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Aggregation of islet amyloid polypeptide: from physical chemistry to cell biology

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Abstract

Amyloid formation in the pancreas by islet amyloid polypeptide (IAPP) leads to β -cell death and dysfunction, contributing to islet transplant failure and to type-2 diabetes. IAPP is stored in the β -cell insulin secretory granules and cosecreted with insulin in response to β -cell secretagogues. IAPP is believed to play a role in the control of food intake, in controlling gastric emptying and in glucose homeostasis. The polypeptide is natively unfolded in its monomeric state, but is one of the most amyloidogenic sequences known. The mechanisms of IAPP amyloid formation *in vivo* and *in vitro* are not understood; the mechanisms of IAPP induced cell death are unclear; and the nature of the toxic species is not completely defined. Recent work is shedding light on these important issues.

Introduction

The presence of amyloid in the pancreatic islets of Langerhans is a pathophysiological feature of type-2 diabetes. Pancreatic islet amyloid deposits were first reported more than 110 years ago [1], but it was not until 1987 that a 37-residue polypeptide hormone, denoted as amylin or islet amyloid polypeptide (IAPP), was shown to be the protein component of islet amyloid [2,3]. IAPP is found in all mammals and is believed to play a role in controlling gastric emptying, glucose homeostasis and in the suppression of glucagon release [4]. IAPP is synthesized as a pre-proform [5], is processed in the Golgi and in the insulin secretory granule (Figure 1), and is released in response to stimuli which trigger insulin release. The concentration of IAPP in the granule is about 1–2% that of insulin. This is much higher than required to lead to rapid amyloid formation *in vitro*, so there must be mechanisms which inhibit irreversible aggregation in the granule [4].

The polypeptide is normally soluble and is natively unfolded in its monomeric state, but forms amyloid in type-2 diabetes (T2D) [2–4]. The process of islet amyloid formation leads to pancreatic β -cell dysfunction, cell death and the loss of islet β -cell mass [6–8]. Islet amyloid is not the cause of T2D; however it contributes to β -cell failure in T2D and the failure of islet cell transplantation [4,9,10*].

There is a large and growing body of work on the biophysics of IAPP amyloid formation and on the biological consequences of islet amyloid deposition. Unfortunately, space limitations prevent a detailed discussion of all aspects of the IAPP field and in this review we focus on the factors which control IAPP amyloid formation, on structural models of

IAPP amyloid fibrils, on the nature of early intermediates, and on mechanisms of IAPP induced cytotoxicity. We provide citations to review articles which cover other topics.

Not all species form islet amyloid and its presence or absence correlates with differences in the primary sequence of IAPP

Mature IAPP is a 37 residue polypeptide that contains an intramolecular disulfide bridge between residues two and seven, and an amidated C-terminus (Figure 1). The only known polymorphism of mature human IAPP (hIAPP) that impacts amyloid formation *in vivo* is a Ser to Gly mutation at position 20, found at low levels in certain Asian populations [4]. This mutation accelerates amyloid formation *in vitro* [4,11]. Other factors leading to accelerated amyloid formation by hIAPP *in vitro* include spontaneous Asn deamidation. Asn deamidation can also lead to changes in the morphology of amyloid fibrils [12].

There is a correlation between the sequence of IAPP and its propensity to form amyloid (Figure 2) [13]. hIAPP, for example, forms amyloid readily while rat/mouse IAPP does not. The differences between the human and rat/ mouse sequences occur at only six out of 37 positions, five of which are located between residues 20–29. Rat/mouse IAPP contains three Pro residues at positions 25, 28 and 29, while the human sequence does not contain any. The inability of rat/mouse IAPP to form amyloid is attributed to the Pro substitutions, consistent with the secondary structure disrupting effect of Pro. A non-aggregating variant of hIAPP, Pramlintide, which contains proline residues at the same positions as found in the rat/mouse sequence, has been approved by the FDA for treatment of diabetes [14].

Multiple Pro substitutions outside of the 20–29 region can abolish amyloid formation by hIAPP, as can replacement of Asn-14 or Asn-21 [15,16]. Conversely, replacement of residues Arg-18, Leu-23, and Val-26 in rat/mouse IAPP by their human counterparts leads to a weakly amyloidogenic polypeptide [17]. These studies show that the 20–29 sequence is not the only region of the polypeptide governing *in vitro* amyloid formation.

