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Membrane Disruption and Early Events in the Aggregation of the Diabetes Related Peptide IAPP from a Molecular Perspective

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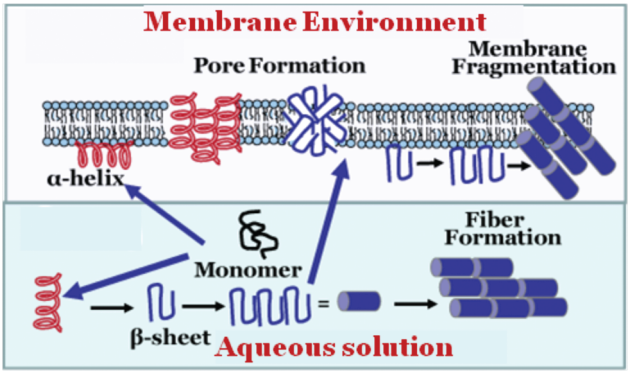

The aggregation of proteins is tightly controlled in living systems, and misfolded proteins are normally removed before aggregation of the misfolded protein can occur. But for reasons not clearly understood, in some individuals this degradation process breaks down, and misfolded proteins accumulate in insoluble protein aggregates (amyloid deposits) over time. Of the many proteins expressed in humans, a small but growing number have been found to form the long, highly ordered β-sheet protein fibers that comprise amyloid deposits. Despite a lack of obvious sequence similarity, the amyloid forms of diverse proteins are strikingly similar, consisting of long, highly ordered insoluble fibers with a characteristic crossed β-sheet pattern. Amyloidogenesis has been the focus of intense basic and clinical research, because a high proportion of amyloidogenic proteins have been linked to common degenerative diseases, including Alzheimer's disease, type II diabetes, and Parkinson's disease.

The apparent link between amyloidogenic proteins and disease was initially attributed to the amyloid form of the protein; however, increasing evidence suggests that the toxicity is due to intermediates generated during the assembly of amyloid fibers. These intermediates have been proposed to attack cells in a variety of ways, such as by generating inflammation, creating reactive oxygen species, and overloading the misfolded protein response pathway. One common, well-studied mechanism is the disruption of the plasma and organelle membranes.

In this Account, we examine the early molecular-level events in the aggregation of the islet amyloid polypeptide (IAPP, also called amylin) and its ensuing disruption of membranes. IAPP is a 37-residue peptide secreted in conjunction with insulin; it is highly amyloidogenic and often found in amyloid deposits in type II diabetics. IAPP aggregates are highly toxic to the β-cells that produce insulin, and thus IAPP is believed to be one of the factors involved in the transition from early to later stages of type II diabetes. Using variants of IAPP that are combinations of toxic or non-toxic and amyloidogenic or nonamyloidogenic forms, we have shown that formation of amyloid fibers is a sufficient but not necessary condition for the disruption of β-cells. Instead, the ability to induce membrane disruption in model membranes appears to be related to the peptide's ability to stabilize curvature in the membrane, which in turn is related to the depth of penetration in the membrane.

Although many similarities exist between IAPP and other amyloidogenic proteins, one important difference appears to be the role of small oligomers in the assembly process of amyloid fibers. In many amyloidogenic proteins, small oligomers form a distinct metastable intermediate that is frequently the most toxic species; however, in IAPP, small oligomers appear to be transient and are rapidly converted to amyloid fibers. Moreover, the aggregation and toxicity of IAPP is controlled by other cofactors present in the secretory granule from which it is released, such as zinc and insulin, in a control mechanism that is somehow unbalanced in type II diabetics. Investigations into this process are likely to give clues to the mysterious origins of type II diabetes at the molecular level.

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Introduction

Islet amyloid polypeptide (IAPP) is a 37 residue amino acid hormone secreted in conjunction with insulin to regulate glucose metabolism (sequence shown in Figure 1).[1] Since its discovery in 1986, IAPP has been intensely investigated for its possible role in the loss of β-cell mass in type II diabetes, an important step in the progression of the disease.[1,2] The reason for this interest is the strong correlation between the formation of IAPP aggregates in β-cells and type II diabetes; IAPP aggregates are found in over 90% of diabetics but only in a minority of nondiabetic subjects.[1] In addition, the loss of β-cell mass appears to be spatially correlated with IAPP protein deposition, because β-cell mass is reduced strongly in islets containing IAPP deposits while neighboring islets lacking the deposits were relatively unaffected. These results suggest that IAPP aggregation is either a possible causative agent or an epiphenomenon associated with type II diabetes. The cytotoxicity of human IAPP (hIAPP) aggregates argues for a causative effect; human IAPP has a strong cytotoxic effect on β-cells, while the nonaggregating rat version of IAPP (rIAPP) has little effect on β-cell survival rates even when massively overexpressed. These findings have led to speculation that a cycle started by the formation of IAPP aggregates may play a central role in the transition from an earlier stage of insulin resistance to the later stages of diabetes mellitus.[2] In the first stage of this cycle, insulin resistance causes a greater demand for insulin. Since the expression of insulin and IAPP are intrinsically linked, a higher production of insulin translates into higher production of IAPP. Due to IAPP's cytotoxicity, higher production of IAPP leads to a greater rate of β-cell apoptosis. The result is a greater rate of production of IAPP in the surviving islets to meet the demand for insulin. IAPP is concentrated in these islets, translating to even greater β-cell death rates. The cycle continues until the pancreas is significantly depleted of β-cells.

