Spatial extent of fragment-ion abundances in electron transfer dissociation and electron capture dissociation mass spectrometry of peptides

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A R T I C L E   I N F O

Article history:
Received 19 April 2012
Received in revised form 17 July 2012
Accepted 17 July 2012
Available online 4 August 2012

Keywords:
Electron transfer dissociation
Electron capture dissociation
Fragmentation patterns

A B S T R A C T

Earlier work from the first author's group has suggested that, in electron capture dissociation (ECD) or electron transfer dissociation (ETD) mass spectrometry experiments, an electron is initially attached into a Rydberg orbital centered at one of the peptide's positive sites (likely a protonated N-terminus, Lysine, Arginine, or Histidine). Moreover, this earlier work predicted that only Rydberg orbitals having principal quantum numbers \(n = 3–6\) are populated in ECD and only \(n = 3\) and 4 in ETD (when an anion donor having an electron binding energy of ca. 0.6 eV is used), and that the populations of these levels are very similar in the nascent charge-reduced peptide. Based upon these predictions, the present paper develops a framework for predicting the abundances of closed-shell \(c\) and open-shell \(z^*\) fragments as a function of distance along the backbone from the site initially holding the attached electron in a Rydberg orbital. The framework is not aimed at differences in branching ratios caused by differences in the physical properties of side chains along the backbone but on the spatial distances between the charged site holding the electron and the backbone amide units. The predictions of this model are tested using ECD and ETD data from experiments carried out using simultaneous infrared photo-activation of the parent ions. Such activated-ion (AI) experiments are thought to disrupt much of the parent ion's secondary structure, which we believe allows us to make more reliable estimates of distances between the charged sites and the various amino acids' amide groups. The abundance patterns predicted based upon the framework described herein are found to be reasonably consistent with the experimental data. However, the data also provide evidence that internal solvation of the peptide's charged sites remains intact even under AI conditions, and that some of these solvated-ion structures (those involving a charged Lys or N-terminal amine) contribute incrementally to the abundances of fragment ions arising from cleaving nearby \(N-C\alpha\) or S-S bonds. As a result, we conclude that ECD and ETD fragment ion abundances are determined by a combination of factors: (i) internal solvation of charged sites, (ii) spatial distributions or Rydberg orbitals charge densities within several residues of charged sites, and (iii) variations induced by differences in physical properties of side chains. It is primarily the first two of these three that the present paper addresses.

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1. Introduction

In electron-transfer dissociation [1–5] (ETD) or electron-capture dissociation [6–9] (ECD) mass spectrometry experiments, an electron attaches to a multiply positively charged gas-phase peptide and induces bond cleavages within the charge-reduced peptide. ECD and ETD are relatively new yet extremely promising analytical techniques that are found to generate significantly higher backbone cleavage fractions than collisional or infrared dissociation techniques, while doing so with great specificity (i.e., in peptides, primarily \(N-C\alpha\) and S-S bonds are cleaved) and leaving post translational modifications intact to a great extent. Why \(N-C\alpha\) and S-S bonds cleave and why they do throughout such a large fraction of the backbone need to be explained, and this has been the focus of much of the theoretical work in this area.

In earlier research [10] efforts, the first author’s group focused on:

1. identifying where in the peptide (and into what kind of orbital and with what cross-section) the excess electron is initially bound,
2. characterizing to where, over what distances, and at what rates the electron may subsequently migrate within the peptide, and
Understanding how the excess electron’s presence causes specific bonds (e.g., disulfide and backbone N–C\textsubscript{\textalpha} bonds are found to preferentially break) to be cleaved and with what intensities.

As a result of our earlier studies and many more from the Turecek and other groups [11–39] and following early mechanistic proposals from the McLafferty and Zubarev groups [6–9], a picture has evolved within which it is the arrival of an electron at an amide π* or disulfide σ* orbital that causes the N–C\textsubscript{\textalpha} or SS bond cleavage, respectively. The amide π* or disulfide σ* orbitals that are amenable to electron attachment are determined by the local intramolecular electrostatic potentials stabilizing the orbitals (if the potential is not sufficiently stabilizing, electron attachment cannot occur). Both Coulombic potentials from charged sites and dipole potentials [40–42] within the peptide have been suggested to play important roles both in guiding the ECD electron or ETD donor anion and in rendering the amide π* or disulfide σ* orbitals capable of accepting an electron.

