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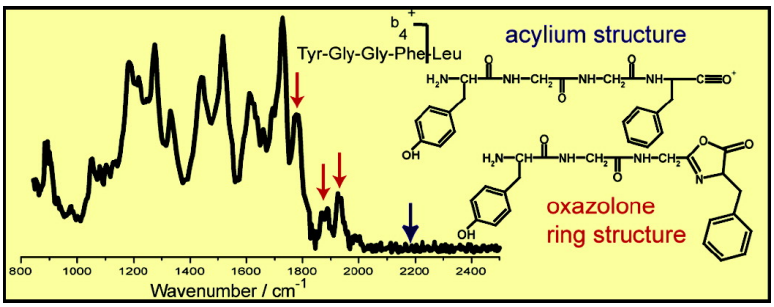

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Spectroscopic and Theoretical Evidence for Oxazolone Ring Formation in Collision-Induced Dissociation of Peptides

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Tandem mass spectrometry (MS/MS) of peptides for the purpose of amino acid sequencing[1] is one of the key techniques of protein identification in the nascent field of proteomics.[2] Peptide dissociation is most commonly effected using collision-induced dissociation (CID) of protonated peptides, yielding predominantly N-terminal *b* and C-terminal *y* fragments, according to the nomenclature given by Roepstorff.[3] MS/MS-based protein identification strongly relies on various bioinformatics tools,[2b] which relate experimental mass spectra to predicted spectra of peptides derived from protein and/or DNA database entries. The "mobile proton"[4] and "pathways in competition" (PIC)[5] fragmentation models provide comprehensive summaries of our present understanding of peptide dissociation chemistry (reviewed recently[5]). While the application and automation of tandem MS-based peptide sequencing strategies have advanced considerably, the dissociation chemistry is still only partly understood, and further developments are necessary to predict fragment ion intensities.

The structure of *b* ions has been the subject of a longstanding debate. Biemann proposed the acylium ion structure in the early days of peptide tandem mass spectrometry.[6] Later studies revealed that the acylium structure is unstable and spontaneously loses a CO to become an *a* ion.[7] To account for their pronounced stability, Harrison proposed the cyclic oxazolone structure for *b* ions.[7a,b] Fragmentation studies by Farrugia et al. on several b_2^+ fragments suggest that some of these form alternative ring structures.[8]

Theoretical mechanistic details of formation of *b* ions have been described only recently,[5,9] suggesting that *b* ions can be formed on the b_n-y_m, cyclic peptide, and amino acid side chain nucleophile activated peptide fragmentation pathways (PFPs) (Scheme S1, Supporting Information). The first two reactions lead to oxazolone and cyclic peptide *b* isomers, respectively. Theoretical studies indicate that the majority of *b* ions are formed via the energetically and entropically favored b_n-y_m PFPs, leading to pronounced oxazolone formation.[9b] Experimentally, the existence of the oxazolone structure is based on indirect evidence. It was shown that a protonated oxazolone structure and a b_2 ion exhibited similar dissociation behavior;[7a] generally, b_1 fragments cannot be formed; however, for N-terminally acylated peptides, this can occur.[10] N-Methylated peptides showed exclusive retention of the proton on the *b* fragment, which was rationalized by the oxazolone structure.[11] Further, for doubly charged larger *b* fragments, Haselmann et al. have suggested acylium structures, based on dissociation with electron capture dissociation (ECD),[12] although recent results by Chen and Turecek suggest that these ECD results are actually consistent with an oxazolone *b* fragment structure.[13]

To recapitulate, no direct experimental information is available on the structure of *b* ions, and a direct structural probe of fragment structures could elucidate some of the questions in the field. It has been shown that infrared multiple-photon dissociation (IR-MPD) spectroscopy[14] can yield important structural information on fragment ions.[14b,c] Here, we demonstrate the application of IR-MPD to the fragment ion b_4^+ (see Figure 1A), which is formed by sustained off-resonance irradiation (SORI) CID from the singly protonated peptide [Leu]-enkephalin (Tyr-Gly-Gly-Phe-Leu) in a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer.[15] The fragmentation of this peptide has already been studied by others and, hence, represents a convenient comparative example.[16] Also,

