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Amylin–Aβ oligomers at atomic resolution using molecular dynamics simulations: a link between Type 2 diabetes and Alzheimer’s disease
Amylin–Aβ oligomers at atomic resolution using molecular dynamics simulations: a link between Type 2 diabetes and Alzheimer’s disease†

Michal Baram,a,b Yoav Atsmon-Raz,a,b Buyong Ma,c Ruth Nussinov,c,d and Yifat Miller*a,b

Clinical studies have identified Type 2 diabetes (T2D) as a risk factor of Alzheimer’s disease (AD). One of the potential mechanisms that link T2D and AD is the loss of cells associated with degenerative changes. Amylin1–37 aggregates (the pathological species in T2D) were found to be co-localized with those of Aβ1–42 (the pathological species in AD) to form the Amylin1–37–Aβ1–42 plaques, promoting aggregation and thus contributing to the etiology of AD. However, the mechanisms by which Amylin1–37 co-aggregates with Aβ1–42 are still elusive. This work presents the interactions between Amylin1–37 oligomers and Aβ1–42 oligomers at atomic resolution applying extensive molecular dynamics simulations for relatively large ensemble of cross-seeding Amylin1–37–Aβ1–42 oligomers. The main conclusions of this study are first, Aβ1–42 oligomers prefer to interact with Amylin1–37 oligomers to form single layer conformations (in-register interactions) rather than double layer conformations; and second, in some double layer conformations of the cross-seeding Amylin1–37–Aβ1–42 oligomers, the Amylin1–37 oligomers destabilize the Aβ1–42 oligomers and thus inhibit Aβ1–42 aggregation, while in other double layer conformations, the Amylin1–37 oligomers stabilize Aβ1–42 oligomers and thus promote Aβ1–42 aggregation.

One of the potential mechanisms that link T2D and AD is the loss of cells associated with degenerative changes.1,2,6 AD is a neurodegenerative disease with extensive neuronal loss resulting from Tau and Aβ aggregation. T2D is also a degenerative disease that results from selective destruction of pancreatic β-cells and associated neuropathies,7–9 which are caused by aggregation of the neuroendocrine hormone named “Amylin”.

Recently, Jackson et al.10 identified Amylin deposits in the temporal lobe gray matter – a major component of the central nervous system – of diabetes patients. In addition to the Amylin deposition in the human brain, Amylin aggregates are co-localized with Aβ aggregates to form the Amylin–Aβ plaques, promoting aggregation and thus contributing to the etiology of AD. Recent in vivo studies investigated the cross-seeding between Aβ and Amylin aggregates.11–13 Yet, the mechanisms by which Amylin co-aggregates with Aβ are still elusive. Both Aβ and Amylin are misfolded peptides. The direct interaction of misfolded peptides, a topic which to date has been poorly explored, could play a major role in the genesis and progression of several pathological conditions. Although not extensively studied, in vitro reports show cross-seeding interactions among several amyloidogenic proteins.14–20 One of these studies12 showed that Aβ1–42 acts as a good seed for Amylin1–37 oligomerization; however, Amylin1–37 aggregates slightly affect soluble Aβ1–42 oligomerization. A recent study applied electrospray ionization-ion mobility spectroscopy-mass

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Introduction

Type 2 diabetes (T2D) is one of the most common metabolic disorders and its prevalence increases with age. Clinical and epidemiological studies identified T2D as a risk factor of Alzheimer’s disease (AD).1–3 Several studies have shown that there are many similarities between T2D and AD, and that both conditions underlie common physiological processes.3 AD is characterized by intracellular neurofibrillary tangles (NFTs), containing an abnormally hyperphosphorylated form of tau protein, and extracellular senile plaques, mainly composed of Amyloid β (Aβ) aggregates. Both Tau and Aβ aggregates which are the pathological hallmarks of AD are found in T2D.4,5
spectroscopy to characterize the dynamics and the kinetics of Amylin1–37 oligomerization, Aβ1–40 oligomerization and Amylin1–37–Aβ1–40 oligomerization. The interactions between Amylin1–37 aggregates and Aβ1–42 aggregates at atomic resolution are still elusive.

