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Oligomer Complexes of the (VQIVYK + NNQQNY) and (VQIVYK + LYQLEN) Mixing Solutions

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Abstract : The π-π interactions of the peptide-dimer and peptide-trimer complexes were investigated in the (VQIVYK + LYQLEN) and (VQIVYK + NNQQNY) mixing solutions. The results showed that tyrosine (Y) residues were critical in the formation of hetero peptide-dimers and -trimers during the early oligomerization process. We used collision-induced dissociation (CID) along with electrospray ionization mass spectroscopy (ESI-MS) to obtain the structural information of the hetero-dimers and -trimers. We chose three amyloidogenic peptides–VQIVYK, NNQQNY, and LYQLEN–from tau protein, yeast prion-like protein Sup35, and insulin chain A, respectively. Hetero-dimer, -trimer, -tetramer, and -pentamer complexes were observed in the mass spectra. The tandem mass spectrum of the hetero-dimer and hetero-trimer showed two different fragmentation patterns (covalent and non-covalent bond dissociation). Y-Y interaction structures were also proposed for the hetero-dimer and -trimer complexes.

Keywords : Peptide oligomer, VQIVYK, NNQQNY, LYQLEN, mass spectrometry (MS), MS/MS

Introduction

Amyloid deposits have been found in a wide variety of diseases including Alzheimer's disease and type 2 diabetes.[1-2] These deposits are formed during the peptide self-assembly process via steric zipper interfaces. They are double beta-sheets, where each sheet is formed from parallel or anti-parallel segments stacked in-register.[3-5] Active short segments from fibril-forming proteins have also demonstrated similar amyloidogenic properties in X-ray fibril diffraction patterns.[6-7] Although the insoluble, mature fibrils have been well-investigated especially in their crystallized states, the soluble oligomers from the early stage of the self-assembly processes are less understood. Unfortunately, the oligomer formation process has proven difficult to investigate owing to the transient and dynamic nature of oligomers.

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Several interactions (hydrogen bonds,[8] hydrophobic,[9] charged groups,[10-12] and π-π interaction[13-16]) have been reported between short active sequences by many research groups. The π-π interactions and tyrosine-mediated two-dimensional peptide assembly were studied via quantum calculation or experimental methods.[17-24] The π-π interactions were reported in the several research methods even though the amount of benzene–benzonitrile interaction energy was proposed as 3.3 kcal/mol in quantum calculation.[17] Three tyrosine (Y)-containing active sequences (VQIVYK from tau protein,[19-20] NNQQNY from the yeast prion protein Sup35,[21-22] and LYQLEN from the human insulin chain A[23]), known to form ordered oligomers with β-strand structures that mature into fibril structures, were investigated to understand the π-π interaction using collision-induced dissociation mass spectroscopy (CID-MS/MS) experiments.[24]

The present paper focuses on the π-π interaction in [peptide-peptide] heterogeneous peptide-dimer (hetero-dimer, peptide-dimer with two different peptide species) and heterogeneous peptide-trimer (hetero-trimer) complexes. We present the results of a tandem mass spectrometry and multiple mass spectrometry study that investigated the interaction of VQIVYK with the NNQQNY or LYQLEN peptides. As a negative control, MS/MS experiments were also conducted on VQIVAK peptides by substituting tyrosine with alanine (VQIVYK → VQIVAK) in order to further characterize the influence of the tyrosine residues on the early oligomerization

process. Homogeneous peptide-dimer (peptide-dimer with the same peptide species) and hetero-dimer complexes were allowed to form in water and electrosprayed onto a quadrupole ion guide. Electrospray ionization mass spectroscopy (ESI-MS) is assumed to produce intact gas-phase dimer or trimer complex ions from the dimer or trimer complexes in solution. Observable dimers and trimers were characterized, mass extracted, and fragmented via a minimal energy CID process using helium particles.

Experimental

Mass Spectrometry

Experimental MS and MS/MS data for fragmentation pattern analysis was obtained using a Thermo Finnigan LTQ mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA). All spectra were acquired in the positive ion mode over an m/z range of 100–2000 by averaging 100–3000 scans. The CID-MS/MS experiments were conducted at capillary temperatures of 150°C, which resulted in the best signal:noise ratios in the MS/MS spectra. The electrospray needle voltage was set between 3.3–3.5 kV and the samples were introduced into the electrospray interface via 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, activation time of 30 ms, and injection time of 100–200 ms. In MS/MS, the parent ion molecules were individually and manually selected and then subjected to CID. Normalized collision energies were optimized for each MS/MS experiment using the minimum collision energy that allowed fragments to be viewed at sufficient signal:noise ratios.