Monomeric IAPP does not adopt a compact globular structure, but it is not a random coil. The region encompassing residues 5–20 transiently populates helical ϕ , ψ angles in aqueous solution even though the level of persistent helical structure is low [18,19]. More persistent helical structure can be induced by negatively charged membranes [20] and NMR studies have delineated the conformation of IAPP in membrane mimetic environments [21].

Models of the hIAPP protofibril propose a parallel in-register β -structure with U-shaped monomers

hIAPP amyloid fibrils consist of a cross- β arrangement of β -strands that run perpendicular to the fibril axis with the hydrogen bonds oriented parallel to the long axis of the fibril. Residues one to seven probably do not participate in β -structure because of conformational constraints imposed by the disulfide bridge. Several atomic models have been proposed for the hIAPP fibril and they share some common features. Two columns of symmetry related hIAPP monomers pack against each other in the basic assembly (Figure 3). Each polypeptide adopts a U-shaped structure and contains two β -strands connected by a bend-loop. There are no intrachain backbone hydrogen bonds. The β -strands form hydrogen bonds with adjacent polypeptide chains within the same column generating an intermolecular, parallel, in-register β -sheet (Figure 3). In the solid state NMR derived model from the NIH group the β -strands are comprised of residues 8–17 and 28–37 while the bend involves residues 18–27 [22].

Two other models have been proposed recently. The UCLA group has developed a model based on crystallographic studies of fragments of hIAPP [23] that shares many features with the solid state NMR model, but differs in how the two columns of hIAPP monomers pack against each other and in the length of the C-terminal β -strand (Figure 3). EPR studies of variants of hIAPP that lack the disulfide bond have led to a variation on these models. These experiments required the use of non conservative nitroxide spin labeled Cys mutants. The fibrils are still built up of U-shaped stacks of monomers, but the planes of the two β -strand regions within one IAPP molecule are staggered with respect to each other; the spacing between the β -strands is also larger than in other models [24].

An interesting feature of all three models is that a significant fraction of the 20–29 region is not part of a β -sheet, but forms a partially ordered bend that connects the two β -strands. This leads to obvious questions about the sensitivity of hIAPP amyloid formation to substitutions in the 20–29 region. In both the UCLA and NIH models, Ser-28 and Ser-29 make key contacts, suggesting that the Pro substitutions in rat/mouse IAPP could disrupt the interface (Figure 3). The bend may also impact the kinetics of amyloid formation, as structure has been postulated to form early in this region [25].

Amyloid formation *in vitro* may proceed via helical intermediates, but other mechanisms have been proposed

In vitro studies provide evidence for the population of helical intermediates during amyloid formation in homogenous solution [18,19,26,27]. Helix formation and self-association are linked in many systems; examples include peptides with a tendency to form amphiphilic helices and coiled coils. hIAPP has the potential to form an amphiphilic helix between residues 5–20 and the initial formation of oligomers may be driven by the linkage between helix formation and association. This would lead to a high local concentration of the amyloidogenic C-terminal region of hIAPP, which could promote intermolecular β -sheet formation. The ability of rat/mouse IAPP and some proline mutants of hIAPP to inhibit amyloid formation by wild type hIAPP are consistent with this model. These peptides have a tendency to form amphiphilic helices, but the prolines in their C-terminal region inhibit formation of β -sheet structure. This suggests that they could bind to helical oligomers and inhibit their conversion to β -structure, but it is important to note that their mechanism is not defined [28,29]. There are also small molecule inhibitors that are designed to target helical structure [30].

Ion mobility mass spectroscopy (IM-MS) combined with MD simulations have led to a different model of early intermediates [31]. The model proposes formation of side by side β -hairpin dimers. This structure requires a significant rearrangement of the backbone hydrogen bonding to form the stacked column structures found in the amyloid fibril models. IM-MS has the important advantage that it can separate different conformers in a heterogeneous mixture, but has the disadvantage that one must assume that conformations detected in the gas phase are representative of those populated by the dynamic peptide in solution.

A third model postulates a head to tail dimerization in which His-18 makes critical contacts with the C-terminal tyrosine [32]. A variant of hIAPP with a free C-terminal carboxylate rather than the physiological C-terminal amidated form was used in the studies to facilitate labeling for NMR, suggesting that there may be non-natural electrostatic interactions between the negatively charged C-terminus and His-18. It will be interesting to see if this model holds for the naturally occurring amidated peptide.

