J. Am. Soc. Mass Spectrom. (2012) 23:1015–1018
DOI: 10.1007/s13361-012-0374-2

SHORT COMMUNICATION

Radical *a*-Ions in Electron Capture Dissociation: On the Origin of Species

Roman A. Zubarev,[1,2] David M. Good,[1] Mikhail M. Savitski[3]

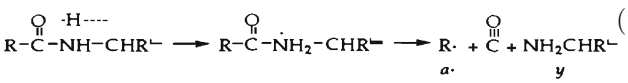
[1]Division of Physiological Chemistry I, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Scheeles väg 2, 171 77 Stockholm, Sweden
[2]Science for Life Laboratory, Stockholm, Sweden
[3]CellZome, Heidelberg, Germany

Abstract

Radical a* ions appear in electron capture dissociation mass spectra sporadically, but sometimes with high intensity. Mechanistically, radical a ions are hypothesized to arise due to thermodynamically disadvantaged charge solvation on the backbone nitrogen (instead of carbonyl), which upon neutralization produces a hypervalent group instantly fragmenting into a radical b* and conventional y' ion. The former species is unstable and, after releasing a CO molecule, decays to an a* ion. Here we validate this scenario by direct observation of the complementarity of a* and y' ions by interrogation of an ECD MS/MS database of >10,000 doubly and >5,000 triply charged tryptic peptides. Intriguingly, the most abundant a*/y' pairs are found to come from the cleavage of the same backbone link as the most abundant c' and z* complementary ions. This result gives strong support to the "local" N-Cα bond cleavage mechanism, in which the dissociation occurs at the site of charge solvation. However, a second strong peak is observed in the c'/z* fragment distribution four residues away from the a*/y' cleavage, which supports the indirect N-Cα bond cleavage mechanism. The size distribution of a ions from doubly (but not triply!) charged precursors shows deficit of a3 ions, and possibly a6 ions.

Key words: Peptide fragmentation, Bond cleavage mechanism

Of the main backbone fragments produced by electron capture dissociation (ECD) [1] and electron transfer dissociation (ETD) [2], radical *a*· ions are probably the most mysterious. They appear in ECD/ETD mass spectra sporadically, but sometimes with high intensity. For instance, activation of the 4+ precursor of melittin using ECD gives at least 20 N–C_α bond cleavages, but only three *a*· ions [3]. Mechanistically, radical *a* ions are hypothesized to arise due to thermodynamically disadvantaged charge solvation on the backbone nitrogen (instead of carbonyl), which, upon neutralization, produces a hypervalent group instantly fragmenting into a radical *b*· and conventional *y*' ion. The former species is unstable and, after releasing a CO molecule, decays to an *a*· ion [4]:



(1)

Unlike the N–C_α bond cleavage mechanism that is still under intensive debates, the scenario (1) of the *a*· ion formation process via the sequential C–N/C_α–C bond cleavage has never been challenged in peer-reviewed literature, and is widely accepted. This, however, does not remove the need to validate (1) by direct observations. One such observation could be the complementarity of the *a*· and *y*' fragments produced in a single bond cleavage event, like the *c*' and *z*· fragments are produced in N–C_α bond cleavage. Because of the sporadic nature of radical *a*-ions in MS/MS spectra, a large MS/MS database is needed for obtaining reliable statistics [5, 6]. We created and interrogated such a

Correspondence to: Roman A. Zubarev; *e-mail:* Roman.Zubarev@ki.se

Received: 30 September 2011
Revised: 2 March 2012
Accepted: 8 March 2012
Published online: 17 April 2012

database, testing the origin of *a*-ions in ECD. They indeed arise from the process (1), which gives complementary $a\cdot$ and y' fragments. Intriguingly, the most abundant $a\cdot/y'$ pairs are found to come from the cleavage of the same backbone link as the most abundant c' and $z\cdot$ complementary ions. This result gives strong albeit indirect support to the "local" ECD mechanism, in which the N–C_α bond dissociation occurs at the site of charge solvation [1]. However, a second, almost equally strong peak is observed in the $c'/z\cdot$ fragment distribution four residues away from the $a\cdot/y'$ cleavage, which supports the indirect mechanism [7–10]. Below, we describe these results and their meaning for the ECD mechanism.

