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How the imidazole ring modulates amyloid formation of islet amyloid polypeptide: A chemical modification study

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ABSTRACT

Background: The misfolding of human islet amyloid polypeptide (hIAPP) is an important pathological factor on the onset of type 2 diabetes. A number of studies have been focused on His^{18}, the only histidine of hIAPP, whose imidazole ring and the protonation state might impact hIAPP amyloid formation, but the exact mechanism remains unclear.
Methods: We used diethylpyrocarbonate (DEPC) to specifically modify His^{18} and obtained mono-ethyloxyformylated hIAPP (DMI). Thioflavin T based fluorescence, transmission electronic microscopy, circular dichroism spectroscopy, fluorescence dye leakage, Fourier transform infrared spectroscopy and replica-exchange molecular dynamics (REMD) simulation were applied to study the impact of DEPC-modification on hIAPP amyloid formation.
Results: After an ethyl-acetate group was introduced to the His^{18} of hIAPP by diethylpyrocarbonate (DEPC) modification, the pH dependent hIAPP fibrillation went to the opposite order and the number of intra-molecular hydrogen bonds decreased, while the possibility of His^{18} participating in the formation of α-helical structures increased. Furthermore, the membrane–peptide interaction and ion–peptide interaction were both impaired.
Conclusions: The intramolecular hydrogen bond formation by His^{18} and the possibility of His^{18} participating in the formation of α-helical structures greatly modulated the manner of hIAPP amyloid formation. The imidazole ring directly participates in the hIAPP–membrane/ion interaction.
General significance: DEPC modification is an alternative approach to investigate the role of the imidazole ring during amyloid formation.

1. Introduction

Amyloid fibrils formed by misfolded proteins are important pathological factors to more than thirty so-called amyloid diseases, including Alzheimer's disease, Parkinson's disease, and type 2 diabetes mellitus (T2DM) [1,2]. Human islet amyloid polypeptide (hIAPP), the major component of amyloid deposits identified in the pancreas of T2DM patients (Fig. 1A), is one of the most amyloidogenic proteins, which readily aggregates into toxic oligomers and mature fibrils that have been regarded to be an important contributor to the death of pancreatic β-cells, possibly through disrupting cellular membranes, increasing endoplasmic reticulum stress, defeating the autophagy and activating the NLRP3 inflammasome [3–5].

In vitro, hIAPP easily aggregates at submicromolar concentrations [6], but in normal healthy β-cells where hIAPP is stored at millimolar concentrations, the aggregation of hIAPP is rarely detected [7]. The microenvironment conditions inside the secretory granule of pancreatic β-cells, including metal ions (especially high concentration of zinc ions), acidic pH and biological membranes, were thought to be major factors influencing hIAPP aggregation [8,9,10]. Understanding how these factors affect hIAPP amyloid formation may provide new angles for T2DM prevention and treatment [11,12].

The only histidine of hIAPP, His^{18}, is important in hIAPP amyloid formation. Studies have shown that the interaction between His^{18} and Tyr^{37} causes fast conversion among hIAPP low-order oligomers [13]. The rate of hIAPP amyloid formation is pH-dependent, fast at neutral pH and slow at acidic pH [14,15], with the protonation state of His^{18}

Abbreviations: IAPP, islet amyloid polypeptide; hIAPP, human IAPP; DEPC, diethylpyrocarbonate; DMI, DEPC-modified hIAPP; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; ThT, thioflavin-T.

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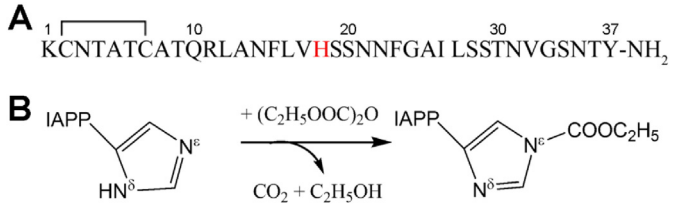

Fig. 1. (A) The primary sequence of hIAPP with His^{18} shown in red; (B) schematic presentation of DEPC modification.

as a major difference in this pH variation. In addition, metal ions such as Zn^{2+} and Cu^{2+} have been shown to affect hIAPP amyloid formation in complex ways [16–18], with His^{18} seeming to be a major metal binding site [19]. His^{18} is also involved in the toxic interaction between hIAPP and cell membrane, an Arg substitution abolished the membrane-penetration effect of hIAPP [20,21], it is also an important binding site of resveratrol, an anti-amyloidogenic compound [22,23]. Multiple studies have focused on the effect of protonation state of His^{18} and its electrostatic interaction after protonation during hIAPP amyloid formation, but the mechanism remained unclear. Here, we introduced an ethylacetate group to the imidazole ring of His^{18} by diethylpyrocarbonate (DEPC) modification to investigate the mechanism of His^{18} in hIAPP amyloid formation from a different angle. The kinetics of fibrillation, the structure of fibrils, the peptide–membrane interaction and the interaction between fibrils and metal ions were studied.

2. Materials and methods

2.1. Materials

Synthetic hIAPP (1-37) was obtained from Genscript Inc. (Piscataway, NJ, USA). Diethylpyrocarbonate (DEPC), hexafluoroisopropanol (HFIP), phosphatidylglycerol (POPG) and thioflavin-T (ThT) were from Sigma-Aldrich (St. Louis, USA). Deuterium oxide (99.9%, D_2O) was from Aladdin Reagents (Shanghai, China). All other chemicals were of the highest grade available.

