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Structural Modulation of Human Amylin Protofilaments by Naturally Occurring Mutations

Florentina Tofoleanu^{*,†,‡}, Ye Yuan^{§,⊥}, Frank C. Pickard IV[†], Bartłomiej Tywoniuk^{§,⊥}, Bernard R. Brooks[†], and Nicolae-Viorel Buchete^{*,§,⊥}

[†]Laboratory of Computational Biology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892, United States [‡]Department of Chemistry, Yale University, New Haven, Connecticut 06520, United States [§]Institute for Discovery, University College Dublin, Belfield, Dublin 4, Ireland [⊥]School of Physics, University College Dublin, Dublin 4, Ireland

Abstract

Human islet amyloid polypeptide (hIAPP), also known as amylin, is a 37-amino-acid peptide, co-secreted with insulin, and widely found in fibril form in type-2 diabetes patients. By using allatom molecular dynamics simulations, we study hIAPP fibril segments (i.e., fibrillar oligomers) formed with sequences of naturally occurring variants from cat, rat, and pig, presenting different aggregation propensities. We characterize the effect of mutations on the structural dynamics of solution-formed hIAPP fibril models built from solid-state NMR data. Results from this study are in agreement with experimental observations regarding their respective relative aggregation propensities. We analyze in detail the specific structural characteristics and infer mechanisms that modulate the conformational stability of amylin fibrils. Results provide a platform for further studies and the design of new drugs that could interfere with amylin aggregation and its cytotoxicity. One particular mutation, N31K, has fibril-destabilizing properties, and could potentially improve the solubility of therapeutic amylin analogs.

Graphical Abstract

^{*}Corresponding Authors florentina.tofoleanu@nih.gov., buchete@ucd.ie.

Supporting Information

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More details on the analysis (PDF)

Notes

The authors declare no competing financial interest.

INTRODUCTION

Co-secreted with insulin by pancreatic islet β -cells,^{1, 2} amylin (or human islet amyloid polypeptide, hIAPP) binds to multiple amylin-specific receptor complexes,³ and under regular conditions, helps insulin decrease the glucose levels after meals.^{4,5} hIAPP is an intrinsically disordered protein and does not have a stable conformation as a monomer.^{6,7} However, under certain conditions, disordered monomers aggregate, become harmful to pancreatic β -cells, and lead to type-2 diabetes.^{8–10}

Amyloid aggregation and toxicity is not fully understood and has been the focus of active study.^{11–14} Despite not having the same primary sequence, amylin shares similar β -sheet structure with amyloid- β (A β), a peptide involved in Alzheimer's disease.^{15–18} More recently, amylin receptors have been identified as potential treatment targets for Alzheimer's disease.¹⁹ Amylin and A β aggregates are co-localized,²⁰ and amylin fibrils can be cross-seeded with A β in vivo.²¹ Such cross-seeding has also been investigated computationally.²²

Amylin isoforms from other mammalian species, such as rat (rIAPP) and pig (pIAPP), presenting several key mutations from hIAPP, have been shown to not aggregate.²³ Cat amylin (cIAPP), on the other hand, although it has three residues in common with both isoforms, does aggregate.²⁴ Pramlintide, an amylin analog containing only three proline substitutions from hIAPP to rIAPP, has been used as an injectable diabetes treatment due to its β -strand fibril-breaking properties.²⁵ These differences have been the object of multiple studies trying to elucidate the propensity to aggregate due to mutations. rIAPP and hIAPP have been shown to have different extents of β -sheet and α -helix structure.^{7,26,27} Extensive computational studies on single-strand and double-strand fibrils of hIAPP and rIAPP investigated how the mutations affect the aggregation process, showing that hIAPP has a higher propensity of forming β -sheets,^{28–34} a hallmark of amyloid fibrils.³⁵ Although rIAPP is nonamyloidogenic, in the presence of hIAPP, it can form hybrid fibrils.^{36,37} Other single point mutations, such as S20G, G24P, or I26P, could lead to different stabilities of hIAPP.^{38–41}

This study investigates the effect of sequence alteration on the dynamics of solution-formed amylin aggregates. Differences in dynamics due to mutations could be crucial in the aggregation propensity.³² We explore how to possibly disrupt the structure of a harmful

aggregate most effectively with minimal mutation, which will potentially help understanding the behavior of hIAPP and type-2 diabetes, and developing new therapies using mutations or biomarkers.

