

Figure 3. Distributions of the number of intra-molecular H-bonds between the amino acid regions 12-22 and 29-40 for each individual chain (hairpin) of the WT (black), A2T (red) and D23N (green) systems at 310 K. Panels A, B, C and D refer to chain 1, chain 2, chain 3 and chain 4.

Figure 4. Distributions of the number of intermolecular H-bonds between the β -strand regions in the four interfaces of the WT (black), A2T (red) and D23N (green) systems at 310 K. Panels A and B show interactions 12-22:12-22, and panels C and D refer to interactions 29-40:29-40.

Figure 5. The intermolecular side chain probability contact maps averaged over the four interfaces of the WT (panel A), A2T (panel B) and D23N (panel C) systems at 310 K.

Figure 6. The distributions of the tilt angles (panel A) and the inner pore diameters (panel B) in the WT (black), A2T (red) and D23N (green) systems at 310 K.

Figure 7. Water molecules interacting with the residues 1-9 and 22-28 using a cut-off distance of 0.3 nm in the WT (black), A2T (red) and D23N (green) systems at 310 K averaged over the four peptides. Note that each chain shows very similar profile.

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