Free Radical Driven Isotopic Scrambling in the Electron Capture Dissociation of Linear Peptides

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Since its introduction as a tandem mass spectrometry (MS) technique, electron capture dissociation (ECD) has proven to be a valuable tool in the structural analysis of peptides and proteins, in that it preferentially breaks the backbone N-Cα bonds while keeping the labile post-translational modifications intact. These unique features lead to the proposed "nonergodic" mechanism, in which single N-Cα cleavage takes place prior to energy randomization. However, in recent ECD studies, extensive secondary cleavages in both cyclic and linear peptides were observed, suggesting a second ergodic fragmentation pathway which appears to be free-radical driven. Here, we present a further investigation of the ECD process by studying the deuterium scrambling in the ECD fragments of a selectively isotope-labeled linear peptide.

All experiments were performed on a home-built 7-Tesla Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS) equipped with an external electrospray ionization (ESI) source. The electrosprayed peptide ions were mass selected via a front-end resolving quadrupole and externally accumulated for ~100 msec before being introduced into the ICR cell. Electrons were generated using an indirectly heated dispenser cathode. Both low energy ECD and hot ECD were performed by biasing the center potential of the dispenser cathode at either -0.2 V or -9 V relative to ground. Collisionally activated dissociation (CAD) experiments were performed both in the Q2 cell and in the ICR cell (SORI-CAD).

The free radical cascade mechanism requires that the radicals formed from the primary cleavage have time to migrate prior to the final, observed fragmentation, with the glycine alpha carbon positions being the preferred migration sites. If such is true, isotopic scrambling in some of the secondary fragment ions would be observed when the alpha hydrogens are replaced by deuteriums. To investigate these possibilities, a pair of peptides, with the sequence of RAAAGADGDGAGADAR, were synthesized in both the normal isotope form and with the alpha carbon sites of the four glycine residues fully deuterated.

In the ECD spectra of both peptides, both the radical (c•) and the even electron forms (c) of the c-type fragment ions were observed, with the former being 1 Da lighter. In the ECD spectra of the deuterated variant only, however, additional peaks (c•-1) about 2 Da lighter than the even electron species can be seen in almost all isotopic distributions detected (see, for example, Figure 1, insert A; for comparison, insert B shows the same c-ion from the nondeuterated variant). Cleavages at sites C-terminal to aspartic acid positions showed effectively no scrambling (Figure 2), which likely results from these being the primary cleavage sites. The c•-1 ions are most likely fragments from long lived radical intermediates which have lost a deuterium and gained a hydrogen, thus supporting the free radical cascade mechanism.

As a control experiment, Q2-CAD and SORI-CAD of these same peptides were performed. With CAD, scrambling still occurred, but to a much lower degree compared to ECD (Figure 2). ECD performed with electron gun mounted on the cell and therefore, hot, showed limited increase in deuterium scrambling, indicating that the internal vibrational energy of the ions is not the major contributing factor in the extent of H/D scrambling and suggests that the scrambling is radical driven.

The c•-1 peaks in the ECD spectrum of peptide BUSM 2 can be explained in several ways. It could result from the intramolecular H/D scrambling of the primary radical fragment, prior to the secondary cleavage (Figure 3A); or it could arise from the intermolecular H/D scrambling between the hydrogen bonded c and z• ion pair formed by primary cleavage, before the resulting c• and z ion pair separates upon breaking of hydrogen bonds (Figure 3B).

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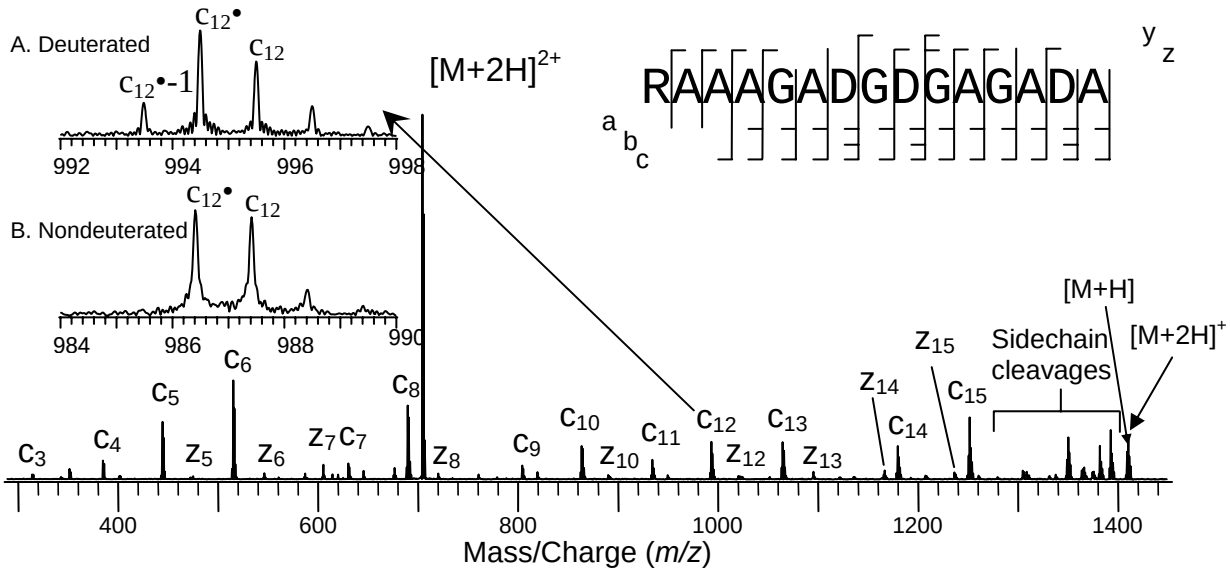