METHODS

Each system consists of five dimeric layers (a 10-mer) of wild type (WT) hIAPP and of mutations to rIAPP, to pIAPP, and to cIAPP (see Figure 1). The disulfide bond between C2–C7 was explicitly modeled. We constructed the hIAPP fibril starting from the NMR structure by Luca et al.,⁴² which has two peptides in each layer and parallel β -sheets. We replicated the dimer shown in Figure 1 to construct the infinite (i.e., using periodic boundary conditions with both dimensions chosen to accommodate an integer number of molecular layers along the fibril axis, see Figure 2) and finite systems (i.e., finite fibril segments solvated uniformly in all directions by explicit water molecules), as shown in Figure 2. The five-layer fibrils, first equilibrated in the infinitely long topology, were subsequently solvated by using the explicit TIP3P water model.⁴³ Protofilaments of this length have been shown to retain their fibrillar state and to gain stability with oligomer growth.⁴⁴ We used periodic boundary conditions with appropriate box sizes to create an “infinite” fibril (see Figure 2), by providing a 4.8 Å distance between layers in adjacent images along the z-axis direction; this arrangement emulates the nearest-neighbor intermolecular distance reported by experiments.⁴² The core of the fibril was initially dry, though it became accessible to water molecules during the simulations. We added Na⁺ and Cl[−] ions to neutralize and provide a 100 mM NaCl solution. The system was then minimized (200 000 steps), heated to 300 K, and equilibrated (700 ps) at the target temperature using the NAMD package^{45,46} with the CHARMM36 force field.⁴⁷ Pressure was maintained constant at 1 atm with the Langevin piston method and the temperature was kept constant at 300 K with a Nosé–Hoover thermostat.^{48,49} We used a time step of 1 fs.

An initial infinite hIAPP system was simulated in an NPT ensemble for 50 ns for equilibration. We then performed mutations on the resulting structure and simulated the systems further, both in an infinite and then in a finite topology. The hydrogen bonds forming between the periodic boxes in the z-axis were used to restrain the mutated systems in an infinite conformation. We generated systems with all single-point mutations to rIAPP, pIAPP, and cIAPP: T4M, R11H, V17D, V17I, H18R, S20R, F23L, A25P, A25T, I26V, L27F, S28P, S29P, and N31K. We also built a fibril with all three mutations to proline found in rIAPP (A25P–S28P–S29P, also known as pramlintide,¹ and henceforth referred to as P3), as well as to all mutations in rIAPP, pIAPP, or cIAPP. As one notices, rIAPP, pIAPP, and cIAPP share three single-point mutations: H18R, F23L, and S29P. Details on system sizes and simulation lengths for WT and mutated fibrils can be found in Table S1. Each finite system was further minimized, equilibrated (as described above), and simulated for 200 ns each, adding up to over 4.5 μ s of total simulation time. For each system, the last 100 ns of simulation were used for analysis. The secondary structure was assessed by using STRIDE,⁵⁰ as implemented in visual molecular dynamics (VMD).⁵¹ The solvent accessible solvent area was assessed by using the VMD plugin; the sphere radius was 1.4 Å. In-house Python scripts were used for calculating the root-mean-square deviation (RMSD) and for performing the principle component analysis, see Supporting Information for details.

RESULTS AND DISCUSSION

We simulated the wild type (WT, hIAPP) and mutant amylin fibrils, first using an infinite fibril topologies for 50 ns (i.e., using periodic boundary conditions with both dimensions chosen to accommodate an integer number of molecular layers along the fibril axis, see Figure 2, in order to equilibrate the systems after the insertion of mutations), followed by simulations of finite amylin fibril segments for 200 ns in each case. The structural models used here were constructed based on the solid state NMR structures by Luca et al.,⁴² in which the fibrils contain two peptides in each layer and consist of parallel β -sheets that are in contact in the C-terminus segments of amylin (Figure 1).

