

Causative factors for formation of toxic islet amyloid polypeptide oligomer in type 2 diabetes mellitus

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Abstract: Human islet amyloid polypeptide (h-IAPP) is a peptide hormone that is synthesized and cosecreted with insulin from insulin-secreting pancreatic β -cells. Recently, h-IAPP was proposed to be the main component responsible for the cytotoxic pancreatic amyloid deposits in patients with type 2 diabetes mellitus (T2DM). Since the causative factors of IAPP (or amylin) oligomer aggregation are not fully understood, this review will discuss the various forms of h-IAPP aggregation. Not all forms of IAPP aggregates trigger the destruction of β -cell function and loss of β -cell mass; however, toxic oligomers do trigger these events. Once these toxic oligomers form under abnormal metabolic conditions in T2DM, they can lead to cell disruption by inducing cell membrane destabilization. In this review, the various factors that have been shown to induce toxic IAPP oligomer formation will be presented, as well as the potential mechanism of oligomer and fibril formation from pro-IAPPs. Initially, pro-IAPPs undergo enzymatic reactions to produce the IAPP monomers, which can then develop into oligomers and fibrils. By this mechanism, toxic oligomers could be generated by diverse pathway components. Thus, the interconnections between factors that influence amyloid aggregation (eg, absence of PC2 enzyme, deamidation, reduction of disulfide bonds, environmental factors in the cell, genetic mutations, copper metal ions, and heparin) will be presented. Hence, this review will aid in understanding the fundamental causative factors contributing to IAPP oligomer formation and support studies for investigating novel T2DM therapeutic approaches, such as the development of inhibitory agents for preventing oligomerization at the early stages of diabetic pathology.

Keywords: amyloid aggregation, causative factor, IAPP, islet amyloid polypeptide, toxic oligomer, T2DM, type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus (T2DM) is caused by the reduction of β -cell mass and accumulation of islet amyloid polypeptide (IAPP, or amylin) in the pancreatic islets, leading to insulin resistance.¹ Islet amyloid deposition could affect and occur in up to 80% of diabetic patients.² IAPP, a 4 kDa peptide hormone composed of 37 amino acids (Figure 1), is synthesized and cosecreted with insulin from pancreatic β -cells. As a glucomodulatory hormone, IAPP is responsible for slowing down gastric emptying and regulating glucose levels. It inhibits the effects of insulin as well as arginine-stimulated glucagon release by α -cells.^{3,4} In addition, IAPP is responsible for other physiological functions such as sleep and appetite regulation via the gut-brain axis, and it functions as a growth factor to maintain β -cell mass.⁵ The incidence of T2DM is considered to increase with age because of the reduced proliferative capacity and increased apoptosis rate of β -cells, which can also lead to higher rates of IAPP aggregation.⁶ The IAPP monomer appears to show normal biological activity

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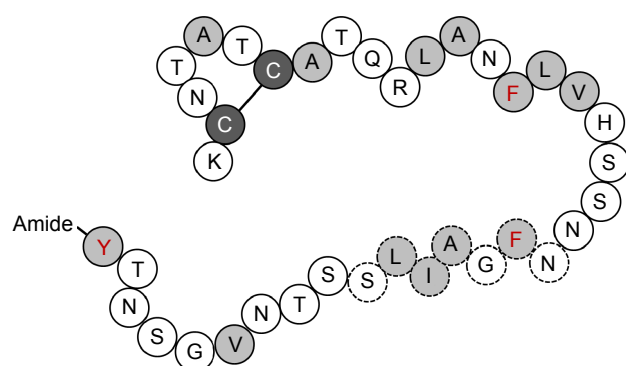


Figure 1 Schematic diagram of the primary sequence of IAPP.

Notes: Black circles indicate the cysteine amino acid C2–C7 disulfide bond. Gray circles indicate amino acid sequences for the hydrophobic regions, with the three aromatic amino acids (F15, F23, and Y37) shown in red. Dashed-circled amino acids are those responsible for amyloid formation.