Since ECD neutralizes one charge, doubly charged precursors may be unsuitable for the test in question, as one of the fragments of any backbone cleavage is a neutral. Indeed, analysis of the SwedECD database [5] of 2+ precursors revealed that the most abundant $a\cdot$ ions are *not* complementary to the most abundant y' ions. But since this was also true for c' and $z\cdot$ fragments, the most likely explanation is that the peptide dications giving rise to y' ions have different gas-phase structure than those producing $a\cdot$ ions. This is why a new database was created in the manner of SwedECD but consisting of ECD mass spectra of 3+ ions formed by 5657 unique tryptic peptide sequences. This new database contains 711 $a\cdot$ and 2149 y' ions from the cleavages of the first 10 N-terminal backbone links. The lower number of $a\cdot$ ions compared to y' ions is explained by the lower stability of $a\cdot$ ions due to the presence of a radical site. Indeed, for the same 10 inter-residue links, 19,303 even-electron c' ions and only 9499 radical $z\cdot$ ions were found. An additional factor disfavoring the formation of the N-terminal $a\cdot$ ions is the proton localization in tryptic peptides on the basic C-terminus. Figure 1 shows the number of spectra with the most abundant y'_{l-n} ions for the most abundant $a_k\bullet$ ($n=1..21$, $k=4..9$). Most frequently, $n=k$ (i.e., the most abundant $a\cdot$ and y' ions are complementary).

For comparison, Figure 2 shows similar distributions for $c'/z\cdot$ fragments. Note that in MS/MS spectra where c_4' is the most abundant c' ion, the complementary $z_{l-4}\bullet$ ion is not even among the top seven most abundant $z\cdot$ ions. The situation is similar for c_5', and only starting from c_5' does the complementary fragment become the most abundant. Since there is no reason to doubt the complementary nature of $c'/z\cdot$ fragments, the observed effect for smaller c' ions is likely due to rapid decomposition of radical $z\cdot$ ions [6]. At any rate, Figures 1 and 2 are qualitatively similar, which testifies to the complementary nature of $a\cdot/y'$ cleavage, and thus to the validity of (1).

The importance of this finding is in the first direct confirmation of a "local" ECD mechanism, albeit the one related to a different backbone bond than the N–C_α bond.