Details of the simulations are summarized in Table S1 and in the Methods Section. The analysis was performed on the last 100 ns of each simulation and was focused on the effects of mutations upon the structure and dynamics of the mutants with respect to the WT solution-formed fibrils.

Deviation from the Initial Structure.

To evaluate how the introduction of mutations affects the fibril structure, we first analyzed the root-mean-square deviation of the C_α atoms, calculated with respect to the initial structure (RMSD₀). For the fibril in the infinitely long topologies, most RMSD₀ versus time values plateaued between 0.5 and 2 Å (Figure S1). Most strikingly, the pIAPP-mutated fibril suffered large structural changes within 50 ns of simulation time, with average RMSD₀ values greater than 4 Å (Table S2). By contrast, for rIAPP sequences, the system with the next largest structural changes, RMSD₀ values plateaued at 2 Å. Experimentally, neither rIAPP nor pIAPP form fibrils, and they perform quite differently during simulations. The fact that pIAPP unravels even as part of an infinite fibril was somewhat unexpected, yet encouraging. The mutations common in both rIAPP and pIAPP peptides are H18R, F23L, and S29P, therefore, the other mutations in pIAPP must account for this difference. The analysis of RMSD per residue of the infinite fibrils (Figure S2) showed that the most perturbed systems were A25P, S28P, S29P, N31K (all four mutations are located in the C-terminus), P3, rIAPP, and pIAPP. P3 (pramlintide) contained all three disrupting mutations to proline, which is a well-known β -sheet-breaker.^{40,52} During the finite simulations, N31K stands out again, with RMSD₀ values larger than for the other systems (Figures S3 and S4). A more detailed discussion of the measured RMSD₀ values is given in the Supporting Information.

Mutation-Induced Variation of the Secondary Structure.

We evaluated the β -sheet content of our molecular models of fibrillar oligomers, a hallmark of amyloid fibrils,⁵³ by assessing the percentage of amino-acids that are in an extended conformation (see the Methods Section). During the simulations of the infinite fibrils, the content of the β -sheet was lowest for N31K and pIAPP, leveling at 0.43 and 0.41, respectively (Figures S5 and S6). For all other systems, the β -sheet content plateaued at values between 0.50 and 0.70 (Figure S5). The average content of the β -sheet for the fully solvated fibrils varied between 0.41 for rIAPP and 0.59 for T4M (Figures S7 and 3). N31K had the next lowest fraction of the β -sheet, at 0.42. Single-point mutations to proline had

lower β -sheet content than WT, with the lowest value for S28P (0.47), followed by S29P (0.48) and A25P (0.51). This finding is consistent with experiments showing that for hIAPP_{20–29}, S28P greatly inhibits fibril formation, whereas A25P and S29P slightly attenuate it.^{54,55} Details on the β -sheet percentage by system are given in Table S3 and in text in the Supporting Information.

We further investigated the effect of mutations on the propensity of each residue to be in a β -sheet conformation. This analysis pinpoints how changes induced by a mutation at a certain position affect different regions of the peptide. Some mutations had a clear stabilizing or destabilizing effect on the secondary structure, but most mutations induced mixed changes (Figure 4). The system with mutation T4M experienced a general increase in the content of the β -sheet compared to that of WT (Figure 3), with the most affected residues being in the N-terminus coil (A5–C7) and the first experimentally determined β -sheet, residues A8–N14 (Figures 1 and 4), with the largest increase for residue A8 of ~0.65. A small increase of the extended structure content was also observed for residues G33–N35 in the second β -sheet. The propensity to form the β -sheet for residue M4 was not affected.

