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Research Article

Inhibitory Activity of Insulin on Aβ Aggregation Is Restricted Due to Binding Selectivity and Specificity to Polymorphic Aβ States

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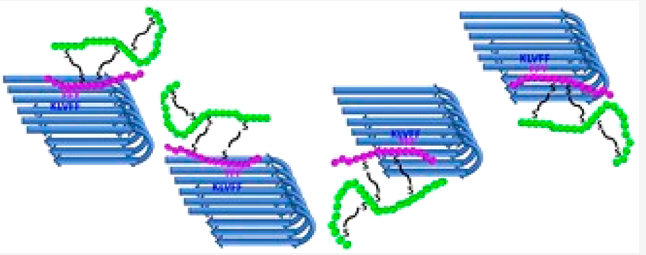
ABSTRACT: Clinical trials of intranasal insulin treatment for Alzheimer's patients have shown cognitive and memory improvement, but the effect of insulin has shown a limitation. It was suggested that insulin molecule binds to Aβ aggregates and impedes Aβ aggregation. Yet, the specific interactions between insulin molecule and Aβ aggregates at atomic resolution are still elusive. Three main conclusions are observed in this work. First, insulin can interact across the fibril only to "U-shape" Aβ fibrils and not to "S-shape" Aβ fibrils. Therefore, insulin is not expected to influence the "S-shape" Aβ fibrils. Second, insulin disrupts β-strands along Aβ fibril-like oligomers via interaction with chain A, which is not a part of the recognition motif. It is suggested that insulin affects as an inhibitor of Aβ fibrillation, but it is limited due to the specificity of the polymorphic Aβ fibril-like oligomer. Third, the current work proposes that insulin promotes Aβ aggregation, when interacting along the fibril axis of Aβ fibril-like oligomer. The coaggregation could be initiated via the recognition motif. The lack of the interactions of insulin in the recognition motif impede the coaggregation of insulin and Aβ. The current work reports the specific binding domains between insulin molecule and polymorphic Aβ fibril-like oligomers. This research provides insights into the molecular mechanisms of the functional activity of insulin on Aβ aggregation that strongly depends on the particular polymorphic Aβ aggregates.



KEYWORDS: *Polymorphism, Alzheimer's disease, amyloid β, insulin, amyloid aggregation, amyloid inhibitors*

INTRODUCTION

Alzheimer's disease (AD) is the most common progressive neurodegenerative brain disorder in man. One of the pathological hallmarks observed in the brain of AD patients is senile plaques that are composed of Aβ peptides. High levels of Aβ peptides accumulate by forming toxic aggregates that lead to neuronal dysfunction and to the progression of AD.[1] The important actions of insulin in the central nervous system such as facilitating memory metabolism were extensively reviewed elsewhere.[2,3] Interestingly, clinical studies reported on low levels of insulin in the cerebrospinal fluid and plasma in patients with AD.[4,5] Later, it was proposed that low levels of insulin enhance Aβ aggregation and consequently the progression of AD.[6] For this reason, in the past decade, in vivo studies, clinical trials,[7] and perspective reviews[8,9] proposed to use intranasal insulin treatment for AD patients. The clinical trials demonstrated improvement memory and enhancement of cognitive function in AD patients.[7,10,11]

Extensive efforts of in vitro studies were implemented to investigate the effect of insulin on Aβ aggregation. While it is known that Aβ oligomers cause toxicity in the synapses and thus synapses' degeneration in AD,[12] insulin was found as an inhibition factor that prevents the binding of small Aβ oligomers to the synapse and consequently protecting the central nervous system against AD.[13] Furthermore, in vivo study exhibited insulin as an inhibitor for aggregation of the toxic $A\beta_{22-35}$ fragment oligomers in rat hippocampal neuron cells[14] and in cell toxic $A\beta_{1-40}$ oligomers.[15] Interestingly, it was shown that insulin decreases the quantity of Aβ peptides binding to the cell surface and hence inhibits Aβ aggregation.[6] It was proposed that the inhibition of Aβ aggregation by insulin is due to the binding of insulin to Aβ peptides and Aβ aggregates.[6,16] Yet, the specific interactions of insulin with Aβ aggregates are still elusive. The long-term effect of intranasal insulin in AD patients showed that insulin is modifying AD-related pathophysiologic processes and not merely improving symptoms of memory impairment.[17] Therefore, it is critical to understand the molecular mechanisms of the effect of insulin on Aβ aggregates at atomic resolution.