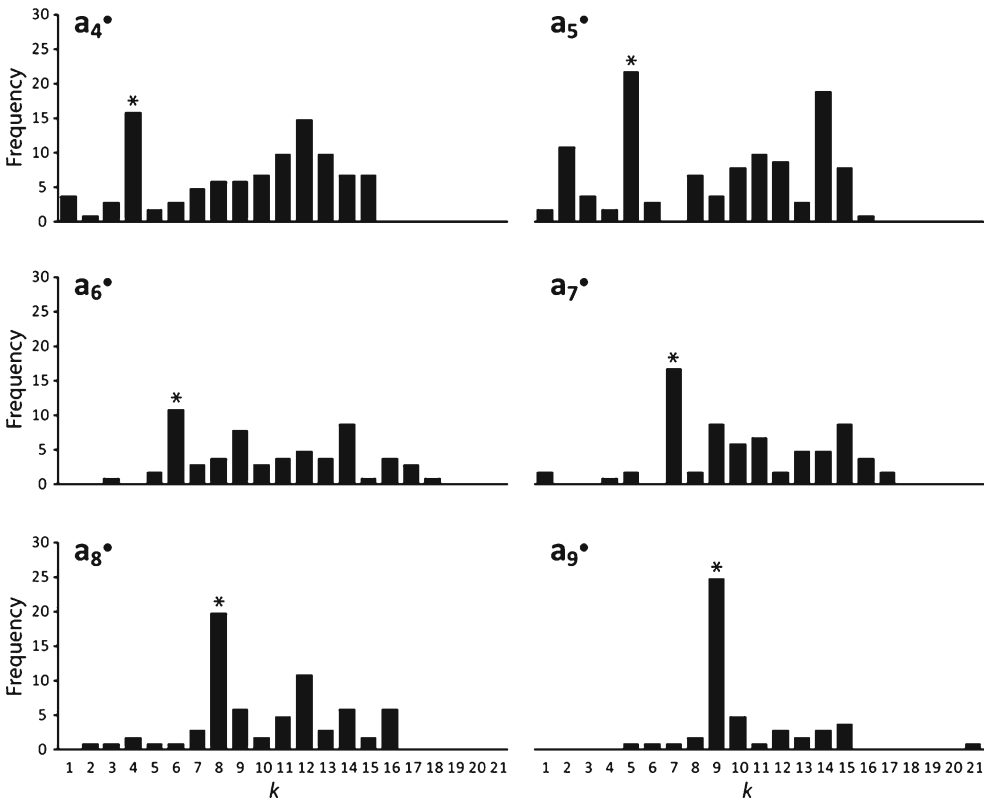


Figure 1. Histograms for ECD MS/MS spectra where the corresponding $a_n\cdot$ ion dominates among other $a\cdot$ ions. The columns represent the occurrences of spectra with the most abundant y_{l-k}' ions for different *k*. The arrows mark the case of $n=k$, when each of the complementary fragments is the most abundant of its kind

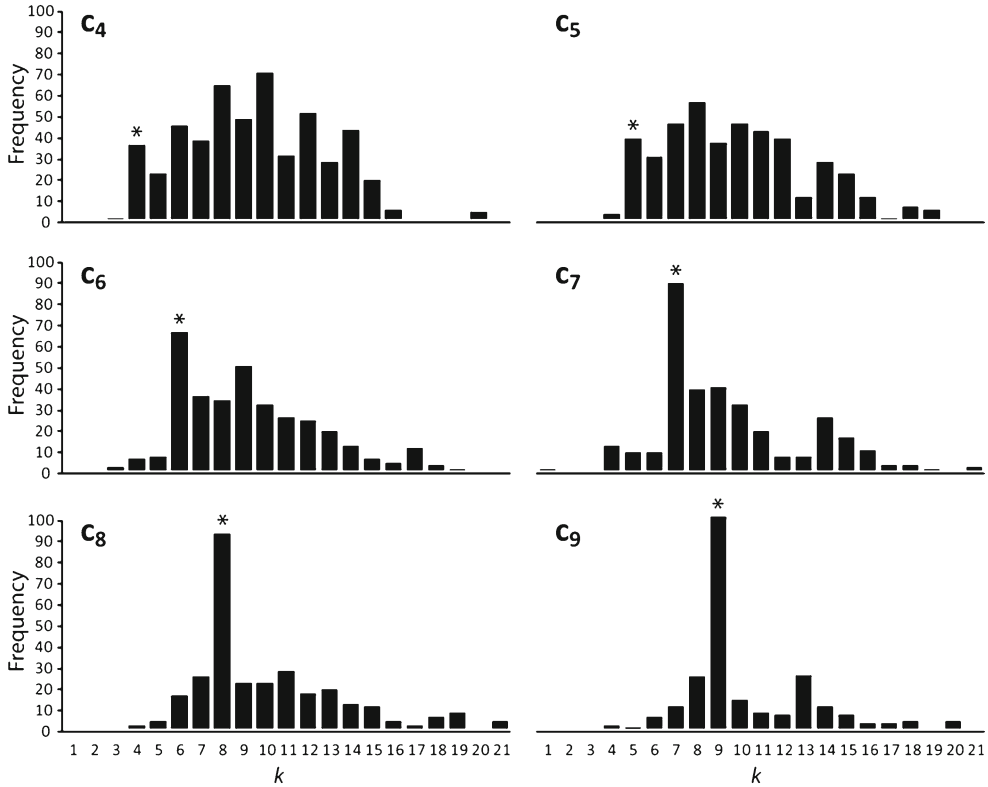


Figure 2. Histograms for ECD MS/MS spectra where the corresponding c_n ion dominates among other c ions. The columns represent the occurrences of spectra with the most abundant $z_{l-k}\cdot$ ions for different k. The arrows mark the case of $n=k$, when each of the complementary fragments is the most abundant of its kind

The local mechanism postulates the electron capture directly onto the charged group, and a bond cleavage next to that group [1].