Abbreviation: IAPP, islet amyloid polypeptide.

in healthy β -cells. Moreover, oligomers can regularly form and degrade in a normal cell system. However, unlike normal monomers and oligomers, toxic oligomers can cause membrane-perforating toxicity, which is a decisive factor that is indicative of the cell cytotoxicity in diabetes models. IAPP oligomers can be generated via the amyloid fibril off-pathway in the fibril assembly process. However, the exact structure and role of toxic IAPP oligomers have been difficult to establish thus far. The toxic amyloid- β oligomer has a cross- β structure,⁷ and it is considered that the IAPP oligomer that is ultimately cytotoxic to pancreatic β -cells would share similar structural elements.⁸ On the bright side, rifampicin has been reported to prevent the formation of human-IAPP (h-IAPP) fibrils. However, rifampicin did not seem to be able to control the production of toxic h-IAPP oligomers from the off-pathway and could therefore not prevent its cytotoxic effects.⁹ Currently, the causative factors of the conversion of soluble IAPP into insoluble fibrils are unknown. However, it is known that these factors lead to the destruction of β -cell function to the point where they cannot properly respond to insulin.²

The final stage of cell disruption involves membrane destabilization and disruption. Hence, it is important to understand how amyloidogenic peptides cause cell membrane disruption. The “carpet” and “barrel-stave” models are used to represent the mechanisms of cell membrane disruption. In the carpet model, monomers interrupt the membrane through nonspecific binding, by dissolving into the membrane in a detergent-like manner and exceeding the concentration threshold value of the membrane.¹⁰ In the barrel-stave model, after assembling on the membrane surface, peptides insert themselves into the membrane, forming channels or pores.¹¹ Thus, membrane disruption from barrier deformation

is observed in these two models. In particular, both models reveal how high cytotoxicity can result from cell disruption induced by a toxic IAPP oligomer. Further in-depth studies with these models are needed to characterize the pore generation induced by h-IAPP, identify the cytotoxic mechanisms, screen for the causative factors that initiate toxic oligomer formation, and ultimately identify how these causative factors can be inhibited.

Factors that cause oligomer aggregation

Environmental condition of the cell

Amyloidogenic protein aggregation can be induced by several factors within the cellular environment. IAPP or amylin, as a potential amyloidogenic protein, was shown to interact with the lipid membrane in one of the pathogenic cytomembrane pathways.¹² IAPP fibrillization was found to play a role in the cytotoxicity in vitro by disrupting the negatively charged phospholipid membrane.¹³ In the same context, it was suggested that a high-fat diet could increase the lipid level, thereby increasing the possibility of oligomer penetration or membrane disruption through increased oligomer–lipid binding. As another possibility, the hydrophobic region of IAPP could be exposed in protein misfolding, which would implicate it in the fibrillation process.¹⁴ Changes in pH were reported to affect the conformation and aggregation patterns of IAPP, where the alkaline pH of 8.8 was suggested to be more favorable for aggregate formation than the acidic condition of pH 4.0.^{15,16} Likewise, chemical modification, salt concentrations, natural ligands, racemization, isomerization, deamidation, oxidation, lipid oxidation, and glycation are factors that could affect the stability of IAPP.²

IAPP sequence

Genetic mutations of IAPP

According to a previous study, a naturally occurring missense mutation (S20G) of the human *IAPP* gene is associated with early onset or more severe types of T2DM.¹⁷ The S20G mutation results in increased hydrophobicity and amyloidogenic characteristics of IAPP, which could increase its fibrillogenic potential.¹⁸ In addition, S20G IAPP showed a nearly twofold increased rate of the formation of amyloid fibrils, resulting in more than threefold greater aggregation and consequently higher cytotoxicity than the wild-type protein.¹⁸ F15, an aromatic residue in IAPP that conserves its hydrophobicity, has been suggested to play a significant role in the amyloid biosynthesis pathway.¹⁹ In an in silico study, an F15L mutation generated from a single-point mutation, which altered the α -helix and β -sheet propensities of the protein, resulted

