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Young Scientist Feature

Multiply charged oligomer complexes composed of the amyloid-forming peptides NNQQNY, VQIVYK, and LYQLEN analyzed by collision-induced dissociation with electrospray ionization mass spectroscopy

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ABSTRACT

Recent research has implicated small, toxic, insoluble oligomeric assemblies as factors in amyloidogenic diseases, but information regarding their biological features, structures, and formation mechanisms has been difficult to obtain. Many shorter but biologically active sequences have also been identified in the larger sequences of amyloid proteins that are themselves capable of forming amyloid fibrils. Here, we used collision-induced dissociation (CID) with electrospray ionization mass spectroscopy (ESI–MS) to gain insights into the self-assembly process and structural information of amyloidogenic oligomers. We selected three tyrosine-containing sequences, NNQQNY, VQIVYK, and LYQLEN, which are known to form ordered β-sheet structures characteristic of amyloid fibrils, and another sequence, YGGFL, which is known to form isotropic structures. Y → A substituted sequences, NNQQNA, VQIVAK, and LAQLEN, were also investigated by CID-MS/MS. Our MS/MS analysis suggests that Y–Y interactions are important in dimer binding, and the charge state of the multiply charged oligomers is related to the formation of β-sheet.

1. Introduction

Aggressive formation of amyloid deposits is found in a wide variety of diseases, including Alzheimer's disease and type 2 diabetes [1,2]. These deposits contain insoluble protein fibrils that are formed by self-assembly of peptides into unbranched elongated structures [3], characterized by a cross-beta spine formed by pairing of beta sheets and dehydrated interdigitation of side chains, known as steric zipper interfaces [4–6]. Recently, shorter active sequences isolated from these proteins have been found to have similar amyloidogenic properties [6–8]. Research so far has largely focused on characterization of structures of insoluble, mature fibrils, especially in their crystallized states [9], but the self-assembly processes of soluble oligomers are less understood. Elucidation of the structure and formation of these oligomers is important, as these prefibrillar oligomers are main factors in amyloid disease pathogenesis [1,9–12]. However, information regarding initial structure and oligomer formation has been difficult to obtain owing to their transient and dynamic nature of the oligomers.

Research on the active sequences has shown that there are several side chain interactions controlling the assembly of these peptides: hydrogen bonds [13], hydrophobic interactions [4,14], aromatic residue interactions [15–18], and interactions between charged groups [19–21]. Previous studies used ion mobility spectroscopy to investigate the collision cross section of each mass extracted aggregation state of short active sequences, and the transition from a globular to an ordered β-strand structure was observed [22].

To further investigate this transition and the known influence of side chain residues, we used collision-induced dissociation (CID) in conjunction with electrospray ionization mass spectroscopy (ESI–MS). Three tyrosine-containing active sequences, known to form ordered oligomers with β-strand structures that mature into fibril structures, were chosen: NNQQNY from the yeast prion protein Sup35 [23–25], VQIVYK from tau protein [13,26–28], and LYQLEN from the human insulin chain A [29]. As a negative control, we included YGGFL, leucine enkephalin, which is known to form only isotropic structures [30,31]. MS/MS experiments were also conducted on peptides with tyrosine residues substituted for alanine residues (NNQQNA, VQIVAK, and LAQLEN) in order to further characterize the influence of the tyrosine residues on the early oligomerization process.

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Oligomer complexes were allowed to form in water and were electrosprayed onto a quadrupole ion guide. ESI–MS is assumed to produce intact gas-phase oligomer complex ions from the oligomer complex in solution [32–35]. Observable oligomers were characterized, mass extracted, and fragmented via a minimal energy CID process through helium particles.

2. Material and methods

Experimental MS and MS/MS data for fragmentation pattern analysis were obtained using a Thermo Finnigan LTQ mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA).

2.1. MS conditions

All spectra were acquired in the positive ion mode over an m/z range of 50–2000 by averaging 100–2000 scans. CID-MS/MS experiments were conducted at capillary temperatures of 100 °C, 150 °C, and 200 °C. Ultimately, capillary temperatures were set to 200 °C, which resulted in the best signal/noise ratios in MS/MS experiments.

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. The MS/MS spectra were acquired under the following experimental conditions: an isolation width of 1–1.5 mass units, an activation time of 30 ms, and an injection time of 100–200 ms. In MS/MS, the parent ion molecules were individually and manually selected and then subjected to the CID process. Normalized collision energies were optimized for each MS/MS experiment using the minimal collision energy that would still allow fragments to be viewed at significant intensities.