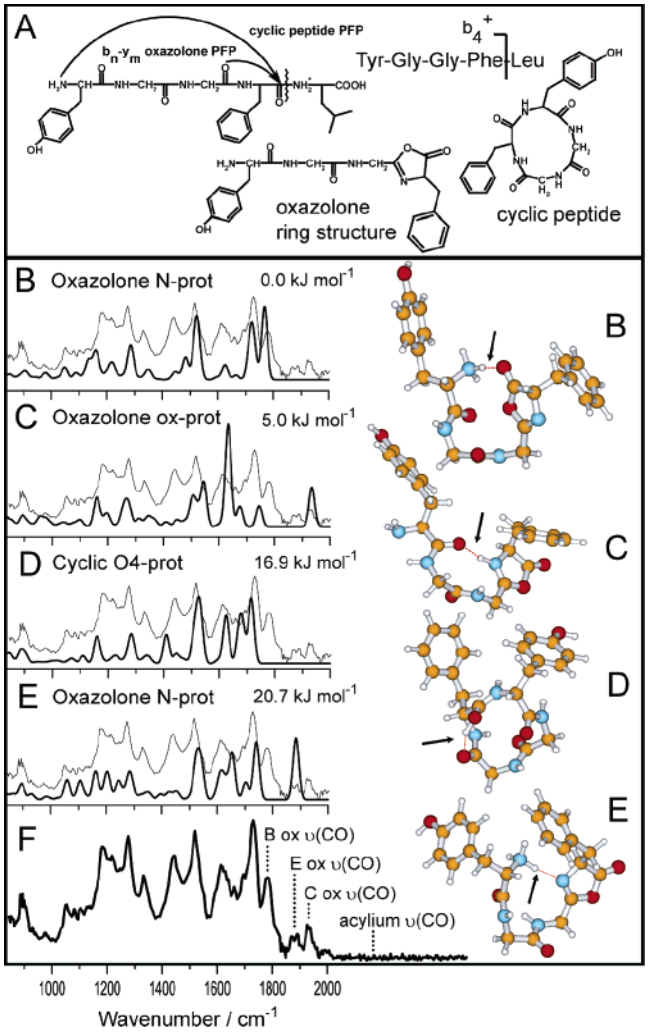

Figure 1. (A) Reaction scheme showing peptide fragmentation pathways (PFP) in CID of Leu-enkephalin to form b_4^+ fragment. Calculated spectra for (B) N-terminal protonated oxazolone, (C) oxazolone ring protonated oxazolone, (D) cyclized peptide protonated on O4, and (E) N-terminal protonated oxazolone. (F) Infrared photodissociation spectrum of Leu-enkephalin b_4^+ fragment depletion. Arrows indicate sites of proton solvation.

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the structure and PFPs of [Leu]-enkephalin and its fragments have recently been studied using modeling techniques.[17]

The trapped ion cloud is overlapped with pulses from the Free Electron Laser for Infrared eXperiments (FELIX) to induce fragmentation (typically 40 macropulses at 10 Hz repetition rate). Infrared spectra are obtained by monitoring the depletion of b_4^+ as a function of wavelength. Note that the photodissociation product ions of b_4^+ include *m*/*z* 120 (Phe immonium), 221 (b_2^+), 278 (b_3^+), and 397 (a_4^+), but that the sum of the photofragments is inferior to the depletion of b_4^+; given the apparent nonconservation of ions in the trap, the depletion spectrum gives a more faithful representation of the IR spectrum of b_4^+. Computationally, candidate structures were determined by a conformational search engine developed in Heidelberg.[5,9b] The energetics and vibrational spectra were obtained at the B3LYP/6-31+G(d,p) level using 0.98 as a scaling factor and the Gaussian program.[18] Further, the calculated stick spectra are convoluted (Figure 1B−E) using a 15 cm^{-1} full-width at half-maximum (fwhm) Gaussian profile.

The IR spectrum of b_4^+ (Figure 1 F) is compared to putative fragment structures (Figure 1B−E) in ascending energy. Since an acylium ion is not a stable structure, no corresponding structure is shown; further, the acylium structure can be discarded based on the absence of a band at ∼2200 cm^{-1}, as previously measured for the benzoyl cation.[14b] Since [Leu]-enkephalin lacks nucleophilic amino acid side chains that can induce formation of b_4^+, only oxazolone and cyclic peptide isomers are possible (Figure 1A). The global minimum structure of b_4^+ is an oxazolone isomer, where the protonation site is on the N-terminus (structure B). Two other major oxazolone conformer families are represented by structure C, which is protonated on the oxazolone ring and merely 5 kJ mol^{-1} higher in energy, and the higher-energy structure E (20.7 kJ mol^{-1}), which is also protonated on the N-terminus, but displays charge solvation by the N atom of the oxazolone ring, as opposed to C═O solvation in the case of structure B. Importantly, structure E could rationalize interconversion of structures B and C by facilitating proton transfer from the N-terminus to the oxazolone ring.

Since the oxazolone ring C═O stretch, υ(CO), is highly susceptible to its chemical environment, all three oxazolone structures show a diagnostic band position (at 1780, 1930, and 1890 cm^{-1} for structures B, C, and E, respectively), which are in fact observed in the experimental spectrum. While band intensities in IR-MPD are not as reliable as in linear absorption techniques, the relative band intensities of these diagnostic bands reflect the relative energies. This is the first direct experimental evidence of oxazolone formation upon CID of oligopeptides, proving that cyclization reactions occur in the fragmentation chemistry of peptides, as proposed by Harrison.[7a,b]

It appears that the calculated spectra for structures B, C, and E cannot explain all the features in the experimental spectrum. In particular, the band at ∼1420 cm^{-1} is much more intense than the corresponding modes for the oxazolone structures. This band could be rationalized by a proton-bound mode of the protonated cyclic peptide (structure D), although the fit is not convincing. Further, given that none of the other modes of structure D are particularly diagnostic, IR spectroscopy can neither confirm nor exclude the cyclic peptide structure. On the basis of computational modeling studies, the cyclic conformer is certainly not expected to form. Thus, while three distinct oxazolone structures have been identified in this study, the unassigned mode at 1420 cm^{-1} suggests the presence of another, yet unidentified b_4^+ conformation. A full study of the structures and fragmentation chemistry of protonated Leu-enkephalin to b_4^+ and a_4^+, as well as proton-transfer pathways, will be presented in a forthcoming paper.

This work demonstrates that IR spectroscopy and computer modeling are powerful tools in the structural elucidation of peptide fragment ions, yielding information that is difficult to ascertain with other techniques; further, the technique is readily applicable to a multitude of systems and dissociation techniques (e.g. ECD).

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Supporting Information Available: Scheme S1, structures, and total energies of the species presented in Figure 1, and complete ref 18. This material is available free of charge via the Internet at http://pubs.acs.org.

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