The polymorphic nature of Aβ aggregates, e.g. oligomers and fibrils, is well-recognized. Thus, one needs to investigate the effect of insulin on each polymorphic state. The current work focuses on probing the effect of binding the insulin molecule to polymorphic Aβ aggregates. Moreover, this study

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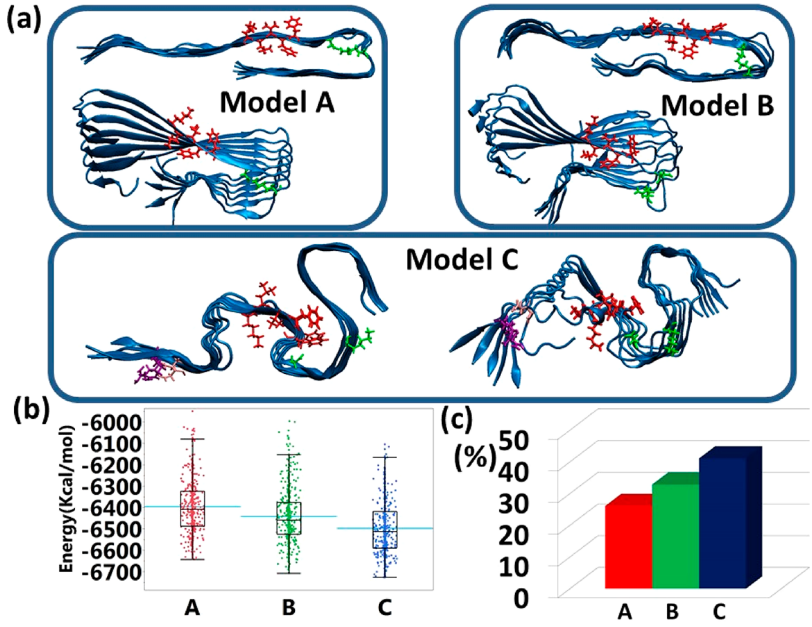

Figure 1. (a) Initial (top) and simulated (bottom) model A of "U-shape" Aβ_{1-42} fibril-like hexamer, based on ref 19. Initial (top) and simulated (bottom) model B of "U-shape" Aβ_{1-42} fibril-like hexamer, based on ref 18. Initial (left) and simulated (right) model C of "S-shape" Aβ_{1-42} fibril-like hexamer, based on ref 20. Red residues exhibit the CHC domain: KLVFF of Aβ. Green residues are D23 and K28. (b) Scatter charts of the 500 conformations obtained from the generalized Born method with molecular volume (GBMV) energy values extracted from the last 5 ns of each model (Supporting Information). The scatter charts represent the "histograms" of the number of conformations in energies' range. The averaged energy values are seen in the "boxes". (c) Populations analysis of models A, B, and C.

demonstrates that binding the insulin molecule at a distinct domain in an individual Aβ aggregate yields a different effect. Some of the polymorphic states are stabilized by the interactions with insulin molecule while others are destabilized. Finally, this work shows for the first time ever the initial seeding of cross-aggregation between an insulin molecule Aβ aggregates at the atomic resolution. The Aβ aggregates that are studied in the current work are fibril-like hexamers. So far, the three-dimensional structure of nonfibrillar disordered Aβ hexamers is still elusive at the molecular level; therefore, this work is focused on the fibril-like oligomers.