System R11H had an overall decrease in the β -sheet content, mostly in the N-terminus region, residues C2–L12, including the mutated residue, and for residue N35. The largest decrease in the β -sheet was within 0.5. The V17D mutation induced an overall decrease in extended conformation for residues assigned to both β -sheet regions (T9, D17, H18) and to coil (C2–T6, S19–N22). Residue C7 has an increase of β -sheet in all three systems. V17I, on the other hand, registered an overall increase of the β -sheet content, with a positive effect on residues in both β -sheet regions, especially residues A8–A13 and G33–T36. Residue I17 itself had a slight decrease in the amount of time it spends in an extended conformation. The most striking difference between mutated systems V17D and V17I was the profile for residues G33–S34 and N35–T36, which experience no change, and a decrease of 0.3, respectively, in V17D, and had an increase of more than 0.5 in V17I.

Mutation H18R decreased the overall β -sheet content compared to WT and had a mixed effect on the individual residues. The largest negative effect was for residues A8, V17, and N35. For residues Q10–A13 and R18 there was a slight increase in β -sheet formation propensity. The hIAPP_{10–19} region is likely important in the formation of interpeptide contacts during aggregation.⁵⁶ In particular, the H18R mutation has been shown to decrease the aggregation propensity for amylin and its potential to disrupt membranes of a hIAPP_{1–19},⁵⁷ pointing toward the effect that a mutation to a charged residue has on the fibril structure and its cytotoxicity. Therefore, disruptions in the sequence by changing the protonation state of the residues in these positions, such as R11H, V17D, and H18R, could explain their relatively large effect on the secondary structure propensity.

System S20R overall had a higher content of residues in an extended conformation than WT. This is not surprising, as it has been shown that S20 does not contribute to fibril formation.⁵⁴ Residues assigned to the first β -sheet (A8–V17) had positive values, whereas residues in the second β -sheet (S28, S29, and N35) had slightly decreased values. Residue F23 had the highest increase in β -sheet content.

Mutation F23L had a mixed effect on residues assigned to the first β -sheet: increased values for Q10-A13, as also observed in H18R, and decreased values for A8-T9 and N14-V17, also similar to H18R. Residue L23 had a higher content of the β -sheet than that of residue F23 in the WT fibril. Residues G33 and S34, assigned to the second β -sheet, were more structured than in the WT fibril. Mutations A25P and A25T both lowered the β -sheet content compared to that of the WT fibril, and the profile of the residues in the two systems was very similar. Residues A5-A8 and, more strikingly, residue N35 had a decrease in β -sheet content. Residues T9-N14 (with a large overlap with residues Q10-A13 in H18R and F23L), P25, and T25, respectively, had an increase in β -sheet content.

System I26 V had a higher content of β -sheet than WT, with the highest increase for β -sheet propensity being for residues A8-T9 and F23. The C-terminus was not affected. System L27F increased the propensity to form the β -sheet for residues in both the N-terminus (A8–A13) and in the C-terminus (G33–S34).

Mutations S28P and S29P had a slight positive effect on residues in the N-terminus, but residues L27–S29 and S28–P29, respectively, had a decrease in β -sheet propensity of almost unity. Residue N35 had a decrease of ~ 0.3 in both systems, the same effect as that in A25P, but of a smaller magnitude. Overall, systems S28P and S29P had a lower β -sheet retention than A25P with average values of 0.47, 0.48, and 0.51, respectively. These results are consistent with previous experimental studies showing that position 25 tolerates substitutions to proline better than positions 28 and 29.^{54,58} System P3 (mutations A25P, S28P, and S29P) had overall a lower β -sheet content than that of WT. It combined characteristics from system A25P, which had an increase in β -sheet propensity for residues T9–A13, and the decrease from systems S28P and S29P for residues L27–P29. It is interesting that the large decrease in the propensity of forming the β -sheet for residue N35 in A25P, S28P, and S29P systems was diminished in system P3.

