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Research paper

Analysis of multiply charged monomers and dimers of human islet amyloid polypeptide by collision-induced dissociation with electrospray ionization mass spectrometry

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HIGHLIGHTS

- Multiply charged monomers and dimers of hIAPP were investigated through CID-MS/MS.
- hIAPP monomers adopt different structures depending on the parent ion charge state.
- hIAPP 1-15 are proposed as an interaction area in dimers in low energy CID conditions.

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ABSTRACT

Collision-induced dissociation (CID) with ESI-MS was used to obtain structural information on early-stage aggregation of hIAPP (human islet amyloid polypeptide) monomers and oligomers. MS analysis showed that the hIAPP monomers and oligomers were multiply charged. MS/MS analysis indicated that the hIAPP monomers adopt different structures depending on the parent ion charge state. The fragmentation patterns indicated structural similarity of M^{2+} & M^{3+} and M^{4+} & M^{5+} (M = monomer). MS/MS analysis of the dimers showed that D^{5+} (D = dimer) comprised M^{2+} and M^{3+} subunits, and the peptide bond dissociated in the 15–37 residue region of the monomer subunit.

1. Introduction

The pathology of type-2 diabetes is attributed to amyloid plaques that contain fibrillar aggregates of human islet amyloid polypeptide (hIAPP), a 37-residue hormone selectively expressed in pancreatic β-cells [1–5]. These fibrillar aggregates are formed by self-assembly of peptides into unbranched elongated structures and have a filamentous morphology, a cross-β spine, steric zipper structures, and are cytotoxic [6–10]. Several studies indicate that the smaller prefibrillar hIAPP oligomers exhibit greater neurotoxicity than the mature fibrils [11–14]. Accordingly, understanding the structure and formation of early hIAPP aggregates is a prospectively important platform for the study of therapeutic agents for inhibiting disease-related structures.

Accordingly, there has been much interest in the early oligomerization of hIAPP [15–21]. Dimerization of hIAPP was observed through biomolecular fluorescence complementation analysis [22]. Crystal structures derived from MBP-hIAPP fusion experiments indicated helix–helix-based interactions between the dimers in the 8–18 residue region [23], which coincides with findings that show a preference for the helical conformation in the N-terminal 5–16 residue region [24]. Further, computational analysis identified strong contributions of the Leu12 and Phe15 residues to the conformation of the helical dimer [25]. ESI-IMS studies suggest β-hairpin monomer structures that form extended dimer conformers [26–28]. Molecular dynamics simulations also suggest that the dimers formed from the β-hairpin monomers feature interactions at residues 11–18 and 23–32 [29]. Other studies indicate different mechanisms of dimerization. Using 2D infrared spectroscopy, transitional oligomers with interactions of the disordered loop region of hIAPP [30] were observed, whereas Wei et. al indicated that the formation of low-order oligomers was associated with direct binding of the monomers involving His18-Tyr37 [31]. Further careful study into the hIAPP monomer and early oligomer structures for better clarification of the early hIAPP oligomerization process is thus merited.

Herein, we use collision-induced dissociation (CID) in conjunction with electrospray ionization mass spectrometry (ESI-MS) to obtain structural information [32–33] on the hIAPP monomers and oligomers

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in the early stages of aggregation. These oligomer complexes are allowed to form in solution and are electrosprayed onto a quadrupole ion guide. ESI-MS is assumed to produce intact gas-phase oligomer complex ions from the oligomer complex in solution. The fragmentation patterns for the structures of hIAPP are investigated in (50/50, H_2O/CH_3OH) solution.

2. Material and methods

2.1. Mass spectrometry

The experimental MS and MS/MS data for the fragmentation pattern analysis were obtained using a Thermo Finnigan LTQ mass spectrometer (Thermo Electron Corp., San Jose, CA, USA). All spectra were acquired in the positive ion mode over the *m/z* range of 50–2000 by averaging 100–2000 scans. The CID-MS/MS experiments were conducted at capillary temperatures of 200 °C, which resulted in the best signal/noise ratios in the MS/MS spectra.

The electrospray needle voltage was set to 3.3–3.5 kV. The samples were introduced into the electrospray interface by a direct infusion method using a microsyringe pump (HAMILTON, USA) at a flow rate of 1–2 μL min^{-1}. The MS/MS spectra were acquired under the following experimental conditions: isolation width: 1–1.5 mass units, activation time: 30 ms, and injection time: 100–200 ms. In the MS/MS analysis, the parent ion molecules were individually and manually selected and then subjected to the CID process. The normalized collision energies were optimized for each MS/MS experiment using the minimal collision energy that would allow the fragments to be viewed at sufficient signal-to-noise ratios.

2.2. Reagents

$hIAPP_{1-37}$ (Bachem, Switzerland) peptides were used in the experiments. HPLC-grade H_2O (Merck Ltd., Korea) and HPLC-grade CH_3OH (Merck Ltd., Korea) were used as the solvents. The peptides were dissolved in H_2O:CH_3OH (1:1, v/v) to prepare 6×10^{-5} M solutions. These specified solutions were prepared for a sufficient D^{5+} ion intensity in CID-MS/MS experiments. The peptide solutions of Ref. [28] were prepared in 100 mM ammonium acetate buffer at pH 7.0 for final peptide concentrations of 20 μM. Experiments were performed within 24 h of sample preparation.

