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Internal Energy Deposition in Electron Capture Dissociation Measured Using Hydrated Divalent Metal Ions as Nanocalorimeters

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Capture of an electron by a multiply charged ion can produce a rich fragmentation spectrum from which ion structural information can be obtained. Electron capture (EC) can occur by collisions between ions and thermally generated free electrons (ECD),[1,2] negative ions (ETD),[3] or atoms (ECID).[4] For peptides and proteins, these methods produce similar fragments from which substantial information can be obtained about both sequence and sites of post-translational modifications. Since the initial report by McLafferty and co-workers on using ECD for "top-down" proteomics,[1a] significant effort has been devoted to understanding how ECD fragmentation occurs. McLafferty and co-workers proposed that fragmentation proceeds via nonergodic processes originating from high-*n* Rydberg states.[1c] Tureček and co-workers have argued that the odd electron ions, resulting from EC by multiply protonated ions, have very low barriers to dissociation and that fragmentation is statistical.[2] Experimental and computational results supporting both mechanisms have been reported.[1,2,5]

A key piece of information that is essential to understanding how ECD occurs is the amount of energy that is deposited into internal modes of the ion. The EC process is exothermic by a value corresponding to the recombination energy,[1a,6] which is estimated to be 4−7 eV for multiply protonated peptides and proteins.[1a,6a] However, the extent to which this recombination energy partitions into internal modes of the precursor ion versus translational modes of the fragmentation products is unknown. In principle, the internal energy deposited into an ion by an activation method can be obtained using "chemical thermometers," which are ions that have known fragmentation energetics.[7] The abundances of fragment ions, formed as a result of activating ions that dissociate via a linear sequence of unimolecular reactions, can be used to directly probe the distribution of internal energy deposition.[7a] A measure of internal energy deposition can also be obtained from the abundance ratio of two or more fragment ions that have different dissociation enthalpies and entropies.[7b] For larger ions, dissociation kinetics can be directly related to effective temperatures from blackbody infrared radiative dissociation (BIRD)[8] measurements and used to determine the extent of activation by other techniques.[7c] Applying these methods to activation by EC is complicated by the requirement for multiply charged ions and fragmentation pathways with known energetics; slow heating methods, such as BIRD, typically produce different fragments than ECD.

Here, extensively hydrated divalent alkaline earth metal ions are used as chemical thermometers to probe, for the first time, the internal energy deposition that occurs in ECD. The thermochemistry of hydrated monovalent ions has been extensively investigated.[9a−c] Castleman and co-workers reported ΔH values for the loss of a water molecule from protonated water clusters, $H(H_2O)_n^+$, $n = 6-28$, and found that these values range between 9 and 11 kcal/mol for clusters with $n > 10$.[9a] The heat of vaporization of water is 10.8 and 9.7 kcal/mol at 0 and 100 °C, respectively.[9d] These results suggest that the energy required for the loss of a water molecule from these large clusters is not significantly influenced by the proton. For hydrated monovalent metal ions,[9b,c] binding energies rapidly decrease with increasing cluster size; the binding energies of the sixth water molecule to hydrated Li^+, Na^+, and K^+ are between 12.1 and 10.0 kcal/mol.[9b] These results suggest that the loss of water from $M(H_2O)_n^+$, M = alkaline earth metal ion, formed by EC from the divalent species, should also require approximately 10 kcal/mol per water molecule that evaporates from the cluster provided that these clusters are sufficiently large. Thus, the internal energy deposition of EC can be estimated from the number and distribution of water molecules that "boil" off from the reduced precursors. Ion relaxation after EC can also occur via collisional or radiative emission processes, although such effects are expected to be small in these experiments due to the low pressure ($<10^{-8}$ Torr) and short time (50 ms) after ECD but prior to ion detection.

Experiments were performed on a 2.75 T Fourier-transform ion cyclotron resonance mass spectrometer with an external nanoelectrospray ionization source and an ion cell cooled to 130 K using a regulated flow of liquid nitrogen.[10] A 1.0 cm diameter heated metal cathode, located 20 cm from the ion cell center, was used to produce electrons for these experiments. A potential of −2 V was applied to the cathode to introduce electrons into the ion cell, and electron radiation times of 40 ms were used.