In addition to contributing to the electrostatic potential within the peptide, the positive sites within the charged peptide provide Rydberg orbitals that act as “antennas” to which the electron is initially attached (either directly in ECD or via transfer from the anion donor in ETD), and subsequently serve to transfer the electron to amenable amide π* or disulfide σ* orbitals over distances determined by the radial sizes of the Rydberg orbitals.

The resulting mechanistic picture describes the ECD or ETD process as follows:

1. In both ECD and ETD, the electron most likely first attaches to a Rydberg orbital localized on a positively charged site (e.g., a protonated side chain or the protonated N-terminus) of the parent peptide as shown in Scheme 1. This positively charged site is often internally “solvated” via hydrogen bonding to, for example, a nearby carbonyl oxygen atom.

2. Within a Landau–Zener model, the initial electron attachment occurs at electron–peptide or anion donor–peptide distances that depend on the electron binding energy of the donor anion 1 and of the Rydberg orbital into which the electron is transferred.

3. Estimates of the electron attachment cross-sections based on such a model suggest that the accessible Rydberg principal quantum numbers (n) are limited to n = 3 and 4 in ETD 1 and n = 3–6 in ECD, and that each of these Rydberg levels is approximately equally populated.

4. After an excess electron is captured into a Rydberg orbital, it can subsequently undergo prompt (i.e., faster than relaxation within the Rydberg orbital manifold) intramolecular electron transfer to any SS σ* or amide π* orbital that lies within the radial shell of that Rydberg orbital but only if the electron binding energy of that Rydberg orbital can be overcome by that of the SS σ* or amide π* orbital. This electron transfer step can be especially facile if the positively charged site on which the Rydberg orbital sits is hydrogen bonded to a nearby carbonyl oxygen atom as suggested in the pathway 2 labeled C in Scheme 1. However, as suggested by the pathway labeled UW in Scheme 1, it is also possible for the electron to transfer to more-distant SS σ* or amide π* orbitals, especially if the electron occupies a Rydberg orbital of large radial extent.

5. Once the electron enters an SS σ* orbital, cleavage of the associated disulfide bond is prompt because the σ*–σ*+1 anionic electronic state is repulsive. If the electron enters an amide π* orbital, cleavage of the N–C\textsubscript{\textalpha} bond can occur by surmounting a thermally accessible barrier that is much smaller than the barrier needed to homolytically cleave this N–C\textsubscript{\textalpha} bond in the absence of the excess electron.

6. After the electron attaches to an amide π* orbital, the N–C\textsubscript{\textalpha} bond is weakened because cleaving it allows a new C= N π bond to form; this is why the barrier to cleavage is reduced. The C=C=N–NH anion site can abstract a proton to form either the enolimine or the more stable amide. The proton is most likely abstracted from a proximal site of low proton binding strength. In either case, a closed-shell c fragment and an open-shell radical fragment are the result of the N–C\textsubscript{\textalpha} cleavage.

In the present paper, we explore the implications of this model’s prediction that n = 3 and 4 Rydberg orbitals will be equally populated in ETD and n = 3–6 Rydberg orbitals will be equally populated in ECD. In particular, we discuss what spatial distribution in the fragment-ion abundance patterns would be expected if these predictions are true, and we examine abundance patterns found in data obtained in experiments carried out under infrared photoactivation conditions where the parent-ion secondary structure is expected to be appreciably disrupted.

2. Theoretical analysis

Rydberg orbital manifolds occur at each positively charged site within a multiply charged polypeptide. Each Rydberg orbital can be characterized by a principal quantum number n that relates (approximately) to its electron binding energy as follows:

\[ BE_{\text{Rydberg}}(n) = 13.6 \text{ eV}/n^2 \]  

and to its radial “size” (i.e., the average value \( \langle r \rangle \) of the distance of the electron from the center of positive charge) as

\[ \langle r \rangle \approx 0.529 \text{ nm} \]  

In atomic cations, each Rydberg level having a given principal quantum number n has a family of orbitals characterized by orbital angular momentum quantum numbers \( L \) and \( m \), with \( L \) running from 0 to \( n−1 \). For molecular ions, the orbitals corresponding to this manifold of \( n \) different \( L \)-values and \( 2L+1 \) different \( m \)-values still exist (i.e., one finds Rydberg orbitals having clear s, p, d, etc. angular nodal patterns), but their energy degeneracies are split by the local potential of the site at which the Rydberg orbitals are bound. Examples of several Rydberg orbitals of NH\textsubscript{4}+, of NH\textsubscript{3}–CH\textsubscript{3}, and of [AR+2H]\textsuperscript{2+} are shown in Fig. 1.

