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Electron capture versus energetic dissociation of protein ions

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Abstract

The identity of neighboring amino acids has little influence on the dissociation of multiply protonated proteins by electron capture dissociation. As exceptions, no cleavage occurs on the N-terminal side of Pro, and little on either side of Cys, whereas the C-terminal side of Trp is heavily favored. The neighboring amino acids have a far greater effect on energetic dissociation, making the combined methods promising for the de novo sequencing of proteins. (Int J Mass Spectrom 182/183 (1999) 1–5) © 1999 Elsevier Science B.V.

Keywords: Electron capture dissociation; Multiply charged gaseous cations; Collisionally activated dissociation; Protein sequencing

1. Dedication

Ben Freiser was certainly one of the most promising mass spectrometrists that our field has ever had. He pioneered whole new areas of exciting research, winning early the American Chemical Society's most prestigious prize for young chemists, The Award in Pure Chemistry. F.W.M. was priviledged to follow Ben's career closely because of overlapping research interests, first meeting him about age 8 at the home of his wonderful parents, Edie and Henry Freiser. One of Ben's many highly original contributions was "EIEIO" (electron impact excitation of ions from organics) [1] that we and many others have found useful for the fragmentation of gaseous cations. Ben's method used energetic electrons to collisionally excite positive ions. We have modified his technique so that electrons are captured by multiply charged ions [2–5]. We dedicate this further report on electron capture dissociation (ECD) to his memory.

2. Introduction

The use of tandem mass spectrometry (MS/MS) to obtain sequence information from proteins has progressed rapidly [6–9]. Electrospray ionization (ESI) [10] is the method of choice, as the multiple protonation of ions formed by ESI provides the electrostatic repulsion necessary for dissociation in the mass spectrometer. Conventional excitation methods such as EIEIO [1], collisionally activated dissociation (CAD) [11,12], or infrared multiphoton dissociation (IRMPD) [13] cause *b*, *y* cleavage [Eq. (1)],

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Dedicated to the memory of Professor Ben Freiser.

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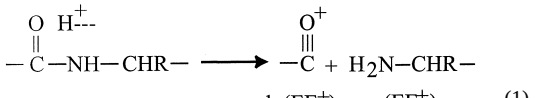
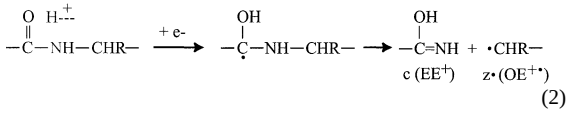


(1)

whereas the recent ECD method [2–5] causes the contrasting c, $z\cdot$ cleavage [Eq. (2)].



(2)

CAD is several times as efficient in producing fragment ions, but ECD can cleave several times as many bonds. The methods are complementary also, as fragment ions from cleavage of the same bond by both methods can then be directionally assigned, as N-terminal b ions [Eq. (1)] are smaller than c [Eq. (2)], and y ions are larger than $z\cdot$.

In the "top down" approach to MS/MS sequencing [14], large fragment ions are first prepared by more gentle dissociation: a complementary set of fragments whose masses sum to those of the molecular ion then define parts of the protein. Their masses can be compared to the predicted sequence, with confirmation by partial MS/MS sequencing; mass discrepancies indicate sequence errors, post translational modifications, a covalently derivatized active site, etc. Restricting the site of such a mass modification within a fragment with MS^3 requires backbone dissociation on either side of the site, whereas de novo sequencing is only as extensive as cleavages can be affected between each pair of amino acids. Although proteolytic enzymes are highly effective for specific cleavages, not only is MS/MS more convenient, it makes possible sample sizes as small as 10^{-17} mol [15].

For the application of these dissociation methods to real protein problems, we examine here the relative effect of each of the common amino acids on the tendency for dissociation of neighboring bonds. For energetic methods (CAD, IRMPD), well-known effects include high tendencies for dissociation on the N-terminal side of Pro and the C-terminal side of Asp. This comparison of CAD and ECD provides statistical data from ESI of a substantial number of proteins, measured on the same instrument.

3. Experimental

As described [4,16], the modified Fourier transform mass spectrometer (6T, Finnigan FTMS, Madison, WI) has a conventional electron filament (movable to on-axis) and extra e^- trapping electrodes 1 mm outside the cylindrical cation trapping electrodes. Samples, electrospray, MS/MS conditions, and electron cooling/ion trapping (gas pulse) in the instrument have been described [2–5,16]. Isotopic cluster identification and mass measurements were made with a new automated program [17].

