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A Two-Site Mechanism for the Inhibition of IAPP Amyloidogenesis by Zinc

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Human islet amyloid polypeptide (hIAPP) is a highly amyloidogenic protein co-secreted with insulin in response to glucose levels. The formation of hIAPP amyloid plaques near islet cells has been linked to the death of insulin-secreting β-cells in humans and the progression of type II diabetes. Since both healthy individuals and those with type II diabetes produce and secrete hIAPP, it is reasonable to look for factors involved in storing hIAPP and preventing amyloidosis. We have previously shown that zinc inhibits the formation of insoluble amyloid plaques of hIAPP; however, there remains significant ambiguity in the underlying mechanisms. In this study, we show that zinc binds unaggregated hIAPP at micromolar concentrations similar to those found in the extracellular environment. By contrast, the fibrillar amyloid form of hIAPP has low affinity for zinc. The binding stoichiometry obtained from isothermal titration calorimetry experiments indicates that zinc favors the formation of hIAPP hexamers. High-resolution NMR structures of hIAPP bound to zinc reveal changes in the electron environment along residues that would be located along one face of the amphipathic hIAPP α-helix proposed as an intermediate for amyloid formation. Results from electrospray ionization mass spectroscopy investigations showed that a single zinc atom is predominantly bound to hIAPP and revealed that zinc inhibits the formation of the dimer. At higher concentrations of zinc, a second zinc atom binds to hIAPP, suggesting the presence of a low-affinity secondary binding site. Combined, these results suggest that zinc promotes the formation of oligomers while creating an energetic barrier for the formation of amyloid fibers.

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Abbreviations used: IAPP, islet amyloid polypeptide; hIAPP, human islet amyloid polypeptide; rIAPP, rat islet amyloid polypeptide; Aβ, amyloid beta; ESI-MS, electrospray ionization mass spectroscopy; ITC, isothermal titration calorimetry; TFE, trifluoroethanol; DMSO, dimethyl sulfoxide; HMQC, heteronuclear multiple-quantum coherence; TOCSY, total correlated spectroscopy; NOE, nuclear Overhauser enhancement; NOESY, NOE spectroscopy.

Introduction

Islet amyloid polypeptide (IAPP) (sequence shown in Fig. 1) is a 37-residue amyloidogenic peptide hormone secreted primarily by pancreatic β-cells in the islets of Langerhans in response to high glucose levels.[1] IAPP is co-secreted with insulin and has been shown to enhance the effects of insulin by slowing gastric emptying, reducing the rate of glucose entering the blood, and signaling the brain to reduce meal size.[1] However, human IAPP

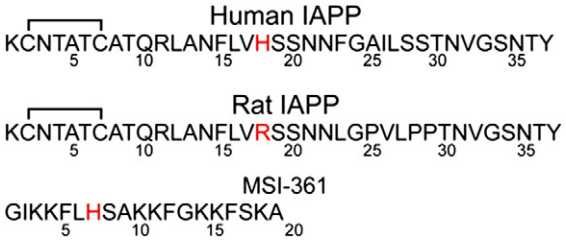

Fig. 1. Amino acid sequences of the peptides used. All unlabeled peptides are amidated and contain oxidized cysteines. The imidazole of His18 can bind to zinc and the substitution of arginine for histidine in rIAPP removes a potential zinc-binding site.

(hIAPP) has a strong amyloidogenic propensity that has been linked to β-cell death in type II diabetics and in transgenic mice overexpressing the hIAPP variant.[2–6] The insoluble plaques are rich in β-sheet character, similar to those found in Alzheimer's, Parkinson's, Huntington's, and prion disease and other degenerative diseases.[7] While hIAPP can aggregate *in vitro* at low micromolar concentrations,[8] the peptide is stored at millimolar concentration *in vivo*.[9–11] The kinetics of fibrillization and fiber morphology are highly dependent on conditions at which fibrillization takes place.[12] Since diabetics and nondiabetics have the same IAPP sequences, the cellular environment must contain factors that either accelerate aggregation or act as chaperones to stabilize the protein at high concentrations.[13–20]

The dysregulation of metal ions in amyloid-related diseases has received particular attention following the discovery of a high prevalence of metal ions in amyloid deposits. It has been shown that amyloidogenic proteins, such as amyloid beta (Aβ), α-synuclein, and mammalian prion protein, have transition metal binding sites that affect aggregation rates.[21–24] Although amyloid proteins are characterized by similar fiber structure and nucleation-dependent kinetics, the effect of metal ions on aggregation is highly dependent on the particular protein and metal.[7] For example, while copper has been shown to promote the formation of Aβ fibers, copper inhibits the formation of IAPP fibers.[25,26] The role of zinc in type II diabetes and hIAPP aggregation is less understood, as both zinc supplements and zinc chelation have been shown to decrease β-cell death and symptoms of type II diabetes.[27–29] These studies suggest that zinc has a variety of effects on β-cells and stress the need for further investigation.

Zinc as a chaperone in regulating hIAPP fiber growth is of particular interest, as the zinc content of β-cell secretory granules, where hIAPP is stored, is among the highest in the body, and the pancreas is particularly sensitive to systemic zinc levels.[30] Although the molecular interaction of zinc to other amyloidogenic proteins, such as Aβ and mammalian prion protein, has been characterized, little is known about how zinc interacts with hIAPP.[31–33] Zinc has already been shown to affect a variety of processes involved in glucose homeostasis, including storage and excretion of insulin, glucose-stimulated secretion cascades, and paracrine communication with α-cells; however, little is known about zinc's effect on IAPP-induced β-cell death.[34–36] Previous studies have shown that zinc accelerates the formation of fibers at pH 3; however, our recent investigation has shown that zinc has a bimodal effect on the aggregation rate of hIAPP at neutral pH.[37] The complex effects of zinc on hIAPP emphasize the need for a more comprehensive study of the molecular basis of the interaction.

In this study, we attempt to further characterize the nature of zinc binding to hIAPP using isothermal titration calorimetry (ITC), dye-binding assays, electrospray ionization mass spectrometry (ESI-MS), and NMR. We show that zinc binds to monomeric IAPP near the His18 residue with low micromolar affinity. The affinity of the fibrillar hIAPP for zinc is significantly less than that of the monomeric form. We also show that zinc is displaced from monomeric IAPP during aggregation. ITC revealed that zinc preferentially binds six hIAPP monomers in a conformationally dependent manner. Concurrently, ESI-MS revealed that the formation of the dimer is inhibited by the presence of zinc. High-resolution NMR structures of hIAPP in the presence and absence of zinc reveal long-range structural variations in the amphipathic region of the previously proposed α-helix conformation of the dimer. The results indicate that zinc inhibits fibrillization by creating a thermodynamic barrier for the formation of mature fiber and dimer while promoting the formation of oligomers.

Results

hIAPP binds zinc with low micromolar affinity

We previously showed that zinc has an overall inhibitory effect on fibril formation while having a concentration-dependent bimodal effect on hIAPP fibrillogenesis.[37] At low concentrations, zinc increases the lag time for fiber formation and decreases the rate of addition of hIAPP to existing fibers, while the effect begins to reverse at higher concentrations. To understand the molecular origin of the inhibitory effect, we first attempted to quantify the binding affinity of hIAPP initially prepared in the monomeric state to $ZnCl_2$ by ITC. However, the rapid aggregation of hIAPP in solution proved to be problematic for the accurate determination of binding constants, as aggregation