Anionic lipids and components of the extracellular matrix accelerate hIAPP amyloid formation *in vitro*

hIAPP has a net positive charge at all biologically relevant pH's and interacts with negatively charged biopolymers, membranes and surfaces. Anionic membranes promote amyloid formation by hIAPP *in vitro* and more highly charged systems have a larger effect for high peptide to lipid ratios [33]. Many of the studies of hIAPP–membrane interactions have used simple model membranes with a mole fraction of anionic lipids that is significantly higher than that found in β -cell membranes, making it difficult to translate the results to the situation *in vivo*. More complicated model membranes containing phospholipids found in β -cell membranes, but which lack cholesterol, have also been shown to accelerate hIAPP amyloid formation, as have heterogeneous anionic model membranes that are capable of forming lipid rafts [34*,35,36]. The mechanism of membrane catalyzed hIAPP aggregation is not completely understood, but helical intermediates appear to be important [20,33,37*].

hIAPP amyloid formation *in vivo* has been proposed to be initiated by the binding of proIAPP processing intermediates to the glycosaminoglycan (GAG) chains of the heparan sulfate proteoglycan (HSPG) perlecan [4]. HSPGs are associated with *in vivo* hIAPP amyloid deposits, and secretion of an incompletely processed proIAPP intermediate, (NproIAPP), containing the N-terminal prosequence is increased in T2D [38,39]. Interactions with model GAGs accelerate amyloid formation by hIAPP and NproIAPP *in vitro* and NproIAPP amyloid can seed amyloid formation by mature hIAPP [40,41*]. Interestingly, NproIAPP interacts more weakly with anionic membranes than the mature sequence [42]. Inhibition of glycosaminoglycan synthesis reduces amyloid deposition in cultured islets, as does overexpression of heparanase in a double transgenic mouse model that overexpresses hIAPP, suggesting that interactions with HSPGs may be important *in vivo* [43,44]. Interactions with membranes and GAGs can impact the efficacy of amyloid inhibitors [45,46].

The mechanism of hIAPP induced β -cell toxicity and the initial site of amyloid deposition are open questions

Amyloid deposits observed in T2D appear to be extracellular and early histological studies with transgenic mouse models are consistent with an extracellular origin. Work with models in which IAPP is over expressed suggests that initial aggregation may occur intracellularly in the secretory pathway [4,47,48]. By contrast, a recent study shows that secretion of IAPP is an important determinant of β -cell toxicity and islet amyloid formation, arguing that islet amyloid has an extracellular origin. In that work, agents that increased secretion increased amyloid formation and toxicity, while inhibition of secretion of IAPP had the opposite effect [49*]. The difference between the studies may be related to the level at which IAPP is produced and the methods used to detect aggregates [4,49*,50]. The debate on an intracellular vs extracellular origin of islet amyloid is important since it impacts treatment strategies.

The process of amyloid formation by hIAPP is toxic to cultured β -cells and induces apoptosis and β -cell dysfunction in isolated human islets [6–8,44,51,52,53**]. The literature strongly suggests that there are multiple mechanisms of hIAPP induced β -cell dysfunction and cell death. Many of these overlap and share the same downstream signaling pathways.

IAPP has been proposed to exert its toxic effects by permeabilizing membranes [54–56]. The ability of IAPP to induce membrane leakage depends on lipid composition and on the

lipid to peptide ratio as well as pH and ionic strength. The fraction of anionic lipids in the β -cell membrane is much lower than that employed in many biophysical studies of membrane leakage and the type of anionic lipids used are often very different [34*]. Most model systems also lack cholesterol and do not contain gangliosides. This may be important since recent work has shown that cholesterol and gangliosides play a role in mediating hIAPP membrane interactions and in the uptake and clearance of hIAPP [35,57–59]. We do not want to leave the impression that loss of membrane integrity is not important; this may indeed be one mechanism of toxicity, especially at high peptide concentrations. A range of studies have shown that hIAPP amyloid fibrils cluster on or near membranes and there is very good evidence that exogenously added IAPP perturbs cell membranes [54–57,60]. We believe that caution should be employed when extrapolating from mechanistic biophysical studies involving simple model membranes to the situation *in vivo*. Particularly since variants of hIAPP which do not induce β -cell death *in vivo* can disrupt some model membranes *in vitro*. More complicated and physiologically relevant model membrane systems are starting to be used and should provide new mechanistic insight [34–36]. The *in vitro* mechanism of hIAPP induced membrane disruption is an interesting open question, but space limitations preclude a detailed discussion [33,56,61].