IAPP aggregates have a characteristic structure shared by other aggregates that commonly occur in Alzheimer's, Huntington's, Parkinson's, and other degenerative diseases and are therefore grouped together as a class called amyloid.[3] Amyloids are defined by their primarily β-sheet structure and their supramolecular organization into long fibers with the protein backbone orthogonal to the fiber axis. Besides these structural characteristics, amyloids share some common ligand binding partners, some common physical properties, and a common general mechanism of assembly despite the lack of sequence similarity among the individual

Human IAPP: KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY
Rat IAPP: KCNTATCATQRLANFLVRSSNNLGPVLPPTNVGSNTY

FIGURE 1. Amino acid sequences of human and rat islet amyloid polypeptides with nonconserved residues shown in red color. Both peptides are physiologically expressed with an amidated C-terminus and a disulfide bridge from C2 to C7.

proteins.[1] Amyloids form through a nucleation-dependent process leading to characteristic sigmoidal type kinetics; in which amyloid formation is minimal (the lag-phase) until a critical concentration of nuclei is reached, at which point amyloid growth proceeds explosively. The specific kinetics of assembly are typically complicated and involve a variety of on- and off-pathway intermediates and differ among amyloidogenic proteins. In addition, almost all amyloids bind a specific set of cellular components and most (but not all) are toxic in some stage of their formation.

The molecular mechanism behind cytotoxicity is not clear; however, several mechanisms have been proposed. The possibilities include the formation of reactive oxygen species caused by metal complexation, endoplasmic reticulum stress caused by the accumulation of misfolded proteins, an inflammatory response induced by amyloid formation, and membrane disruption among others.[1] In the absence of convincing cellular data, it is difficult to ascertain which of the mechanisms is most prevalent; it is possible that all of the above mechanisms and others play a role in β-cell death. The membrane disruption hypothesis remains the most studied for IAPP and will be the main focus of this Account, with a special focus on the structural changes that occur in IAPP and the membrane upon membrane binding and aggregation as researched in our laboratory.

Mechanism of Membrane Disruption

There are essentially two general theories of membrane disruption by hIAPP and other amyloid proteins, although the mechanisms are not exclusive and may be both operative at once.[4] In the pore theory, amyloid proteins form discrete pores within the membrane, whether of a traditional ion channel type ("barrel stave") or in localized ruptures of the membrane that resemble discrete pores in some respects. In the nonspecific model, the collective action of large oligomeric species reduces the structural integrity of the membrane resulting in either membrane fragmentation or membrane thinning and greatly increased membrane conductance. Controversy over the mechanism is not merely of theoretical importance but has great practical implications for the prevention of membrane damage by amyloidosis. Membrane damage primarily mediated by discrete ion

channels can be alleviated by channel blockers designed to plug the channels formed by toxic amyloid species, while a nonspecific mechanism requires a different approach.

The strongest evidence for a pore-like mechanism of membrane disruption by hIAPP comes from single channel recordings of hIAPP on planar membranes. The addition of hIAPP at low (10 mM KCl) salt concentrations causes step-like fluctuations in membrane conductance that are reflective of the opening and closing of individual channels.[5−7] The channels can be reversibly blockaded by zinc,[6] indicating that the conductance jumps are likely related to a specific pore structure. Additionally, permeabilization by hIAPP is strongly selective for small molecules, arguing against the large-scale disruptions predicted by detergent-like effects.[8]

On the other hand, a detergent-like mechanism with mosaic-like opening and closing of transient defects within the membrane is supported by AFM studies, which show macroscale defects in the lipid bilayer upon prolonged exposure to hIAPP.[9] The time course of membrane disruption is strongly correlated with amyloid fibril formation and can be altered by seeding amyloid formation, implying a direct link between fibril formation and membrane disruption.[10] It is difficult to reconcile this observation with a pore-dominated mechanism, because the dimensions of a fiber or protofiber are too large to form pores of a traditional type.