in rapid amyloid formation.²⁰ In another in silico study, the Y37L and F23L IAPP mutations resulted in decreased rates of amyloid fibril formation.²⁰ The replacement of tyrosine (Y) with leucine (L) resulted in greater flexibility of the C-terminus, with loss of the steric zipper interactions, and the F23L mutation, in which phenylalanine (F) was replaced with leucine (L), showed slower amyloid formation.²¹ Overall, the rate of aggregate formation was reduced significantly in these two mutations compared to other mutants and the wild-type protein.²¹ Interestingly, the single point mutants G24P and I26P also showed potential for inhibiting amyloid aggregation.^{22–24} A list of the genetic mutations of IAPP is shown in Figure 2. Mutations by displacement of one amino acid residue could greatly affect the rate and property of amyloid formation. These aforementioned studies described suggest a close correlation for balancing the reversal or the rate of fibril formation in reducing or increasing cytotoxicity.

Comparison of IAPP sequences among species and establishment of transgenic rodent models

IAPP residues at the N-terminus and C-terminus are conserved in mammals, whereas the amyloidogenic core region is species specific (Figure 3). Sequence homology has been found between primate and human IAPPs, and these peptides form islet amyloids that lead to the development of T2DM. In contrast, the presence of three proline residues in rat and mouse IAPPs renders the protein water soluble, thereby giving it nonamyloidogenic properties by providing a water-soluble environment; as a consequence, T2DM does not typically occur in rodents.^{25,26} Since the cytotoxicity of IAPP seems to be dependent on its propensity for oligomer formation, the prevention of fibril formation by the proline residues of IAPP 20–29 in the rat and mouse is very likely to be the cause of their reduced IAPP cytotoxicity, which is hardly detected in these species.²

Since pancreatic β -cell apoptosis and the induction of diabetes have been confirmed in h-IAPP transgenic rat and mouse models, it is a viable hypothesis that amyloids

are associated with the induction and progression of diabetes.²⁷ Indeed, an h-IAPP transgenic model formed toxic IAPP oligomers that eventually generated endoplasmic reticulum stress-induced apoptosis and T2DM characteristics such as hyperglycemia, impaired insulin secretion, insulin resistance, and hyperglucagonemia.^{28,29}

IAPP processing

IAPP is encoded by an 89-residue coding sequence that produces the 67-amino acid pro-IAPP residue. Mature IAPP is then generated by the following process. First, pro-IAPP is formed after cleavage of the 22-amino acid signal peptide before it undergoes proteolysis and posttranslational modification. Sixteen amino acids at the C-terminus are removed by proprotein convertase 1/3 (PC1/3), followed by truncation of eleven amino acids at the N-terminus by proprotein convertase 2 (PC2).³⁰ Then, the C-terminus carboxypeptidase E removes the terminal lysine and arginine residues,³¹ and peptidylglycine α -amidating monooxygenase (PAM) adds an amine group to the C-terminus. Finally, a disulfide bond is generated between the cysteine 2 and cysteine 7 residues to produce the mature IAPP protein made up of 37 amino acids (IAPP sequence: KCNTATCATQRLANFLVHSSN NFGAILSSTNVGSNTY).³² Both an intact intramolecular disulfide bond and C-terminal amidation are required for IAPP to have normal biological activity. Therefore, the factors that prevent the formation of the disulfide bond and/or cause deamidation, as well as the absence of PC2, contribute to the aggregation of IAPP.

Absence of PC2

Impaired processing of pro-IAPP can affect amyloid formation in T2DM. The first enzyme in the pro-IAPP processing pathway is PC1/3, which plays a crucial role in removing the amino acids of the C-terminus. In the absence of PC1/3, PC2 takes over at both the N-terminal and C-terminal cleavage sites as part of normal posttranslational modification.³³ In contrast, because PC2 usually only functions after PC1/3 in the

	1	10	20	30	37																																			
Wild-type	K	C	N	T	A	T	C	A	T	Q	R	L	A	N	F	L	V	H	S	S	N	N	F	G	A	I	L	S	S	T	N	V	G	S	N	T	Y			
S20G	G	→ Rapid amyloid formation
F15L	L	→ Slow amyloid formation
Y37L	L	→ Inhibit amyloid formation	
F23L	
G24P	P	
I26P	P	

Figure 2 List of genetic mutations of IAPP determined from in silico studies, and naturally occurring (eg, S20G) mutations.