2.2. Reagents

NNQQNY (>95%, Peptron, Daejeon, Korea), VQIVYK (>95%, Peptron, Daejeon, Korea), LYQLEN (>95%, Peptron, Daejeon, Korea), NNQQNA (>95%, Peptron, Daejeon, Korea), VQIVAK (>95%, Peptron, Daejeon, Korea), LAQLEN (>95%, Peptron, Daejeon, Korea), and YGGFL (>95%, Peptron, Daejeon, Korea) synthetic peptides and HPLC-grade H_2O (Merck Ltd. Korea) were used in the experiments. These peptides were dissolved in H_2O to prepare 5×10^{-4} M solutions. The experiments were performed within one day of sample preparation.

3. Results and discussion

3.1. Peptide MS spectra and MS/MS spectra of peptide monomers

Under our ESI–MS experimental conditions, clear oligomerization of the three amyloidogenic peptides was observed in the positive ion mode. Mass spectra of the three amyloidogenic peptides are shown in Fig. 1, and their relative intensities are summarized in Table S1 (Supplementary information). In the case of NNQQNY and LYQLEN, 2/1, 3/2, 4/3, 5/2, 5/3, 5/4, 7/3, 7/4 charged oligomers (n/z where n = number of peptides and z = charge state) were observed. In the case of VQIVYK, 4/3 and 5/4 charged oligomers were not observed.

MS/MS analysis through collision-induced dissociation (CID) was used to obtain fragmentation pattern data for n/z oligomers, as shown in Fig. 1, and to acquire structural information of amyloidogenic oligomers. MS/MS analysis of monomer (n/z = 1/1) CID experiments are shown in Fig. 2. Fragment ions are represented as $(M\text{-}y_1)^{1+}$, $(M\text{-}y_2)^{1+}$, $(M\text{-}y_3)^{1+}$, and $(M\text{-}b_1)^{1+}$, $(M\text{-}b_2)^{1+}$, $(M\text{-}b_3)^{1+}$, where M is the monomer, $-y_1$ represents 'y_1 loss', and 'xx loss' is used to emphasize a fragmentation pattern. Conventional notation (y''_n and b_t, as proposed by Roepstorff and Fohlman [36,37]) in MS/MS is typically employed to elucidate a linear monomer sequence instead of a peptide assembly. However, the use of conventional notation in the present work would lead to ambiguity in data analysis, particularly in the analysis of fragment ions lost from oligomers. For convenience, the neutral species $[y''_n\text{-}2H]^0$ and $[b_t]^0$ are represented as y_n and b_t, respectively. Series of single b_1, b_2, b_3... loss fragment ions are represented in curly brackets, e.g. {b_t loss} series. Likewise, y_n loss fragment ions were represented as {y_n loss} series. The proposed nomenclature is summarized in Table 1.

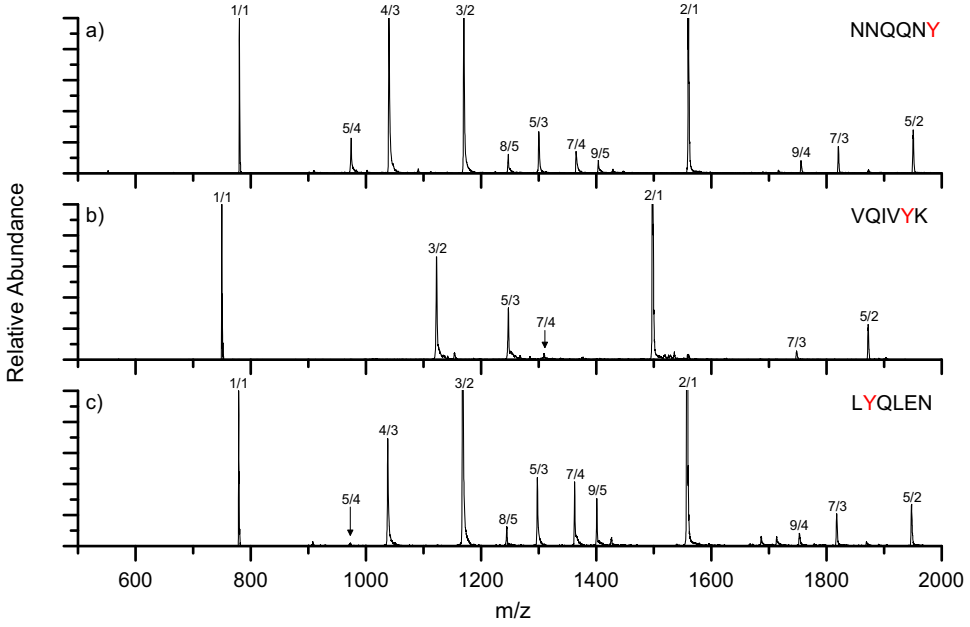

Fig. 1. ESI–MS spectra of (a) NNQQNY, (b) VQIVYK, and (c) LYQLEN. The peak intensity between m/z = 900 and 2000 was amplified by a factor of 30. Peaks are labeled with their respective oligomer-to-charge ratios (n/z).