4. Results

All CAD and ECD spectra were measured in this laboratory. CAD data were from the following proteins: carbonic anhydrase B, melittin, and porcine serum albumin [18]; rabbit and human brain [19,20] creatine kinase; ubiquitin [12,13]; cytochrome c [21]; thiamin biosynthetic enzymes C, D, E, F, S, G, H, K [22,23]; and prolyl-4-hydroxylase B [24] that contained a total of $\sim$ 4500 amino acids. Any product (e.g. b or y) resulting from cleavage of a specific backbone bond is credited to the amino acids on both the N-terminal side (. . . XX?$\overline{\text{X}}$XX. . .) and C-terminal side (. . .XX$\overline{\text{X}}$?XX. . .) of the cleavage, irrespective of the fragment ion abundance. The sums for each residue are divided by its number of occurrences in these proteins. The average of 8% for all amino acids (Fig. 1) would be much higher if smaller proteins were chosen; note that albumin, the creatine kinases, ThiC, Thi H, and P4HB are $>$ 40 kDa.

The ECD data were from the following proteins: ubiquitin, cytochrome c, apomyoglobin [2,4,5], and peptides containing 12, 12, 15, 17, 17, 21, and 24 residues [3]. These contained a total of 451 amino acids and produced 514 ECD fragments, indicative of the far greater extent of dissociation by ECD for smaller proteins (myoglobin, 17 kDa, only exhibits 33 ECD cleavages, and none has been observed for proteins $>$ 20 kDa [2–5]. Because of this high cleavage tendency, it was necessary to use also relative product abundance in comparing the influence of the

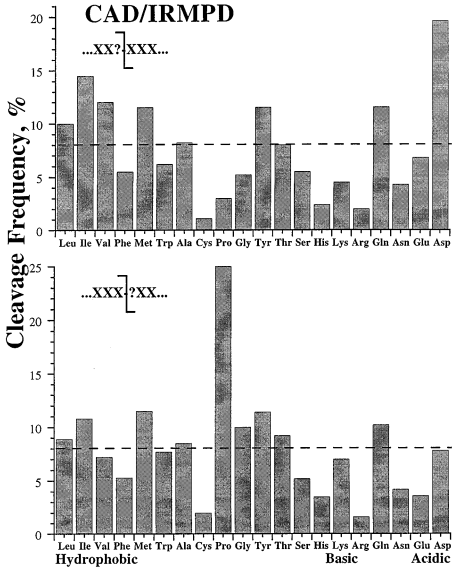


Fig. 1. For CAD and IRPMD spectra of multiply protonated peptides and proteins, frequency of cleavage (%) on the C-terminal (top) and N-terminal sides of the designated amino acids.

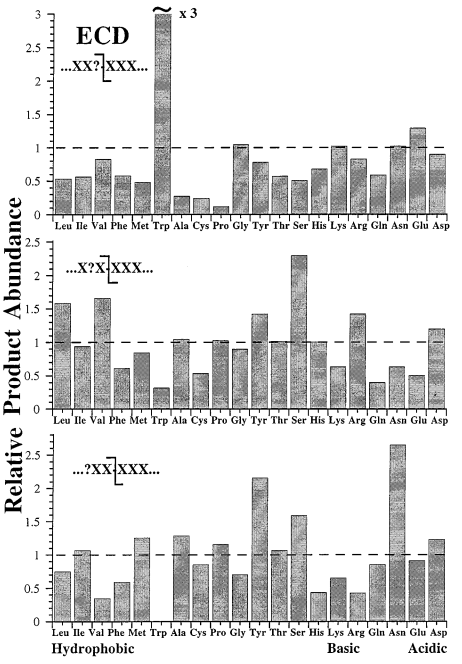


Fig. 2. For the corresponding ECD spectra, the average relative abundance of product ions from cleavage on the C-terminal side of the designated amino acids.

individual amino acids. In addition, some residues appeared to have an influence on the backbone cleavage even if placed one or two residues away from the cleavage site (Figs. 2 and 3).

5. Discussion

5.1. Energetic dissociation

Many CAD [11,12] and IRMPD [13] spectra of multiply protonated proteins (Fig. 1) have been reported [6–9,12–15,18–26]. The most widely recognized correlations are the high tendencies for cleavage on the N-terminal side of Pro (here three times the average) and C-terminal side of Asp (three times that of Glu). In addition Cys on either side of the amide backbone bond greatly stabilizes it. This is true to a lesser extent for the basic amino acids His, Lys, and Arg, possibly through solvation of their protonated side chains to neighboring backbone carbonyl groups [7,21,27]. The most hydrophobic residues show close to average influence, consistent with a dominant effect, either positive or negative, by the amino acid on the other side of the bond.