In addition to their low electron binding energies, which are even smaller than the valence electron binding energies of alkali cations, Rydberg orbitals are characterized by large radial extents (e.g., a 6s orbital has its highest density near 30 Å). To achieve a more accurate description than provided by Eq. (1) for molecular Rydberg orbitals, it is common to characterize the electron binding energy in terms of a so-called quantum defect \( \delta \) and a principal quantum number \( n \)

\[ BE_{\text{Rydberg}}(n) \approx 13.6 \text{ eV}/(n \− \delta)^2 \]  

The quantum defect is introduced to (approximately) account for the fact that the Rydberg orbital is not fully screened from the

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1 In this paper, we assume a value for the electron binding energy \( BE_{\text{Donor}} \) of 0.6 eV (i.e., that of the commonly used fluoranthene anion) to characterize ETD, and a value of 0.0 eV is used to characterize the free electron used in ECD.

2 This step can alternatively be viewed as involving transfer of a hydrogen atom from the hypervalent –NH\textsubscript{3} radical to the nearby carbonyl oxygen atom.

3 It is possible that the proton is abstracted prior to cleavage of the N–C\textsubscript{\textalpha} bond, in which case formation of the enolimine would be favored. Some experimental infrared spectroscopic evidence exists [34] providing evidence that the amide is formed, but this is not conclusive support proving that the proton is transferred after N–C\textsubscript{\textalpha} bond cleavage because the enolimine, if formed first, could subsequently rearrange to the thermodynamically more stable amide.
Scheme 1.

Fig. 1. 3s, 3p, 3d, 4s, 4p, and 5s Rydberg orbitals of NH₄⁺ (left); the lowest (3s) Rydberg orbital of NH₃—CH₃ (middle); and the lowest Rydberg orbital of the dipeptide (AR+2H)²⁺ (right). The latter figure is taken from Fig. 3 in Ref. [43].
underlying nuclear charges by the inner-shell orbitals. For example, in NH₄⁺, we view the lowest Rydberg state as having \( n = 3 \) because nitrogen's 7 and the four protons' 4 positive charges are partially screened by the 10 total (1s² and 4e²) inner-shell electrons. That is, NH₄⁺ is similar to Na⁺ whose lowest unoccupied orbital is its 3s orbital. The energy needed to remove an electron from the 3s orbital of Na is 5.1 eV. To obtain this binding energy from Eq. (3) for \( n = 3 \) would require a quantum defect of 1.37. The binding energy of the lowest Rydberg orbital of NH₄ is ca. 4 eV, which is not close to the estimate 13.6/(2²) = 1.5 eV obtained from the hydrogenic expression of Eq. (1). However, if we take \( \delta = 1.16 \), Eq. (3) gives the correct 4 eV electron binding energy for the lowest \( n = 3 \) state of NH₄. This same quantum defect of 1.16 then predicts binding energies of 1.7, 0.9, and 0.6 eV for the \( n = 4 – 6 \) levels of NH₄, respectively.

Because the radial extent of Rydberg orbitals also varies with \( n \) (see Eq. (2)), introduction of the quantum defect also alters estimates of these orbitals' sizes. Again using \( \delta = 1.16 \) to characterize NH₄⁺, we find ratios in the radial sizes of \( n = 3 – 6 \) Rydberg orbitals of \( (n – \delta^2)/n^2 = 0.4, 0.5, 0.6, \) and 0.6, compared to the size estimates obtained from the purely hydrogenic Eq. (2). That is, partial screening by inner-shell electrons radially contracts the \( n = 3 – 6 \) Rydberg orbitals by 40–60%. In the discussion given below, we use a factor of 50% to be representative of the average radial-scaling appropriate to the range of \( n = 3 – 6 \) Rydberg orbitals.

In the top portion of Fig. 2 we show the radial probability densities \( \rho^2 \) to \( \sin \theta \rho^r (\theta, \phi) \rho (\theta, \phi) dr d\phi \) for hydrogen (i.e., assuming \( \delta = 0 \)) 3s, 4s, 5s, and 6s orbitals. Plots of Rydberg orbital densities appropriate to NH₄⁺ (and, we believe, reasonably appropriate for protonated amine sites) would have their radial extents contracted by ca. 50% as explained above.