In contrast, the non-local mechanisms [7–9] assume electron capture on a non-charged atom near, but not immediately at, the charge location. One of the objections to a local mechanism is that direct electron-proton recombination releases too much energy to be accommodated by the system on the time scale of electronic transitions, which enhances the probability of the reversed reaction–electron release to the continuum [9]. However, if (1) is validated, then this objection is lifted.

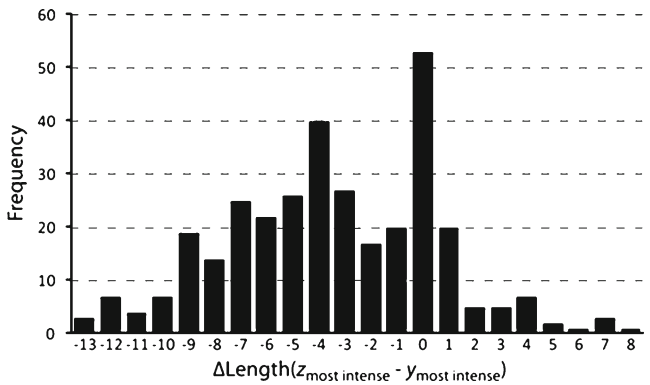


Figure 3. The number of mass spectra plotted against k-n, where k is the number from the N-terminus of the cleaved backbone link which gives a $c_k'/z_{l-k}\cdot$ pair with the most intense $z_{l-k}\cdot$ fragment; n is a similar index for the $a_n\cdot/y_{l-n}'$ pair with the most abundant y_{l-n} fragment

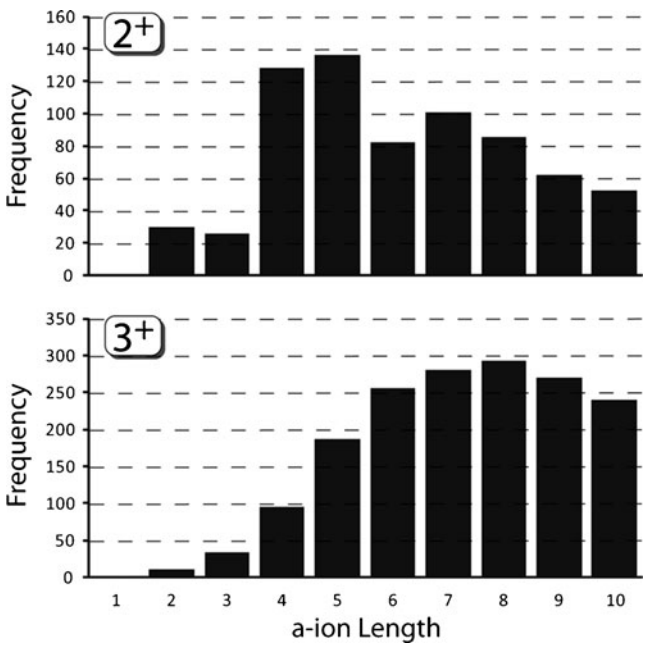


Figure 4. Size distribution of $a_n\cdot$ ions from 2+ and 3+ precursors

It is possible to estimate the relative contributions of the local versus non-local mechanism in N–C_α bond cleavage. To this end, only MS/MS spectra were selected in which both $c'/z\cdot$ and $a\cdot/y'$ complementary pairs were present. The local model predicts that the most abundant $c'/z\cdot$ and $a\cdot/y'$ complementary pairs will come from the cleavage of the same backbone link.