Several studies proposed that the sequences of Aβ1–42 and Amylin1–37 have 25% identity and 50% similarity and thus some domains in Aβ and some in Amylin participate in the co-assembly of Aβ–Amylin. Yet, these studies do not provide the atomic resolution of the molecular structures of Aβ1–42–Amylin1–37 aggregates. Recently, Berhanu et al. investigated the molecular structures of Aβ15–40–Amylin10–35 oligomers at atomic resolution. They explored an Aβ15–40 oligomer fragment of the ssNMR model of Aβ17–42, not considering the toxic full-length Aβ1–42 oligomer, arguing that residues 1–16 in the N-terminus of Aβ are in a disordered domain and thus unlikely to play a role in aggregation. However, previous studies have shown that residues 1–16 in the N-terminus of Aβ can play important roles in fibrilization and form a well-organized β-sheet structure. It is known that several mutations in the N-terminus accelerate amyloid formation, such as the English (H6R) mutation, in addition, mutating Ala2 to Thr or Val modifies the β-aggregation landscape. Amylin10–35 oligomers of one of the two structures proposed by the Eisenberg group, which differ in the orientation of the residues along the U-turn region and thus can strongly affect the interaction between Aβ and Amylin, were explored, but not those proposed by the Tycko group. Recently, our group illustrated that Amylin 1–37 oligomers have four variant conformations that differ in the orientation of the residues along the β-strands and the turn region of the β-arch. The interactions between Aβ and Amylin may differ due to these different orientations. Although residues 1–7 in the N-terminus of Amylin are not part of the β-sheet of the experimental models and the disulfide bridge between Cys2 and Cys7 does not contribute to aggregate assembly, it may have an effect on Aβ–Amylin aggregation when considering single and double layer conformations. Residues Ala8 and Thr9 in Amylin that were overlooked in earlier studies may also contribute to Aβ–Amylin aggregation. Finally, the N-terminus of Aβ, residues Asp1–Lys16, may interact with Amylin and thus affect cross-seeding of Aβ–Amylin aggregation. Here we study the interactions between Aβ1–42 oligomers with each of the four models of the full-length Amylin1–37 oligomers at atomic resolution. Our results led to two important conclusions. First, all four variant models of the full-length Amylin1–37 oligomers prefer to interact with Aβ1–42 oligomers to form single layer conformations. Second, interactions between the cross-seeded species in the single layer and the double layer conformations affect differently the flexibility (or rigidity) of the turn region of the self-assembled β-arch amyloid. Finally, residues in the N-termini of Aβ and Amylin contribute to the cross-seeding Aβ–Amylin aggregation; therefore it is important to consider the full-length Aβ and Amylin.

Materials and methods

Molecular dynamics protocol

MD simulations of the solvated oligomers were performed in the NPT ensemble using NAMD with the CHARMM27 force field. The oligomers were energy minimized and explicitly solvated in a TIP3P water box with a minimum distance of 15 Å from each edge of the box. Each water molecule within 2.5 Å of the oligomers was removed. Counter ions were added at random locations to neutralize the charge of the oligomers. The Langevin piston method with a decay period of 100 fs and a damping time of 50 fs was used to maintain a constant pressure of 1 atm. The temperature (330 K) was controlled by a Langevin thermostat with a damping coefficient of 10 ps. The short-range van der Waals (VDW) interactions were calculated using the switching function, with a twin range cutoff of 10.0 and 12.0 Å. Long-range electrostatic interactions were calculated using the particle mesh Ewald method with a cutoff of 12.0 Å. The equations of motion were integrated using the leapfrog integrator with a step of 1 fs.

The solvated systems were energy minimized for 2000 conjugated gradient steps, where the hydrogen bonding distance between the β-sheets in each oligomer is fixed in the range 2.2–2.5 Å. The counter ions and water molecules were allowed to move. Hydrogen atoms were constrained to the equilibrium bond using the SHAKE algorithm. The minimized solvated systems were energy minimized for 5000 additional conjugate gradient steps and 20 000 heating steps at 250 K, with all atoms allowed to move. Then, the systems were heated from 250 K to 300 K and then to 330 K for 300 ps and equilibrated at 330 K for 300 ps. Simulations ran for 30 ns for each variant model, with a total run of 960 ns for all models. The structures were saved every 10 ps for analysis. These conditions were applied to all models.

