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Research paper

Residue specific effects of human islet polypeptide amyloid on self-assembly and on cell toxicity

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ABSTRACT

Type 2 diabetes mellitus is characterized histopathologically by the presence of fibrillary amyloid deposits in the pancreatic islets of Langerhans. Human islet amyloid polypeptide (hIAPP), the 37-residue pancreatic hormone, is the major constituent of these amyloid deposits. The propensity of IAPP to form amyloid fibrils is strongly dependent on its primary sequence. An intriguing example is His at residue 18. Although H18 is located outside the amyloidogenic region, it has been suggested that this residue and its charge state play an important role in the kinetics of conformational changes and fibril formation as well as in mediating cell toxicity. To gain more insight into the importance of this residue, we have synthesized four analogues (H18R-IAPP, H18K-IAPP, H18A-IAPP and H18E-IAPP) and we performed a full biophysical study on the properties of these peptides. Kinetic experiments as monitored by thioflavin-T fluorescence, transmission electron microscopy, circular dichroism and cell toxicity assays revealed that all variants are less fibrillogenic and less toxic than native hIAPP both at neutral pH and at low pH. This demonstrates that the effect of H18 in native IAPP is not simply determined by its charge state, but rather that residue 18 is important for specific intra- and intermolecular interactions that occur during fibril formation and that may involve charge, size and hydrophobicity. Furthermore, our results indicate that H18R-IAPP has a strong inhibiting effect on native hIAPP fibril formation. Together these results highlight the large impact of modifying a single residue outside the amyloidogenic domain on fibril formation and cell toxicity induced by IAPP, opening up new avenues for design of inhibitors or modulators of IAPP aggregation.

1. Introduction

Amyloid is a fibrous quaternary structure formed by the aggregation of monomeric protein or peptide into a cross β-sheet fold. A wide range of pathologies, including Alzheimer's disease, Parkinson's disease, Huntington disease and type 2 diabetes mellitus (T2DM), is associated with the formation of amyloid fibrils [1]. For each of these diseases, a specific protein is involved in the amyloid assembly, leading to cell degeneration and cell death.

In T2DM the main constituent of islet amyloid is the islet amyloid polypeptide, IAPP, which displays amino acid sequence homology with calcitonin gene-related peptides [2,3]. IAPP is a 37-residue polypeptide pancreatic hormone coproduced and consecrated with insulin in a molar ratio of about 1:100 in healthy

Abbreviations: IAPP, Islet Amyloid Polypeptide; CD, circular dichroism; T2DM, type 2 diabetes mellitus; TEM, transmission electron microscopy; ThT, Thioflavin T.

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individuals, but in a ratio of about 1:20 in T2DM [4,5]. The formation and the deposition of IAPP amyloid in the islets of Langerhans of the pancreas is a typical pathological feature of T2DM. A strong correlation was shown between the occurrence of T2DM in a particular species and the primary sequence and amyloid forming propensity of the IAPP that is produced by that species. For example, rat or mouse IAPP (rIAPP or mIAPP) differs from human IAPP by six residues out of 37, does not form fibrils and rats or mice do not spontaneously develop T2DM, whereas cat IAPP differs from human IAPP by four residues, does form amyloid fibrils and cats do develop the disease [6,7]. Therefore, understanding particular sequence/structure relationships is likely to be essential in defining why some amyloids do form toxic amyloids and others do not.

In general, the ability of a peptide to form amyloid fibrils is dependent on i) the presence of hydrophobic amino-acids with a high β-sheet propensity, ii) the presence of aromatic residue especially phenylalanine and tryptophan, iii) the lack of prolines and iv) the presence of electrostatic interactions [8–12]. To investigate possible amyloidogenic regions in full length human IAPP (hIAPP), several research groups have synthesized truncated and mutated peptides and studied their amyloidogenic properties using biophysical and biological techniques [13–15]. It was shown that the mutations within the 20–29 region induce an important alteration on IAPP amyloid formation. Nevertheless, this segment is not the sole domain determining its amyloidogenic propensity. Multiple proline substitutions outside this region could also induce a loss in IAPP amyloid formation, as for example, mutations within the N14, F15, L16, V17 residues [8,14,15]. Previous studies suggest that residue 18 may have a critical role in mediating cell toxicity [16,17]. In addition, studies at different pH indicated that the ionization state of histidine 18 significantly affects the kinetics of conformational changes and concomitant fibril formation [18]. Importantly, one difference between the sequence of hIAPP and non-amyloidogenic mIAPP is the substitution of histidine 18 of hIAPP with an arginine in mIAPP. Green and co-workers have synthesized a mutated mIAPP where arginine 18 has been replaced by histidine 18, and reported that this modified mIAPP was able to form fibrils, underlining the importance of residue 18 and the potential role of its charge state for hIAPP structure and fibril formation [19].