RESULTS AND DISCUSSION

"S-Shape" Aβ_{1-42} Fibril-like Oligomer Is Stabilized by Extensive Interactions within the Fibril Compared to the "U-Shape" Aβ_{1-42} Fibril-like Oligomers. The current work focuses on three polymorphic structural Aβ_{1-42} fibril-like oligomers that were solved by ssNMR: models A, B, and C (Figures 1a).[18−20] In two Aβ_{1-42} fibril-like oligomers (models A and B) the structural fibril is characterized by a "β-turn-β structure", i.e. β-arch structure.[18,19] These two Aβ_{1-42} fibril-like oligomers are named as "U-shape" and mainly differ in the shape of the turn and the location of the residues along the two β-strands (Table S1). The third Aβ_{1-42} fibril-like oligomer (model C) is characterized by a "β-turn-β-turn-β-turn-β-turn-β" structure (Table S1), named as "S-shape".[20] The three Aβ_{1-42} fibril-like oligomers were simulated, and all three models exhibited converged structures (Figures S1 and S2). Conformational energy and population analyses demonstrate that the "S-shape" Aβ_{1-42} fibril-like oligomer is more stable and populated than the two "U-shape" Aβ_{1-42} fibril-like oligomers (Figures 1b and 1c).

Recent studies investigated the structural and the mechanical properties of the "U-shape" Aβ_{17-42} fibril-like oligomer versus the "S-shape" Aβ_{17-42} fibril-like oligomer.[21,22] The "S-shape" fibril-like oligomer of Aβ_{17-42} was taken from a similar ssNMR structure[23] to the "S-shape" fibril that is applied in the current work for Aβ_{1-42} fibril-like oligomer. Yet, while the "S-shape" Aβ_{17-42} fibril-like oligomer is based on ssNMR that exhibits only three β-strands, herein the "S-shape" Aβ_{1-42} fibril-like oligomer is based on ssNMR that shows five β-strands. Interestingly, the structural stability of the "S-shape" Aβ_{17-42} fibril-like oligomer versus "U-shape" Aβ_{17-42} fibril-like oligomer also has shown that the "S-shape" is more stable than the "U-shape" Aβ_{17-42} fibril-like oligomer, due to structural characterization analyses. Herein, we apply not only structural analyses but also relative conformational stabilities analyses between the "U-shape" full-length Aβ_{1-42} fibril-like oligomers and the "S-shape" full-length Aβ_{1-42} fibril-like oligomer. Moreover, the root-mean-square-fluctuations (RMSFs) analyses for Aβ_{17-42} fibril-like oligomers differ from the RMSFs analyses for the full-length Aβ_{1-42} fibril-like oligomers (Figure S3). Obviously, the N-termini domains affect the structural stability along the fibrils' domains. While the "S-shape" Aβ_{17-42} fibril-like oligomer exhibits disruptions of β-stands along the fibril, in the "S-shape" Aβ_{1-42} fibril-like oligomers the β-strands were conserved along the MD simulations. The locations of the β-strands of the original "S-shape" Aβ_{1-42} fibril that was solved by ssNMR are similar to those obtained in the simulated Aβ_{1-42} fibril-like oligomer. However, comparing the simulated "S-shape" Aβ_{17-42} fibril to the original ssNMR Aβ_{1-42} fibril indicated the loss of one β-strand and loss of residues that are located along the other two β-strands.

Yet, both the simulated Aβ_{17-42} fibril-like oligomer and Aβ_{1-42} fibril-like oligomer of the "S-shape" are stable due to the extensive interactions in the hydrophobic core of the fibrils (Figure S4). Specifically, extensive clusters of hydrophobic

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interactions along the sequence of L17-I41 and the special intra- and interpeptide $\pi-\pi$ interactions between F19 and F20 along the fibril stabilize the "S-shape" fibril (Figure S4). These $\pi-\pi$ interactions are known to stabilize Aβ fibrils and play a crucial role in initial seeding of Aβ aggregation.[24] Finally, intra- and interpeptide electrostatic interactions between R5 and D7 stabilize the "S-shape" Aβ_{1-42} fibril (Figure S4). It is more likely that the formation pathway of "S-shape" fibrils requires overcoming energy barriers in order to produce such extensive interactions that stabilize the fibrils, and this issue necessitates further future studies.