**Generalized Born method with molecular volume (GBMV) and population analysis**

To obtain the relative structural stability of the models, the trajectories of the last 5 ns were extracted from the explicit MD simulation excluding water molecules. The solvation energies of all systems were calculated using the generalized Born method with molecular volume (GBMV). In the GBMV calculations, the dielectric constant of water was set to 80.0. The hydrophobic solvent-accessible surface area (SASA) term factor was set to 0.00592 kcal mol⁻¹ Å². Each variant was minimized for 1000 cycles and the conformation energy was evaluated by grid-based GBMV. The minimization does not change the conformations of each variant, but only relaxed the local geometries due to thermal fluctuation which occurred during the MD simulations.

A total of 16 000 conformations (500 conformations for each of the 32 examined conformers) were used to construct the free energy landscape of the conformers and to evaluate the conformation probabilities by using Monte Carlo (MC) simulations. In the first step, one conformation of conformer i and one conformation of conformer j were randomly selected. Then, the Boltzmann factor was computed as e⁻⁽Ej⁻Ei⁾/kT, where Ei and Ej are the conformational energies evaluated using the GBMV calculations for conformations i and j, respectively, k is the Boltzmann constant and T is the absolute temperature (298 K used here). If the value of the Boltzmann factor was larger than the random number, then the move from conformation i to conformation j was allowed. After 1 million steps, the visited conformations for each conformer were counted. Finally, the
relative probability of model $n$ was evaluated as $P_n = N_n/N_{\text{total}}$, where $P_n$ is the population of model $n$, $N_n$ is the total number of conformations visited for model $n$, and $N_{\text{total}}$ is the total steps. The advantages of using MC simulations to estimate conformer probability lie in their good numerical stability and the control that they allow for transition probabilities among several conformers.

Using all 32 models and 16,000 conformations (500 for each model) generated from the MD simulations, we estimated the overall stability and populations for each conformer based on the MD simulations, with the energy landscape being computed with GBMV for these 32 models. The group that these 32 models are likely to represent may be only a very small percentage of the ensemble. Nevertheless, the carefully selected models cover the most likely structures. It should be noted here that the results obtained in this study depend on the initial structures and the initial conditions.

### Analysis details

We examined the structural stability of the models by following the changes in the number of the hydrogen bonds between $\beta$-strands, with the hydrogen bond cut-off set to 2.5 Å. In addition we followed the root-mean square deviations (RMSDs) and root-mean square fluctuations (RMSFs) of all structures. The $\psi$ and $\varphi$ angles of each residue in the Amylin models were computed for the last 5 ns to estimate the secondary structure of the self-assembled models.

### Reaction coordinates for the formation of Amylin–Aβ oligomer structures

To investigate the stability of each soluble Amylin–Aβ oligomer structure, the conformational energies were computed for all Amylin oligomers and for the Aβ oligomer (Table S1, ESI†). The conformational energies for each model are based on the energy computed with the GBMV method. For each model, a total of 500 conformations from the last 5 ns of the simulations were used to evaluate the conformational energy.

We estimated the relative stability of each Amylin–Aβ model by comparing its energy with the energies of its two types of components, the Amylin model and the Aβ oligomer, as illustrated by the following chemical “reaction”:

$$\text{(Amylin)}_n + (\text{Aβ})_n \leftrightarrow \text{(Amylin)}_n (\text{Aβ})_n$$

where $n$ indicates the number of monomers within the Amylins’ model and the Aβ oligomer. In the current study $n = 6$.

Obviously, single and double layer models may have different interaction types. Yet, as we previously demonstrated, one can compare the relative conformational energies between the single and the double layer models to provide insight into these different interactions. The differences in the interaction types may explain the differences in the relative energies.