Reassuringly, system rIAPP combined the features of system P3 and mutations H18R, F23L, and I26 V, and had the lowest percent of β -sheet content of all simulations of finite fibrils, as expected. The negative effect of the P3 mutations was the most prevalent, with the largest decrease in the β -sheet propensity for residues L27–P29. The next affected residue was N35, an effect common to systems H18R, A25P, S28P, and S29P. The negative effect on C7–A8 could be attributed to A25P, which had the largest decrease for residue C7 out of all six mutations. The positive effect of mutations I26V and L27F was overwritten, but the increase in β -sheet propensity for mutation F23L was still present. There was also a slight increase for residue R18, also seen in the H18R system. A study showing that P3, with reverse mutations to hIAPP, R18H, L23F, and V26I, can still form amyloid fibrils,⁵⁹ suggests that there is a synergetic mechanism by which rIAPP is rendered non-amyloidogenic.

Out of all mutations, N31K negatively affected the β -sheet propensity of the largest portion of the C-terminus. Besides residues L27–S28, the next most affected residues were K31, V32, N35, and T36. The N31K mutation also slightly increased the β -sheet propensity for residues in the N-terminus. The delocalized effect of the mutation on the entire second β -sheet could be due to the fact that the K31 residue is located at the interface between the two strands. The large effect for N31K could be attributed to the change of the protonation state

at a position in the C-terminus and at the strand-strand interface, where it can disrupt stacking for the β -sheet and the interstrand contacts, as previously inferred for H18R.⁵⁹

System cIAPP had a lower β -sheet content than that of WT, and the most obvious negative effect was due to mutation S29P, with a decrease in the propensity to form the β -sheet for residues S28–P29. Residues T9–Q10 and A13–N14 had the next most pronounced decrease, which was not common to any of the systems that contain single-point mutations; this feature was specific to cIAPP. The decrease in β -sheet propensity for N35 could be attributed to S29P. R18 had a higher increase in the propensity to form the β -sheet in cIAPP than in the H18R system. The fact that S29P had the least effect on the secondary structure is consistent with an experimental study showing that position 29 is not critical for amyloid formation.⁵⁴ Therefore, cIAPP is still able to form fibrils, even with a mutation to proline in its C-terminus.

Identifying which Mutations Have the Highest Impact on Structure.

We performed a principal component analysis (PCA) on the propensity to form β -sheet for each mutated residue with respect to the WT. We chose to use the first three PCs, which accounted for 72.6% of the total variance in the data (mode detail in Supporting Information), as they are easy to visualize. PCA allows for interpretation of data from the point of view of the most correlated changes that occurred in all systems during simulations. Principal component 1 classified the systems in two major groups, as seen in the top row of Figure 5. Systems S28P, S29P, N31K, P3, rIAPP, and cIAPP had characteristics distinct from the rest of the systems. PCA grouped hIAPP and V17D together, with V17I closeby, as shown in the visualization of any of the principal components. Systems T4M and I26V, and A25P and A25T were also similar.

Clustering by the Euclidean distance in the space of the three PCs distinguished the two major families (as seen in Figure 5), each with two subfamilies, but also identified the subtler connections between the different systems (see Figure 6). The clustering indicated that changes in the β -sheet propensity in rIAPP were most correlated to changes in N31K. This is also intuitive, as both systems experienced the most drastic decrease of β -sheet in the C-terminus. As a reminder, rIAPP was the system with the lowest content of β -sheet. P3 and S28P formed another cluster, as supported by the correlation of the decrease in the propensity to form the β -sheet for residues L27–P29 and N35–T36, and increase for residues R11–N14, also as seen in Figure 4. The fact that S29P and cIAPP were clustered together in the second subfamily is also intuitive, as the largest decrease in the β -sheet in cIAPP was due to the S29P mutation in its C-terminus.

The third subfamily had WT and V17D as its closest members, although they had different contents of β -sheet, at 0.54 and 0.51, respectively. However, out of all mutated systems, changes in β -sheet propensity per residue had the lowest magnitude in V17D (see Figure 4). F23L and H18R formed another pair, due to correlated changes for residues A8–V17, as mentioned in the previous section. R11H was a distant cousin, clustered here due to a lack of variation in the extended structure for residues in the C-terminus, similar to other members of the family, but also due to the correlation of residues experimentally assigned to coil, rather than of those assigned to β -sheet. Farther off were A25P and A25T, which had a low

β -sheet content, similar to members in the third subfamily, but also had similar characteristics to H18R and F23L for region T9–A14.

