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IRMPD spectroscopy reveals a novel rearrangement reaction for modified peptides that involves elimination of the N-terminal amino acid

Michael J. van Stipdonk[a,*], Khiry Patterson[a], John K. Gibson[b], Giel Berden[c], Jos Oomens[c,d]

[a] Department of Chemistry and Biochemistry, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15282, USA
[b] Chemical Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720-1150, USA
[c] Radboud University Nijmegen, Institute for Molecules and Materials, FELIX Laboratory, Toernooiveld 7, 6525ED Nijmegen, The Netherlands
[d] University of Amsterdam, Science Park 904, 1098XH Amsterdam, The Netherlands

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ABSTRACT

In this study, peptides were derivatized by reaction with salicylaldehyde to create N-terminal imines (Schiff bases). Collision-induced dissociation of the imine-modified peptides produces a complete series of *b* and *a* ions (which reveal sequence). However, an unusual pathway is also observed, one that leads to elimination of the residue mass of the N-terminal amino acid *despite* the chemical modification to create the imine. This pathway was investigated further using infrared multiple-photon dissociation (IRMPD) spectroscopy and density functional theory with alanine-glycine-glycine (AGG) as the test peptide. The IRMPD spectrum for the product generated by loss of 71 from modified AGG (Sal-AGG) matches one predicted for protonated Sal-GG, as well as the IRMPD spectrum of glycine-glycine derivatized independently to produce a Schiff base. We conclude that the residue mass of the N-terminal amino acid is likely eliminated through a pathway that involves nucleophilic attack by an amide N atom and possible formation of an imidazole-4-one intermediate.

1. Introduction

Tandem mass spectrometry (MS/MS) and collision-induced dissociation (CID) remain two of the most important tools used for peptide and protein identification in proteomics [1–13]. Sequencing, whether done by comparison to known peptide fragmentation patterns, or by searching sequence databases with bioinformatics tools that attempt to predict fragmentation, is dependent, in part, on product ion distributions generated by CID [14]. Therefore, a clear understanding of peptide fragmentation mechanisms, energetics and dynamics is necessary to maximize the effectiveness of MS/MS based identification.

Under low-energy CID conditions, fragmentation of protonated peptides typically involves charge (proton) mediated reactions, in which cleavage of amide bonds leads to generation of *b*, *y*, and *a* ions [14–17]. Development of the mobile proton model [18,19] of peptide fragmentation, and related amide bond cleavage pathways [19–28], has been focused primarily on proton mobilization. The pathways in competition (PIC) fragmentation model [14] uses the mobile proton model as a foundation for understanding, but takes into account the structures and reactivity of key reactive configurations and primary fragments as well as transition states and their energies.

It has been shown that small (i.e. three or fewer residues within the fragment) N-terminal b_n type fragment ions have structures that include C-terminal oxazolone rings [20,21,23,29–34] and retain the primary sequence of the precursor peptide ion. More recent experiments [35–57] strongly suggest that macro-cyclic *b* ion isomers, or intermediates, can arise through cyclization of linear, oxazolone-terminated *b* ions. For example, in a detailed experimental and theoretical study [37], the gas-phase structures and fragmentation pathways of the N-terminal *b* and *a* fragments of YAGFL-NH_2, AGLFY-NH_2, GFLYA-NH_2, FLYAG-NH_2, and LYAGF-NH_2 were investigated using CID and detailed molecular mechanics and density functional theory (DFT) calculations. It was shown that low-energy CID of the b_5 fragments of the pentapeptide amides produced nearly the same dissociation

* Corresponding author. Tel.: +1 412 396 4923.
E-mail address: vanstipdonkm@duq.edu (M.J. van Stipdonk).

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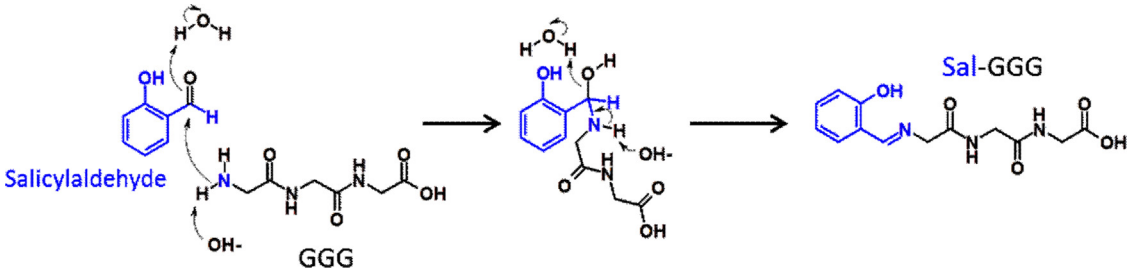

Scheme 1. Condensation reaction to convert peptide to N-terminal imine.

patterns. Furthermore, CID of protonated cyclo-(YAGFL) produced the same fragments with nearly identical ion abundances to those observed for the b_5 ions when using similar experimental conditions. The results strongly suggested that rapid cyclization of the primarily linear b_5 ions can take place, and that the CID spectrum is influenced by the fragmentation behavior of the cyclic isomer.

