A Review of Mass Spectrometry Studies on Noncovalent Interactions Between Peptides

Noncovalent interactions between peptides—such as hydrogen bonding, hydrophobic interactions, electrostatic forces, and van der Waals forces—are pivotal factors determining their secondary structure formation, molecular recognition, assembly, and function. Traditionally, research on these weak interactions has primarily relied on methods like nuclear magnetic resonance (NMR), X-ray crystallography, and calorimetry. In recent years, mass spectrometry (MS), particularly soft-ionization MS and its coupled techniques, has emerged as an indispensable and powerful tool for investigating noncovalent interactions between peptides. This advancement stems from its high sensitivity, rapid analytical capabilities, and direct access to stoichiometric information of complexes. This paper aims to provide a systematic review of key MS techniques applied in this research field, major research strategies, and the progress achieved.

I. Core Mass Spectrometry Technology: How to "Gently" Detect Weak Interactions?

The primary challenge in studying noncovalent complexes is ensuring

that their fragile noncovalent bonds remain intact during the transition from the solution phase to the gas phase and subsequent ionization detection. The following techniques are key to achieving this goal.

1. Soft Ionization Techniques: Preserving Complex Integrity

Electrospray Ionization (ESI): This remains the most widely used ionization technique for studying noncovalent interactions. ESI enables the "whole" entry of peptide monomers or their noncovalent complexes from solution into the gas phase as multiply charged ions under mild conditions. Optimizing ion source parameters minimizes disruption to noncovalent bonds. The observed mass-to-charge ratio of complex ions directly reveals their precise stoichiometric ratio.

Matrix-Assisted Laser Desorption/Ionization (MALDI): Certain MALDI conditions (e.g., using mild matrices, reducing laser energy) can also study some stable noncovalent complexes. However, this process is more aggressive than ESI and has a relatively narrower application range.

2. Ion Mobility Spectrometry: Separation and Measurement of Shape

The integration of ion mobility spectrometry (IMS) with mass spectrometry (MS) represents a significant recent advancement. IMS separates ions within a drift tube based on the ratio of their shape, size, and charge. For peptide noncovalent complexes, IMS-MS provides:

Collision cross section (CCS): Reflects the three-dimensional size and compactness of complexes in the gas phase, enabling differentiation

between distinct assembly conformations.

Conformation separation: Even complexes with identical mass-to-charge ratios (m/z) but different spatial structures may be separated based on varying CCS, revealing multiple coexisting conformational states in solution.

3. Hydrogen/Deuterium Exchange Mass Spectrometry: Probing Dynamic Structures

HDX-MS serves as a powerful tool for investigating the conformational dynamics of proteins/peptides in solution. Its principle relies on the exchange rate between main-chain amide hydrogen and solvent deuterium, a rate protected by hydrogen bonds and solvent accessibility. When coupled with mass spectrometry, HDX enables precise mapping of noncovalent interaction interfaces and detection of binding-induced conformational changes by comparing deuteration rates between the free peptide and its various fragments after complex formation.

4. Collision-Induced Dissociation and Surface-Induced Dissociation: Assessing Interaction Strength

CID: By applying collision energy to complex ions and observing their dissociation thresholds and fragmentation pathways, the relative stability of different complexes can be qualitatively compared, and sites of noncovalent binding inferred.

SID: Compared to CID, SID offers more controlled and uniform

energy transfer, providing more precise information on complex dissociation energies and aiding in the quantitative assessment of interaction strength.

II. Key Strategies and Application Directions in Mass Spectrometry Research

Based on the aforementioned techniques, mass spectrometry is employed to analyze noncovalent interactions between peptides from multiple dimensions.