The last subfamily contained members S20R, L27F, V17I, I26V, and T4M, which all had a higher content of β -sheet than WT and all other systems (between 0.57 and 0.59). The closest two members were I26V and T4M, with the highest values for β -sheet content and correlated changes for residues T6–L16. L27F and V17I were the next closest, due to similar changes for residues in the first β -sheet, for residues N21–F23, and except for A25, for the rest of the C-terminus. S20R was connected to the L27F–V17I pair due to the first β -sheet.

Behavior of Structural Components Is Correlated.

Using the principal component vectors, we analyzed the β -sheet character of each residue in the mutants with respect to the WT, and we identified how changes in certain regions of the peptide were correlated. The first principle component indicated that changes of residues L27–S29 were highly correlated (Figure 7). The decrease of the propensity to form β -sheet for these residues was also correlated to a slight decrease in the distal end of the C-terminus. The next strongest correlation was for residues T9–N14, for which positive changes were also related to negative changes in residues N35–T36. The third principal component suggests that the change in β -sheet content was positively correlated for residues in the N-terminus (C2–V17) and residues G33–N35.

Mutations that Increase Solubility.

One might expect that the more disordered the fibril became during the MD simulations, the more it was solvent exposed, such that the loss of β -sheet structure would be correlated with an increase in the solvent accessible surface area (SASA). Indeed, N31K, one of the systems with the lowest β -sheet content, stood out as being one of the most solvent-exposed systems (Figure 8). Out of other mutations to charged residues, H18R had a higher SASA value than that of WT, V17D and S20R, surprisingly, had lower SASA values than that of WT. A25T, another mutation in the C-terminus, had one of the lowest values for β -sheet content and also a high SASA value. However, overall, we saw a weak negative relationship between the content of β -sheet and SASA values for each finite system, with a Pearson's r -value of -0.50 and a Kendall's τ -value of -0.37 (Figure S8). There was no clear correlation between the propensity to be in an extended conformation and the SASA value for each residue in the finite systems (Figure S9).

CONCLUSIONS

We have investigated how naturally occurring mutations of the amylin peptide corresponding to three different species, rat, pig, and cat, affect the structure of solution-formed human amylin (hIAPP) fibrillar oligomers. We identified and analyzed the mutations that disrupt the structure the most and render the fibril more solvent accessible, in an effort to identify an isoform with similar drug properties to pramlintide,²⁵ but with increased solubility.

By performing a principal component analysis on the β -sheet content of the fibrils, we were able to establish the correlated effects of mutations. Our analysis (see Figure 7) indicated

that certain mutations actually enhanced the extended fibril structure, such as T4M, V17I, S20R, I26V, and L27F. Others had a similar β -sheet profile as that of the WT amylin sequence, such as R11H, V17D, H18R, and F23L, and more remotely, A25P and A25T. Another distinct family comprised of mutations that decreased the β -sheet content. In this family, N31K was clustered with rIAPP, P3 with S28P, and S29P with cIAPP, which indicated that each two systems had correlated disruptive mutations. None of these results were surprising; S29P had the most striking effect within cIAPP, S28P within P3, and N31K and rIAPP both had the largest negative effect on the C-terminus (see Figure 4). Both S29P and N31K are most disruptive in pIAPP, which became unstable in the infinite conformation. The fact that, for the infinite systems, the content of the β -sheet for both N31K and pIAPP had similar values, indicated that the largest contribution to the disruption of the pIAPP fibril was from N31K.

A recent study targeting the optimization of pramlintide,⁶⁰ investigated, in detail, the amylin 20–29 segment and proposed S20R as a possible mutation to enhance the solubility. However, in the full-length amylin fibril, this mutation is located in the turn region (see Figure 1). The S20–P29 segment was once believed to be the amyloidogenic core, but this hypothesis disagrees with solid-state NMR experimental data.^{42,61} Interestingly, our analysis also points to the fact that S20R does not negatively affect the β -sheet structure. On the contrary, the S20R mutation enhances the extended structure and is less soluble than the WT fibril.