Insulin Binds across the Fibril of "U-Shape" Aβ_{1-42} Fibril-like Oligomers and Not of "S-Shape" Aβ_{1-42} Fibril-like Oligomer. Experimental studies proposed that insulin affects Aβ aggregation via interactions between insulin and Aβ.[25,26] One of the most challenging issues in investigating the molecular mechanisms of the interactions between insulin and Aβ is to identify the specific interactions between them, i.e. to determine the recognition site motif. The recognition motif between insulin and other amyloid proteins was proposed previously by experimental studies.[24,26] Furthermore, we previously investigated this recognition motif between insulin and amylin fibril-like oligomers.[27] Herein, we applied the recognition motif of the insulin with the homology sequence of the amyloids, similarly as proposed by the previous experimental studies.[24,26] It was proposed that insulin chain B binds to the central hydrophobic core (CHC) domain of Aβ that contains the diphenylalanine motif.

To interact insulin chain B with Aβ, we have taken into account the recognition motif in two orientations between insulin and Aβ_{1-42} fibril-like oligomers across the fibril (Figures 2a and 2b), similarly as we previously examined for the insulin-amylin fibril-like oligomer recognition motif.[27] While the CHC domains in the "U-shape" fibril-like oligomers are exposed and allow the insulin to bind the insulin, in the "S-shape" fibril-like oligomer the CHC domains are buried inside the hydrophobic core of the fibril and thus do not allow for interactions with insulin (Figure S4). The initial interactions of insulin with model A in two orientations exhibited models A1 and A2 (Figure S5), and the interactions with model B are illustrated in models B1 and B2 (Figure S6). These four simulated models are shown in Figure 3 and demonstrate converged structures (Figures S7 and S8) and similar fluctuations of residues along the sequence of Aβ_{1-42} fibril-like oligomers (Figure S9).

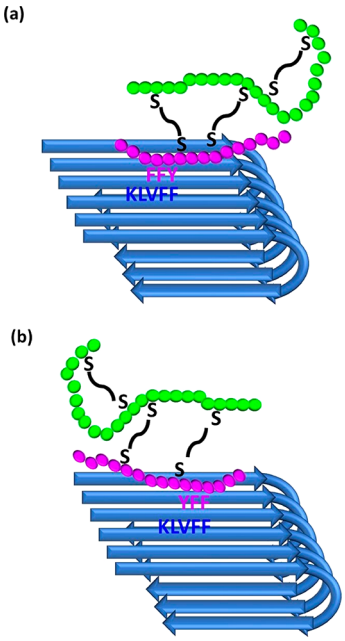

Figure 2. Schematic representation of the initial interactions in the recognition motif between insulin chain B (residues F24−F25−Y26) and the Aβ domain (residues K16-L17-V18-F19-F20). Chain A is colored in green, and chain B is colored in purple. The interactions of insulin molecule are across the fibril in the central domain of the fibril in two orientations.