Our first steps into investigating membrane disruption by hIAPP was to examine the oriented ^{31}P spectra of DMPC membranes after addition of hIAPP$_{20-29}$, using an aluminum oxide matrix for alignment of the membrane.[11] The 20−29 segment of hIAPP has been shown to be critical for the aggregation of the peptide into amyloid fibers; the majority of the sequence differences between nonamyloidogenic variants of IAPP (such as rIAPP) and amyloidogenic versions of IAPP are found within this sequence. Like full-length hIAPP, hIAPP$_{20-29}$ inserts into lipid bilayers, albeit with a lower binding affinity and without the preference for anionic lipids shown by full-length hIAPP.[12]

If hIAPP$_{20-29}$ fragments the bilayer into vesicle-like structures, the rapidly tumbling the vesicles should give rise to a motionally averaged isotropic ^{31}P NMR signal near 0 ppm. This isotropic ^{31}P signal was indeed detected for a narrow concentration range of hIAPP$_{20-29}$, suggesting that a detergent-like mechanism of membrane disruption is involved.[11] The absence of membrane fragmentation below a threshold concentration of peptide is consistent with the requirement for a threshold concentration of membrane defects to be achieved before fragmentation and is predicted by the standard detergent-like model for membrane disruption.[13] However, the disappearance of the isotropic peak at higher concentrations is unusual and suggests that highly cooperative aggregation on the membrane surface can interfere with membrane disruption by hIAPP$_{20-29}$.

Linkage between Amyloidogenicity and Membrane Disruption

The isotropic ^{31}P NMR signals were not detected for the nonamyloidogenic rIAPP$_{20-29}$ sequence and disappeared when an inhibitor of amyloidogenesis, Congo Red, was added.[11] This finding suggests an intimate connection between amyloidogenesis and membrane damage. This connection was noted based on the observation of amyloid fibrils located at the surface of islet cells *in vivo* that are correlated with membrane morphology irregularities.[14] The morphological irregularities are absent when cells are incubated with nonamyloidogenic rIAPP, apparently corresponding to the nontoxic nature of this variant. In addition, inhibition of hIAPP fiber formation generally (but not always) inhibits hIAPP induced membrane damage, leading to the hypothesis that amyloidogenesis and membrane disruption are linked processes. The strongest evidence for this hypothesis is that the kinetics of membrane disruption by hIAPP in mixed DOPC/DOPG membranes largely matches that of amyloid fiber formation, suggesting a link between these two processes.[10]

If membrane disruption is linked to amyloidogenicity, it is reasonable to assume that nonamyloidogenic rIAPP will have little affinity for membranes. However, rIAPP binds to membranes at a similar surface pressure as hIAPP[12] and can disrupt model membranes at high concentrations.[15] Interestingly, disruption by rIAPP is linked to the formation of nonamyloid helical aggregates, suggesting that aggregation on the membrane, but not amyloid formation *per se*, is a prerequisite for membrane disruption.[15] Furthermore, the membrane binding site of hIAPP is largely localized to the N-terminal 19 residues of the peptide, which are similar in the human and nontoxic rat versions of the peptide.[12] Given the involvement of the N-terminus in membrane binding and its apparent low amyloidogenicity, hIAPP$_{1-19}$ provides a reasonable test for the influence of amyloidogenicity on membrane disruption. hIAPP$_{1-19}$ causes severe membrane disruption of anionic model membranes despite not forming fibers when bound to the membrane (hIAPP$_{1-19}$ slowly forms fibers in the absence of the membrane).[16] This finding suggests that amyloid formation may be a sufficient but not necessary condition for membrane disruption. Since hIAPP

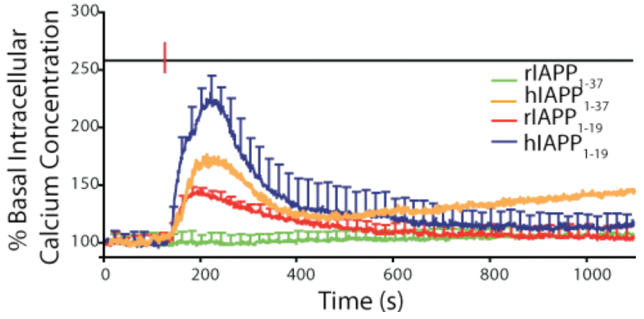

FIGURE 2. Membrane disruption caused by IAPP variants in islet cells. Disruption of the cellular membrane of intact islet cells causes an influx of extracellular calcium detected by the dye Fura-2AM localized in the cell. Human $IAPP_{1-37}$, $hIAPP_{1-19}$, and $rIAPP_{1-19}$ cause a significant amount of influx immediately after addition (red line), while $rIAPP_{1-37}$ does not, largely correlating with their ability to disrupt model membranes. The decrease in the signal after 250 s is most likely related to the escape of Fura-2 from the cell. Adapted from ref 17.

