Probing Long-lived Radical Intermediates in Electron Capture Dissociation with a Double Resonance Experiment

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Introduction

Despite its growing application in the field of proteomics, the mechanism of electron capture dissociation (ECD)[1] is still not thoroughly understood. Recent calculations and experiments suggested that in addition to a rapid, possibly non-ergodic primary cleavage, ECD generates a metastable radical cation as one of the product species.[2-4] ECD of cyclic peptides showed extensive secondary sidechain and backbone cleavages, which was postulated to be the result of free radical cascade.[2] Radical rearrangement was also observed in ECD of linear peptides in the form of H/D scrambling.[4] The presence of reaction intermediates can sometimes be revealed via double resonance (DR) experiments,[5] where resonant ejection of an intermediate during the dissociation event will modulate the peak intensity of all secondary ions derived from it.

Methods

Experiments were performed on a home-built hybrid Fourier-transform mass spectrometer (FT-MS) with an electrospray ionization (ESI) source.[6] A single charge state of peptide/protein ions of interest were isolated and externally accumulated in the front end quadrupole before entering the cell, where they were trapped and subjected to ~2-10 millisecond irradiation of low energy electrons from an indirectly heated dispenser cathode. In DR experiments, ions of interest were resonantly ejected by applying single frequency excitation with a peak-to-peak amplitude of 5 to 40 volts during the electron irradiation period.

Results

ECD of fibrinopeptide B (EGVNDNEEGFFSAR) generated a complete z-ion series, with the peak intensity of every z-ion smaller than z_9 decreasing sharply upon resonant ejection of the charge reduced molecular ions, while that of z_9 and larger ions remaining roughly the same (Figure 1). It is well known that after the initial N-C_α bond cleavage, the c/z$^\bullet$ ion pair may still be held together by noncovalent interactions. These ion pairs may be ejected out of the cell before they break up, causing the drop in the peak intensity of the individual fragment ions detected. In fibrinopeptide B, the C-terminal arginine can form hydrogen bonds with the Asp, Asn and Glu sidechains. It is thus of no surprise that smaller z-ions showed steeper drop in their peak intensities, as their complementary c-ions have more such sidechains available to interact with arginine. Since the ejection time directly relates to the ejection voltage, it is possible to find the lifetime of these intermediates. These lifetimes are often in the millisecond range, a time sufficiently long to facilitate multiple free radical rearrangements. DR-ECD experiments on BUSM1 (RAAAGADGDGAGADAR) peptide showed that odd electron c• ions have much smaller survival ratio than the corresponding even electron c ions, marking their different origins, with the former likely being the result of inter-molecular hydrogen transfer between c/z• ion pairs.

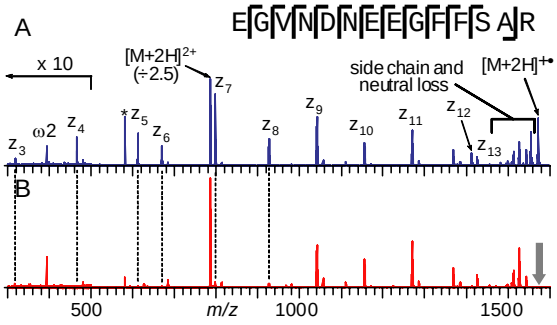

Figure 1. ECD (A) and DR-ECD (B) spectra of fibrinopeptide B.

Fragment ions from ECD of cyclosporin A (a cyclic peptide) showed virtually no change in their peak intensities in the DR-ECD experiment (spectra not shown), even though almost all fragment ions are secondary ions. Thus the secondary backbone cleavage via free radical cascade must occur on a timescale shorter than the ejection time, and the resulted secondary fragment ion pairs fell apart readily, due to lack of strong noncovalent interactions of this aliphatic residue rich peptide.