1. Direct Characterization of Complex Stoichiometry and Assembly Pathways

Mass spectrometry enables direct measurement of the mass-to-charge ratio of complexes in the gas phase, thereby determining the composition of peptide oligomers. For example, for the self-assembling peptide RADA16, mass spectrometry clearly demonstrates the presence of dimers, tetramers, and even higher-order oligomers, providing direct evidence for understanding its stepwise assembly pathway from monomers to nanofibers. By monitoring changes in the abundance of oligomer peaks at different concentrations or under varying solution conditions, the dynamic equilibrium process of assembly can be inferred.

2. Localization of Interaction Interfaces and Key Residues

CID/MS-based Fragmentation Analysis: In non-covalent complexes, non-covalently bound subunits may dissociate during CID before covalent bond

cleavage occurs. Analyzing dissociation products reveals which peptide segments are critical for complex stability.

HDX-MS-based dynamic protection mapping: Currently one of the most precise interface mapping methods. By comparing mass spectra of peptide monomers and their complexes after identical deuteration times, the deuteration difference for each fragment region can be calculated. Regions protected by complex formation (showing significantly reduced deuteration rates) represent the core noncovalent interaction interface.

3. Analysis of Conformational Heterogeneity and Dynamic Changes

The integration of ion mobility spectrometry (IMS) enables researchers to identify and separate different conformational isomers within the same stoichiometric complex. For example, certain amyloid-derived peptides may coexist as disordered oligomers and β-sheet-rich ordered oligomers. IMS-MS can resolve these conformational isomers, allowing separate studies of their stability and toxicity.

Time-resolved HDX-MS or native MS can monitor kinetic processes in peptide interactions, such as the speed of binding events and the temporal sequence of conformational changes.

4. Evaluation of Environmental Factors Affecting Noncovalent Interactions

Mass spectrometry provides a convenient approach to investigate how environmental factors—including pH, ionic strength, organic solvent ratio,

and small-molecule ligands—influence the stability and composition of peptide noncovalent complexes. By observing changes in the intensity of specific complex ion peaks or alterations in the Co-Co Shift (CCS), one can assess whether these factors enhance or weaken the interactions.

III. Challenges and Prospects

Despite significant advantages, this field still faces several challenges:

1. Gas-phase versus liquid-phase correlation: Mass spectrometry detection occurs in the gas phase, while interactions take place in the solution phase. Whether structures in the gas phase faithfully reflect the true state in solution is a fundamental issue requiring careful evaluation. Validation using complementary techniques (such as solution NMR) is crucial.

2. Quantification of weak interactions: Accurately measuring absolute binding constants for non-covalent interactions remains challenging, requiring complex experimental designs and data processing models.

3. Resolution Capabilities for Complex Systems: Assigning and resolving mass spectrometry signals becomes exceptionally difficult for extremely large, highly heterogeneous, or non-specifically aggregated peptide systems.

Future development trends will focus on:

4. Deepening of Multimodal Integration: Tighter integration of IMS-MS, HDX-MS, high-resolution mass spectrometry with advanced fragmentation techniques (e.g., electron capture/transfer dissociation) to

provide multidimensional structural information.

5. Integration of computational modeling: Combining mass spectrometry experimental data (e.g., CCS values, HDX rates) with molecular dynamics simulations to construct and validate atomic-level structural models of peptide complexes.

6. In situ and spatiotemporal resolution studies: Developing faster sampling and monitoring techniques to capture transient intermediates during peptide interactions and assembly processes.

IV. Conclusion

Mass spectrometry, particularly modern platforms centered on electrospray ionization and ion mobility separation, has significantly advanced our understanding of noncovalent interactions between peptides. It not only "weighs" complexes and determines their chemical composition but also probes their three-dimensional conformations, interaction interfaces, and dynamic properties by measuring parameters such as collision cross sections and hydrogen-deuterium exchange rates. Despite inherent challenges such as the representativeness of the gas-phase state, ongoing technological advancements and interdisciplinary approaches will ensure mass spectrometry remains a cutting-edge tool for unraveling the microscopic physical mechanisms of peptide recognition and self-assembly. It provides indispensable insights for rationally designing peptide therapeutics and functional materials, as well as for understanding

related pathological processes.