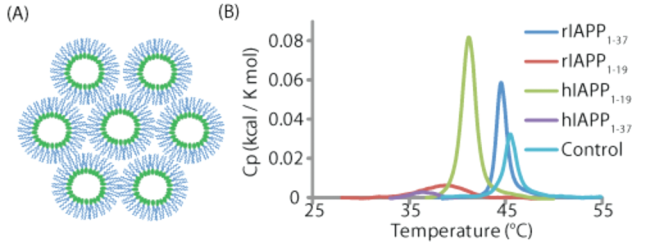

FIGURE 3. Toxic variants of IAPP stabilize negative curvature: (A) cartoon of the inverted hexagonal phase, showing the highly curved surface around the lipid headgroup; (B) DSC chromatograms showing a significant lowering of the phase transition of DiPOPE to the inverted hexagonal phase for the toxic variants of IAPP ($hIAPP_{1-37}$, $hIAPP_{1-19}$, and $rIAPP_{1-19}$) but not for the nontoxic $rIAPP_{1-37}$, suggesting the ability to stabilize curvature of the membrane may be related to IAPP's toxicity. Adapted from ref 19.

differs from rIAPP at only one residue in the 1–19 region, it is useful to compare the abilities of the 1–19 fragments from both peptides to disrupt membranes. While $hIAPP_{1-19}$ caused leakage at low concentrations, $rIAPP_{1-19}$ was only able to disrupt membranes at higher peptide to lipid ratios.[17] This finding was mirrored in tests measuring the calcium influx caused by IAPP into islet cells (see Figure 2), suggesting that it is not an artifact of the model vesicle system used, and $IAPP_{1-19}$ has a similar effect on β-cell membranes.[17] Interestingly, while $hIAPP_{1-19}$ efficiently disrupts anionic liposomes and cellular membranes, it does not have that effect on primarily zwitterionic membranes.

IAPP May Disrupt Membranes by Imposing Excessive Curvature on the Membrane

What force drives the disruption of the membrane by IAPP? The fragmentation of the bilayer by $hIAPP_{20-29}$ suggests that binding of the amphipathic peptide to the membrane creates an unfavorable stress in the membrane, which is relieved by the release of the peptide into peptide–lipid micelles. Several groups have reported distortions of lipid vesicle shape when the vesicle is bound to hIAPP amyloid fibers.[10,18] This finding has led to the theory that the growth of the fiber on the membrane generates an excess of curvature strain on the membrane, possibly due to the inability of a flat membrane surface to accommodate the twist found in β-sheet fibers.[18]

The ability of proteins to induce curvature can be measured by monitoring the transition temperature of DiPOPE from the liquid crystalline (L_α) to the inverted hexagonal state (H_{II}). The inverted hexagonal state is highly curved (see Figure 3A), and it is expected that ligands that stabilize highly curved lipid structures will lower the temperature of this phase transition. Human $IAPP_{1-37}$, $hIAPP_{1-19}$, and $rIAPP_{1-19}$, all of which caused calcium influx in β-cells (see Figure 2), caused a significant decrease in the transition temperature (see Figure 3B), while $rIAPP_{1-37}$, which failed to cause such an increase, had much less of an effect on the transition temperature.[19]

While this correspondence is indirect evidence that curvature strain is directly related to membrane disruption by IAPP, it is complicated somewhat in that the inverted hexagonal phase transition temperature is dependent not only on the spontaneous curvature of the membrane but also on the bilayer elasticity, as a more flexible membrane favors curvature in both directions.[20] To more directly probe the role of curvature in IAPP membrane binding, we examined the binding of IAPP to bicelles at the atomic level. Bicelles are a membrane mimetic system composed of mixtures of short (DHPC) and long (DMPC and DMPG) chain lipids. At certain ratios of short to long chain lipids, they form perforated lamellar bilayers in which the curved perforations are lined with the short chain DHPC lipid molecules (see Figure 4C). Because the short and long chain lipids localize into the regions of high and low curvature, respectively, bicelles can be used to test the binding preferences of peptides for curved vs flat regions.

For the two IAPP variants that could be successfully incorporated into the bicelle, solid-state NMR shows that the toxic $rIAPP_{1-19}$ fragment strongly associates with DHPC in the highly curved perforation, while nontoxic $rIAPP_{1-37}$ is mostly associated with DMPC and DMPG in the flat lamellar region (Figure 4), in agreement with the DSC results.[19] Preliminary results also indicate that membranes incorporating POPE, a lipid favoring negative curvature, also favor a

