Published in final edited form as:

J Am Chem Soc. 2011 May 18; 133(19): 7240–7243. doi:10.1021/ja1081537.

The Amyloid Formation Mechanism in Human IAPP: Dimers have β -strand Monomer-Monomer Interfaces

Nicholas F. Dupuis, Chun Wu, Joan-Emma Shea, and Michael T. Bowers*

Department of Chemistry and Biochemistry, University of California Santa Barbara, Santa Barbara, California 93106

Abstract

Early oligomerization of human IAPP (hIAPP) is responsible for β -cell death in the pancreas and is increasingly considered a primary pathological process linked to Type II Diabetes (T2D). However, the assembly mechanism remains poorly understood, largely due to the inability of conventional techniques to probe either distributions or detailed structures of early oligomeric species. Here, we describe the first experimental data on the isolated and unmodified dimers of human (hIAPP) and non-amyloidogenic rat IAPP (rIAPP). The experiments reveal that the human IAPP dimers are more extended than those formed by rat IAPP and likely descend from extended monomers. Independent all-atom molecular dynamics simulations show that rIAPP forms compact helix and coil rich dimers, whereas hIAPP forms β -strand rich dimers that are generally more extended. Additionally, the simulations reveal that the monomer-monomer interfaces of the hIAPP dimers are dominated by β -strands and that β -strands can recruit coil or helix structured regions during the dimerization process. Our β -rich interface contrasts with an N-terminal helix-to-helix interface proposed in the literature but it is consistent with existing experimental data on the self-interaction pattern of hIAPP, mutation effects and inhibition effects of the N-methylation in the mutation region.

Protein aggregation and fibril formation are central processes in many age related diseases including Alzheimer's Disease, Parkinson's Disease and Type II Diabetes (T2D).¹ In 1987, Islet Amyloid Polypeptide (hIAPP) was identified as the primary component of the amyloid deposits found in and around the β -cells in patients with T2D.² Although a structure of hIAPP fibrils has been modeled from experimental distance constraints, structures of early oligomeric aggregates and the mechanism of β -sheet formation remain poorly understood for this 37 residue peptide (KCNTATCATQRLANFLVHSSNFGAILSSTNVGSNTYNH₂).³ Further, early oligomers have been implicated as the primary cytotoxic components of the aggregation pathway.⁴ Thus, characterizing the structures of early hIAPP oligomers is critical for a better understanding of the aggregation process and these structures might be good drug targets.

In our previous study,⁵ ion mobility spectrometry combined with mass spectrometry (IMS-MS) was used to characterize the size distribution of monomeric hIAPP and the non-amyloidogenic rat IAPP (KCNTATCATQRLANFLVRSSNNLGPVLPPTNVGSNTYNH₂), which differs from hIAPP by only 6 amino acids mainly located between residues 20–29, called the “mutation region”.^{6, 7} Experiments indicated hIAPP has an extended, large cross section structural family not found in rIAPP.⁵ By direct comparison with replica exchange molecular

* bowers@chem.ucsb.edu .

SUPPORTING INFORMATION: Experimental details and mass spectra of rIAPP. Analysis of full MD simulation trajectories and dimer structures (PDB files are available upon request).

dynamics (MD) simulations, two experimental hIAPP conformer families were identified: a compact helix-coil family and an extended β -hairpin family, with the mutation region adopting coil and β -strand conformations respectively. In contrast to hIAPP, the simulations indicated that rIAPP only populates compact coil-rich and helix-coil families (with coiled mutation regions) in agreement with experiment.^{5, 8, 9} Based on these results, a mechanism of hIAPP aggregation was proposed with direct side-by-side assembly of β -hairpin monomers to form β -sheet rich oligomers.⁵ This “early conformation transition” mechanism highlights conversion of monomers into β -sheet rich oligomers, which can be considered assembly-prone structures.^{5, 9–11} This model contrasts with a paradigm of fibril formation where the N-terminal helix interactions drive assembly to form helix-rich oligomers, followed by a “phase transition”, to β -sheet structured aggregates, later in the aggregation cascade.^{12–15} Transient increase of helical content from NMR¹⁴ and CD data¹² were used to infer the helix-helix assembly model. However, these techniques provide an average picture of peptide aggregation since neither method can distinguish oligomers from monomers. Additionally, by fusing a full length hIAPP to a 370-residue maltose binding protein (MBP), Eisenberg and coworkers were able to obtain a crystal structure of a hIAPP homodimer with residues 8–18 in helical conformations at the monomer-monomer interface.¹⁶ However, this helix-helix interface may well be a result of crystal packing of the large fusion protein. This possibility is consistent with a lack of a helix-helix interface in the crystal of the second fusion protein (MBP fused with the N-terminal fragment (1–22) of hIAPP).¹⁶