2.2. Synthesis of DEPC-modified IAPP

hIAPP was dissolved in HFIP and sonicated for 2 min, then diluted with a DEPC stock solution (pH 6.5) to a final concentration of 10 μM peptide and 20 mM DEPC [24]. After a 5 min reaction, two major products were identified by ESI-MS. The mono-acylated ethyl product (DMI) was purified and collected by reverse phase HPLC. hIAPP and DMI were qualified by HPLC, aliquoted and freeze-dried, and then stored at −80 °C till use as we previously described [25].

2.3. Nuclear magnetic resonance (NMR) spectroscopy

hIAPP and DMI were dissolved in 25 mM phosphate buffer (pH 7.4) and lyophilized 3 times with deuterium oxide (99.9%). The samples were dissolved in D_2O to a final concentration of 1 mg/mL, the NMR spectra were collected at 25 °C on an Agilent DirectDrive2 running at 600 MHz.

2.4. Protein aggregation

For amyloid formation, peptides were first dissolved in HFIP and sonicated for 2 min to produce a homogeneous solution, and then diluted in 25 mM phosphate buffer (pH 7.4), or in 25 mM phosphate buffer (pH 8.8), or in 25 mM NaAc buffer (pH 4.0) or in 25 mM phosphate buffer in D_2O with 50 mM NaCl, to a final concentration of 10 μM peptide and 1% HFIP. Stock solutions of $CuCl_2$ and $ZnCl_2$ were freshly prepared. Peptide solutions with or without metal ions (1:1000) were incubated at 25 °C without agitation. After co-incubation, the aggregated solutions were centrifuged (16,000 g × 30 min), the supernatant was collected and mixed with 8 M guanidine hydrochloride for HPLC analysis to assure that no soluble peptides existed (Fig. S1).

2.5. Thioflavin-T (ThT) fluorescence assays

ThT assays were performed as we previously described [26]. 10 μL of aggregated peptide solution was aliquoted at designated time points, and thioflavin-T based fluorescence assays were used to detect the formation of amyloid. Fluorescence was measured using a Hitachi FL-2700 fluorometer. The excitation and emission wavelengths were set at 450 nm and 482 nm respectively, and the slits were both 10 nm. The assay solution contains 25 mM PBS, 50 mM NaCl, pH 7.4 and 20 μM thioflavin-T. All experiments were performed in triplicate. The following formula was used to fit the kinetics curves, the lag time is described as $t_{50} - 2/k$, and the slope is described as k:

$$Y = Y_0 + (Y_{max} - Y_0) / [1 + \exp - (t - t_{50})k].$$

2.6. Far-UV circular dichroism (CD) spectroscopy

The CD spectra was recorded at 25 °C under a constant flow of N_2 using a JASCO-810 spectropolarimeter. Data was recorded from 260 to 190 nm with a 1 mm path length. hIAPP or DMI was dissolved in 25 mM phosphate buffer (pH 7.4), or in 25 mM NaAc buffer (pH 4.0) with 50 mM NaCl and 1% HFIP to a final concentration of 10 μM. For CD spectra with POPG vesicles, POPG was first dissolved in chloroform at a concentration of 20 mg/mL. After removing chloroform under a stream of nitrogen gas followed by an overnight drying under vacuum, the dry lipid film was rehydrated to make vesicles to a concentration of 40 mg/mL [27]. The vesicles were then subjected to 10 freeze–thaw cycles and filtered 21 times through a 220 nm polycarbonate filter to produce lipid membrane. 10% POPG lipid membrane (V/V) was added to the solution of 25 mM phosphate, 50 mM NaCl buffer (pH 7.4) or in 25 mM NaAc, 50 mM NaCl buffer (pH 5.5) and 1% HFIP to a final concentration of 10 μM peptide for CD spectra. The spectra were recorded at the time intervals indicated with a scanning speed of 50 nm/min, a response time of 1 s and a bandwidth of 2 nm. Each result is given as the average of three measurements. The data were converted to mean residue ellipticity [θ] and were further analyzed with the software CDPro as previously described [28].

2.7. Dye leakage measurement

Fluorescein was added to the POPG vesicles to a final concentration of 20 mg/mL. The solution was passed through a PD-10 column (Sangon, Shanghai, China) to remove fluorescein-nonencapsulated POPG vesicles, and the fluorescein-containing POPG vesicles were collected in a buffer containing 25 mM PBS and 50 mM NaCl (pH 7.4) for the dye leakage assay [29]. 1 mM stock solutions of hIAPP or DMI in DMSO were added to the fluorescein containing POPG vesicles to a final concentration of 1 μM. The fluorescence was recorded with the excitation and emission wavelengths set at 493 nm and 518 nm respectively, and the slits were both 10 nm. The fluorescence was recorded at 100 s after the addition of the peptide as pore formation is believed to be mediated by helical oligomers [30,31], complete disruption of the lipid vesicles was achieved by adding Triton X-100 to a final concentration of 0.2% (V/V) as described [32]. All experiments were performed in triplicate.

2.8. Transmission electronic microscopy (TEM)

5 μL of samples after aggregation was applied onto a 300-mesh Formvar–carbon coated copper grid (Shanghai, China) followed by