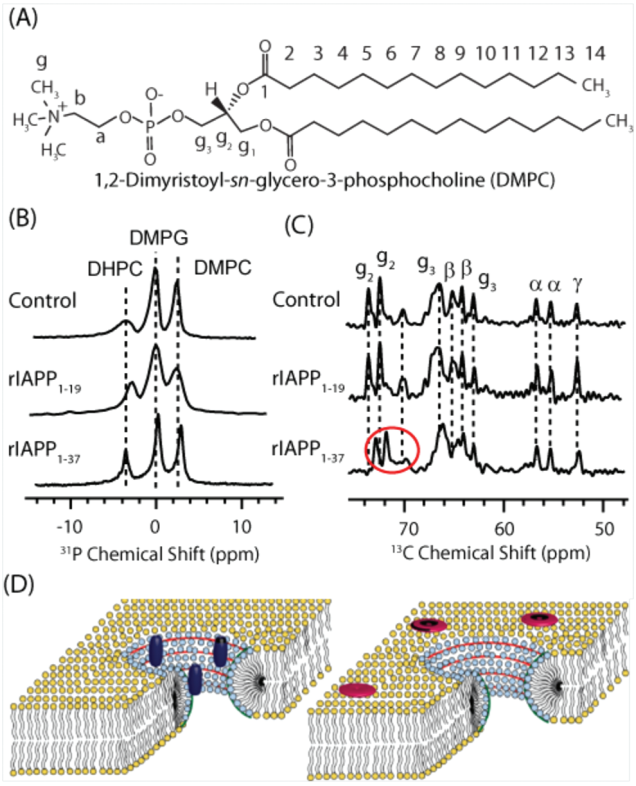

FIGURE 4. Toxic variants of IAPP: (A) Structure of DMPC; (B) ^{31}P NMR spectra showing binding of toxic rIAPP$_{1-19}$ to DHPC in bicelles; (C) ^{13}C NMR spectra showing binding of nontoxic rIAPP$_{1-37}$ to DMPC and DMPG; (D) cartoons of bicelles showing rIAPP$_{1-19}$ (dark blue) localized to DHPC in the curved perforation (light blue) and rIAPP$_{1-37}$ (red) localized to DMPC and DMPG in the flat lamellar region (yellow). Adapted from ref 19.

detergent-like membrane disruption by hIAPP. Taken together these results indicate that the excess curvature caused by membrane binding of toxic IAPP variants destabilizes the membrane.

IAPP Does Not Form a Substantial Population of the Small Oligomers Typical of Other Amyloidogenic Proteins

Substantial controversy exists about the nature of the membrane disruptive species. Amyloid proteins typically form a variety of amyloid-like and nonamyloid oligomeric intermediates along the aggregation pathway. Some of these species, such as the mature amyloid fiber, are known to have a low toxicity, while others are believed to be strongly toxic. Attention has focused on a particular set of small oligomers of approximately 5 nm in size due to the apparently high toxicity displayed by these species (for most amyloid proteins) and their pore-like morphology. Small oligomers of this type are typically formed in solution after a lag period but before amyloid fibers are formed. Although oligomers of this type have been heavily studied for Aβ_{1-40} and α-synuclein (among other amyloidogenic proteins),[21] data for oligomers of this specific type has been relatively scant for IAPP.[22]

Since the presence of membranes can strongly affect the pathway of aggregation, it is appropriate to ask whether the membrane disruptive species form on the membrane or in solution before inserting. To address this question, we tracked the size of particles generated during IAPP amyloidogenesis by PFG-NMR spectroscopy. In the PFG ^{1}H proton spectra, most of the resonances decay rapidly with increasing field gradient consistent with rapid diffusion, as would be expected for monomeric hIAPP.[23] However, a broad peak near 0 ppm corresponds to a large, slowly tumbling oligomeric species with a radius around 100 nm corresponding to approximately 1 million monomers.[23] Similar peaks have also been detected for other amyloidogenic proteins including Aβ_{1-40} and calcitonin,[24] and the peak has been used as a diagnostic for oligomeric species in coexistence with the monomer. Interestingly the peak is absent for PAP$_{248-286}$, an amyloidogenic protein with a long lag-time for amyloid formation.[25] The experiment was repeated over 10 h without changes in the spectra that would be reflective of aggregation.[23] This result is somewhat surprising in the context of nucleation-dependent aggregation theory, which predicts that once a nucleus is formed aggregation should occur rapidly. The absence of a substantial degree of aggregation in these samples argues that the large aggregates detected by PFG are not likely to be nuclei for further aggregation.