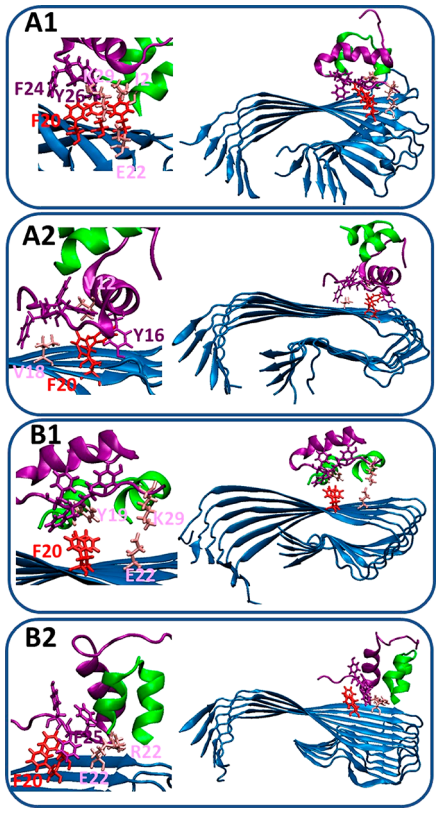

Figure 3. Simulated models A1, A2, B1, and B2 of insulin molecule binding to Aβ fibril-like hexamer across the fibril. A close picture of the interactions (left) is seen for each model. The residues in the recognition motif in insulin are colored in purple. Pink residues exhibit interactions that were produced during the simulations.

Insulin Prefers to Interact across the Fibril Axis of "U-Shape" Aβ Fibril-like Oligomers in a Distinct Orientation and Only with the Recognition Motif in Insulin Chain B. Conformational energy and populations analyses demonstrated that the insulin is slightly more preferred to interact in one orientation (as seen in Figure 2b) than the other orientation (as seen in Figure 2a) for both models A and B of Aβ_{1-42} fibril-like oligomers (Figures 4). Therefore, models A2 and B2 are preferred compared to models A1 and B1. Interestingly, the interactions that stabilize the contacts between insulin and Aβ_{1-42} fibril-like oligomer of models A1 and B1 contain not only chain B of insulin but also chain A of insulin (Figure 3, Figures S10 and S11). Obviously, the initial orientation between insulin and Aβ_{1-42} is similar for both models A1 and B1; therefore, this specific orientation allows chain A of insulin to interact with Aβ_{1-42}, in addition to chain

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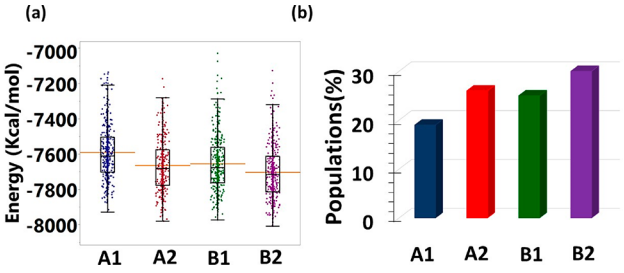

Figure 4. (a) Scatter charts of the 500 conformations obtained from the generalized Born method with molecular volume (GBMV) energy values extracted from the last 5 ns of each model (Supporting Information). The values indicate conformational nonbonded interaction energies. The scatter charts represent the "histograms" of the number of conformations in energies' range. The averaged energy values are seen in the "boxes". (b) Populations analysis of models A1, A2, B1, and B2.

B. The contacts between insulin and Aβ_{1-42} in model A1 include a cluster of $\pi-\pi$ interactions, hydrophobic interactions, and salt-bridge interactions (Figure S10). In model B1 the contacts contain $\pi-\pi$ interactions and salt-bridge interactions (Figure S11). We thus propose that because chain A of insulin is not part of the recognition motif, the models A1 and B1 are less populated (Figure 4). The recognition motif was conserved in models A2 and B2, and the chain A in insulin does not participate with the interactions with Aβ_{1-42} (Figures S12 and S13); therefore, these two models are more populated (Figure 4).

The interactions between the insulin molecule with Aβ_{1-42} fibril-like oligomers across the fibril of models A and B in a specific orientation (as seen in Figure 2a) yield the disruption of β-sheet structure along the sequence of the β-arch. The insulin molecule does not disrupt the β-sheet structures along the CHC domain of model A1 (Figure S14). In model B1, the β-sheets in the second hydrophobic core (SHC) domains, residues[30]AIIGL,[35] were disrupted (Figure S15). Therefore, we propose that when insulin binds across Aβ_{1-42} fibril-like oligomers, it may inhibit not all polymorphic Aβ fibrils but only those based on a specific orientation (as seen in Figure 2a) and those that are less populated.