### Results and discussion

**Experiment-based Amylin$_{1–37}$ oligomer model, Aβ$_{1–42}$ oligomer model and Aβ$_{1–42}$–Amylin$_{1–37}$ oligomer model construction**

Previously, we illustrated four models of Amylin$_{1–37}$ that differ in the orientation of the residues along the backbone of the $\beta$-strands and along the turn domain of the $\beta$-arch structure. These four models (M1–M4, Fig. S1, ESI†) were based on the experimental structures of Tycko$^{41}$ and Eisenberg. Herein, on the basis of Tycko’s two-fold Aβ$_{1–40}$ model, we extended the C-terminus by two residues to form Aβ$_{1–42}$, which is more toxic than Aβ$_{1–40}$. For each of the four Amylin$_{1–37}$ oligomers we constructed eight models of Aβ$_{1–42}$–Amylin$_{1–37}$ oligomers, where each model consists of six Aβ$_{1–42}$ monomers and six Amylin$_{1–37}$ monomers. Four were single layer conformations and four double layer conformations. Therefore, we constructed a total of 32 Aβ$_{1–42}$–Amylin$_{1–37}$ models. Table 1 details all 32 Aβ$_{1–42}$–Amylin$_{1–37}$ models: B1–B8, C1–C8 and D1–D8. Fig. 1 illustrates the eight initial models of Aβ$_{1–42}$–Amylin$_{1–37}$ oligomers of one of the four Amylin$_{1–37}$ models (model M1): B1–B8. Similarly we constructed the arrangements between Aβ$_{1–42}$ oligomers and Amylin$_{1–37}$ oligomers (models M2, M3 and M4). Fig. S2–S4 (ESI†) demonstrate the other 24 initial Aβ$_{1–42}$–Amylin$_{1–37}$ models and Fig. S5–S8 show the 32 simulated Aβ$_{1–42}$–Amylin$_{1–37}$ models. Finally, we simulated the Aβ$_{1–42}$ oligomer model which is based on Tycko’s two-fold Aβ$_{1–40}$ model (Fig. S9a, ESI†). Interestingly, the simulated Aβ$_{1–42}$ oligomer model illustrated a

<table>
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<tr>
<th>Model</th>
<th>Amylin type</th>
<th>Orientations between Aβ and Amylin</th>
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<tbody>
<tr>
<td>B1</td>
<td>M1</td>
<td>Parallel</td>
</tr>
<tr>
<td>B2</td>
<td>M1</td>
<td>Parallel</td>
</tr>
<tr>
<td>B3</td>
<td>M1</td>
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</tr>
<tr>
<td>B4</td>
<td>M1</td>
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</tr>
<tr>
<td>B5</td>
<td>M1</td>
<td>N(Aβ)–N(Amylin)</td>
</tr>
<tr>
<td>B6</td>
<td>M1</td>
<td>N(Aβ)–C(Amylin)</td>
</tr>
<tr>
<td>B7</td>
<td>M1</td>
<td>C(Aβ)–N(Amylin)</td>
</tr>
<tr>
<td>B8</td>
<td>M1</td>
<td>C(Amylin)–C(Aβ)</td>
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<tr>
<td>C1</td>
<td>M2</td>
<td>Parallel</td>
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<tr>
<td>C2</td>
<td>M2</td>
<td>Antiparallel</td>
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<tr>
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<td>M2</td>
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<tr>
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<td>N(Aβ)–N(Amylin)</td>
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<tr>
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<td>M3</td>
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<td>D2</td>
<td>M3</td>
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<td>D3</td>
<td>M3</td>
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<td>D5</td>
<td>M3</td>
<td>N(Aβ)–N(Amylin)</td>
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<td>D6</td>
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<td>N(Aβ)–C(Amylin)</td>
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<td>E8</td>
<td>M4</td>
<td>C(Amylin)–C(Aβ)</td>
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third β-sheet at the C-termini of the monomers (Fig. S9b, ESI†) leading to a new structural model of the αβ oligomer. Recently, a novel structural model of the αβ oligomer provided further evidence for the highly polymorphic nature of the αβ peptide fibril. Polymorphism was also obtained in some of the simulated αβ−Amylin1−37 models. Structural comparison suggests that the structural similarity between the αβ−Amylin1−37 oligomer and amylin oligomers is lower than with the model of the αβ oligomer in the current study. It will be interesting to examine the cross-seeding αβ−Amylin1−37 oligomers using this new αβ structure as well as with additional polymorphic states such as the triangular structure. Previous ssNMR studies have shown α-helical structures of Amylin and not cross-β structures. Recent ssNMR studies presented unstructured αβ oligomers and Amylin oligomers that form large micelles, which may be a general phenomenon for natively unstructured Amylin. We did not apply these α-helical and the unstructured amylin, or the unstructured αβ oligomers in the current study, because of the lack of the PDB coordinates. Future work would need to solve these oligomer structures in order to study the cross-seeding of αβ−Amylin1−37 oligomers. Finally, the RMSDs and the hydrogen bond analysis illustrate that the simulated αβ−Amylin1−37 models are structurally stable (Fig. S10–S15, ESI†).