While generally not observed in the DR-ECD spectra of the doubly charged peptide ions, some fragment ions from the quadruply charged melittin ion showed increase in their abundance upon $[M+4H]^{3+\bullet}$ ejection, e.g. z_{24}^{3+} in Figure 2. This is due to prevention of secondary electron capture of the $[M+4H]^{3+\bullet}$ ion (a competing channel to the z_{24}^{3+} ion formation) as well as the z_{24}^{3+} ion itself (a z_{24}^{3+} ion consumption process) upon resonant ejection of the $[M+4H]^{3+\bullet}$ ion. It can be shown that ions which increase in intensity in DR-ECD spectra derive from intermediates with very short lifetimes (<100 μs).

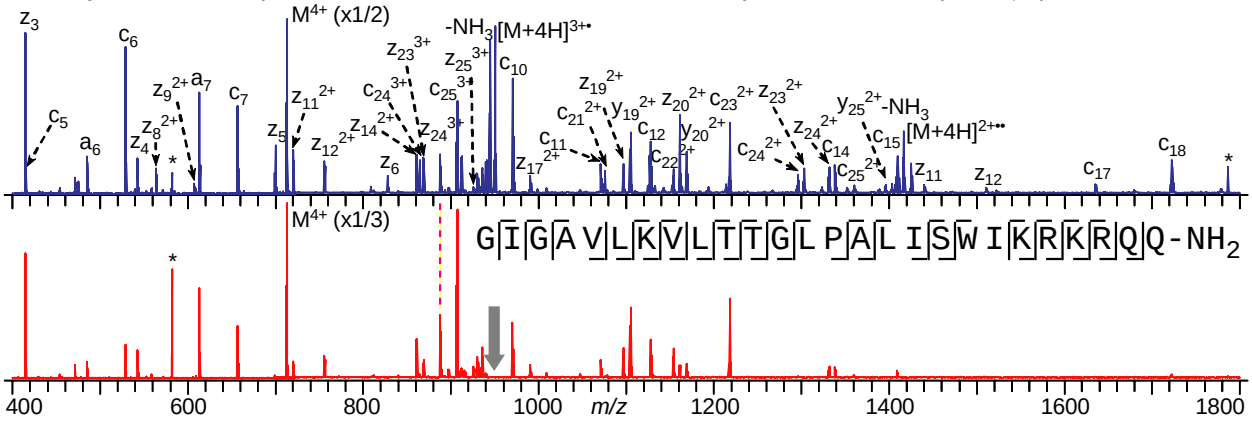

Figure2. Melittin 4+ ECD spectrum (top) and DR-ECD spectrum (bottom).

The fibrinopeptide B example above demonstrated that DR-ECD can reveal non-covalent intramolecular interaction in peptide ions, which, in turn, may be used to deduce their gas phase conformations. As an example for extending this approach to larger protein ions, most ECD fragment ions from the 7+ ion of bovine ubiquitin decreased in their abundances with resonant ejection of the charge reduced molecular ion, while many from 11+ ion showed little change (Figure 3), indicating that the former is of a compact form, and the latter is more unfolded in the gas phase, agreeing with previous ECD studies.[7]

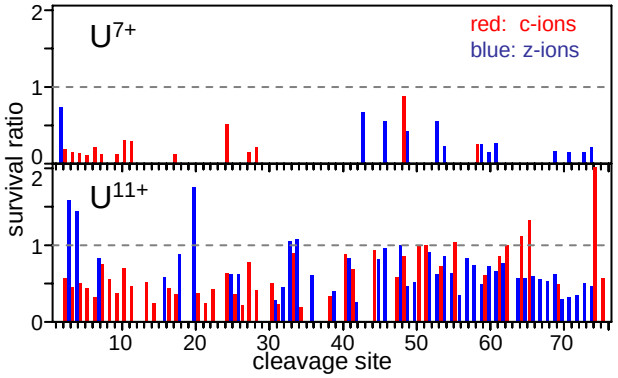

Figure 3. Survival ratio of fragment ions in bovine ubiquitin DR-ECD spectra.

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