Here, the combined IMS-MS and MD modeling approach⁵ is used to investigate the structure of unmodified, isolated dimers of rIAPP and hIAPP. This study breaks new ground in amyloid formation mechanisms as currently there are no detailed experimental descriptions of dimer structure in the literature for amyloid systems with the complexity of hIAPP. The dimer is crucial as it provides the first, and best, opportunity to study the dynamics and structure of the monomer-monomer interface that drives further oligomer growth. This interface will be a focal point of this study and it will provide insight into why hIAPP proceeds to form β -sheet fibrils and rIAPP does not. Additionally, it will shed light on the self-interaction pattern between 10-residue fragments of hIAPP and the full length hIAPP determined by fluorescence titration binding assays¹⁷ and the working mechanism of the designed peptide inhibitors based on N-methylations in the mutation region.^{18–20}

The hIAPP and rIAPP peptides came from two sources: Bachem company (Torrance, CA) and the Raleigh group (SUNY Stony Brook).²¹ The peptides were dissolved in 100% hexafluoroisopropanol at 1 mM. Aliquots of the stock solutions were dried and re-dissolved in 100 mM ammonium acetate buffer (pH 7.4) yielding 20 μ M peptide concentrations. Samples were then analyzed on a home built nano-ESI ion mobility mass spectrometer.²² Full details of sample preparations are included in the Supplementary Information (SI).

The nano-ESI mass spectrum of hIAPP is shown in Figure 1A. The $z/n = +3$ and $+4$ monomers are at 1302 m/z and 977 m/z respectively. A small amount of the $z/n = +5/2$ is found state at 1562 m/z , resulting from a dimer with five positive charges, $[2hIAPP]^+5$. The rIAPP mass spectrum is nearly identical to the hIAPP spectrum and is shown in the SI.

Arrival time distributions (ATDs) of the $z/n = +5/2$ peaks of hIAPP and rIAPP are shown in Figure 1B. The ATDs were fit with multiple features, using the procedure described in the SI. Collision cross sections and relative abundances are listed in Table 1. The rIAPP ATD was best fit with two components. The compact species (1033 $\text{\AA}^2$) comprises ~88% of the ion intensity and is broader than expected for a single conformation, suggesting there may be multiple families contributing to this peak. The hIAPP ATD was best fit with three features. In this ATD the 1150 $\text{\AA}^2$ peak is most abundant, contributing ~75% of the ion intensity.

Comparison of the two most abundant features for each peptide shows that the hIAPP dimer is ~12% larger in cross section than the rIAPP dimer ($1033 \text{ \AA}^2 \rightarrow 1150 \text{ \AA}^2$). The larger hIAPP dimer cross section is consistent with the previous observation of hIAPP β -hairpin monomers that are ~17% larger than the coil-rich rIAPP monomers.⁵ The dimer cross sections can be estimated from monomers, and should be larger by a factor of $2^{2/3}$, assuming no change in the monomer structure on forming the dimer.^{22, 23} From this approximation, dimers formed from compact hIAPP and rIAPP monomers, are expected to have cross sections of $\sim 1030 \text{ \AA}^2$ ($650 \text{ \AA}^2 \times 2^{2/3}$). However, dimers assembled from β -hairpin monomers are expected to have cross sections of $\sim 1220 \text{ \AA}^2$ ($770 \text{ \AA}^2 \times 2^{2/3}$). This simple analysis suggests that some of the hIAPP dimers, with experimental cross sections of $1225 \text{ \AA}^2$, may be dimers, with experimental cross sections of $1225 \text{ \AA}^2$, may be directly assembled from β -hairpin like monomers. The major dimer structural family, with a cross section of $1150 \text{ \AA}^2$, also appears to have significant extended β -strand character. Overall, the extended conformations of the hIAPP monomers and dimers, compared with rIAPP, suggest that extended hIAPP conformations may play a critical role in the early stages of amyloid fibril formation and that β -strand character emerges early in the aggregation cascade.