Notice that each Rydberg orbital has an outermost major radial peak and a series of minor peaks at smaller distances, that the location \( \langle r \rangle \) and width \( \Gamma \) of the Rydberg orbitals' major radial peaks increases as \( n \) increases, and that the height of these major peaks decreases with \( n \) (e.g., \( n = 3 \) is more strongly peaked near 7 Å than \( n = 5 \) near 14 Å). Orbitals with non-zero angular momentum have fewer radial nodes and are a bit more radially contracted than their corresponding \( s \)-orbitals described in Fig. 2. Moreover, as discussed earlier, limited screening as embodied by a non-zero quantum defect, causes the Rydberg orbitals of, for example, NH₄, to be radially contracted relative to what is shown in Fig. 2. However, at all levels of theory, Rydberg orbitals are radially large, have small electron binding energies, and have major peaks whose width increases with \( n \) and whose height decreases with \( n \). These attributes of Rydberg orbitals will be seen to be of much importance when predicting the spatial distribution of abundances of bond-cleavage fragment ions.

As noted earlier, our earlier mechanistic studies concluded that

1. \( n = 3 \) and 4 Rydberg orbitals for ETD and \( n = 3 – 6 \) orbitals for ECD are approximately equally populated in the initial electron attachment event, and
2. the rate of electron transfer from such a Rydberg orbital to any SS \( \sigma^* \) or amide \( \pi^* \) orbital lying within that Rydberg orbital's major radial peak is fast compared to rates of relaxation among the Rydberg orbital manifold.

This is why we display radial electron densities for ensembles with (i) equal populations of \( n = 3 \) and 4 Rydberg orbitals (for ETD) or with (ii) equal populations of \( n = 3 – 6 \) Rydberg orbitals (for ECD) in the bottom of Fig. 2.

The range of residues covered by the \( n = 3, 4 \) and \( n = 5, 6 \) Rydberg densities shown in the bottom part of Fig. 2 derive from assuming (i) a spacing of ca. 3 Å between neighboring backbone amide groups, (ii) a contraction of ca. 50% in the orbitals' radial extent when a quantum defect of 1.16 is used as discussed earlier, and (iii) that the charged side chain's flexibility allows it to hydrogen bond to its nearest carbonyl site thus making the location of the positive site close to the side chain's neighbor's carbonyl oxygen. It is this assumption of internal solvation of the charged site that allows us to estimate the location of the Rydberg-bound electron relative to the backbone amide sites independent of the length of the side chain possessing the charged site.

The 3 Å inter-residue distance may be appropriate for a spatially extended polypeptide, but not if the peptide is highly folded or globular. Moreover, each of the positive sites within a peptide sample used in ECD or ETD adopts a dynamically changing range or positions (e.g., the basic side chains and even the N-terminus are flexible) and the electron density experienced by any given SS \( \sigma^* \) or amide \( \pi^* \) orbital will depend on its instantaneous distance from the positive site holding the attached electron. For these reasons, it is best to average the density plots in Fig. 2 over a range of distances characterizing the location of the charge site holding the electron. With these caveats in mind, we think it still reasonable that these plots predict high cleavage rates between 1 and 5 residues from positive sites whose \( n = 3, 4 \) Rydberg orbitals are populated and lower cleavage rates beyond the 5th residue out to ca. 9 residues in ECD where the \( n = 5, 6 \) orbitals are also populated.

In summary, we expect certain major features contained in the idealized data of Fig. 2 (with radial distances scaled to reflect the quantum defect) to persist in real Rydberg orbital densities:

1. The \( n = 3, 4 \) density has high amplitude between residues 1 and 5.
2. The \( n = 5, 6 \) density (i.e., the difference between the red and yellow plots in the bottom of Fig. 2), which we suggest accounts for density present in ECD but not in ETD, arises mainly between residues 4 and 9.
3. The intensity of the \( n = 3, 4 \) density is higher within its primary range than is the \( n = 5, 6 \) density within its primary range.

With this picture of the \( n = 3, 4 \) and \( n = 3 – 6 \) electron densities in mind, we now put forth the main predictions of this paper.

1. In ETD,¹ the abundance of \( c \) or \( z^* \) fragments resulting from \( N-C_{\alpha} \) cleavage should reflect that spatial distribution of \( n = 3 \) and \( n = 4 \) Rydberg orbitals around each charged site, and
2. In ECD, the abundance of \( c \) or \( z^* \) fragments resulting from \( N-C_{\alpha} \) cleavage should reflect that spatial distribution of \( n = 3 \) through \( n = 6 \) Rydberg orbitals around each charged site, with higher abundance in the range of the \( n = 3, 4 \) Rydberg orbitals.
3. Because electron attachment to various charged sites need not occur with equal probability, the total abundances associated with these sites can differ.