Notes: Sequence alignment between mutants and wild-type IAPP revealed the effect of single-point mutations (S20G, F15L, Y37L, F23L, G24P, and I26P). The S20G and F15L mutations result in higher rates of amyloid formation and amyloidogenesis. In contrast, the Y37L, F23L, G24P, and I26P mutations result in a reduction in the amyloidogenic property. Dots indicate identical residues, and blue letters indicate aromatic regions (F15, F23, and Y37).

Abbreviation: IAPP, islet amyloid polypeptide.

	1	10																20										30	37									
Human	K	C	N	T	A	T	C	A	T	Q	R	L	A	N	F	L	V	H	S	S	N	N	F	G	A	I	L	S	S	T	N	V	G	S	N	T	Y	
Rat	R	.	.	.	L	.	P	V	.	P	P
Mouse	R	.	.	.	L	.	P	V	.	P	P
Cat	I	R	.	.	.	L	P
Monkey	R	T
Dog	R	T	.	.	.	L	P

Figure 3 Alignment of IAPP amino acid sequences from different species.

Notes: Amino acid sequences of the N-terminus and C-terminus are conserved, but region 20–29 is species specific. Amino acid alignment of IAPP identified slight differences between human, rat, mouse, cat, monkey, and dog sequences. Dots represent the conserved amino acids compared with human IAPP. Red letters denote the amyloidogenic regions.

Abbreviation: IAPP, islet amyloid polypeptide.

pro-IAPP processing pathway, its absence creates a block at the level of the pro-IAPP convertase intermediate (NH₂-terminally extended pro-IAPP). This intermediate acts as a critical initiating factor to induce intracellular and extracellular amyloid formation, and finally induces β -cell death.^{34,35}

Deamidation

Deamidation is an important factor in amyloid formation and affects protein structure, folding, stability, and aggregation.^{36,37} Deamidation of the side chain at Asn and Gln is a spontaneous reaction and a nonenzymatic posttranslational modification.³⁸ There are six Asn residues and one Gln residue in the IAPP sequence that can be subject to deamidation effects. In addition, for IAPP to properly perform its biological function, it should have some form of C-terminal amidation. Thus, once the C-terminal amide group is exposed to deamidation, the protein structure will be affected. Deamidation may influence the protein structure and stability by adding a negative charge, and thus change the amyloid formation kinetics such that unmodified IAPP (deamidated form) accelerates amyloid formation, creating the seeds for aggregation.³⁹ In other words, if an impurity is produced by deamidation, it induces amyloid formation, thereby affecting the purified peptide's secondary structure and aggregation behavior.⁴⁰

Reduction of disulfide bond formation

In one study, the intramolecular disulfide bond of C2–C7 was shown to be the critical site for the formation of IAPP fibrils; however, the role of the intrinsic disulfide bond in the IAPP monomer structure as well as the specific mechanism of aggregation remain unclear.⁴¹ The N-loop that is generated by the disulfide bond (C2–C7) of IAPP is highly conserved in the rigid structure.^{42,43} Although the N-loop is not involved in direct molecular interactions, it nevertheless affects the IAPP aggregation kinetics indirectly, since N-loop removal was found to change the mass/length distribution and kinetics

of the h-IAPP fiber.^{44,45} The absence of the disulfide bond decreases the extent of the helix at the N-terminal region, but favors random coiling and β -sheet formation, which can affect the protein kinetics such as reducing the rate of IAPP aggregation by secondary nucleation.⁴¹ In fact, early oligomerization is connected to helix formation. The S–S bridge, which stabilizes the N-terminal helix of h-IAPP, collapses the protein into an amorphous aggregate with less β -sheets. These factors were shown to facilitate the initialization of h-IAPP aggregation, encoded at the monomeric level.^{41,46,47} Likewise, the disulfide bond determines the morphology of the fibril (eg, stabilizing the amyloid fibril in the folded state), and also plays a role in limitation by topologically restraining the polypeptide during amyloid fibril arrangement.⁴⁸ Therefore, the disulfide bond may ultimately reduce the toxicity of the amyloid fibril.⁴⁹