Independent from the experiment, the hIAPP and rIAPP dimers were modeled with all-atom molecular dynamics simulations using the AMBER protein force field (ff96) coupled with a recent generalized Born implicit solvent (IGB=5). Recent achievements of this ff96/IGB5 combination include the successful *ab initio* folding of α , β , and α/β proteins^{24–27} and amyloid peptides including a prion fragment²⁸ and IAPP.⁵ For all trajectories, the most populated monomer structural families identified in the previous study were used as the starting structures.⁵ Each trajectory was run for 400 ns, both with generalized Born implicit solvent and without solvent. The latter simulations are useful for comparison with cross sections obtained from experiment to ascertain whether equilibrium gas phase structures contribute to the measured ATDs. Full details of the modeling are included in the SI. The hIAPP dimer simulations were run in both the +3 and +4 monomer charge states, generating +6 and +8 dimers, as both charge states are physiologically relevant. The simulations show that the hIAPP dimerization process is largely independent of charge state, producing +6 and +8 dimers with very similar structural motifs. The average binding energies for each peptide are listed in Table 1 and the +6 dimer structures, formed from +3 monomers, are given in Figure 2. The mutation regions containing residues 22–29 of hIAPP and rIAPP are colored red to identify their role in the monomer-monomer interface.

The gas phase dimerization trajectory of rIAPP (RGP, Figure 2A) readily formed a dimer with a cross section of $1018 \text{ \AA}^2$. In the solution simulations, stable dimers were formed in all three trajectories. The R1 trajectory, starting with two coil-rich monomers formed a coil-rich dimer with a cross section of $1079 \text{ \AA}^2$. The mixed R2 trajectory formed a dimer with a cross section of $1157 \text{ \AA}^2$. And the R3 trajectory, starting with two helix-coil monomers formed a dimer with a cross section of $1116 \text{ \AA}^2$, where the peptides interact at the coil-rich C-termini. Overall, the dimers produced by the rIAPP peptide appear to be largely coil rich with some partial conservation of the N-terminal helix from the monomers.

The +6 hIAPP dimers (Figure 2B) show that the gas phase trajectory (HGP) produces a coil-rich dimer with a cross section of $1050 \text{ \AA}^2$. The H1 solution phase trajectory formed a dimer via a side-by-side association of two β -hairpin monomers with a cross section of $1150 \text{ \AA}^2$. The interface contains two β -strands, one of which contains the mutation region. The mixed H2 solution phase trajectory with one helix-loop-hairpin and one β -hairpin also produced a dimer where the two β -hairpins are stacked against each other with a cross section of $1172 \text{ \AA}^2$. A similar structure was observed for simulations initiated from hairpins.⁹ Lastly, the H3 solution trajectory formed a dimer with a cross section of $1105 \text{ \AA}^2$. In this dimer structure the two hairpins contribute significantly to the binding interface, more so than the helices.

Again, the two interfacing strands contain the mutation region. The dimer structures generated in the +8 hIAPP simulations are all similar to the +6 dimers and are located in the SI.

The simulations here highlight four new insights about the assembly of both IAPP peptides. First, in all of the hIAPP dimers, the binding interface occurs almost exclusively between β -strand secondary structural segments containing the mutation region, rather than between the N-terminal helices. Additionally, side-by-side assembly between the β -hairpins is the major binding mode, whereas stacking between the β -hairpins is a more minor contributor. This observation holds for both the +6 and +8 dimers. Interestingly, two strand regions of our hairpin structure (i.e. residues 11–18 and 23–32) in the monomer-monomer interface of H1 are coincident with the hot regions (i.e. residues 8–18 and 22–28) of hIAPP-hIAPP interaction interface as determined by fluorescence titration binding assays.¹⁷ Thus, independent measurements of the interaction interface support the results of our modeling. In contrast, rIAPP forms almost no β -strand and the dimers predominantly interact through the coil secondary structural segments. Together these simulations suggest that α -helix stacking is likely not a primary mode of peptide assembly.