Because the \( n = 3, 4 \) distribution has higher intensity for \( r \)-values falling within its major peak than does the \( n = 5, 6 \) distribution within its major peak, we predict more bond cleavage 1–5 residues from a positive site holding the attached electron and, for ECD but not ETD, relatively less cleavage 6–9 residues from this site. Moreover, for reasons explained earlier, we do not expect the cleavage patterns to display the fine details (e.g., nodes and oscillations) shown in Fig. 2.

The predictions just put forth do not take into consideration the nature of the spatial distribution of amino acid side chains within the peptide. They focus only on the \( n = 3, 4 \) or \( n = 3 – 6 \) Rydberg densities' spatial extent as a measure of the potential of these densities to deliver the attached electron to various amide \( \pi^* \) orbitals. As we will see later, the physical characters of the side chains can indeed alter the product ion abundance patterns beyond what the current model suggests. Within our model, this means that the ability of a particular amide \( \pi^* \) orbital to accept the attached electron from a
Rydberg orbital depends on the nature of the side chain attached to that amide’s ω carbon atom. Other studies [44–47] have examined how various physical properties of the side chains can alter the cleavage rates of nearby N–Cα bonds and have even made use of these effects by introducing artificial (predator) side chains [48] that capture ETD or ECD electrons thus interrupting the usual N–Cα bond cleavages.

In the following section, we examine selected experimental data to ascertain whether the abundances of c or z fragments do or do not agree with the predictions about the range (e.g., 1–5 residues for ETD) and spatial distribution (e.g., peaking around residues 1–3 for ETD) of fragment ions around each positive site. We focus on experiments in which infrared photo-activation steps are taken in conjunction with the ETD or ECD as a means for disrupting the secondary structure (i.e., folding, internal solvation, and hydrogen bonding) of the parent ion. This is done because we believe it is under such conditions that we can reasonably estimate the distances between the sites holding the Rydberg electron and the backbone amide sites. As will be seen, the spatial distribution of fragment ions that we are considering tells only part of the story, and the nature of the side chains as well as residual parent-ion structure that remains, even when infrared activation techniques have been employed, are part of the full story.

3. Analysis of experimental data

In Fig. 3 we display experimental data [49] taken from the Coon laboratory at Madison, Wisconsin, where the second author is actively involved. At the top of the figure, data obtained by first carrying out ETD (using azulene, which has an electron binding energy of 0.8 eV, close to the 0.6 eV value assumed in this paper, as the donor anion [50]) and subsequently submitting the non-dissociated electron transfer species to gentle resonant-excitation collisionally activated dissociation (the combined process termed ETcaD [51]). At the bottom of the figure, we see data on the same system obtained by simultaneously subjecting the parent-ion sample to infrared photons and to azulene anions, thus achieving so-called activated-ion ETD (AI-ETD). As explained in Ref. [49], the infrared activation is assumed to heat the parent ions sufficiently to disrupt secondary structure, thus (presumably) rendering the ensemble of
ions that subsequently undergo ETD fragmentation more spatially extended than in the original sample. Of course, the average gas phase conformation of precursor peptides is not expected to be rigidly linear; however, based on observations related to the degree of H-atom transfers between newly formed c/\(z\) ion pairs noted among ETD, AI-ETD, and ETDaD spectra in Ref. [49], it is our expectation that data on extended structures are more likely to follow the predictions we made earlier because, for such structures, the distances from charged sites to backbone amide units can be estimated with reasonable confidence.

ETDaD spectra typically comprise a mixture of odd and even electron product ions for both c- and z-type product ions. While ETD typically results in the exclusive production of c- and z-fragment ions, ETDaD also produces c\(^*\) and z\(^*\)-ions, nominally shifted \(\sim 1\) Da from their predicted mass. It is believed that these shifted ions arise via an H-atom transfer occurring between c- and z-ions that are bound together immediately following dissociation [52,53] (note that such H-atom transfers have been found to take place on an extremely rapid timescale [54]).

In contrast, spectra arising from Al-ETD data are dominated by c/\(z\)\(^*\) fragments, which we believe indicates that fragmentation occurred promptly after N-\(\mathrm{Ca}\) bond cleavage. Assuming that the infrared excitation indeed disrupts much of the peptide secondary structure, such data should give us the best chance for testing the predictions made earlier in this paper. For all Al-ETD data shown, photon wavelength was \(\sim 10.6\) \(\mu\)m, with a photon flux of 40–50 W unless otherwise indicated.