3. Results and discussion

3.1. Mass spectrum

Under the present ESI experimental conditions, the mass spectrum of hIAPP indicated the presence of multiply charged monomers and oligomers (Fig. 1). In the hIAPP MS spectrum, monomer peaks were observed at *m/z* 1951.4, 1301.3, 976.2, and 781.2, indicating that the monomers had multiple charges ranging from 2+ to 5+. The hIAPP sequence has three basic residues (Lys1, Arg11, His18) and an N-terminal position available for protonation. Depending on the pk_a value, we speculate that protonation occurs at Lys1 and Arg11 for M^{2+} (M = monomer, *m/z* 1951.4), with incremental protonation at the N-terminal amide for M^{3+} (*m/z* 1301.3), and at His18 for M^{4+} (*m/z* 976.2) [27]. The monomers with charges of 3+ and 4+ show particularly high intensity peaks. The intensity of the signals from the M^{5+} (*m/z* 781.2) monomer was lower than that of the other monomer peaks, and it is unclear where the 5th protonation occurs. In the case of the oligomers, peaks were observed at *m/z* 1561.4, 1301.3, 1115.5, 1672.8, and 1463.8, corresponding to D^{5+}, D^{6+}, D^{7+}, T^{7+}, and T^{8+} (D = dimer and T = trimer), respectively.

3.2. MS/MS spectra of the monomers

CID-MS/MS experiments were conducted to obtain structural information on the hIAPP monomer and dimer in the early stages of aggregation. For the MS/MS spectra, the fragment ions are labeled using different colors and shapes based on the region of the sequence in order to compare the fragmentation pattern of each parent ion. We observed three main b_u ion fragmentation regions at u = 11–19, u = 20–29, and u = 30–37, which we termed ***A***, ***B***, ***C***, respectively. The MS/MS spectra of the hIAPP monomers are shown in Fig. 2. The fragment ion assignments for Fig. 2 are presented in Supplementary Tables S1–4.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.cplett.2018.08.001.

In the MS/MS spectrum of hIAPP M^{2+} (Fig. 2a), we observed the fragmentation of all three regions, i.e., ***A*** (b_u series, u = 11–19), ***B*** (b_u series, u = 20–29), and ***C*** (b_u series, u = 30–37). The fragments in the ***A*** region were singly charged positive ions, in which one charge is removed from the parent ion. The fragments in the ***B*** and ***C*** regions were doubly charged positive ions. The MS/MS fragmentation patterns are summarized in Table 1. Fragments in which one charge is removed from the parent ion are underlined.

In the MS/MS spectrum of hIAPP M^{3+} (Fig. 2b) fragmentation of the three regions was observed, as in the case of M^{2+}. In all fragmentation regions, fragment ions were observed with a charge of 2+, where one charge was removed from the parent M^{3+} ion. The peaks with the highest intensities in the three fragmentation regions were those of the b_{17}, b_{18}, b_{26}, b_{27}, and b_{32} fragment ions. The patterns of the high intensity peaks in each region were similar to the pattern of M^{2+}. Thus, we speculate that the structure of hIAPP M^{3+} is similar to that of M^{2+} and has weak bonds at V17-H18-S19, I26-L27-S28, and V32-G33. In addition, $b_u{}^{3+}$ fragment ions that retained the valence of the parent ion were also found in the ***C*** region.

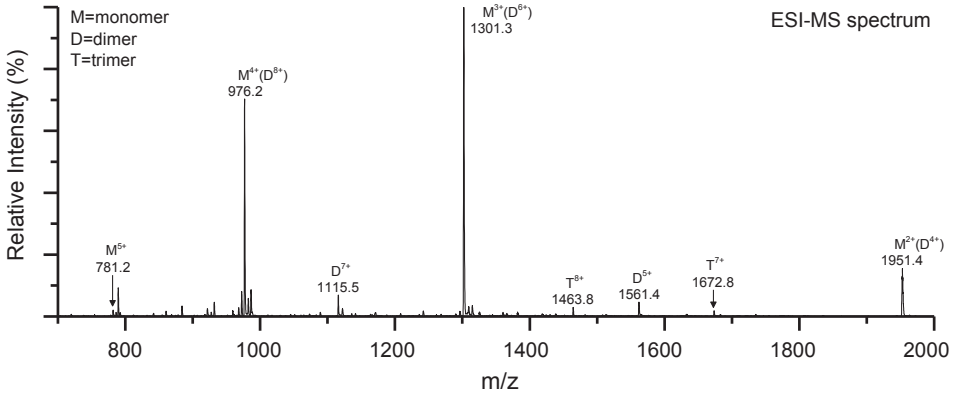

Fig. 1. ESI-MS spectrum of human islet amyloid polypeptide (hIAPP). Multiply charged monomers and oligomers are represented as M^{z+}, D^{z+}, and T^{z+} (M = monomer, D = dimer, T = trimer, and z = charge state).