Although the PFG experiment is not sensitive enough in that size range to determine the exact size of the large oligomers, a lower limit of approximately 50 nm can be definitively established.[23] Surprisingly, oligomers of less than 50 nm in radius could not be detected, suggesting that they do not accumulate to a significant amount during aggregation.[23] These findings are consistent with a study that showed by sedimentation velocity experiments under a different set of conditions that oligomers composed of <100 monomers form <1% of the total population of hIAPP before fibrillation,[26] in marked contrast to Aβ, α-synuclein, and others in which small oligomers play a crucial role in aggregation.[21] The aggregation of IAPP in solution appears to be largely an all or nothing process; once small oligomers are formed they appear to be quickly converted into large protofibrillar aggregates, and therefore a significant population of small oligomers is never accumulated (see Figure 5). It

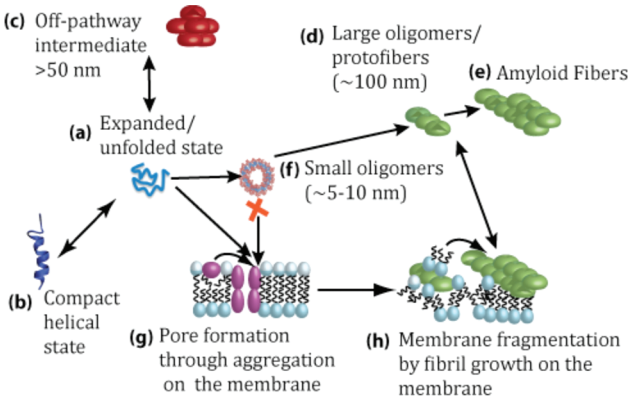

FIGURE 5. Hypothetical mechanism for membrane disruption by IAPP. In solution, IAPP initially exists in equilibrium between expanded (a) and compact (b) monomer states as well as disordered large aggregates (c). In solution, IAPP aggregates rapidly to form amyloid fibers (d, e), likely through a helical intermediate (b). Small oligomers (f) are rapidly converted to large oligomers (d). Alternatively, the monomeric IAPP can bind to the membrane and aggregate to form pores (g). Continued aggregation results in amyloid formation and membrane fragmentation (h). Drawing is not to scale. Adapted from ref 23.

is a reminder that, despite their many similarities, substantial differences exist among amyloidogenic proteins.

Structures of Membrane-Bound IAPP

Aggregation of IAPP on the membrane proceeds through a different pathway than in solution. Both hIAPP and rIAPP initially bind to the membrane in a largely helical state before hIAPP aggregates on the surface of the membrane to form amyloid. To investigate the initial steps of this process, we, along with others, have solved the structures of IAPP variants of varying degrees of toxicity and amyloidogenicity in detergent micelles, where the low number of peptide molecules per micelle keeps the peptide in a monomeric conformation. The structures of $hIAPP_{1-19}$ and $rIAPP_{1-19}$ are very similar in DPC micelles (see Figure 6),[27] suggesting that a structural difference is not responsible for the difference in the behavior of the two peptides. However, while $hIAPP_{1-19}$ and $rIAPP_{1-19}$ adopt similar α-helical folds, they differ in their orientation of binding to the membrane. Rat $IAPP_{1-19}$ binds near the surface of the membrane while human $IAPP_{1-19}$ penetrates deeper into the hydrophobic core.[17,27] This change is entirely dependent on the charge of H18 as $hIAPP_{1-19}$ binds near the surface similar to $rIAPP_{1-19}$ at acidic pH.[17,27] This change in orientation is correlated with a change in the potential for membrane disruption, as $hIAPP_{1-19}$ at pH 6 causes a low level of membrane disruption almost identical to $rIAPP_{1-19}$.[17]

The similarities between the membrane-bound structures of $hIAPP_{1-19}$ and $rIAPP_{1-19}$ are preserved in full-length hIAPP

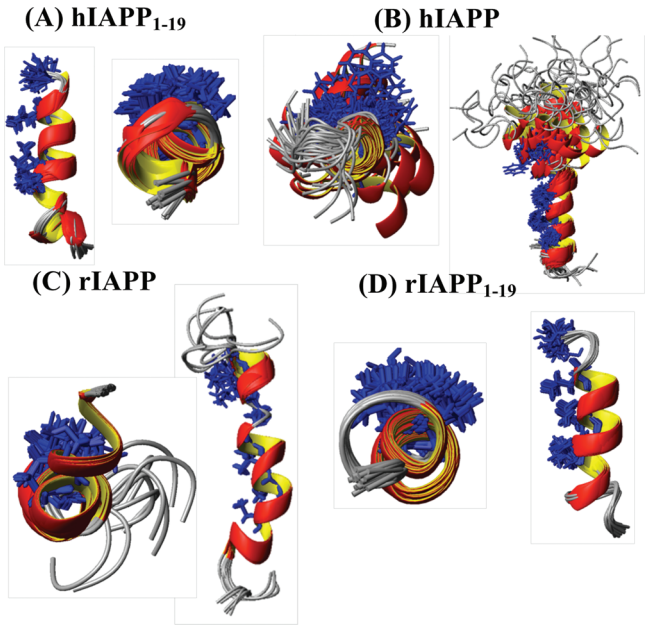

FIGURE 6. NMR structures of $hIAPP_{1-19}$ (A), hIAPP (B), rIAPP (C) and $rIAPP_{1-19}$ (D). The hydrophobic residues that have been implicated in coiled-coil interactions and stabilize the IAPP oligomer are colored in blue. Adapted from ref 29.

