

Electron Capture Dissociation of Multiply Charged Protein Cations. A Nonergodic Process

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Neutralization-reionization mass spectrometry (MS)¹ is of unique value for preparing and characterizing highly reactive and unstable neutral species, such as the intermediate in the dissociative-recombination reaction $\text{H}_3\text{O}^+ + \text{e}^- \rightarrow \text{H}_2\text{O} + \text{H} + 6.4 \text{ eV}$.² Following an earlier suggestion,³ using neutralization accompanying surface-induced dissociation (SID)⁴ to form an unstable site did not yield new cleavage reactions⁵ in multiply charged protein cations from electrospray ionization (ESI) with Fourier transform (FT) MS.⁶ Serendipitously, we now find that one or more charges on such protein cations can be neutralized with low-energy electrons to cause specific cleavage of the amine bond to form *c*, *z* products,⁷ in contrast to the amide cleavage *b*, *y* products formed by collisionally activated dissociation (CAD),⁸ infrared multiphoton (IRMPD)⁹ and UV¹⁰ photodissociation, 70 eV electron impact excitation,¹¹ and SID.⁵

The *b*, *y* products are formed by the lowest energy backbone cleavage of ESI protein ions.^{6–9} An attempt to cleave stronger bonds using high-energy (6.4 eV) 193 nm photons gave mainly *b*, *y* products for 2 kDa protein ions,¹⁰ but for 2.8 and 8.6 kDa protein ions¹² gave small yields of *c*, *z* amine bond cleavage products not previously observed. In this further investigation, extra electrodes were placed outside the ion cell electrodes that trap the positively charged ions. With the outside electrodes at +9 V,¹³ extensive 193 nm laser irradiation of SWIFT-selected¹⁴ $(\text{M} + 11\text{H})^{11+}$ ubiquitin ions (8.6 kDa) only produces *b*, *y*, not *c*, *z*, ions. However, with the outside electrodes at –1 V, the *c*, *z* products are formed along with 10+ molecular ions; unexpectedly,

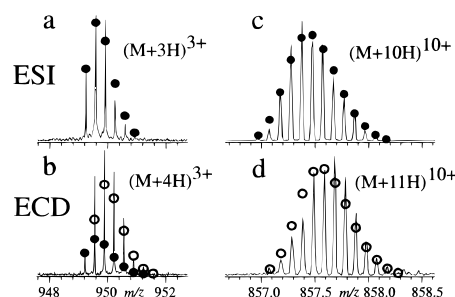
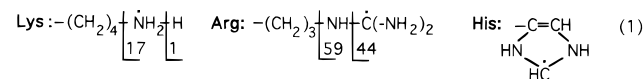


Figure 1. Isotopic distributions of (a, b) melittin 3+ and (c, d) ubiquitin 10+ ions obtained by (a, c) ESI and (b, d) ECD. Closed and open circles, theoretically predicted isotopic abundance distributions for $(\text{M} + n\text{H})^{n+}$ and $(\text{M} + n\text{H})^{(n-1)+}$, respectively;⁶ those of 1b should sum to the measured abundances.

these are mainly $(\text{M} + 11\text{H})^{10+}$ ions (Figure 1d), 1 Da heavier than the $(\text{M} + 10\text{H})^{10+}$ ions formed by ESI (Figure 1c). The 4+ melittin ion spectrum measured under the same conditions (Figure 1b) similarly contains $(\text{M} + 4\text{H})^{3+}$, consistent with capture of secondary electrons formed by the 193 nm photons impinging on metal surfaces and trapped by the –1 V electrodes: $(\text{M} + 4\text{H})^{4+} + \text{e}^- \rightarrow (\text{M} + 4\text{H})^{3+}$.¹⁵