Interactions of Insulin along the Fibril Axis of Aβ Fibril-like Oligomers Induce Aβ Aggregation Due to Cross-seeding. Recent efforts were performed to investigate the link between type 2 diabetes (T2D) and AD.[28−31] It has been proposed that insulin molecules can bind to Aβ oligomers and fibrils.[15] Furthermore, it has been shown that Aβ fibrils promote insulin aggregation.[15] Yet, the specific interactions between insulin and Aβ at the atomic resolution are still elusive. Specifically, so far, the interactions of the insulin molecule with Aβ fibril-like oligomers had not been investigated.

Therefore, the insulin molecule interacted with each one of the polymorphic Aβ fibrils in two orientations along the fibril axis. (Figures 5a and 5b) The specific interactions between insulin and Aβ were chosen initially as the recognition motif that was previously proposed by experimental studies.[24,26] Six models were constructed to produce these interactions along the fibril axis: A3, A4, B3, B4, C1, and C2 (Figures S16−S18). The simulated models are seen in Figure 6. The interactions in the recognition motif were conserved along the MD simulations for four models: A3, A4, B4, and C2 (Figures S19−S22). For models B3 and C1, these interactions were not

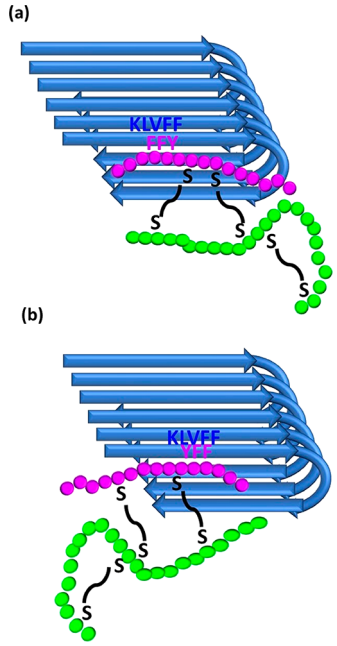

Figure 5. Schematic representation of the initial interactions in the recognition motif between insulin chain B (residues F24-F25-Y26) and the Aβ domain (residues K16-L17-V18-F19-F20). Chain A is colored in green, and chain B is colored in purple. The interactions of the insulin molecule are along the fibril axis at the edge of the fibril in two orientations.

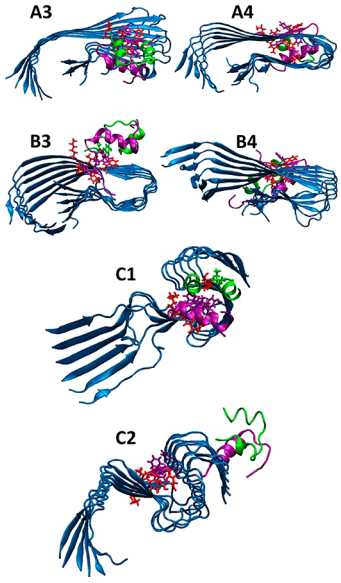

Figure 6. Simulated models A3, A4, B3, B4, C1, and C2 of insulin molecule initially binding to Aβ fibril-like hexamer along the fibril axis. The residues in the recognition motif in insulin are colored in purple. The residues in the recognition motif in Aβ are colored in red. Green residues in models B3 and C1 exhibit hydrophobic and $\pi-\pi$ interactions that were produced during the simulations, between Chain A (green) and Aβ.

conserved (Figures S23 and S24). Interestingly, the interactions that were conserved along the MD simulations in these four models demonstrated a formation of β-strands along the sequence of insulin chain B that participates in the recognition motif (Figure 7a). In these models, the interactions are only between the insulin chain B and Aβ_{1-42} fibril-like oligomers. In