Second, the simulations reveal conversion of α -helix and coil secondary structures to β -strand during hIAPP dimerization. In the H3 trajectory (Figure 2B), an increase of β -strand from 16% to 33% was observed, with a corresponding decrease of α -helix from 27% to 13%. This recruitment effect was especially apparent in the +8 dimers; the coil structure (residue 19–37) of the helix-coil monomer was converted into β -strand in three separate trajectories. Again, the mutation region is located directly at the interface. This process is illustrated for a single trajectory in Figure 3.

Third, our side-by-side sheet assembly around the mutation region may explain the working mechanism of the N-methylated hIAPP in which two methyl groups were added to the amide nitrogens of G24 and I26 located within the mutation region causing a dramatic reduction of amyloid fibril formation. N-methylation may prevent interstrand hydrogen bond formation and block the side-by-side sheet assembly. In a similar way, our model may explain the inhibition mechanism of the designed inhibitors containing NF(N-Me)GA(N-Me)IL^{19, 20}. One side of these inhibitors might bind to the native hIAPP and the other side with N-methylated groups preventing further side-by-side β -sheet formation.

Lastly, the simulations show that the hIAPP dimer binding energies (–59.5 kcal/mol, Table 1) are, on average, larger than the rIAPP dimer binding energies (–38.3 kcal/mol). The trend from our implicit solvent calculations is consistent with that from the potential of mean force calculations of dimer formation of hIAPP(20–29) and rIAPP(20–29) in explicit solvent.²⁹ The helix-helix model of association,¹⁶ yielded a binding energy of the helical dimer structure (PBD ID 3G7V) of –26.2 kcal/mol, which is far less favorable than the –59.5 kcal/mol binding energy of β -strand bound hIAPP dimers. These calculations indicate that the β -strand motif, rather the helix motif, is generally a more stable and favorable interface for dimer formation and β -sheet nucleation than other secondary structures. This result is generally consistent with the observed differences in aggregation behavior of the two peptides.³⁰ When comparing the experiments and simulations of the rIAPP peptide in this study, the gas phase dimer (RGP) and the compact coil-rich dimer (R1) are in closest agreement at 1.5% below and 4.5% above the experimental cross section of 1033 Å². Dimers from the other two trajectories (R2 and R3) may also make contributions to the experimental ATD as they fall within the limit of the largest experimental cross section (1170 Å²). For the hIAPP peptide, the +6 dimers are considered because they are closest to the experimentally observed +5 charge state. Overall, all three trajectories with implicit solvent produced structures with cross sections (1105 Å², 1150 Å² and 1172 Å²) that were

in reasonably good agreement with the experimental value of 1150 Å². The gas phase dimer structure at 1050 Å² is also close to the most compact feature in the ATD at 1024 Å², which it should be noted is very minor comprising only 7% of the total conformations observed experimentally. The simulations indicate that the hIAPP dimers are more energetically stable than the rIAPP dimers suggesting that the hIAPP dimers are more likely to retain more solution character than rIAPP.

In summary, experiments show hIAPP forms dimers that are significantly more extended than those formed by rIAPP, suggesting they have a high percentage of β -sheet content and may descend from β -hairpin monomers. The data is supported by models that reveal three routes to β -sheet formation in the dimers: 1) two β -hairpins associate side-by-side to form four strand β -sheet (H1), 2) the hairpins stack, forming a two layer structure (H2),⁹ and 3) the α -helix or coil structures are recruited to form β -strands and β -sheet. Furthermore, β -strands including the mutation region play a critical role in the monomer-monomer interface. In contrast, the rIAPP modeling shows compact and disordered dimers are formed from coil-rich structures with cross sections in good agreement with experiment.