There clearly are significant differences between the ETDaD and Al-ETD data even if we focus on the c/\(z\)\(^*\) fragment ions that are presumed to result from prompt cleavage of N-\(\mathrm{Ca}\) bonds. To a reasonable extent, the Al-ETD abundances appear to be in line with what our model predicts:

i. 1–5 residues from the K site, fragments c\(_1\)z, c\(_1\)z, c\(_1\)z, c\(_1\)z + z\(_2\)\(^*\), and z\(_2\)\(^*\) comprise one spatial distribution;

ii. 1–5 residues from the N-terminus, fragments z\(_2\)\(^*\), z\(_1\)\(^*\), z\(_1\)\(^*\), z\(_2\)\(^*\), and z\(_2\)\(^*\) form another.

Both of which have low abundance one residue displaced from the respective charged site, a maximum in abundance one or two residues more distant, and then decreasing abundance (except for z\(_6\)\(^*\) and z\(_7\)\(^*\), which we discuss below).

Before discussing the two features that do not fit our expectations, let us reflect on what the data in Fig. 3 might suggest about when proton transfer is taking place relative to when N-\(\mathrm{Ca}\) bond cleavage occurs. First, we note that the Al-ETD spectrum shows far more z\(^*\) than c fragment ions. Likely, this is because formation of c\(^*\) ions requires a proton to transfer from the less acidic protonated K residue than the more acidic protonated N-terminus. To form the closed-shell c\(_12\) through c\(_9\) ions within the mechanism in Scheme 1, a proton must be transferred from the C-terminal direction, presumably from the protonated Lys. To form the open-shell z\(_2\)\(^*\) ion, proton transfer from the Lys must not be taking place (otherwise, c\(_8\) would form). So, it appears that proton transfer from the Lys site is limited to distances corresponding to ca. 4 residues. However, two of the most abundant ions in Fig. 3 are z\(_6\)\(^*\) and z\(_7\)\(^*\), which lie 7 and 6 residues from the protonated N-terminus and 6 and 7 residues from the Lys, respectively. Although not conclusive, these observations seem to be most consistent with a view in which the proton transfer takes place after cleavage of the N-\(\mathrm{Ca}\) bond. For z\(_6\)\(^*\) or z\(_7\)\(^*\) to be formed with proton transfer taking place prior to bond cleavage would require either the Lys or the protonated N-terminus to transfer its proton over 6 or 7 residues, which one would expect to be less likely than transfer over fewer residues and which seems inconsistent with the high abundances of z\(_6\)\(^*\) and z\(_7\)\(^*\).

As noted earlier, a feature of the abundance pattern in Fig. 3 that does not seem to fit in with our predictions is the high intensities of the c\(_12\) and z\(_7\)\(^*\) fragments. These enhanced intensities suggest that both the protonated N-terminus and the C-terminal charged K are internally solvated to proximal amide carbonyl groups and thus may generate cleavage of the N-\(\mathrm{Ca}\) bonds closest to them as the pathway labeled C in Scheme 1 describes. In such a case, attachment of an electron into an \(n = 3\) Rydberg orbital at either of these sites could, via this Cornell-like mechanism [6] (which is why we used the label C in Scheme 1) generate facile cleavage to generate the observed c\(_12\) and z\(_7\)\(^*\) fragments. It would have to be an \(n = 3\) Rydberg orbital because this orbital has the correct radial size and thickness to connect the protonated amine and the solvated amide \(n^+\) orbital and because it is known [55] that this \(n = 3\) state undergoes facile H atom loss, whereas the states with \(n > 3\) do not.

In summary, it may well be that cleavage of N-\(\mathrm{Ca}\) bonds within amide units not intimately involved in internal solvation occurs via the pathway labeled UW in Scheme 1 while cleavage of N-\(\mathrm{Ca}\) bonds whose amide carbonyls are involved in internal solvation follows the pathway labeled C in Scheme 1. It makes sense that the efficiency of N-\(\mathrm{Ca}\) bond cleavage when internal solvation is present should be especially high because of the small distance between the charged site (from which the proton can also come) and the amide unit. Moreover, we note that internal solvation of charged Lys (either to the C-terminal COOH group or to a carbonyl unit in the opposite direction) and of the protonated N-terminus (to a nearby carbonyl) are commonly encountered.