Reagents

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

Results and discussion

MS Spectra

A previous study indicated the importance of Y residues in the early oligomerization process of single tyrosine-containing active sequences.[24] Therefore, three amyloidogenic peptides containing Y residues at varying locations were selected to investigate its effect on the Y-Y interactions of [peptide-peptide] complexes. Figure 1 shows the MS spectra of two mixing peptide solutions, (VQIVYK + NNQQNY) and (VQIVYK + LYQLEN). The MS spectra of single peptide solutions of the three amyloidogenic peptides can be found in ref. 24. The peak intensity between m/z = 900 and 2000 was observed to be amplified by a factor of 30. The peaks are labeled with their respective (peptide①:peptide②) ratios. The $2^{1+}(20)$ represents a single charge of the (two peptide①:zero peptide②) homo-dimer complex. In Figure 1a, the $2^{1+}(11)$ notation shows that the hetero-dimer complex is composed of (one VQIVYK peptide① + one NNQQNY peptide②) with a single positive charge. The $4^{2+}(31)$ notation means that the hetero-tetramer complex is composed of (three VQIVYK peptide① + one NNQQNY peptide?) with double positive charges.

In general, the intensities of the homo-dimer and homo-oligomer spectral peaks decrease with an addition of the second different Y-containing peptide. It is assumed that the added Y-containing peptide plays a role in decreasing the population of the early homo-dimer, $2^{1+}(20)$ and $2^{1+}(02)$, or homo-oligomer complexes. Here, the homo-dimer intensities decreased prominently when compared to those in ref. 24. As shown in Figure 1, the hetero-dimer, -

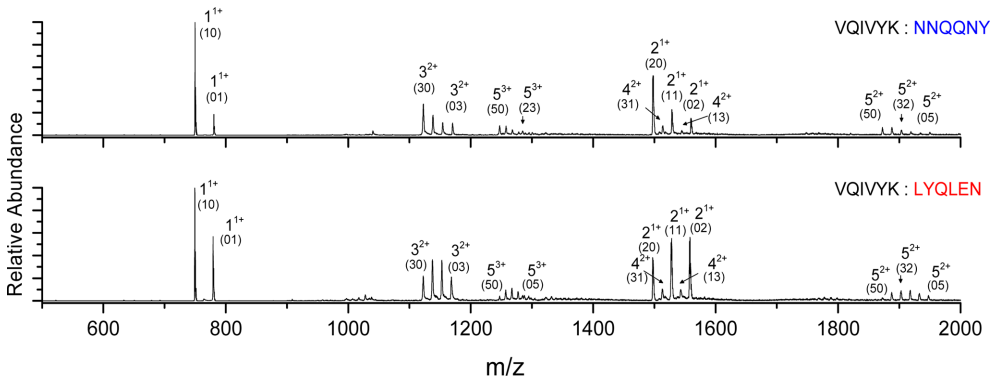


Figure 1. ESI-MS spectra of the a) (VQIVYK + NNQQNY) solution and b) (VQIVYK + LYQLEN) solution. The peak intensity between m/z = 900 and 2000 is amplified by a factor of 30.