Figure 1. ECD mass spectra of BUSM 2.

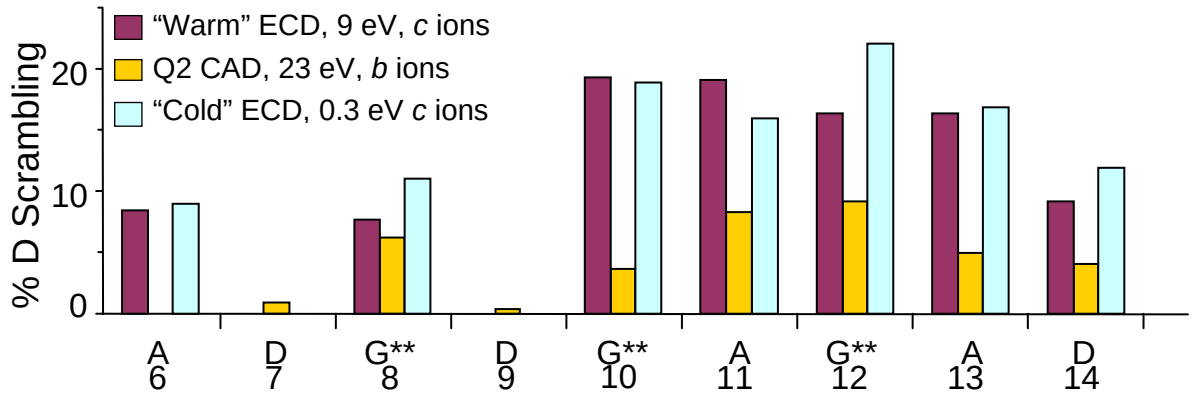

Figure 2. Site specific Deuterium scrambling ratios for BUSM 2 under ECD and CAD conditions.

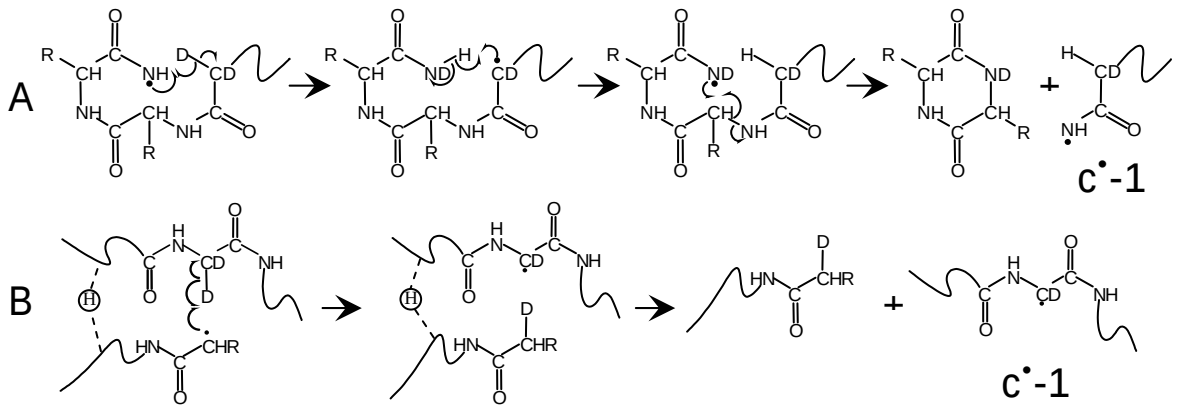

Figure 3. Possible mechanisms to describe the formation of (+H-D) scrambling *c•* fragments: A) intra- and B) intermolecular H/D scrambling.