Based on these results an updated assembly mechanism for hIAPP is shown in Figure 4. In this mechanism, the monomer can interconvert between a structure with an N-terminal helix and a β -hairpin structure. Simulations predict significant heterogeneity in the C-terminus of the α -helical monomer.⁵ A β -sheet rich dimer is now included in the aggregation pathway. Even though the hIAPP dimer may have multiple contributing conformers, the parallel side-by-side dimer is specifically included in the figure as it appears to have the greatest likelihood of propagating the β -sheet motif to form larger β -structured aggregates and fibrils. We note that further structural reorganization in forming multi-layered β -structured aggregates is necessary to produce final mature fibrils such as that illustrated by Tycko's fibril model structure.³ Overall, both the experiment and simulation results provide further support for a route to the fibril state through an "early conformational transition" to a β -stranded conformer, a result that contrasts with the current "phase transition" paradigm via coiled or helix-rich oligomers for fibril formation in amyloid systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding for this study was provided by the National Institutes of Health, the National Science Foundation under grants # CHE-0909743 (MTB) and MCB-0642088 (JES) and the David and Lucile Packard Foundation (JES). The computer time was provided by Texas Advanced Computing Center (LRAC MCA 05S027). We are grateful to Dan Raleigh for supplying us with samples of hIAPP and rIAPP (funded by the National Institutes of Health under grant NIH-GM078114).

References

- (1). Sipe, JD. Amyloid proteins: the β -sheet conformation and disease. Wiley-VCH; Weinheim: 2005.
- (2). Westermark P, Wernstedt C, Wilander E, Hayden DW, O'Brien TD, Johnson KH. P Natl Acad Sci USA. 1987; 84(11):3881–3885.
- (3). Luca S, Yau WM, Leapman R, Tycko R. Biochemistry-Us. 2007; 46(47):13505–13522.
- (4). Lin CY, Gurlo T, Kaye R, Butler AE, Haataja L, Glabe CG, Butler PC. Diabetes. 2007; 56(5): 1324–1332. [PubMed: 17353506]
- (5). Dupuis NF, Wu C, Shea J-E, Bowers MT. J Am Chem Soc. 2009; 131(51):18283–18292. [PubMed: 19950949]