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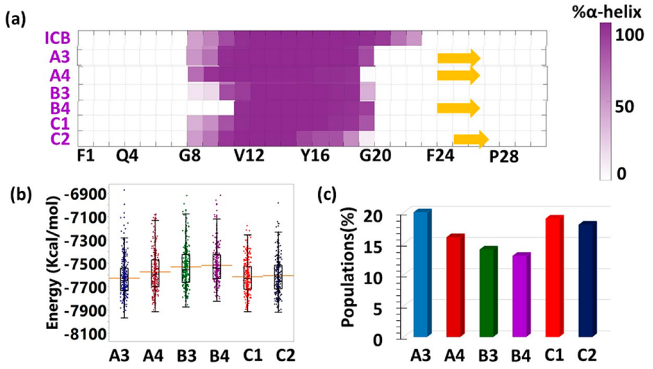

Figure 7. (a) Location of helical structure along the sequence of insulin in simulated models A3, A4, B3, B4, C1, and C2. β-Strands are presented in yellow arrows. The analysis of the secondary structure was calculated by the algorithm defining secondary structure of proteins (DSSP). (b) Scatter charts of the 500 conformations obtained from the generalized Born method with molecular volume (GBMV) energy values extracted from the last 5 ns of each model (Supporting Information). The values indicate conformational nonbonded interaction energies. The scatter charts represent the "histograms" of the number of conformations in energies' range. The averaged energy values are seen in the "boxes". (c) Populations analysis of models A3, A4, B3, B4, C1, and C2.

models B3 and C1, chain A of insulin participates; therefore, there is a lack of β-strands along the sequence of insulin chain B. We thus propose that insulin is more prone to coaggregate with model A in two orientations, while in models B and C it is prone to aggregate only in one orientation along the fibril axis. The coaggregation between amylin and Aβ has been extensively investigated in solution[32] and in membrane[33,34] environments with various orientations. Moreover, it was found that Genistein exhibited inhibitory effects for both amylin and Aβ aggregation.[35]

Finally, insulin does not disrupt the β-strands of the "U-shape" fibril-like Aβ_{1-42} oligomers for both models A and B, i.e. models A3, A4, B3, and B4 (Figure S25 and S26). However, in the "S-shape" fibril-like Aβ_{1-42} oligomer, insulin disrupts the β-strands only when it binds initially in one orientation—model C2—as seen in Figure 5b (Figure S27). The effect of the disruption of the β-strands may not be due to the loss of the interactions of chain B of insulin with the "S-shape" fibril-like Aβ_{1-42} oligomer (Figure S22) but due to the shifting of the other domains of the insulin from the recognition motif (Figure 6). Interestingly, although insulin chain A interacts with the "U-shape" fibril-like Aβ_{1-42} oligomers of model B, i.e. model B3 (Figure S23), the β-strands were not disrupted.

Interestingly, due to conformational energy and populations analyses, model A3 is more stable and populated than model A4 (Figure 7b and 7c). We hence propose that insulin prefers to interact with model A in the orientation as seen in Figure 5a compared to in the orientation that is seen in Figure 5b. Previously, it was demonstrated by experimental techniques that Aβ fibrils promote insulin aggregation;[15] however, herein we show that this phenomenon is limited for only specific polymorphic Aβ fibrils and not for all polymorphic Aβ fibrils. Obviously, our simulations cannot demonstrate well-organized insulin fibrils, but the initial seeding of the fibrillation of insulin that was proposed in the recognition motif may provide primary steps for understanding the molecular mechanisms of the cross-seeding between Aβ fibrils and insulin.

CONCLUSIONS

Clinical trials applied intranasal insulin as a therapeutic strategy for AD and showed improved memory and cognitive function.[36−40] The activity effect of insulin has been investigated by in vitro studies,[15,16] and it was proposed that the activity depends on the binding of with Aβ oligomers/fibril. Long-term effects of insulin on the symptoms of AD patients were not observed. Therefore, research with regard to the success of insulin as an inhibitor for Aβ aggregation necessitates further studies. Yet, the specific interactions between insulin and Aβ oligomers/fibrils are still elusive. It is aimed to provide an insight into the molecular mechanism of the effect of insulin on Aβ aggregation.