Electrons were produced instead (no laser) by a conventional heated filament source outside the FTMS magnet opposite the ESI source.¹¹ With a 10^{-5} Torr Ar pulse for e^- cooling (energy < 0.2 eV; an SF_6 pulse lowered the efficiency), the 11+ ions of ubiquitin gave a spectrum that showed *c*, *z* cleavage of 50 out of 75 backbone positions; CAD⁸/IRMPD⁹ gave *b*, *y* cleavage of eight of these positions plus seven others. Cooled electrons plus the 15+ ions of Fe^{III} equine cytochrome *c*¹⁶ produced (Figure 2) *c*, *z* fragment ions from cleavages at all but 40 of the 103 possible backbone sites (e.g., N-terminal side of Pro, none; of Ile, Leu, Val, few); CAD produces *b*, *y* cleavages (total 19) at eight additional sites. The 21+ apomyoglobin (17 kDa) yielded 33 *c*, *z* cleavages, but the 34+ ions of bovine carbonic anhydrase (29 kDa) as yet has given only 33+, 32+, and 31+ molecular ions.

Electron capture dissociation (ECD)^{1–3} rationalizes these results. The capture cross section should be proportional to the ionic charge squared, consistent with the minimal secondary fragmentations to produce internal ions and the predominance of cleavages in the central ~70% of the protein chain. Charge values and masses¹⁷ of the complementary product ions are consistent with dissociation *after* e^- capture, such as $\text{c}_{35}^{5+}/\text{z}_{37}^{5+}$ from the 76-residue ubiquitin 11+ ions and $\text{c}_{69}^{7,8,9+}/\text{z}_{35}^{4,5+}$ from the 104-residue cytochrome *c* 15+ ions. The most favored protonation sites are the side chains of Lys, Arg, and His,¹⁸ neutralization to form hypervalent species^{1–3} at Lys and Arg would account for ions (Figure 2) representing losses of 17, 44, and 59 Da from $(\text{M} + n\text{H})^{(n-x)+}$ (eq 1; neutralization of protonated His gives a more stable radical site).



However, these products are less abundant than the *c*, *z* species for the larger molecular ions. Their side chain protonated sites

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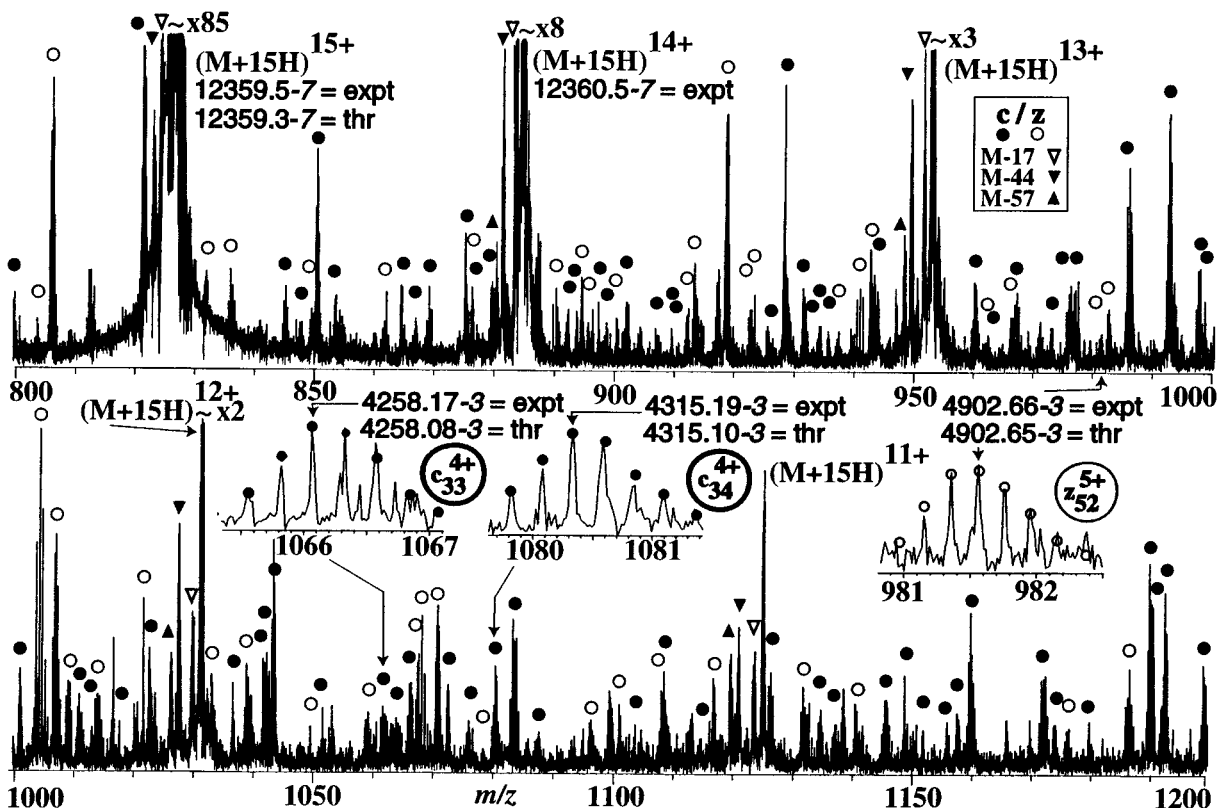
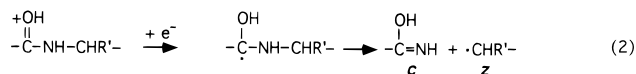