ER stress and defects in ERAD have been proposed to be important factors in hIAPP induced β -cell death [47,48,62]. ProIAPP and not mature hIAPP may be the culprit, as the processing of proIAPP to mature hIAPP is completed in the Golgi and secretory granule [4]. Exogenously added hIAPP has also been reported to induce ER stress [63]. The exact role of ER stress in hIAPP mediated toxicity *in vivo* is currently unclear. Some of the studies that support a role for ER stress made use of transgenic rodent models that significantly over express hIAPP. By contrast, no ER stress was detected in cultured islets that produce IAPP at lower levels, and the overproduction of hIAPP has been suggested to be responsible for the differences [64].

Other proposed mechanisms of hIAPP toxicity include defects in autophagy, the enhanced production of pro-inflammatory cytokines, mitochondrial membrane damage and receptor-mediated mechanisms linked to oxidative stress and activation of signaling pathways leading to cell death [65–69,70**]. These pathological cellular processes can be triggered by either intracellular or extracellular aggregates. The pathways that mediate β -cell apoptosis in response to hIAPP amyloid formation are not completely characterized, but recent work has shed light on this important point. The cJUN N-terminal kinase (JNK) pathway is a critical pro-apoptotic pathway in β -cells and is activated by a range of stress stimuli. These include ER stress, oxidative stress, exposure to pro-inflammatory cytokines and high glucose. JNK mediates β -cell apoptosis in cultured cells and in islets exposed to high concentrations of hIAPP; it has recently been shown to do the same in response to amyloid generated from endogenous hIAPP [70**]. Downstream mediators have been identified in both intrinsic (Bim) and extrinsic (Fas, Fadd) pathways. Aggregation of both endogenous and exogenous hIAPP upregulates Fas and activates caspase pathways [65,71*], and deletion of Fas protects β -cells from hIAPP toxicity [71*]. hIAPP aggregation has also been shown to upregulate the terminal effector Casp3, and *in vivo* studies have shown that preventing Casp3 activation protects β -cells from hIAPP amyloid induced apoptosis [72].

Macroautophagy and chaperone-mediated autophagy lead to clearance of ubiquitinated proteins and autodigestion of abnormal or aged organelles by degradation in the lysosome. Defects in autophagy play a role in the toxicity of other amyloidogenic proteins. Recent studies have shown that over expression of hIAPP in β -cells leads to impaired autophagy; this effect has been demonstrated to occur before the development of hyperglycemia [66**, 73]. Inhibition of autophagy/lysosomal degradation promotes hIAPP induced β -cell apoptosis while stimulation of autophagy protects β -cells from IAPP toxicity [66**].

hIAPP aggregates may promote β -cell dysfunction by triggering a localized inflammatory response, as well as by acting directly on β -cells [67**,69]. Recent reports highlight the role of inflammasomes in metabolic syndrome and provide evidence that hIAPP can stimulate their activity [67**]. Inflammasomes are protein complexes that recognize a range of pro-inflammatory stimuli and control the production of key pro-inflammatory cytokines, such as Interleukin-1 β (IL-1 β). Studies support a role for IL-1 β in hIAPP-induced β -cell dysfunction and cell death.

New biophysical approaches hold the promise of a high resolution view of *in vitro* amyloid formation

Advances in 2DIR and isotopic labeling offer the prospect of obtaining site specific information about aggregation, but currently require high sample concentrations [25,74**]. Single particle methods provide insight into hIAPP membrane interactions [37*,75], while the development of non-invasive fluorescence probes allows the study of side chain solvation and backbone compaction in real time [76]. Advances in mass spectroscopy provide information about heterogeneous populations [31]. Continued refinement of these methods should lead to further advances. A key challenge will be to connect *in vitro* biophysical studies of model systems to the situation *in vivo*.

Conclusions

Progress has been made in understanding hIAPP amyloid formation, but important challenges remain. These include elucidating the mechanisms of islet amyloid formation *in vivo* and *in vitro*; identifying the initiation sites of amyloid formation *in vivo*; defining the nature of the toxic species and the mechanisms of cell death; elucidating the mechanisms of hIAPP clearance *in vivo* and the role such processes play in islet amyloid deposition. The development of inhibitors of hIAPP toxicity is also an area that warrants further effort, especially since many studies have relied on *ex vivo* assays using cultured cells and islets.