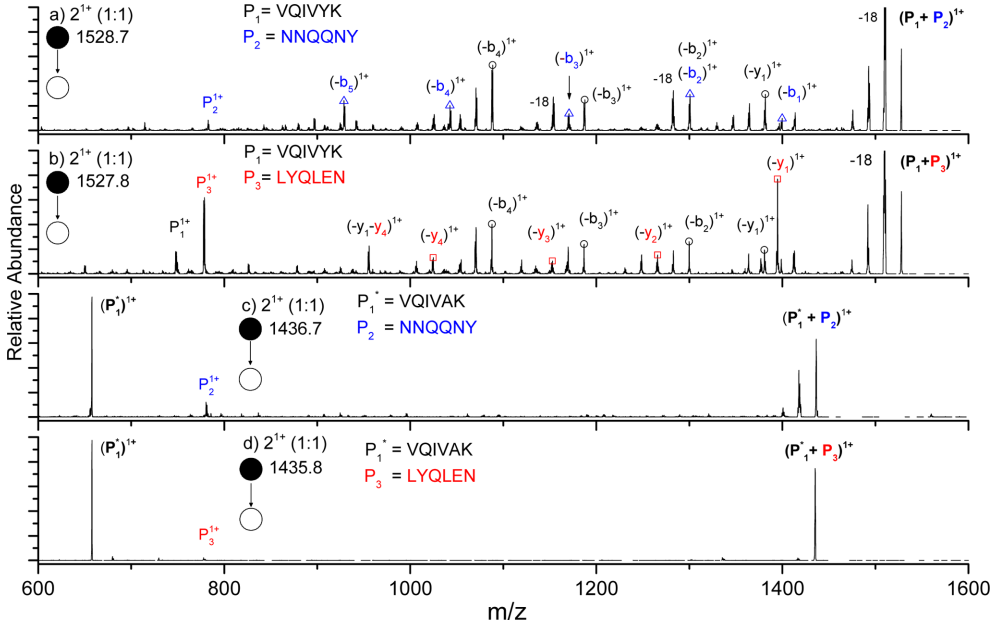


Figure 2. MS/MS spectra of the 2^{1+}(11) hetero-dimer in the a) (VQIVYK + NNQQNY), b) (VQIVYK + LYQLEN), c) (VQIVAK + NNQQNY), and d) (VQIVAK + LYQLEN) solutions. Peaks are labeled as $(-B_u)^{1+}$ or $(-y_n)^{1+}$ for space.

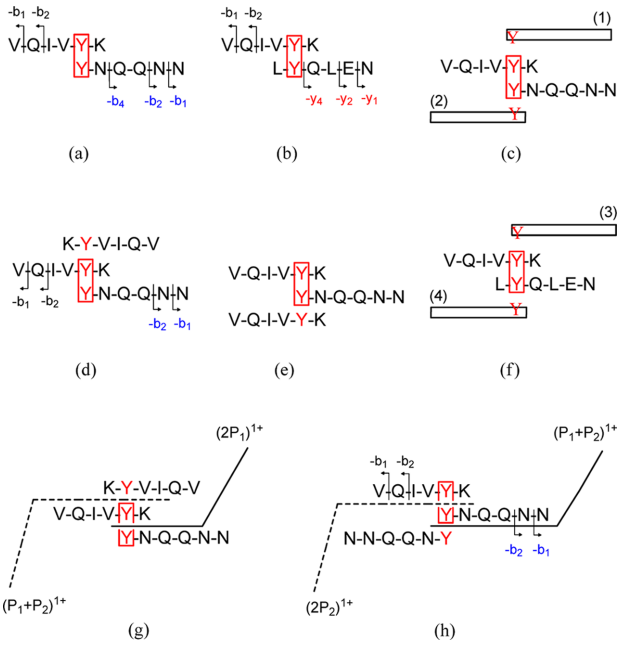
trimer, and -tetramer complexes are observed as 2^{1+}(11), 3^{2+}(21), 3^{2+}(12), 4^{2+}(31), and 4^{2+}(13). The observed complexes up to their pentamer configuration and m/z values of the peptide complex peaks are listed in the Electronic Supplementary Information.[†]

MS/MS Spectra

MS/MS spectra of peptide dimers

CID experiments were conducted to understand the possible structures of the hetero-dimer and hetero-trimer complexes. For the MS/MS spectra, fragment ions were assigned in the 'xx loss' form to emphasize the patterns of fragment loss in the covalent bond dissociation process. Fragment ion nomenclature has been described previously.[24-25] For convenience, the neutral species $[y_n"\text{-}2H]^0$ and $[b_u]^0$ are represented as y_n and b_u, respectively. Series of single b_1, b_2, b_3… loss fragment ions are represented within curly brackets as in the {b_u loss} series. Likewise, y_n loss fragment ions are represented as {y_n loss} series. Moreover, the fragmentation patterns of the three peptides, VQIVYK, NNQQNY, and LYQLEN, were observed via the {b_u or y_n loss} series in the covalent bond dissociation process of peptide monomers and homo-dimers.[24]

In the MS/MS spectra of 2^{1+}(11) hetero-dimers shown in Figure 2a-2b, two different fragmentation patterns–non-covalent and covalent bond dissociation–were observed. Non-covalent bond dissociation channels occurred between the monomer subunits and were observed as 1^{1+} peaks in the 2^{1+}(11) MS/MS spectra. Covalent bond dissociation



Scheme 1. Schematic diagram of the proposed structures for a) 2^{1+}(11) hetero-dimer in the (VQIVYK + NNQQNY) solution and b) 2^{1+}(11) hetero-dimer in the (VQIVYK + LYQLEN) solution. c) Two potential locations for an additional third VQIVYK peptide on the 3^{2+}(21) hetero-trimer complex are indicated as (1) and (2), leading to two potential structures as shown in d) and e). f) Two potential locations for an additional third peptide on the 3^{2+}(21) hetero-timer complex are indicated as (3) and (4) in the (VQIVYK + LYQLEN) solution.

channels occurred along the peptide bonds and were observed as the {b_u loss} or {y_n loss} series in 2^{1+}(11) MS/MS spectra. The fragmentation patterns of the (VQIVYK + NNQQNY) hetero-dimer 2^{1+}(11) MS/MS spectra are shown in Figure 2a.

