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New peptide inhibitors modulate the self-assembly of islet amyloid polypeptide residues 11–20 in vitro

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ABSTRACT

The structural transition and misfolding of human islet amyloid polypeptide may cause a common metabolic disease Type 2 diabetes mellitus. Seventeen peptides have been synthesized, possessing different lengths, compositions, and peptide conformation. In this study, the mechanism of these peptides on inhibiting the formation of $hIAPP_{11-20}$ amyloid fibrils was investigated using a conventional ThT fluorescence assay and microscale thermophoresis. The results showed that short peptides AT, SA, RF, KS, KT and KN, and cyclic peptides cyclic-KS, cyclic-KT and cyclic-KN displayed considerable inhibitory effect on $hIAPP_{11-20}$ fibril formation and a strong affinity for $hIAPP_{11-20}$. The detailed investigation indicated that the phenylalanine residue and some special peptide composition significantly inhibit amyloid formation. The peptide conformation of the designed peptide inhibitors may also play an important role. Microscale thermophoresis quantified the binding affinities between $hIAPP_{11-20}$ and the peptides; and revealed that high affinity binding more likely leads to inhibiting fibril formation of $hIAPP_{11-20}$ and vice versa, which is in accordance with the results from the ThT assays. These findings suggest a feasible model of peptide inhibitor design for inhibiting amyloid formation. In addition, microscale thermophoresis was proven as a promising rapid method for preliminarily screening inhibitors of $hIAPP_{11-20}$.

1. Introduction

Amyloid deposits formed by related amyloidogenic proteins or peptides, such as the prion protein, amyloid-β peptide, and human islet amyloid polypeptide (hIAPP), are associated with several neurodegenerative diseases involving spongiform encephalopathies, Alzheimer's diseases and type 2 diabetes mellitus (T2DM) (Selkoe, 2003; Ahmad et al., 2011). Diabetes is characterized by a sustained high level of blood glucose, depending on the decline in pancreatic β-cell function caused by metabolic disorders (Defronzo, 1988). hIAPP is a kind of hormone, containing 37 residues, co-secreted with insulin by pancreatic β-cells (Tomita, 2012). Fibrillation of hIAPP may deposit in the islets of Langerhans of the pancreas, damaging the cytoskeleton and triggering cellular apoptosis, which is found in over 90% of T2DM cases (Westermark et al., 1987; Scrocchi et al., 2002; Anguiano et al., 2002). Therefore preventing the formation of amyloid is a novel therapeutic approach for T2DM (Farah and Donangelo, 2006). The aggregates formed by hIAPP residues 11–20 (RLANFLVHSS) are shown to be similar to those generated by full-length hIAPP in vitro (Ahmad et al., 2011). $hIAPP_{11-20}$, accordingly, could be regarded as a simple template for investigation of inhibitors.

Some researchers have speculated that π-stacking interactions and hydrogen bonding forces may play a significant role in the self-assembly processes that lead to amyloid formation (Gazit, 2002; Kapurniotu et al., 2002). Inhibition of hIAPP fibril formation by peptides and small compounds has been reported in vivo and in vitro (Meng et al., 2010; Cheng et al., 2011, 2013; Kamihira-Ishijima et al., 2012; Figueroa et al., 2012).

As various peptide inhibitors (Scrocchi et al., 2002; Kapurniotu et al., 2002; Muthusamy et al., 2010; Potter et al., 2009; Tatarek-Nossol et al., 2005) come from some fragment regions of the full-length hIAPP, most of which are rich in specific residues (Ala, Leu, and Val), aromatic residues (Phe), and cationic residues (His), we designed and synthesized a series of peptides to study the inhibitory effects of different types of amino acid residues.

Since aggregation of hIAPP could be inhibited through π-stacking interaction, different aromatic residues (Phe and Trp) have been introduced in peptide inhibitors design. Different cationic residues

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(Arg, His, and Lys) also have been introduced in the designed peptides, because electrostatic repulsion may exert some effects on inhibition of fibril formation of hIAPP.

Peptide inhibitors reported in previous research (Scrocchi et al., 2002; Kapurniotu et al., 2002; Muthusamy et al., 2010; Potter et al., 2009; Tatarek-Nossol et al., 2005) are all short chain peptides. Therefore a series of short chain peptides, containing five amino acid residues, have been designed such as KT, KS and KN with the same cationic residues (K), the same aromatic residues (F) and different middle residues, which are hydrophilic amino acids to improve the solubility. As the π bond is delocalized in a wider region in tryptophan than in phenylalanine, the phenylalanines in KT, KS, and KN are replaced by tryptophans to design new peptides KW and RW to investigate the effect of aromatic residue on inhibition of $hIAPP_{11–20}$ aggregation. As the positive charges of arginine display larger delocalization degree compared to lysine, RW and RF have been designed to compare the inhibitory effect of different cationic residues. Furthermore, peptides (FR and RH) containing multi-cation residues were also synthesized to enhance the electrostatic repulsion. To sum up some reported peptide inhibitors (Scrocchi et al., 2002; Kapurniotu et al., 2002; Muthusamy et al., 2010; Potter et al., 2009; Tatarek-Nossol et al., 2005), most of which are rich in A, N, F, L, V, H, S and G, and therefore peptides (AT and SA) containing some of these reported residues were also investigated, but AT and SA did not have any cationic residues and aromatic residues.