One might be tempted to suggest that all of the N-\(\mathrm{Ca}\) bond cleavages occur along the C pathway. However, this would require that the protonated N-terminus or K be involved in solvation events with amide carbonyl groups over 6 residues and to occur with a population that mimics the product ion abundance pattern seen in Fig. 3. It is difficult to imagine why a solvation pattern like this would occur, so we believe this proposal is unlikely true.

So, if formation of c\(_12\) and z\(_7\)\(^*\) is dominated by the Cornell pathway and the UW pathway of Scheme 1 (with the associated spatial distribution of the \(n = 3, 4\) Rydberg densities) accounts for c\(_12\), c\(_11\), c\(_10\), c\(_9\) + z\(_4\)\(^*\), and z\(_5\)\(^*\) as well z\(_12\)\(^*\), z\(_11\)\(^*\), z\(_10\)\(^*\), z\(_9\)\(^*\), and z\(_8\)\(^*\), why are the intensities of z\(_6\)\(^*\) and z\(_7\)\(^*\) so high? One possibility is the presence of the acidic Glu side chains on the amides giving rise to these fragments. The Tsybin group has found [56] enhancements in product
of the lower case ‘s’ denotes a phosphorylated serine residue. For AI-ETD, a laser power transfer takes place after N

c2 specifically, from 7 residues away from R again suggests that this transfer takes place after N–Cα bond cleavage rather than before.

In Fig. 4, we see another set of data from the Coon lab in which ETD, AI-ETD and resonant-excitation collision-activated dissociation (CAD) data were obtained. As expected, the CAD data differ tremendously from either the ETD or AI-ETD data and display a fragmentation pattern more typical of collisional dissociation. The main difference between the ETD and AI-ETD data is the bond cleavage rather than before.

The somewhat enhanced c9 abundance could be due to electron attachment to the internally solvated protonated K terminus generating enhanced cleavage via a Cornell-type mechanism as discussed earlier. The fact that c9 is not correspondingly enhanced would be consistent with the protonated R, even if solvated to the proximal amide carbonyl, not being able to take part in the Cornell mechanism. Indeed, in Ref. [16], evidence is discussed that supports this view; the fact that R is considerably more basic than K or than the N-terminal amine causes the protonated R’s ground Rydberg state to have a substantial energy barrier for transferring an H atom to solvation-partner carbonyl oxygen.

In Fig. 5, we display data on substance P collected by Tsybin et al.[57] using ECD methods with simultaneous infrared excitation [58,59], the latter being utilized for reasons similar to the use of IR radiation in AI-ETD to disrupt secondary structure.

In this data, we see a pattern of c ions (c4 through c9 with a peak at c5) that is consistent with the shapes of the n = 3, 4 distributions surrounding the R or K sites. The c9 and c10 ions could come from the n = 5, 6 distribution or from the tail end of the n = 3, 4 distribution if dynamical motion of the C-terminus of the peptide (see the next paragraphs for more detail) caused these residue to approach the R or K site. The fact that primarily c rather than z∗ ions are observed is not surprising in this case (to form z∗ ions would require transfer of a proton from the positively charged c residue to the neutral z∗ fragment) and sheds no light on the issue of when and if proton transfer is taking place. Moreover, the lack of enhancement in cleavages proximal to but in the C-terminal direction from R or K (i.e., enhancement due to involvement of a Cornell mechanism) is also not surprising because the neighboring Pro groups block backbone cleavage at these sites.

Next, in the bottom part of Fig. 6, we display ECD fragment ion data from Tsybin and co-workers for a doubly charged REYPLLIR-terminus doubly charged ion labeled in blue and the ranges of the n = 3, 4 and n = 5, 6 Rydberg densities shown with red and yellow brackets, respectively. Data taken from Fig. 3 in Ref. [57]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
In Fig. 7, we see another set of Al-ETD data from the Coon laboratory on the doubly charged DNIQGITKPAIR.

The abundance pattern for $z^*_n$ through $z^*_{11}$ seems to fit the $n = 3, 4$ density profile for Rydberg orbitals centered on the K site. The somewhat enhanced intensity of $z^*_5$ could arise via a Cornell-type mechanism resulting from the protonated K site being solvated to a nearby carbonyl oxygen. For reasons explained earlier, it is unlikely that $c^*_5$ could arise from analogous solvation of the charged R site in a Cornell-like mechanism.

Let us finish with an example that could be used to further test the ideas about fragment ion abundance patterns put forth here.