This prediction is based on the assumption that the basicities of the nitrogen and oxygen atoms in a particular backbone amide are linked together since they are similarly affected by the environmental factors, such as the nature of neighboring side chains and folding of the polypeptide chain. Therefore, the nitrogen atom with the most frequent charge solvation among the backbone nitrogens should belong to the same amide as the carbonyl oxygen with a preferred charge solvation among the backbone carbonyls. Thus, the prediction is that for the most abundant complementary pair $c_k'/z_{l\text{-}k}\bullet$ and the most abundant $y_{l\text{-}n}'$ with a complementary $a_n\bullet$ in the same ECD MS/MS spectrum, k will tend to be equal to n. To test this prediction, the number of MS/MS spectra is plotted in Figure 3 against k-n. The maximum peak at zero validates the prediction of the local model, but the second most intense peak at –4 indicates, within the above assumptions, that many N–C_α cleavages occur one helix turn away from the charge solvation site, as predicted by non-local models [7–9] and a later variant [10] of the local model. Therefore, ECD appears to produce N–C_α cleavages of two types, according to the two distinctly different mechanisms. To estimate the ratio between the frequencies of the two N–C_α cleavage types is difficult because the peaks in Figure 3 are merged. However, given that the local mechanism predicts a narrow peak at 0 and non-local, a broad peak at a non-zero value, it appears that the latter mechanism dominates at least 2- to 3-fold.

The size distribution of $a_n\bullet$ ions from 2+ precursors (Figure 4, upper panel) carries a signature of the deficit of $a_3\bullet$ ions, the phenomenon observed earlier for even-electron a ions in CAD [11]. There is also a hint on the deficit of $a_6\bullet$ ions, a previously unreported observation. Remarkably, 3+ precursors produce a smooth distribution of larger fragments (the most probable length of $a_n\bullet$ ions is eight residues as opposed to five residues for 2+ precursors), without any obvious signs of deficits.

Acknowledgment

The authors acknowledge support for this by the Swedish Research Council and KAW Foundation. D.M.G. is grateful for support from a Wenner-Gren postdoctoral fellowship.

References

1. Zubarev, R.A., Kelleher, N.L., McLafferty, F.W.: Electron capture dissociation of multiply charged protein cations. A nonergodic process. *J. Am. Chem. Soc.* **120**, 3265–3266 (1998)
2. Syka, J.E., Coon, J.J., Schroeder, M.J., Shabanowitz, J., Hunt, D.F.: Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 9528–9533 (2004)
3. Zubarev, R.A., unpublished data.
4. McLafferty, F.W., Zubarev, R.A., Kruger, N.A., Fridriksson, E.K., Lewis, M.A., Horn, D.M., Carpenter, B.K.: Electron capture dissociation of gaseous multiply-charged proteins is favored at disulfide bonds and other sites of high hydrogen atom affinity. *J. Am. Chem. Soc.* **121**, 2857–2862 (1999)
5. Falth, M., Savitski, M.M., Nielsen, M.L., Kjeldsen, F., Andren, P.E., Zubarev, R.A.: Analytical utility of small neutral losses from reduced species in electron capture dissociation studied using SwedECD database. *Anal. Chem.* **80**, 8089–8094 (2008)
6. Savitski, M.M., Nielsen, M.L., Zubarev, R.A.: Side-chain losses in electron capture dissociation to improve peptide identification. *Anal. Chem.* **79**, 2296–2302 (2007)
7. Syrstad, E.A., Turecek, F.: Toward a general mechanism of electron capture dissociation. *J. Am. Soc. Mass Spectrom.* **16**, 208–224 (2005)
8. Anusiewicz, I., Jasionowski, M., Skurski, P., Simons, J.: Backbone and side-chain cleavages in electron detachment dissociation (EDD). *J. Phys. Chem. A* **109**, 11332–11337 (2005)
9. Patriksson, A., Adams, C., Kjeldsen, F., Raber, J., van der Spoel, D., Zubarev, R.A.: Prediction of N–C_α bond cleavage frequencies in electron capture dissociation of Trp-cage dications by force-field molecular dynamics simulations. *Int. J. Mass Spectrom.* **248**, 124–135 (2006)
10. Breuker, K., Oh, H., Lin, C., Carpenter, B.K., McLafferty, F.W.: Nonergodic and conformational control of the electron capture dissociation of protein cations. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 14011–14016 (2004)
11. Savitski, M.M., Falth, M., Fung, Y.M., Adams, C.M., Zubarev, R.A.: Bifurcating fragmentation behavior of gas-phase tryptic peptide dications in collisional activation. *J. Am. Soc. Mass Spectrom.* **19**, 1755–1763 (2008)