**Single layer conformational arrangements of αβ−Amylin1−37 oligomer models are preferred over double layer conformational arrangements**

In order to compare the 32 αβ−Amylin1−37 oligomers we generated 500 conformations for each arrangement by MD simulations and estimated the conformational energies and the populations (Table S1, ESI†). Fig. 2 demonstrates the populations of all 32 αβ−Amylin1−37 oligomers. One can see that single layer conformations of αβ−Amylin1−37 oligomers with parallel and antiparallel arrangements (B1, B2, C1, C2, D1, D2, E1 and E2) show the highest populations and thus are preferred over the two single layer conformations and all the double layer conformations. We previously showed that in the cross-seeded αβ−mutated Tau R2 oligomers the double layer conformations are preferred over the single layer conformations. The preference of the cross-seeding of some conformations over others may be due to the interactions between residues along the sequences of the various types of amyloids. The interactions that stabilize structurally and energetically the cross-seeding amyloid oligomers will yield preferred organizations.

We computed the secondary structures of αβ−Amylin1−37 oligomers in all 32 cross-seeding Amylin1−37−αβ−Amylin1−37 oligomers (Fig. S16–S23, ESI†). Interestingly, one can see from Fig. S16–S21 (ESI†) that in the single layer simulated αβ−Amylin1−37 oligomer models and in the double layer simulated αβ−Amylin1−37 oligomer models (in which the C-termini of αβ monomers do not interact with Amylin) residues Val39–Ala42 in the C-termini of αβ−Amylin1−37 showed formation of a third β-sheet and a second turn region (residues Gly37–Gly38). The original experiment-based αβ−mutated oligomer has two β-strands connected by a U-turn; however, herein our simulations demonstrate that the αβ−Amylin1−37 oligomer has three β-strands connected by two turns both when it does not interact with Amylin1−37 oligomers and when it does. A previous study has shown that isoforms of Tau repeats form triple-stranded and two-turn structures.

**Common mechanisms between various types of Amylin1−37 oligomers and αβ−Amylin1−37 oligomers**

To investigate the mechanisms by which Amylin1−37 oligomers interact with αβ−Amylin1−37 oligomers to form the cross-seeded Amylin1−37−αβ−Amylin1−37 oligomers, we estimated the “reaction coordinate” in which Amylin1−37 oligomers interact with αβ−Amylin1−37 oligomers. To this aim, we computed the relative conformational energies of the separated oligomers and the cross-seeding Amylin1−37−αβ−amylin1−37 oligomers.
oligomers using the GBMV method for each of the four Amylin1–37 oligomers (Fig. 3). We previously studied similarly the cross-seeding interactions between two types of amyloids and two types of Amylin1–37.

Interestingly, the common mechanisms by which Amylin1–37 oligomers interact with Aβ1–42 oligomers to form the cross-seeded Amylin1–37–Aβ1–42 oligomers illustrate that each of the four Amylin1–37 oligomers prefers to interact with an Aβ1–42 oligomer to form a single layer conformation in parallel and in antiparallel orientation, yielding an ‘exothermic reaction’. In some of the four Amylin1–37 oligomers there are other mechanisms that illustrate the formation of double layer conformations, but in all four Amylin1–37 oligomers the common mechanisms show the formation of the single layer conformation. Therefore, the preference of the single layer conformation indicates a strong cross-seeding tendency between Aβ1–42 and Amylin1–37 peptides.

