

New insights into the mechanism of Electron Capture Dissociation

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Electron Capture Dissociation (ECD) is an activation technique used for the analysis of peptides or proteins. In ECD, multiply charged even-electron cations are partially reduced by one electron and thus are converted to intermediate radical-cations which fragment by backbone N-CD bond cleavage to give c and z-type ions. A systematic study using various fragmentation techniques on a single five residue AGWLK peptide led to the observation of unusual ECD fragments: only b/y and w fragments were observed [1]. Stemming from this observation, we pursued a more systematic investigation of ECD applied to a series of similar peptides (of the type AGXLK, where X is one of the naturally occurring amino acids, as well as larger peptides). Since the current mechanism leading to the formation of c/z fragments is still a question of debate, the study of peptides leading to competing fragmentation pathways provides insights on the early steps of the reaction.

Doubly protonated pentapeptides AGXLK (X=A, S, W, E, D) and related peptides such as AKGEL, AGKEL, KAGEL, etc. were subjected to ECD FT-ICR mass spectrometry. In contrast with the typical ECD patterns from peptides of more than 10 amino acids and proteins, c and z-type ions were rarely observed for these peptides, and the fragments were mostly b/y with also intense w fragments. Complementary studies on a TOF/TOF instrument, in the high collision energy regime, ruled out the w ions as originating from a population of protonated peptides having a high internal energy content.

Interestingly, an ETD ion trap mass spectrometry study on the same peptides revealed a regular behaviour, with abundant c/z fragments, as expected for such experiments. Although ETD and ECD are two similar activation methods, based on the reactivity of an intermediate radical cation, our results suggest that the difference in energetics for both techniques lead to different fragmentation pathways.

To explore further why these pentapeptides do not follow the typical dissociation mechanism of ECD, we resorted to computational studies and database analysis. Computational modelling results, based on the doubly protonated AGSLK-NH₂ peptide indicate that hydrogen loss after the electron capture, which can lead to the formation of b/y fragment ions, is a low

energy pathway, competing with N-CD cleavage. The analysis of the SwedECD database (comprised of 11 492 ECD spectra) was also performed and showed that the observed behaviour of our pentapeptides upon ECD was not unusual but rather systematic for peptides of this particular size.

Taken together, these results suggest the existence of at least three separate competing pathways in the ECD/ETD fragmentation of peptides: classical N-CD cleavage, H[•] loss followed by b/y fragmentation and direct formation of w ions from the radical cation. Computational modelling suggests that the prominence of these pathways relies on different factors such as the initial electron attachment site, the internal energy deposited, the electronic states assessed after the electron capture/transfer and the structure of the initial peptide (and of its hydrogen bonding network).

[1] R. Antoine et al., *Rapid Comm. Mass Spectrom.* 20, 1648-1652 (2006).