and rIAPP, as the N-terminus adopts a similar α-helical fold in both structures in detergent micelles (see Figure 6).[28−30] Instead, the structural differences between hIAPP and rIAPP largely lie at the C-terminal end of the peptide. In rIAPP, the C-terminus is almost completely disordered.[29] Human IAPP, on the other hand, adopts a helix−kink−helix structure in micelles in which the C-terminus is largely helical. The degree of helicity in the C-terminus is higher in the amidated form of hIAPP compared with the free acid form of hIAPP, correlating with the greater amyloidogenicity of the former.[28] Another correspondence between structure and membrane activity is the degree of exposure of the hydrophobic face of the N-terminal helix, which is largely occluded by the N-terminal disulfide ring (Cys2−Cys7) in nontoxic variants of IAPP but is exposed in toxic ones.[29]

Structures of Membrane-Bound IAPP Share Many Similarities with Those of the Putative Helical Intermediates of hIAPP in Solution

It is useful to compare the membrane bound structures of IAPP to the structural propensities of the peptide in solution. In solution, both hIAPP and rIAPP are predominantly unstructured. However, the absence of a significant degree of secondary structure does not mean that the peptide is completely unfolded, because a variety of techniques have shown that IAPP adopts a more compact structure than

would be predicted for a random polymer, with hIAPP being substantially more compact than rIAPP.[23,31,32]

The compaction of the peptide and possible secondary structure formation is important because a partially folded conformation is intrinsically more amyloidogenic than a completely folded or unfolded conformation. In a partially folded conformation, aggregation prone sequences can be favorably positioned to interact with each other, which is difficult to achieve in completely unfolded and folded states.[33] In particular, there is evidence for a mechanistic role for helical intermediates in amyloid aggregation.[3] Helical intermediates have not been directly observed as major species along the aggregation pathway for hIAPP, as they have been for the Alzheimer's amyloid Aβ_{1-40},[34] possibly because the highly transitory nature of the species prevents a stable population from being formed.

Solution NMR suggests that the monomeric states of both hIAPP and rIAPP transiently sample helical states.[35,36] Unlike CD, solution NMR directly reports on the monomeric state because the long rotational correlation times of large oligomers broadens their signal beyond detection. The transient nature of the helical state is reflected in chemical shifts but an absence of the medium range (i,i+3) NOEs indicates a lack of stable secondary structure.[35,36] The helical state of hIAPP is apparently a low-lying excited state conformer, with the helical state lower in energy in hIAPP than rIAPP. The importance of a low percentage of helical conformers of IAPP is reflected by the influence of the helix-inducing solvent HFIP on aggregation. A low percentage of HFIP (1%) has a strong catalytic effect on aggregation, while higher percentages trap the protein in a helical monomeric state.

Some specific details of the membrane-associated IAPP structures appear to be preserved in the helical state as well. A comparison of chemical shifts indicates that the helical propensity is greatest in the N-terminus (residues 8−18) and decreases near the C-terminus for both hIAPP and rIAPP.[35,36] Similar to what is observed in membranes, this difference between the two termini is much more pronounced in rIAPP than in hIAPP. The relative orientations of the disulfide ring may be preserved in solution as well. In hIAPP, the disulfide ring of monomeric hIAPP is positioned toward the helical face in models of both the solution and membrane structures.[36] It is interesting to note that corresponding NOEs were not found in the NOESY spectra of rIAPP, suggesting that the disulfide ring in the rIAPP structure is not as constrained in both solution and the membrane as it is in hIAPP.[35,36]

Influence of Secretory Granule Components on Human IAPP Aggregation and Toxicity Pathways. The strong amyloidogenic propensity and membrane disruptive characteristics of hIAPP suggest that there must be factors for attenuating these effects *in vivo*. IAPP is stored at millimolar concentrations and would be expected to aggregate in minutes under these conditions, yet the lifetime of the secretory granule in which it is stored is on the order of hours.[37] Several factors can explain this discrepancy including pH (IAPP amyloid formation is strongly disfavored at low pH) and other components of the secretory granule.[38] In particular, IAPP is stored with high (millimolar) concentrations of zinc and insulin, both of which impact amyloidogenesis as explained below.