5.2. Electron capture dissociation

Product abundances were tabulated instead for ECD (Figs. 2 and 3). These should show greater variations as a function of the neighboring amino acid than just a yes/no tabulation (as used for Fig. 1); in many of the examples (e.g. peptides, ubiquitin) $> 90\%$ of the bonds are cleaved, so most cases are "yes." Despite this, all but one of the 20 neighboring amino acids give abundances less than 2.5 times the average, and all but two of Fig. 2, and three of Fig. 3, are ≥ 0.4 times the average. This is consistent with the proposal [5] that electron capture at a protonated site releases an energetic H· atom that, in turn, is captured (the S–S bond is a favored site). For c, z· cleavage [Eq. (2)], the H· affinity of the backbone carbonyl groups of the different amino acids are sufficiently similar to give, for most cases, nearly

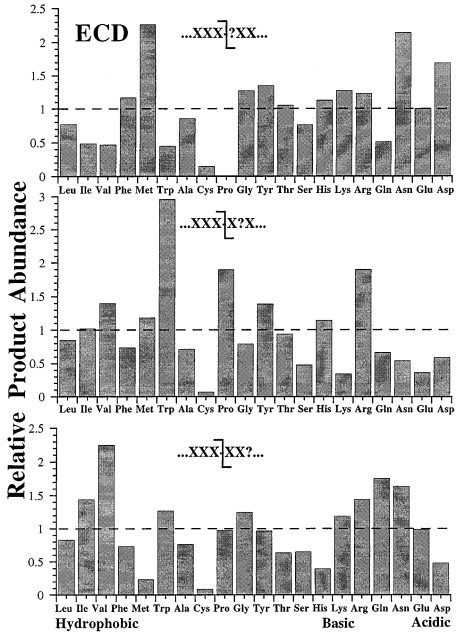


Fig. 3. As in Fig. 2, but ECD cleavage on the N-terminal side.

equivalent H· capture probabilities. Rice–Ramsberger–Kassel–Marcus (RRKM) calculations indicate that the resulting backbone cleavage is nearly instantaneous (nonergodic) [5].

As noted previously [2–5], the most dramatic influence is that of Pro; contrary to its acceleration of CAD/IRMPD cleavages, Pro completely blocks *c*, *z*· cleavage [Eq. (2)] on its N-terminal side (Fig. 3). This is consistent with its unique tertiary nitrogen at this cleavage site, so that dissociation of a single N–C bond will not affect backbone cleavage. It is less clear why Pro also exhibits the least cleavage of all amino acids on its C-terminal side, but both negative correlations are useful for sequencing [2–5].

By far the most dramatic positive correlation is that of Trp [5]; cleavage on its C-terminal side yields 9 times more abundant fragment ions (Fig. 2). This is consistent with its high H· affinity, similar to that of the S–S bond; attack of its new radical site on its own carbonyl, or donation of H· to that carbonyl, could result in *c*, *z*· cleavage [5]. Although this nearly eliminates *c*, *z*· cleavage at the next two C-terminal sites (Fig. 2), this has a surprising (next largest, 3 times) positive effect at the second site towards the N-terminus. Because the proteins examined contained a total of only three Trp residues, the ECD spectrum of the 15-mer CLKNGPTRWQYKRT-NH_2 with one Trp (W) was measured, and it confirmed these correlations. The somewhat higher abundance from N-terminal cleavage by the S-containing Met (Fig. 3) could be explained also by a somewhat higher H· affinity, but this rationale does not apply to Cys, which has the opposite effect. Cys also greatly reduces cleavage one and two residues away towards the N-terminus as well as on its C-terminal side; perhaps its –SH group can interact with the intermediate radical –CHRC(OH)NH– of Eq. (2). Cleavages do occur on the exterior sides of the two cysteine S–S linkages between the A and B chains of insulin [5]. Except for Trp, all other amino acids cause C-terminal cleavage that is average or below, with the least for hydrophobic residues (Fig. 2), with some tendency for this shown also by the N-terminal cleavages (Fig. 3). Of the seven (of 75) bonds in ubiquitin not cleaved by ECD, three are N-terminal to Pro and the other four are adjacent to Leu or Ile.

6. Conclusions

Energetic cleavages (CAD, IRMPD, even EIEIO [28]) of multiply protonated proteins are far more affected by the identity of the neighboring amino acids than those from ECD. Thus, ECD provides far more sequence information, but the complementary CAD/IRMPD data makes even more complete sequencing possible. For ECD the most influential amino acids are Pro (no N-terminal cleavage), Trp (9 times C-terminal cleavage), and Cys (little N- or C-terminal cleavage). With the more efficient data reduction and interpretation algorithms [17], far more complete de novo sequencing [14] at the subfemtomol level [15] should be relatively routine in the future.

Acknowledgements

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