Comparing the inhibitory effects on $hIAPP_{11–20}$ fibril formation by these short chain peptides, some effective peptides have been selected for further improvement. In details, two identical short peptides have been connected by the GSG amino acid sequence to enhance the interaction with $hIAPP_{11–20}$. This approach has led to the synthesis of homodimers double-KS, double-KT, and double-KN. For comparison, the homodimer of the less effective inhibitor double-RW was also prepared.

The double peptides are linear peptides, possessing flexible conformation which has been shown in the previous report (Yu et al., 2013), and may hide their effective residues and hinder their interaction with targets. Therefore , we further designed cyclic peptides with relatively rigid structures to study their effect on the inhibition of $hIAPP_{11–20}$ aggregation. Disulfide bonds were introduced to reduce the flexibility of the double peptides. The two terminal residues of these double peptides were connected with two cysteines, forming cyclic peptides through the intra-disulfide bond formed by the thiol group of the two cysteines. Double-KN, double-KS and double-KT were changed into cyclic-KN, cyclic-KS and cyclic-KT, respectively. All these designed peptides are listed in Table 1.

Microscale thermophoresis (MST) was applied to study the inhibitor-$hIAPP_{11–20}$ interaction and to speculate on possible inhibition mechanism. This technique is an immobilization-free method in which thermophoresis, the directed movement of fluorescently labeled molecules in a temperature gradient, is monitored to determine binding curves (Baaske et al., 2010). In contrast to some common methods, such as enzyme-linked immunosorbent assays, surface plasmon resonance, and fluorescence resonance energy transfer, MST allows characterizing ligand-target interactions with advantages in terms of simple set-up, immobilization-free, sample-efficient, and time-saving properties (Mao et al., 2015; Lippok et al., 2012).

In this study, some peptides were designed so as to have different lengths, compositions, and conformations. Using the $hIAPP_{11–20}$ modle, these inhibitors were assessed for their ability to decrease aggregation. The inhibitory effects of different types of amino acid residues were investigated. To further understand the inhibition mechanism, MST was utilized to quantify affinities between amyloidogenic peptides and inhibitors, deducing a feasible model of peptide inhibitor design for hIAPP.

Table 1
The sequence of potential peptide inhibitors.

Peptide abbreviation	Peptide sequence
AT	Ac-AMVGT-NH_2
SA	Ac-SSVMA-NH_2
FR	Ac-FRWWRR-NH_2
RH	Ac-RHWWRF-NH_2
KS	Ac-KFSFK-NH_2
KW	Ac-KWTWK-NH_2
KT	Ac-KFTFK-NH_2
KN	Ac-KFNFK-NH_2
RF	Ac-RFTFR-NH_2
RW	Ac-RWTWR-NH_2
double-KT	Ac-KFTFKGSGKFTFK-NH_2
double-KN	Ac-KFNFKGSGKFNFK-NH_2
double-KS	Ac-KFSFKGSGKFSFK-NH_2
double-RW	Ac-RWTWRGSGRWTWR-NH_2
cyclic-KS	Ac-Cys-KFSFKGSGKFSFK-Cys
cyclic-KN	Ac-Cys-KFNFKGSGKFNFK-Cys
cyclic-KT	Ac-Cys-KFTFKGSGKFTFK-Cys

2. Material and methods

2.1. Apparatus

An LS55 Fluorescence Spectrofluorometer system (PerkinElmer Inc., UK) was employed for the fluorescent measurements. Time curves of thermophoresis were conducted using the microscale thermophoresis instrument (Monolith NT.115 system, NanoTemper Technologies GmbH, Germany). Affinities of molecules were calculated by the NT ANALYSIS SOFTWARE provided by NanoTemper Technologies GmbH. All the pH values were measured with a PHS-3C precision pH meter (Leici Devices Factory of Shanghai, China), which was calibrated with a standard buffer solution each day.

2.2. Materials and reagents

$hIAPP_{11–20}$ and fluorescein isothiocyanate (FITC) labeled $hIAPP_{11–20}$ were purchased from GL Biochem Ltd. (Shanghai) with >95% purity. Thioflavin-T (ThT), hexafluoroisopropanol (HFIP), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Aladdin Reagent (Shanghai, China). Seventeen peptides were synthesized by GL Biochem (Shanghai) Ltd with purity >90% (Table 1) and used without further purification. HEPES buffer 10 mmol/l pH 7.4, containing 10.0 mmol/l NaCl was used for all experiments unless otherwise specified. Ultrapure water was obtained from Molgene 1830I ultrapure water system (Chongqing Molecular Water System Co. LTD., China). All other chemicals and reagents were of analytical grade and used without further purification.

2.3. Background of thermophoresis

MST (Duhr and Braun, 2006), a new proposed biophysical method, monitors the directed movement of molecules along a temperature gradient through fluorescence intensity of the FITC label on the $hIAPP_{11–20}$ (FITC-labeled $hIAPP_{11–20}$). The FITC-labeled $hIAPP_{11–20}$ in the capillaries was excited and the fluorescence was detected using the same objective. In MST measurements, capillaries containing a series of concentration gradient of $hIAPP_{11–20}$ and constant concentration of the labeled $hIAPP_{11–20}$ were scanned and detected. The thermophoresis of a molecule differs significantly from that of a molecule-partner complex depending on binding-induced changes in conformation. The differences in thermophoresis of molecules could be used to quantify the binding affinities (the dissociation constant, K_d) by titration experiments.

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