Copper(II) ion effect

Copper ions have been associated with many diseases.⁵⁰ Furthermore, calcium and zinc ions were shown to be involved in maintaining the native structure of IAPP, by inhibiting amyloid aggregation.^{51,52} Although the precise impact of copper ions on IAPP is still a matter of debate,⁵³ copper metabolism is thought to be associated with the pathological mechanism of amyloidosis in diabetes.⁵⁴ In one previous study, copper inhibited IAPP fibrillization,⁵² whereas in another study, copper contributed to h-IAPP aggregation and cytotoxic oligomer formation.⁵⁵ Copper can possibly interact with the C2, C7, H18, and Y37 regions in the IAPP sequence.⁵⁵ These copper–IAPP complexes have metalloprotein complex structures with low aggregation potential⁵⁶ and may instead produce granular oligomers, which are the main cause of increased copper-mediated h-IAPP cytotoxicity. Granular oligomers are expected to show the characteristics of toxic oligomers, inducing membrane destabilization and, ultimately, cell apoptosis.^{55,57,58} In addition, copper-promoted reactive oxygen

species (ROS) generation, such as H_2O_2 , and mitochondrial disruption affect the degeneration of islet cells, leading to the production of caspase-3 and poly (ADP-ribose) polymerase (PARP), which in turn promote apoptosis.^{59–61} Furthermore, noncomplexed copper ions (ie, free copper ions) also show toxicity and can easily turn into reactive copper ions that generate cell-damaging ROS.⁵⁶ Therefore, treatment with a copper-chelating agent has a beneficial effect against the pathogenesis of T2DM and diabetes complications, by lowering the copper levels, and hence the ROS levels.⁶²

Heparin

The heparin-binding property of amyloid β -protein is dependent on the state of aggregation of this amyloidogenic protein since heparin does not interact with nonfibrillar forms.⁶³ A similar heparin study was conducted with IAPP. Heparin enhanced IAPP fibrillization, and the binding property was found to be dependent on the length of the heparin polysaccharide

fragment and the aggregation state. The negatively charged heparin helix essentially binds with the positively charged N-terminal cross β -sheet of the IAPP fibril.⁶⁴ Short heparin fragments of 2–8 saccharides showed a better protective effect against cell cytotoxicity than fragments of 20 saccharide or longer, which exhibited no protective function.⁶⁵

Conclusion

A wide range of diabetes-specific biomarkers has been identified. However, it appears that it is the unusual protein properties induced by such factors and the effect of protein aggregation on β -cell apoptosis that ultimately induce the development of diabetes. In particular, IAPP oligomers generated from IAPP monomers lead to β -cell-specific toxicity, ultimately causing diabetes. This review presented the known factors that appear to trigger IAPP aggregation. Figure 4 demonstrates how pro-IAPP decomposes into the IAPP monomer through the posttranslational process,