Based on our simulation results, we proposed that N31K (a mutation in the C-terminus) is not only one of the most disruptive mutations for the amyloid fibril structure, but it also renders the fibril's molecular surface more solvent accessible. Another mutation observed to decrease the overall β -sheet content and increases fibril solubility was A25T. Both these mutations are in the C-terminus region of the peptide and at the interface between the two fibril strands (see Figure 1). The F23–L27 region has been shown to form β -sheet intermediates, which can be disrupted to form a loop in the fibril.⁶² In another study, residues F23–S28 are part of the β -sheet core,⁶¹ as part of a polymorphic variation of the amylin fibril. However, here we show that A25T disrupts the amylin fibril, not by affecting the β -sheet propensity of residue 25, but that of residues in the N-terminus and at the G35 location.

In this study we simulated amylin in solution, however, the biological function of amylin is also strongly related to its interaction with cellular membranes.^{63–65} We have previously shown how the structure of fibrillar oligomers of a related amyloid, Alzheimer's A β peptide, is affected by the lipid composition of model membranes, and how, in return, the amyloid fibril induces local structural perturbations in the membrane itself.^{66–69} Further study is necessary to test whether the fibril-disruptive mutations proposed in this study are able to also affect the toxicity of amylin peptides in a more complex and crowded cellular environment, and in the vicinity of lipid membranes in particular. The mutation effects revealed in this study may be probed in more detail (though also more computationally demanding) through enhanced sampling simulation, focused on characterizing the underlying molecular mechanisms of their kinetic and structural effects by using recently developed master equation approaches.^{70–73}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) Amino acid sequence of human (hIAPP), cat (cIAPP), rat (rIAPP), and pig amylin (pIAPP). In the wild type hIAPP (WT), residues involved in β -sheet are marked in blue, the rest are gray. Residues C2 and C7 form a disulfide bridge. Mutations from hIAPP to cIAPP, rIAPP, and pIAPP are shown in yellow, green, and red, respectively. (b) Secondary structure of a cross-section of double-strand amylin fibrils based on the NMR model.⁴² Same color scheme as in (a).

Figure 2. Building infinite and finite molecular fibril system in an explicit water environment. (a) Cross-section through an infinitely long system of WT hIAPP. (b) Fibril along the growth direction, surrounded by water. (c) The finite fibril model surrounded by water in all three dimensions.

Figure 3.

Finite fibril segments. The average percentage of β -sheet content for each system. The WT hIAPP is in black, mutations to pIAPP, cIAPP, and rIAPP are in coral, yellow, and green. H18R, F23L, and S29P are common to pIAPP, cIAPP, and rIAPP. Values for each mutated fibril are compared to the WT value (horizontal black line).

Figure 4.

Finite systems. The difference between the propensity to form β -sheets for each residue in each system and the propensity for each residue in the hIAPP (WT) system. Systems R11H, A25T, N31K, and those systems containing mutations to proline had the largest decrease in the β -sheet content. Residues assigned to β -sheet by NMR are in blue, the rest are in gray. The mutated residues in each system are colored in black.

Figure 5.

Principle component analysis (PCA) for the propensity to form β -sheet conformations for each mutated residue with respect to the WT. Systems S28P, S29P, N31K, P3, rIAPP, and cIAPP form a distinguished group from the rest by PC1.

Figure 6. Clustering the finite systems based on the Euclidean distance in the principal component space. The dendrogram on the left is based on distances between the centroids of each corresponding cluster. On the right, are shown distances between the corresponding system pairs.

Figure 7.

First three principal components of the variation in β -sheet character for the mutated finite systems with respect to WT. Residues assigned to β -sheet by NMR are colored in blue, the rest of the residues are in gray.

Figure 8. Probability density of the solvent accessible surface area (SASA) for each simulated finite system. For each system, the three red dashes indicate the lowest, average, and highest values. The horizontal dotted line is the average value for the WT system.