The different fragmentation patterns of the (VQIVYK + LYQLEN) hetero-dimer 2^{1+}(11) MS/MS spectra are shown in Figure 2b. A different {y_n loss} series up to n = 4 is observed for the LYQLEN covalent bond dissociation channels because of the Y2 position of the N-terminal part in the LYQLEN peptide. This observation of {y_n loss} series for LYQLEN dissociation is in contrast to the {b_u loss} series for NNQQNY dissociation in Figure 2a. However, the {b_u loss} series can still be observed for VQIVYK dissociation. These results suggest a Y5-Y2 interaction i.e., residue Y5 in VQIVYK and Y2 in LYQLEN, in the hetero- dimer 2^{1+}(11) structure as shown in Scheme 1b.

Non-covalent bond dissociation into monomer unit fragments was also observed in the MS/MS spectra of hetero-dimer 2^{1+}(11) parent ions (Figure 2a-2b). However, it is difficult to deduce hetero-dimer 2^{1+}(11) structural information from non-covalent bond dissociation data because the unit fragmentation process is commonly possible from any reported dimer structure (e.g., parallel, anti-parallel, steric zipper, and Y-Y interaction). In Figure 2c-2d, the monomer unit fragment is observed as the main fragment in the MS/MS spectra of the hetero-dimer 2^{1+}(11) complex in the Y→A substituted VQIVAK, (VQIVAK + NNQQNY), and (VQIVAK + LYQLEN) mixing solutions. Therefore, we speculate that the Y residue in the VQIVYK peptide plays an important role in the interaction process at the hetero-dimer 2^{1+}(11) complex structure. The importance of aromatic side group residues in π-π stacking interactions was reported in the amyloid formation of NFGAILSS sequences. Modification of phenylalanine into aliphatic amino acid residues significantly reduced the ability of the peptide to form typical amyloid fibrils. In contrast, modification of phenylalanine residues to tryptophan or tyrosine (another aromatic amino acid) allows the formation of amyloid-like structure.[26-28]

MS/MS spectra of peptide trimers

The double charged hetero-trimers were analyzed via MS/MS spectra using the (VQIVYK + NNQQNY) and (VQIVYK + LYQLEN) mixing solutions. Similar to the hetero-dimer MS/MS spectra, the 3^{2+}(12 or 21) MS/MS spectra showed two different fragmentation patterns, covalent and non-covalent bond dissociation (Figure 3a-3d). Covalent bond dissociation channels occurred along peptide bonds and were observed as{b_u loss} or {y_n loss} series (Figure 3). It appears as though the Y-Y interaction structures are maintained in the double charged hetero-trimer structures. The possible schematic structures are