The current research reported here focuses on characterizing the specific interactions between insulin and polymorphic Aβ_{1-42} fibril-like oligomers. Our work led us to three main conclusions. First, insulin can interact across the fibril only to Aβ fibrils with "U-shape" structures and not to "S-shape" structures. Therefore, there are no effects of insulin on "S-shape" Aβ fibril-like oligomers, because it does not bind across the fibril. Second, insulin may disrupt β-strands along Aβ fibril-like oligomers via interaction with chain A, which is not a part of the recognition motif. It may affect as an inhibitor of Aβ fibrillation, but it is limited due to the specificity of the polymorphic Aβ fibril-like oligomer. Third, insulin may promote Aβ aggregation, when interacting along the fibril axis of Aβ fibril-like oligomer. The coaggregation may be initiated via the recognition motif. The lack of the interactions of insulin in the recognition motif impede the coaggregation of insulin and Aβ.

The complexity of the functional activity of insulin on Aβ aggregation may be due to polymorphic Aβ states that may be produced in the brain of AD patient. The polymorphic Aβ fibrils imply distinct patterns due to intra- and inter-residues interactions. Polymorphic Aβ fibrils were also solved by cryo-EM,[41,42] in which one of them demonstrated a "C-shape" structure of Aβ_{1-40} fibrils.[41] In some cases, insulin may inhibit Aβ aggregation, while in other cases, it may promote Aβ aggregation or will coaggregate with Aβ or will not have any effect. Future studies are essential to investigate the functional activity effect of insulin on these polymorphic Aβ fibrils and to forthcoming Aβ fibrils that will be solved by experimental studies. It has been shown that Aβ oligomers are highly toxic.[43] Therefore, further studies are required to investigate the effect of insulin on Aβ oligomers associated with membranes[44] and nanodiscs.[45] There are challenges to investigate the specific interactions of insulin with disordered nonfibrillar disordered hexamers. Recently, our group illustrated the three-dimensional structure of disordered nonfibrillar Aβ dimers.[46] It is expected that insulin may interact with the CHC, C-terminal, and SHC domains of Aβ dimers—domains that play an important role in the nucleation of Aβ aggregation. Future studies are required to investigate the effect of insulin on these early stage oligomers.

METHODS

Molecular Dynamics (MD) Simulations Protocol. The constructed models of Aβ fibril-like hexamers, insulin, and insulin-Aβ fibril-like hexamers are detailed in the Supporting Information. MD simulations of the constructed models of insulin, Aβ hexamers, and insulin-Aβ fibril-like hexamers were performed in the NPT ensemble using NAMD[47] with the CHARMM27 force-field.[48,49] The models were energy minimized and explicitly solvated in a TIP3P

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water box[50] with a minimum distance of 15 Å from each edge of the box. Each water molecule within 2.5 Å of the models was removed. Counter ions were added at random locations to neutralize the charge of the models. The Langevin piston method[47,51,52] 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.[47] 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.[53,54] The equations of motion were integrated using the leapfrog integrator with a step of 1 fs. The pH of each system was set to physiological pH.

The solvated systems were energy minimized for 2000 conjugated gradient steps, where the hydrogen bonding distance between the β-sheets in the Aβ aggregates is fixed in the range 2.2−2.5 Å. The counterions and water molecules were allowed to move. The hydrogen atoms were constrained to the equilibrium bond using the SHAKE algorithm.[55] 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 to 300 K and then to 330 K for 300 ps and equilibrated at 330 K for 300 ps. These conditions were applied to all variant models. Simulations ran for 100 ns for each variant model, and a total ran for 1 μs for all variant models. These time scales of simulations were chosen after examining the convergence of the simulated models, using hydrogen bond analysis and root-mean-square deviation (RMSD) analysis (Supporting Information). The simulated structural models were saved every 10 ps for analysis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschemneuro.9b00645.

Computational and structural analyses (PDF)

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Author Contributions

M.B. constructed the models, ran the simulation, collected the data, analyzed the data, and wrote the original draft. Y.M. designed the research and revised the manuscript.

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Notes

The authors declare no competing financial interest.

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