Insulin and IAPP are intimately linked biologically. IAPP works in tandem with insulin to regulate glucose levels at the molecular level, and for this reason its production is tightly coordinated with insulin. Insulin and IAPP are both initially produced as immature hormones in the secretory granule that are cleaved by the same enzyme to produce the active form and are released nearly simultaneously from the secretory granule. Insulin has a strong inhibitory effect on the fibrillization of hIAPP at substoichiometric levels by preventing fiber elongation by blocking the reactive ends of the fiber.[39]

The affect of insulin on hIAPP disruption of cellular membranes has yet to be determined; however, model membranes provide evidence that insulin has a complicated effect on membrane-mediated aggregation and membrane disruption by hIAPP. While insulin is a strong inhibitor of IAPP fiber formation in the absence of membranes,[39] its effect on membrane- mediated aggregation is greatly attenuated.[40] Insulin's effect on membrane disruption is similarly complex. Membrane disruption by hIAPP in the absence of insulin is two-phased, with a first phase occurring in the lag time of fiber formation and a second phase strongly correlated with fibrillization.[10,41] Insulin completely blocks the second fiber-dependent phase of membrane disruption but has essentially no effect on the first.[41] In addition, by blocking the formation of the inactive fiber form, insulin maintains hIAPP in the membrane disruptive form for a longer period of time.[41] Insulin's ability to block the time-dependent decrease in hIAPP permeabilization activity and only the secondary membrane disruption phase indicates that the interaction is relatively complex and makes it difficult to extrapolate the results from model systems to *in vivo* situations.

Zinc is another factor found in millimolar concentrations in the secretory granule and has been shown to decrease the aggregation propensity of human IAPP *in vitro*.[38] Zinc binds unaggregated hIAPP at micromolar concentrations at a 1:6 ratio despite hIAPP lacking a traditional zinc-binding motif

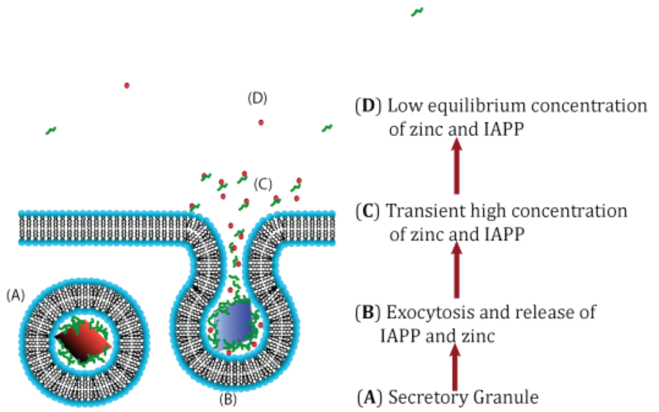

FIGURE 7. A cartoon schematic of how zinc may influence IAPP aggregation and toxicity. Adapted from ref 38.

and having only one residue, H18, that should bind zinc tightly.[42] By contrast, the amyloid form of hIAPP has low affinity for zinc, a difference that should disfavor amyloid formation in the presence of zinc.[42] This difference is reflected in strongly decreased elongation rates that favor the formation of smaller oligomers in the presence of zinc. Physiologically, zinc may chaperone hIAPP in the dangerous phase following secretion where hIAPP local concentrations are high and pH values are neutral, because zinc is released slowly in comparison to IAPP from the secretory granule due to the slow dissolution of zinc binding insulin hexamers (see Figure 7).[38]

Integrating Biophysics and Cellular Biochemistry: The Future of IAPP and Amyloid Research. We have shown that by isolating specific steps in the aggregation process a reductionist approach can yield useful information on the extremely complicated behavior of IAPP at the molecular level. However, the reductionist approach used here can run into complications when confronted with the complexity of the cell's biochemistry. As an example of the complications that can arise, Figure 2 shows the influx of calcium caused by the exogenous addition of $IAPP_{1-19}$ to islet cells,[17] suggesting that $IAPP_{1-19}$ is disrupting the plasma membrane in agreement with results obtained in anionic model membranes.[16,17] However, $hIAPP_{1-19}$ does not cause membrane disruption in mixed model membranes with a lower anionic content intended to more accurately mimic cell surfaces.[43] This discrepancy suggests that either (a) $IAPP_{1-19}$ does not directly induce membrane disruption in β-cells but instead activates an unidentified endogenous calcium channel or (b) $IAPP_{1-19}$ does induce membrane disruption in cells and anionic membranes but does not in simple mixed zwitterionic/anionic model membrane systems. Some evidence for the latter has emerged with studies showing the importance of lipid rafts and gangliosides for the membrane interaction of IAPP.[44,45] Further work on IAPP in cells and more realistic model systems, in particular using advanced tools like in cell NMR and advanced imaging techniques,[46,47] is likely to yield the answer to this and other questions. The integration of biophysical and *in vivo* techniques remains an outstanding frontier in IAPP research and will likely be a fruitful area of research in the future.

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BIOGRAPHICAL INFORMATION

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LIPID ABBREVIATIONS:

DMPC 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine
DHPC 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine
DMPG 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol)
DPC *n*-dodecylphosphocholine
DiDOPE 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine

OTHER ABBREVIATIONS:

PFG pulsed field gradient
HFIP hexafluoroisopropanol
DSC differential scanning calorimetry

FOOTNOTES

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