- (6). Bernstein SL, Dupuis NF, Lazo ND, Wytttenbach T, Condron MM, Bitan G, Teplow DB, Shea JE, Ruotolo BT, Robinson CV, Bowers MT. *Nat Chem*. 2009; 1(4):326–331. [PubMed: 20703363]
- (7). Wytttenbach T, Bowers MT. *Top Curr Chem*. 2003; 225:207–232.
- (8). Reddy AS, Wang L, Lin YS, Ling Y, Chopra M, Zanni MT, Skinner JL, De Pablo JJ. *Biophys. J*. 2010; 98(3):443–451. [PubMed: 20141758]
- (9). Reddy AS, Wang L, Singh S, Ling Y, Buchanan L, Zanni MT, Skinner JL, De Pablo JJ. *Biophys. J*. 2010; 99(7):2208–2216. [PubMed: 20923655]
- (10). Kaye R, Bernhagen J, Greenfield N, Sweimeh K, Brunner H, Voelter W, Kapurniotu A. *J Mol Biol*. 1999; 287(4):781–796. [PubMed: 10191146]
- (11). Straub J, Thirumalai D. *Curr Opin Struc Biol*. 2010; 20(2):187–195.
- (12). Abedini A, Raleigh D. *Phys Biol*. 2009; 6(1):015005. [PubMed: 19208933]
- (13). Chiti F, Dobson CM. *Annu Rev Biochem*. 2006; 75:333–366. [PubMed: 16756495]
- (14). Williamson JA, Miranker AD. *Protein Sci*. 2007; 16(1):110–117. [PubMed: 17123962]
- (15). Wiltzius JJW, Sievers SA, Sawaya MR, Cascio D, Popov D, Riekel C, Eisenberg D. *Protein Sci*. 2008; 17(9):1467–1474. [PubMed: 18556473]
- (16). Wiltzius J, Sievers S, Sawaya M, Eisenberg D. *Protein Sci*. 2009; 18(7):1521–1530. [PubMed: 19475663]
- (17). Andreotto E, Yan L, Tatarek-Nossol M, Velkova A, Frank R, Kapurniotu A. *Angew Chem Int Edit*. 2010; 49(17):3081–3085.
- (18). Kapurniotu A, Schmauder A, Tenidis K. *J Mol Biol*. 2002; 315(3):339–350. [PubMed: 11786016]
- (19). Tatarek-Nossol M, Yan L, Schmauder A, Tenidis K, Westermark G, Kapurniotu A. *CHEM BIOL*. 2005; 12(7):797–809. [PubMed: 16039527]
- (20). Yan LM, Tatarek-Nossol M, Velkova A, Kazantzis A, Kapurniotu A. *P Natl Acad Sci USA*. 2006; 103(7):2046–2051.
- (21). Abedini A, Singh G, Raleigh DP. *Anal Biochem*. 2006; 351(2):181–186. [PubMed: 16406209]
- (22). Wytttenbach T, Kemper PR, Bowers MT. *Int J Mass Spectrom*. 2001; 212(1–3):13–23.
- (23). Bleiholder C, Dupuis NF, Wytttenbach T, Bowers MT. *Nat Chem*. 2011; 3(2):172. [PubMed: 21258392]
- (24). Shell MS, Ritterson R, Dill KA. *J Phys Chem B*. 2008; 112(22):6878–6886. [PubMed: 18471007]
- (25). Voelz V, Bowman G, Beauchamp K, Pande V. *J Am Chem Soc*. 2010; 132(5):1526–1528. [PubMed: 20070076]
- (26). Voelz V, Singh V, Wedemeyer W, Lapidus L, Pande V. *J Am Chem Soc*. 2010; 132(13):4702–4709. [PubMed: 20218718]
- (27). Wu C, Shea J-E. *PLoS Comput Biol*. 2010; 6(11):e1000998. [PubMed: 21124953]
- (28). Grabenauer M, Bernstein SL, Lee JC, Wytttenbach T, Dupuis NF, Gray HB, Winkler JR, Bowers MT. *J Phys Chem B*. 2008; 112(35):11147–11154. [PubMed: 18693700]
- (29). Rivera E, Straub J, Thirumalai D. *Biophys. J*. 2009; 96(11):4552–4560. [PubMed: 19486677]
- (30). Goldsbury C, Goldie K, Pellaud J, Seelig J, Frey P, Muller SA, Kistler J, Cooper JS, Aebi U. *J. Struct Biol*. 2000; 130(2–3):352–362. [PubMed: 10940238]

Figure 1.

A) The nano-ESI mass spectrum of hIAPP. B) Rat and Human IAPP +5 dimer ATDs. Each ATD is fit with multiple features using the procedure described in the SI. Each feature is fit with a different color for clarity.

Figure 2.

Representative dimerization trajectories of rIAPP (A) and hIAPP (B) in gas phase and solution. Residues 22–29 are colored in red. The secondary structure is coded by color: coil in silver, β -sheet in yellow, α -helix in purple, π -helix in pink, 3–10 helix in blue and turn in cyan. The N-terminus is indicated by a red ball. Binding energies by MM-GBSA and collision cross sections under dehydration for solution dimers are noted.

Figure 3.
The hIAPP +8 (HH) trajectory shows conversion of coil into β -strand upon dimerization.

Figure 4.

An updated proposed assembly mechanism hIAPP is shown including a β -sheet rich dimer. This addition supports the β -structured aggregates rather than the disordered aggregates as “on” pathway species. Note that further H-bond pattern reorganization in forming the large β -structured aggregates is necessary to produce mature fibrils like the Tycko model.³

Table 1

Collision cross sections for monomers and dimers and the relative contributions of the features in the +5/2 ATDs. The average dimer binding energies from the simulations are included for each peptide.

	Monomer ^{a)} σ ($\text{\AA}^2$)	Dimer σ ($\text{\AA}^2$)	Dimer % Contribution	Ave. Binding E (kcal/mol)
<i>Human IAPP</i>	653	1024	7.4	
	770	1150	74.7	−59.5
		1225	17.9	
<i>Rat IAPP</i>	644	1033	87.8	
		1170	12.2	−38.3

^{a)}_{ref 5}