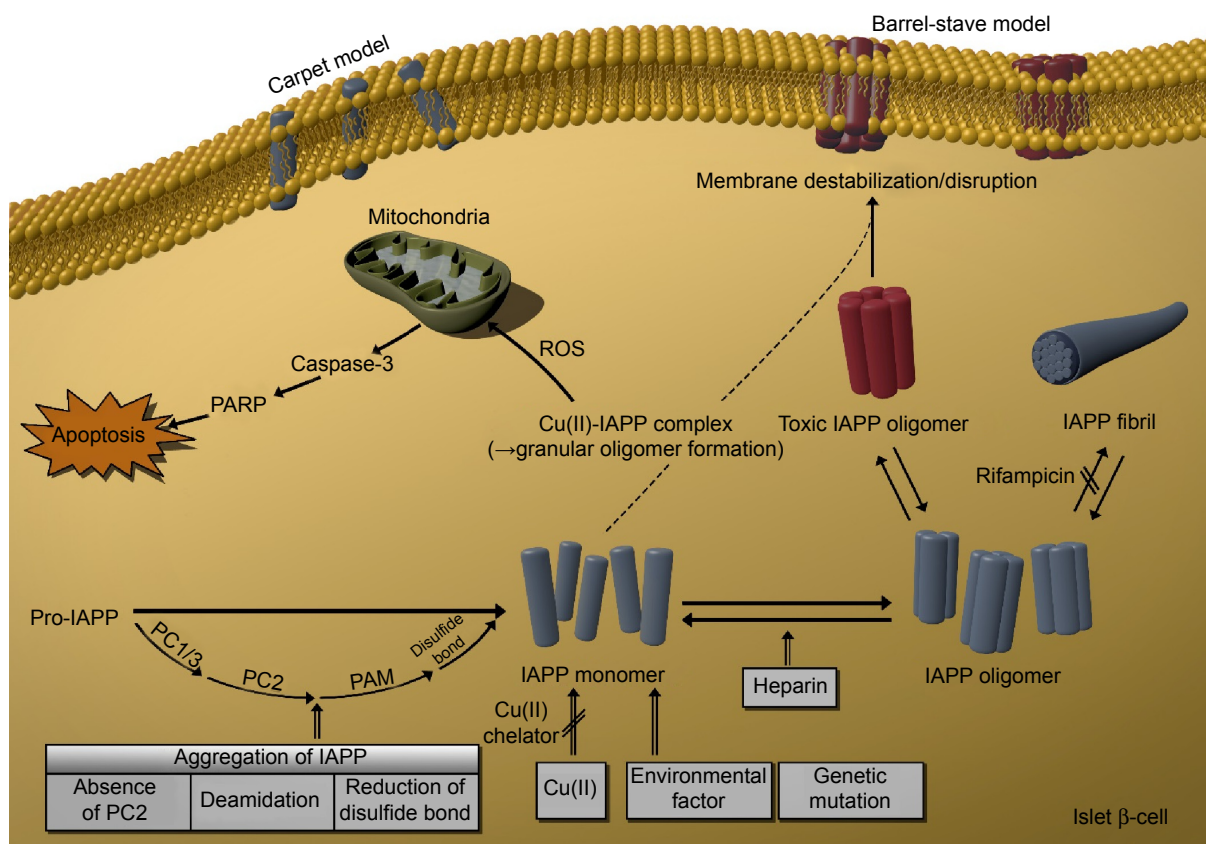


Figure 4 Causative factors of toxic oligomer formation.

Notes: Mechanistic factors that result in the generation of oligomers from pro-IAPP include the absence of PC2 enzyme, deamidation, reduction of the disulfide bond, environmental factors in the cell, genetic mutations, copper metal ions, and heparin-induced cell apoptosis. The barrel-stave model and carpet model demonstrate known methods by which amyloidogenic peptides can trigger membrane destabilization and disruption. In addition, both intra- and extracellular aggregation may be involved in these cytotoxic processes, although they most likely occur intracellularly. The mechanisms contributing to inhibitory effects such as copper ion chelators and rifampicin are indicated.

Abbreviations: IAPP, islet amyloid polypeptide; PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species; PC1/3, proprotein convertase 1/3; PC2, proprotein convertase 2; PAM, peptidylglycine α -amidating monooxygenase; Cu(II), copper (II) ion.

and how these IAPP monomers can create and organize into toxic IAPP oligomers in β -cells. As illustrated in Figure 4, the mechanistic factors that influence IAPP aggregation include the absence of PC2 enzyme, deamidation, reduction of disulfide bonds, environmental factors in the cell, genetic mutations, copper metal ions, and heparin. Other factors can also affect amyloid aggregation. Further research on these factors could ultimately aid in the inhibition of amyloid aggregation, which would be a breakthrough for the treatment of diabetes. To date, lack of knowledge about the toxic IAPP oligomer, the ultimate causative factors for reduction of β -cell mass, and the cause of cell death has hindered the ability in treating diabetes and limiting its progression, even in the prediabetic stage, and for selecting or developing novel therapeutic agents. Therefore, determining the mechanism by which the formation of toxic IAPP oligomers leads to T2DM should be an important research goal with the aim of elucidating the pathogenesis of this disorder and establishing new treatments.

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Disclosure

The authors report no conflicts of interest in this work.

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