The extent to which sequence scrambling reactions influence the accuracy of peptide and protein identification by tandem MS has been debated [58–61]. It is important to recognize that the (negative) influence of sequence scrambling on peptide identification is likely to be highest during multiple-stage (MS^3 or higher) CID experiments in 3D or linear ion traps. Indeed, the first identification of potential scrambling pathways was made at the MS^3 stage [62]. It has been noted that modification of the N-terminus, such as through acetylation [37,38] or conversion to trimethylpyridinium ion [63], reduces or eliminates the cyclization that leads to sequence scrambling. Our past examination using YAGFL-NH_2 and permuted sequence isomers suggested that sequence scrambling is initiated by nucleophilic attack by the N-terminal amine, upon a carbon atom within the oxazolone ring of a b_n ion, to produce a macrocyclic isomer [37]. Opening of the macrocycle at various positions leads to the loss of sequence information. The prevention of sequence scrambling with acetylation, for example, can be attributed to the fact that the nucleophilic amino group is converted to an amide (or otherwise functionalized if other modification is made).

In an attempt to identify ways to mitigate effects of sequence scrambling pathways and enhance MS^n approaches to direct peptide identification, we have been investigating several derivatization strategies. Our goal is to derivatize the N-terminus of peptide, efficiently and quantitatively (thus eliminating the amine nucleophile responsible for the scrambling reactions) while maintaining a basic site to enhance ionization (protonation) efficiency. As part of these studies, one approach taken was to convert the N-terminus to an imine (Schiff base) by condensation with an aldehyde (Scheme 1). In our preliminary experiments, we found that an extensive series of b_n and a_n ions are produced by CID, and that the N-terminal product ions dominate C-terminal fragments such as the y_n ions (likely reflecting the high basicity of the imine group). In addition, sequence scrambling pathways were apparently suppressed or eliminated altogether. More importantly, we also observed a fragmentation reaction that appeared to involve elimination of the residue mass of the N-terminal amino acid, despite the chemical modification to create the imine. The pathway therefore reflects another interesting and unanticipated rearrangement reaction and is the subject of the present study. Here, we report the general fragmentation patterns of the derivatized peptides that helped identify the new rearrangement, and the use of IRMPD spectroscopy and density functional theory calculations to shed light on the reaction pathway that leads to the elimination of the residue mass of the N-terminal residue.

2. Experimental methods

2.1. Sample preparation

Peptides (glycine-glycine-glycine, alanine-glycine-glycine, glycine-glycine-alanine, valine-alanine-alanine-phenylalanine and tyrosine-glycine-glycine-phenylalanine-leucine) were purchased from Sigma Aldrich (St. Louis, MO) and used as received. Peptide imines were generated by reacting peptides with salicylaldehyde (1:1 mole ratio) in methanol for 2–6 h at 37 °C. The reaction mixture was then diluted with 50:50 methanol/water and then acidified with 10 μL of 1% acetic acid in H_2O. Modification of the respective peptides was confirmed by a mass shift of 104 mass units (u), the expected change due to the condensation reaction. Final peptide concentrations for ESI-MS and IRMPD studies were $\sim 10^{-4}$ M.

2.2. Collision-induced dissociation experiments

Ion trap multiple-stage CID experiments were conducted on a ThermoScientific LTQ-XL linear ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA). The atmospheric pressure ionization stack settings for the LCQ (lens voltages, quadrupole and octapole voltage offsets, etc.) were optimized for maximum $(M+H)^+$ transmission to the ion trap mass analyzer using the auto-tune routine within the LTQ Tune program. Following the instrument tune, the spray needle voltage was maintained at +5 kV, the N_2 sheath gas flow at 10 units (arbitrary for the ThermoScientific instruments) and the capillary (desolvation) temperature at 200 °C. Helium was used as the bath/buffer gas to improve trapping efficiency and as the collision gas for CID experiments.

The CID studies (MS/MS and MS^n) were performed as follows. The $(M+H)^+$ ions were isolated for the initial CID stage (MS/MS) using an isolation width of 0.9–1.2 m/z units. The exact width was chosen empirically and reflected the best compromise between high $(M+H)^+$ abundance and the isolation of a single isotopic peak. The (mass) normalized collision energy, which defines the amplitude of the R.F. energy applied to the end cap electrodes in the CID experiment, was set between 20% and 25%, which corresponds roughly to 0.80–0.99 V with the instrument calibration used in this study. The activation Q (as labeled by ThermoFinnigan, used to adjust the q_z value for the precursor ion) was set at 0.30. The activation time employed at each CID stage was 30 ms.

2.3. ESI FT-ICR mass spectrometry

Previously established methods were used for generation of ions and the subsequent collection of IRMPD spectra [26–30]. Briefly, ESI was performed using a Micromass Z-Spray source. Ions were injected into a home-built Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer described in detail elsewhere [64]. Instrument operating parameters, such as desolvation temperature, cone voltage, and ion accumulation and transfer optics