The effect of cross-seeding on the structural features of Amylin1–37 oligomers and Aβ1–42 oligomers

One of the interesting topics in studying the cross-seeding between amyloids is to investigate the effect of cross-seeding on the structural features of the amyloids. The cross-seeding between Amylin1–37 oligomers and Aβ1–42 oligomers is of particular interest, because there are four variant models of Amylin1–37 oligomers that differ in the orientation of the residues along the β-arch structures and thus we expect that the effect on the structural features of the various cross-seeding Amylin1–37–Aβ1–42 oligomers may be different.

We first examined the effect on the β-strand of the β-arch structures of Aβ1–42 oligomers. The secondary structures of these oligomers in all 32 cross-seeding Amylin1–37–Aβ1–42 oligomers were computed (Fig. S16–S23, ESI†). In Aβ1–42 oligomers residues D1–F20 and residues A30–A42 show the properties of β-strand, but in the cases (models B6, C6, D6 and E6) where the N-termini of Aβ1–42 oligomers interact with the C-termini of Amylin1–37 oligomers to form double layer conformations these residues do not demonstrate β-strand properties. Also, in some cases (models C5 and D5) where the N-termini of Aβ1–42 oligomers interact with the N-termini of Amylin1–37 oligomers in one case (model B7) where the C-termini of Aβ1–42 oligomers interact with the N-termini of Amylin1–37 oligomers these residues do not show β-strand properties. In such cases the cross-β structures that characterize the fibrillation of amyloids yield structurally less stable cross-seeding.

We then examined the fluctuation of the backbone of Aβ1–42 oligomers and Amylin1–37 oligomers for each of the 32 cross-seeding Amylin1–37–Aβ1–42 oligomers using RMSF calculations (Fig. 4 and 5). Interestingly, in cases (models B6, C6, D6 and E6) where the N-termini of Aβ1–42 oligomers interact with the C-termini of Amylin1–37 oligomers to form double layer conformations, the turn regions of Aβ1–42 oligomers fluctuate relatively more than in other cross-seeding models. These models demonstrated no β-strand properties, because the interactions in the double layer conformations do not allow structurally stable structures. One can see from the simulated models (Fig. S5–S8, ESI†) that Aβ1–42 oligomers do not show cross-β structures, and are structurally unstable oligomers. Finally, those simulated models that do not exhibit cross-β structures (models C5, D5 and B7) also fluctuate in the turn region of the Aβ1–42 oligomers.

Our results suggest that among the double layer conformations of the cross-seeding oligomers, the stability of the turn region domain in the Aβ1–42 oligomers may be affected by the

Fig. 3 The relative conformational energies of separated Aβ1–42 hexamers and Amylin1–37 hexamers (M1–M4) and Aβ1–42–Amylin1–37 dodecamers.
interactions with all four Amylin\textsubscript{1–37} oligomer variants suggesting that the turn region may affect the fibrillation (or the self-assembly process) of A\textsubscript{\textbeta 1–42} oligomers. In cases where the turn regions in A\textsubscript{\textbeta 1–42} oligomers are destabilized due to the interactions with Amylin\textsubscript{1–37} oligomers, these interactions may inhibit aggregation of the A\textsubscript{\textbeta 1–42} oligomers. Zanni’s group proposed an aggregation pathway for amylin in which the turn regions of amylin play a role as initial seeding for aggregation.\textsuperscript{66} We thus suggest that in some cases destabilization of the turn regions of A\textsubscript{\textbeta 1–42} oligomers may inhibit A\textsubscript{\textbeta 1–42} aggregation. However, in some cases the turn regions in A\textsubscript{\textbeta 1–42} oligomers are stabilized by these interactions and therefore we expect that these interactions will induce aggregation of A\textsubscript{\textbeta 1–42} oligomers.