Figure 2. ECD spectra of 15+ ions of cytochrome *c* (Fe^{III}),¹⁶ 75 scans: ▽, -17 Da; ▼, -44 Da; ▲, -59 Da; ●, *c* ions, ○, *z* ions. Mass values are for the neutral molecule's most abundant isotopic peak; the mass difference (units of 1.0034 Da) between this and the monoisotopic peak is shown by the italicized final digit.⁶ Circles, as in Figure 1.

apparently are stabilized by solvation to backbone functionalities;^{16,18} an electron approaching such a proton-bound species $A\cdots H^+\cdots B$ would tend to give $A-H + B$ if the electron affinity (EA) of $A+H$ is greater than that of HB^+ . EA of a protonated nitrogen (amine, amide) is ~ 4 eV versus ~ 6 eV for a protonated carbonyl,^{1,2} suggesting a radical site reaction (eq 2) that should require less energy than *b*, *y* cleavage. Also, e^- neutralization of



the protonated carbonyl would be ~ 6 eV *exothermic* versus ~ 1 eV for surface neutralization (in which *c*, *z* cleavage was not observed);⁵ this apparently represents nonergodic dissociation of some $(M + nH)^{(n-1)+}$ ions before they can be stabilized by randomization of this energy.¹⁹ Consistent with this, SWIFT isolation of the reduced $(M + 8H)^{7+}$ ions from ECD of ubiquitin followed by CAD gave only *c*, *z* products; however, many of these were not in the ECD spectrum, resulting presumably from intramolecular electron transfer (a fast reaction)²⁰ to a more stable backbone site before dissociation. Other intramolecular reactions also appear slower than ECD. Equine cytochrome *c* 15+ ions exposed to 10^{-7} Torr D₂O for 30 min exchanged 125 D atoms, as expected;¹⁶ under e^- capture conditions that halved the 15+ molecular ion abundance, reexposure to D₂O only increased this to 130–135 D atoms in the 15+, 14+, and 13+ molecular ions. Thus, dissociation is also faster than H/D scrambling, in contrast to CAD or IRMPD excitation that gave 170 ± 10 D atoms in

similar experiments,¹⁶ compromising MS/MS location of D sites. ECD of deuterated 15+ ions gave z_{55} ions with 52 D at protein sites vs 59 D for equivalent ions from CAD.¹⁶

To date, the highest yield from ECD of 15+ cytochrome *c* ions is 27% fragment ions plus 9% reduced molecular species, with 25% of 15+ ions undissociated; CAD⁸ and IRMPD⁹ give far higher efficiencies, especially above 20 kDa. CAD, IRMPD, and proteolysis of proteins as large as 67 kDa (even at the 10^{-17} mol level)²¹ have provided valuable information on DNA-derived sequence errors, posttranslational modifications, and covalently derivatized active sites.²² More complete sequencing is obtained more efficiently with a "top down" initial dissociation producing larger (5–25 kDa) fragment ions or peptides;²³ subjecting these to ECD, as well as CAD, will greatly increase the specificity of such MS/MS studies. Such unique radical cation species of other large molecules should provide characteristic new reactions (inter- as well as intramolecular) along with more definitive data on nonergodic dissociations.

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