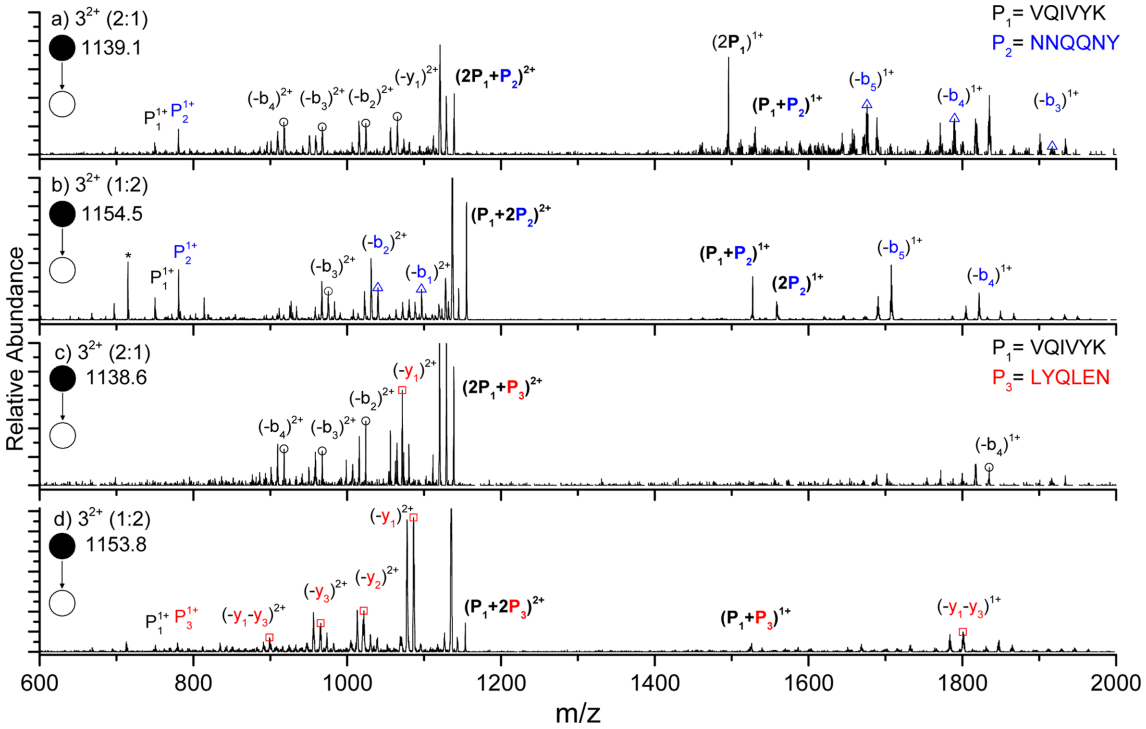


Figure 3. MS/MS spectra of the hetero-trimers of a) 3^{2+}(21) in (VQIVYK + NNQQNY) solution, b) 3^{2+}(12) in (VQIVYK + NNQQNY) solution, c) 3^{2+}(21) in (VQIVYK + LYQLEN) solution, and d) 3^{2+}(12) in (VQIVYK + LYQLEN) solution. Peaks are labeled as $(-b_u)^{2+}$ or $(-y_n)^{2+}$ for space.

shown in Scheme 1c. Positions (1) and (2) are available as the third peptide position in the (VQIVYK + NNQQNY) mixing solution for hetero-trimer formation in which the Y-Y interaction structure is maintained.

Two potential structures of the 3^{2+}(21) hetero-trimer for the (VQIVYK + NNQQNY) mixing solution are shown in Scheme 1d-1e. Similarly, positions (3) and (4) are available in the (VQIVYK + LYQLEN) mixing solution (Scheme 1f) for hetero-trimer formation in which the Y-Y interaction structure is maintained. The fragment ions are observed as {b_u loss} series for VQIVYK (or NNQQNY) and {y_n loss} series for LYQLEN in Figure 3a-3d because the N-terminal part of NNQQNY (or VQIVYK) and the C-terminal part of LYQLEN are open for the collision process via the CID-MS/MS. The VQIVYK b5 ion, NNQQNY b6 ion, or LYQLEN y5 ion (Y-containing fragment ion) are not observed in Figure 3a-3d.

Non-covalent bond dissociation channels occurred between the monomer subunits and were observed as 1^{1+} or 2^{1+} peaks in the 3^{2+}(12 or 21) MS/MS spectra of the (VQIVYK + NNQQNY) mixing solution. It is difficult to deduce the 3^{2+}(12 or 21) structural information from the 1^{1+} non-covalent bond fragment ions because several different trimer structures (from the aforementioned parallel, anti-parallel, steric zipper, and Y-Y interaction) could account for the 1^{1+} non-covalent bond fragment ions. On the contrary, two types of 2^{1+} fragment ions (homo-dimer and hetero-dimer ions that are coincidently observed in Figure 3a-3b) provide some clue in deducing the 3^{2+}(12 or 21) structural information. Interestingly, the $(2P_1)^{1+}$ and $(P_1+P_2)^{1+}$ non-covalent bond fragment ions in Figure 3a or $(P_1+P_2)^{1+}$ and $(2P_2)^{1+}$ in Figure 3b could be formed in the schematic dissociation channels (Scheme 1g-1h) if the Y-Y interaction dimer structures are maintained in the 3^{2+}(12 or 21) hetero-trimer structure.

Conclusions

The intensities of the homo-dimer and -trimer spectral peaks decrease with the addition of the second different Y-containing peptide. The added Y-containing peptide, as a limiting agent, is proposed to play a role in decreasing the population of the early homo-dimer and -trimer complexes by forming new hetero-dimer and -trimer complexes. The tandem mass spectrum of the hetero-dimer and -trimer shows two different fragmentation patterns (covalent and non-covalent bond dissociation). The Y-Y interaction structures, Y5-Y6 interaction between VQIVYK and NNQQNY and Y5-Y2 interaction between VQIVYK and LYQLEN, are proposed to explain the MS/MS fragmentation patterns of the 2^{1+}(11) hetero-dimer complex. The 3^{2+}(12 or 21) MS/MS spectrum shows that the Y-Y interaction structures, formed in the hetero-dimer complex, are maintained in the 3^{2+}(12 or 21) hetero-trimer structures.

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