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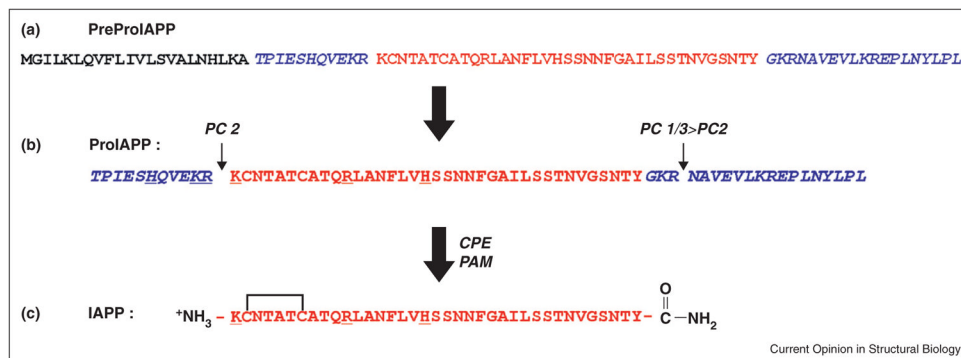
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**Figure 1.**

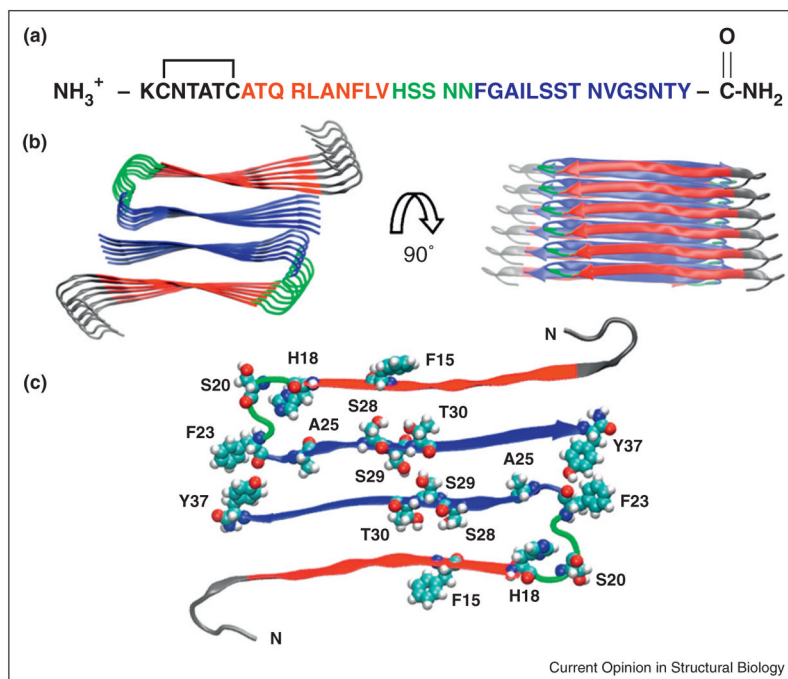
Post translational modification of human PreProIAPP to form the mature IAPP sequence: **(a)** The primary sequence of the 89-residue human PreProIAPP. The 22 residue signaling sequence is shown in black, the N-terminal and C-terminal proIAPP flanking regions are shown in blue, and the mature IAPP sequence is shown in red. **(b)** The primary sequence of the 67-residue human proIAPP. Before secretion, proIAPP is cleaved by the prohormone convertases PC2 and PC(1/3) at two dibasic sites, indicated by arrows. Further processing by the CPE/PAM complex results in an amidated Tyr at the C-terminus of mature IAPP. **(c)** The mature 37-residue human IAPP. The biologically active peptide has an intramolecular disulfide bridge between Cys-2 and Cys-7 and an amidated C-terminus. Positively charged residues are underlined in the ProIAPP and mature IAPP sequences.