To gain more insight into the importance of residue 18 for the intermolecular IAPP interactions that are essential for fibril formation, we here have synthesized four mutated peptides where histidine 18 has been replaced by arginine (H18R-IAPP), lysine (H18K-IAPP), glutamic acid (H18E-IAPP) and alanine (H18A-IAPP) to achieve variations in charge, size and polarity. We report a full biophysical study of the behavior of these mutated peptides in comparison with that of native hIAPP. We determined the ability of the mutated peptides to form fibrils, their secondary structure and their toxicity towards INS-1 β-cells. Finally, the properties of the peptides at pH 5.5 and pH 7.4 were compared to allow comparison with effects of pH on native hIAPP [18]. It also should be noted that both pH values are physiologically relevant: mature hIAPP is stored in the β-cell granules of the pancreas at a pH of approximatively 5.5 and released into the extracellular compartment, which has a pH of 7.4. Our results show that all mutated peptides are less prone to aggregation and less toxic to INS-1 β-cells than wild-type hIAPP, even though the mutation is outside the amyloidogenic domain. This was observed for both values of pH, indicating that the previously observed pH dependent differences in aggregation of hIAPP [18] are not solely due to differences in the charge state of H18. Rather, the results suggest that residue 18 is involved in specific intra- and intermolecular interactions that are important for fibril formation and that involve charge, size and hydrophobicity.

2. Materials and methods

2.1. Peptide synthesis

All peptides were synthesized with a CEM Liberty Blue (CEM corporation, Matthews, USA) automated microwave peptide synthesizer using standard reaction cycles at the Institut de Biologie Intégrative (IFR83-Université Pierre et Marie Curie) as described [20]. The synthesis of all IAPP with an amidated C-terminus and a disulfide bridge were performed using Fmoc chemistry and a PAL Novasyn TG resin. Two pseudoproline dipeptides were chosen for the synthesis Fmoc-Ala-Thr(ΨMe,MePro)-OH replaced residues Ala-8 and Thr-9, and Fmoc-Leu-Ser(ΨMe,MePro)-OH replaced residues Leu-27 and Ser-28. Double couplings were performed for the pseudoprolines and for the residues following the pseudoprolines and for every β-branched residue. The peptides were cleaved from the resin and deprotected using standard TFA procedures with 1,2-ethanedithiol, water, and triisopropylsilane as scavengers. The peptides were purified by reverse phase high-performance liquid chromatography (HPLC) with a Luna C18(2) column (Phenomenex, USA). A two-buffer system was used. Buffer A consisted of 100% H2O and 0.1% TFA (vol/vol), and buffer B consisted of 100% acetonitrile and 0.07% TFA (vol/vol). Linear peptides were dissolved in aqueous DMSO (33%) and oxidized with air to the corresponding disulfide bond. Purity of peptides was higher than 95% as determined by analytical HPLC and identity of peptides was confirmed by MALDI-TOF mass spectrometry.

2.2. Sample preparation

An essential criterion for measuring aggregation kinetics of amyloid peptides is to start with a monomeric form of the peptide. Therefore, peptide stock solutions were freshly prepared prior to all experiments using the same batch. Peptide stock solutions were prepared as described previously [21]. Briefly, stock solutions obtained by dissolving the peptide at a concentration of 1 mM in hexafluoroisopropanol (HFIP) and by leaving it to incubate for an hour. Then, HFIP was evaporated and the sample was dried by vacuum desiccation for at least 30 min. The resulting peptide film was dissolved at a concentration of 1 mM in DMSO for the fluorescence experiments (final DMSO concentration of 2.5% v/v) and then diluted in 10 mM sodium phosphate buffer, 100 mM NaCl (pH 7.4 or pH 5.5). Both DMSO and NaCl interfere with the circular dichroism experiments, and therefore in these experiments the peptide film was directly dissolved in a 10 mM sodium phosphate buffer, 100 mM NaF (pH 7.4 or pH 5.5). It is important to note that NaF may slow down the β-sheet transition as compared to NaCl [22]. For the cytotoxicity assay the peptide film was directly dissolved in the culture media.

2.3. Circular dichroism

The secondary structure of peptides was measured using a Jasco J-815 CD spectropolarimeter with a Peltier temperature-controlled cell holder over the wavelength range 190–260 nm. Measurements were carried out in cells of 0.1 cm path length at 25 °C in 10 mM phosphate buffer, 100 mM NaF (pH 7.4 or pH 5.5). Measurements were taken every 0.2 nm at a scan rate of 20 nm/min. Each spectrum reported is the average of four scans. Peptide concentration was 25 μM. The background spectrum was subtracted and the results were expressed as mean residue molar ellipticity [θ].