1. The Coulomb and dipole stabilizations of the protonated Lys residues and of the two oppositely directed Ala α-helices, respectively, cause electron attachment to the SS $σ^*$ orbital to be 4 eV exothermic for the $j = 15$ species. Although a bit less so for the $j = 20$ peptide, it is still more than enough to allow the SS $σ^*$ orbital to extract an electron from an $n = 5$ or $n = 6$ Rydberg orbital on either Lys site. Moreover, as seen in Fig. 2, the distance between either Lys and the SS bond lies within the radial extent of the $n = 5, 6$ Rydberg orbitals’ density but not within that of the $n = 3, 4$ density. As a result, it makes sense that disulfide cleavage is observed for $j = 10, 15$, or 20 under ECD conditions where the $n = 5, 6$ density is operative.

2. The electrostatic stabilization near any of the four C-terminal amide groups exceeds 2 eV, as a result of which electron attachment to any of their $π^*$ orbitals is exothermic. The distance between the Lys and any of these four amide units falls within the $n = 3, 4$ Rydberg orbital density. So, it is also reasonable that cleavage of the four C-terminal $N–C_\alpha$ bonds is observed under ECD. It is also reasonable that a Cornell-type mechanism could be operative in cleaving some of the C-terminal N–Cα bonds since the protonated Lys sites are, as shown in Fig. 8, involved in hydrogen bonds to nearby carbonyl oxygen atoms.

However, the model put forth in this paper predicts that, under ETD conditions, where the $n = 5, 6$ Rydberg orbital density is not operative, cleavage of the disulfide bond should not be observed although cleavage at the four C-terminal $N–C_\alpha$ bonds should still be found. Thus, a good test of our predictions would involve carrying out ETD experiments on species like that shown in Fig. 8 using a donor anion that can populate $n = 3, 4$ Rydberg orbitals, but not $n = 5, 6$ orbitals.

Another interesting feature of the ECD data reported in A. Marshall, K. Håkansson (personal communication to J.S.) is that...
N–Ca was limited to the four C-terminal residues. This is surprising because the radial extent of the n = 3, 4 Rydberg orbitals is larger than four residues, as evidenced by the data reported in Figs. 3–7, especially because the α-helices shorten distances between amide groups along the backbone. Therefore, it would be helpful to carry out an Al-ETD experiment on (AcCAK+H)2+ to see whether infrared activation could either disrupt some of the α-helix structure and expose more of the N–Ca bonds to cleavage.

4. Summary

Predictions from earlier work by the Simons group suggest that n = 3–6 Rydberg orbitals are populated in ECD and n = 3, 4 orbitals in ETD using anion donors having electron binding energies near 0.6 eV, and that the populations of these orbitals are approximately equal. Based on these predictions, in the present manuscript we postulate that the distribution of abundances in N–Ca bond cleavages along the backbone of a multiply charged parent peptide ion will mimic the spatial distribution of the n = 3, 4 (for ETD) or n = 3–6 (for ECD) Rydberg orbitals on the charged sites. These predictions are tested on a series of ECD and ETD data obtained under conditions (i.e., simultaneous infrared activation) that are believed to disrupt much of the parent ion’s secondary structure thus rendering it maximally extended, which makes distance estimates based on location of various amide units along the backbone most reliable.

It is found that the fragment ion abundance patterns do mimic the spatial extents and general shapes of the Rydberg orbitals’ densities, but with two additional features. First, there appears to be enhanced N–Ca bond cleavage immediately proximal to charged Lys or N-terminal amine sites; such enhancement is rationalized in terms of contributions from a so-called Cornell mechanism when these charged sites are internally solvated to a nearby amide carbonyl group. Second, there are a few variations within the general outline of the Rydberg orbitals’ density patterns that we attribute to effects from the physical nature of the associated side chains. This effect seems to be most pronounced for acidic side chains.

References


[58] Earlier, the McLafferty group showed that collisional activation carried out simultaneously with ECD could also serve to exposing a larger fraction of the backbone to bond cleavage. See, for example D.M. Horn, Y. Ge, F.W. McLafferty, Analytical Chemistry 72 (2000) 4778–4784.

[59] Breuer and co-workers also showed how increasing the charge state of a large peptide can expose a higher fraction of backbone residues to ECD cleavage presumably by disrupting the secondary structure (e.g., folding, α helices, salt bridges) of the peptide K. Brueker, S. Brüschweiler, M. Tollinger, Angewandte Chemie International Edition 50 (2011) 873–877.