Our study illustrates for the first time the importance of investigating the cross-seeding between full-length A\textbeta and full-length Amylin and that the N-termini play a role in some cases in the stabilization of the cross-seeding of A\textbeta–Amylin oligomers. One can see that interactions between residues in the N-termini of A\textbeta and Amylin (those that had not been considered earlier\textsuperscript{27}) stabilize both single and double layer conformations (Fig. S24–S26, ESI\textsuperscript{†}). On the other hand, interestingly, in some cases the interactions between the Val12 of A\textbeta (which is located in the N-terminus) and the C-terminus of Amylin (residues Ile26 and Leu27) destabilize the A\textbeta oligomers but do not affect the stabilization of Amylin (Fig. S27, ESI\textsuperscript{†}). Therefore, this is the first study that illustrates the role of the interactions of the N-termini in cross-seeding A\textbeta–Amylin aggregation at atomic resolution. In some cases, the N-termini are favorable for cross-seeding and in some other cases the N-termini are unfavorable for cross-seeding.

Finally, we examined the effect of the interactions of A\textsubscript{\textbeta 1–42} oligomers on the fluctuation of the turn regions of Amylin\textsubscript{1–37} oligomers. Interestingly, one can see from Fig. 5 that the interactions of A\textsubscript{\textbeta 1–42} oligomers with the variant models of Amylin\textsubscript{1–37} Oligomers (M3 and M4) result in fluctuations of the turn regions of Amylin\textsubscript{1–37} oligomers, \textit{i.e.} flexibility of the turn regions, which is in contrast with the interactions of A\textsubscript{\textbeta 1–42} oligomers with the variant models of Amylin\textsubscript{1–37} oligomers (M1 and M2) which result in more rigid turn regions. Previously we showed that the variant models M1 and M2 of Amylin\textsubscript{1–37} oligomers illustrated rigid turn regions, while with the variant models of Amylin\textsubscript{1–37} oligomers M3 and M4 showed flexible turn regions.\textsuperscript{42} We thus suggest that the interactions of A\textsubscript{\textbeta 1–42} oligomers do not affect the structural features of Amylin\textsubscript{1–37} oligomers; rather, in some cases Amylin\textsubscript{1–37} oligomers affect the structural features and the stability of A\textsubscript{\textbeta 1–42} oligomers.

Conclusions

Many, though not all, clinical studies indicate that individuals with T2D are at higher risk of eventually developing AD or other dementia.\textsuperscript{1–3} but the connection between these two diseases is not understood. Recently, Amylin deposits were found in the temporal lobe gray matter – a major component of the central nervous system – of diabetes patients.\textsuperscript{10} In addition to the Amylin deposition in the human brain, Amylin aggregates were found to co-localize with A\textbeta aggregates to form Amylin–A\textbeta plaques, promoting aggregation and thus contributing to the etiology of AD. The mechanisms by which Amylin co-aggregates with A\textbeta are still elusive. Herein, we present for the first time the co-aggregation between the full-length A\textsubscript{\textbeta 1–42} oligomer and each of the four variants of the full-length Amylin\textsubscript{1–37} oligomers that we have recently established.\textsuperscript{42} Here, we focus on the parallel \textbeta-sheet structure of the A\textsubscript{\textbeta 1–42} oligomer, although the antiparallel \textbeta-sheet structure of A\textsubscript{\textbeta 1–40} oligomers has also been considered as a toxic species.\textsuperscript{67} Yet, it is known that A\textsubscript{\textbeta 1–42} is more toxic than A\textsubscript{\textbeta 1–40}. The toxicity of A\textbeta oligomers to neuronal cells has been demonstrated to occur via a two-step mechanism of membrane disruption.\textsuperscript{68}

Two important observations emerge from this study. First, all four variant models of the full-length Amylin\textsubscript{1–37} oligomers preferred to interact with A\textsubscript{\textbeta 1–42} oligomers to form polymorphic single layer conformations. Second, the interactions between the cross-seeding Amylin\textsubscript{1–37}A\textsubscript{\textbeta 1–42} oligomers both in single
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