	1	10	20	30	37
Human CGRP1:	<u>AC</u> <u>D</u> TATCVT	HRLA <u>G</u> LLSR <u>S</u>	<u>G</u> <u>G</u> VVKNNFVP	TNVGS <u>K</u> A <u>F</u>	
Human CGRP2:	<u>AC</u> <u>N</u> TATCVT	HRLA <u>G</u> LLSR <u>S</u>	<u>G</u> <u>G</u> MVKSNEFVP	TNVGS <u>K</u> A <u>F</u>	
Human:	KCNTATCAT	QRLANFLVHS	SNNFGAILSS	TNVGSNTY	
Monkey:	KCNTATCAT	QRLANFLV <u>R</u> S	SNNFG <u>T</u> ILSS	TNVGS <u>D</u> TY	
Macaque:	KCNTATCAT	QRLANFLV <u>R</u> S	SNNFG <u>T</u> ILSS	TNVGS <u>D</u> TY	
Baboon:	<u>I</u> CNTATCAT	QRLANFLV <u>R</u> S	SNNFG <u>T</u> ILSS	TNVGSNTY	
Porcine:	KCN <u>M</u> ATCAT	Q <u>H</u> LANFL <u>D</u> RS	<u>R</u> NN <u>L</u> G <u>T</u> I <u>F</u> SP	<u>T</u> KVGSNTY	
Cow:	KC <u>G</u> TATC <u>E</u> T	QRLANFL <u>A</u> PS	<u>S</u> N <u>K</u> L <u>G</u> A <u>I</u> FSP	<u>T</u> KVGSNTY	
Cat:	KCNTATCAT	QRLANFL <u>I</u> RS	SNN <u>L</u> GAIL <u>S</u> P	TNVGSNTY	
Dog:	KCNTATCAT	QRLANFLV <u>R</u> T	SNN <u>L</u> GAIL <u>S</u> P	TNVGSNTY	
Rat:	KCNTATCAT	QRLANFLV <u>R</u> S	SNN <u>L</u> G <u>P</u> V <u>L</u> PP	TNVGSNTY	
Mouse:	KCNTATCAT	QRLANFLV <u>R</u> S	SNN <u>L</u> G <u>P</u> V <u>L</u> PP	TNVGSNTY	
Guinea Pig:	KCNTATCAT	QRL <u>T</u> NFLV <u>R</u> S	<u>S</u> H <u>N</u> L <u>G</u> A <u>A</u> L <u>L</u> P	<u>T</u> DVGSNTY	
Hamster:	KCNTATCAT	QRLANFLVHS	<u>N</u> NN <u>L</u> G <u>P</u> V <u>L</u> SP	TNVGSNTY	
Degu:	KCNTATCAT	QRL <u>T</u> NFLV <u>R</u> S	<u>S</u> H <u>N</u> L <u>G</u> A <u>A</u> L <u>L</u> P	<u>T</u> KVGSNTY	
Ferret:	KCNTATCVT	QRLANFLV <u>R</u> S	SNN <u>L</u> GAIL <u>L</u> P	<u>T</u> DVGSNTY	
Rabbit:	CNT <u>V</u> TCAT	QRLANFL <u>I</u> HS	SNNFGA <u>F</u> L <u>P</u> P	<u>S</u>	
Hare:		<u>T</u> QRLANFL <u>I</u> HS	SNNFGA <u>F</u> L <u>P</u> P	<u>T</u>	

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Figure 2.

Primary sequences of IAPP from different species: residues that differ from the human sequence are underlined and highlighted in red. Only partial sequences are available for rabbit and hare. The biologically active mature sequence has a disulfide bridge between Cys-2 and Cys-7 and an amidated C-terminus. Primates and cats have been reported to form islet amyloid while dogs, rodents and cows do not. Porcine and ferret IAPP are significantly less amyloidogenic than human IAPP. The degu forms islet amyloid, but the deposits are derived from aggregation of insulin, not IAPP.

**Figure 3.**

The structure of human IAPP: **(a)** The primary sequence of human IAPP. Residues 8–17 form intermolecular β -sheets in models of the IAPP amyloid fiber and are color coded in red. Residues 18–22, colored in green, form a bend in the UCLA model while residues 23–37, colored in blue, take part in an intermolecular β -sheet. The color coding corresponds to the IAPP amyloid structural model developed by the UCLA group [23]. The first 7 residues do not take part in β -sheet structure in existing models of IAPP amyloid. **(b)** A structural model of the IAPP fiber developed by the UCLA group [23]. Two views are shown: a top down view and an image rotated by 90 degrees. The color coding corresponds to that used in panel A. **(c)** A view of one layer of the stacked structure shown in panel **(b)**. His-18, Ser-20, Ser-28, Ser-29, Thr-30 are shown in space-filling representation. The protonation state of His-18 affects the rate of amyloid formation [77]. A Ser-20 to Gly mutant is the only reported mutation in mature IAPP found in humans and has been shown to accelerate amyloid formation [4,11]. Ser-28, Ser-29 and Thr-30 form key inter-peptide contacts in the UCLA model. IAPP contains three aromatic residues (Phe-15, Phe-23, and Tyr-37) which are also highlighted.