In these experiments, ions dissociate via two pathways. The precursor can be activated by BIRD, collisions with residual background gas (CAD), or by inelastic non-capture collisions with electrons (EIEIO)[11] resulting in the loss of a single water molecule. The precursor may also capture an electron resulting in formation of a singly charged ion that undergoes loss of between 9 and 11 water molecules. These processes are illustrated in Figure 1. The spectrum of isolated $Ca(H_2O)_{32}^{2+}$, measured with identical experimental conditions as the ECD spectra but with no electrons introduced into the cell (the cathode is still heated), is shown in Figure 1 (top). Loss of a single water molecule (∼6% total ion abundance) resulting from precursor activation by BIRD/CAD is observed. An ECD spectrum of this same ion is shown in Figure 1 (bottom). No additional water loss from the precursor occurs, indicating that vibrational excitation of the ions by EIEIO is negligible. Additional water loss from EIEIO can occur when higher energy electrons are used. In ECD of proteins, the observation of ***b*** and ***y*** ions, corresponding to cleavage of the amide bond of the protein backbone, has been attributed to the EIEIO process.[6a] With our nanocalorimetry method, the extent of activation by EIEIO in an ECD experiment can be quantified. Also clearly observed in this spectrum is a remarkably narrow distribution of singly charged ions that correspond to the capture of a single electron by the doubly charged precursor and loss of either 10 or 11 water molecules. The absence of an ion corresponding to loss of 12 water molecules indicates that the maximum energy deposited into these ions is approximately between 110 and 120 kcal/mol (4.8 and 5.2 eV).

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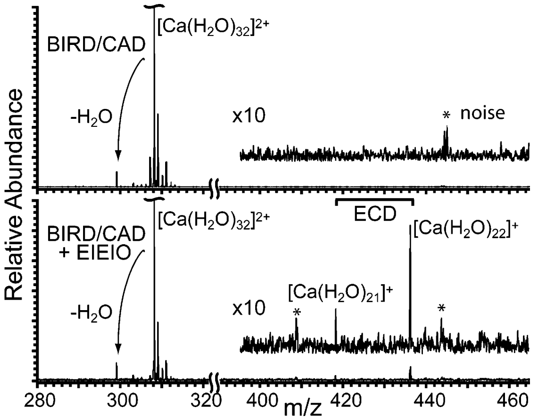

Figure 1. ECD spectrum of $Ca(H_2O)_{32}^{2+}$ (bottom) showing reduced precursor ions that have lost 10 or 11 water molecules and reference spectrum (top) measured under identical conditions but with no electrons injected into the cell showing extent of BIRD and/or CAD that occurs.

Table 1. Normalized Abundances of ECD Product Ions from $M(H_2O)_{32}^{2+}$, Weighted Average Number of Water Molecules Lost from the Reduced Precursor, and These Latter Values Corrected for BIRD/CAD Dissociation

	Number of Molecules Lost			Weighted Average	
$M(H_2O)_{32}^{2+}$	–11	–10	–9	ECD	corrected
Mg	61	100	<9	10.4	10.3
Ca	30	100	<7	10.2	10.2
Sr	34	100	20	10.1	10.0
Ba	25	100	<10	10.2	10.1

The second ionization energy of these alkaline earth metal atoms ranges from 15.0 eV for Mg to 10.0 eV for Ba. Results of the ECD experiments with clusters of each of these divalent metal ions with 32 water molecules are given in Table 1. An estimate of the average energy deposited as a function of metal ion size was obtained from a weighted average number of water molecules lost from the reduced precursor calculated using the ECD fragment ion abundances (Table 1). These values range from 10.4 water molecules lost for Mg to 10.2 for Ba (corresponding to 4.5 and 4.4 eV, respectively), indicating that the internal energy deposition depends only slightly on metal ion size for these extensively hydrated ions, despite the large difference in second ionization energies of the isolated atoms. This suggests that an ion–electron pair is formed in the reduced cluster. The water loss from the reduced clusters that is attributable to BIRD/CAD was estimated to be the same as that from the precursor. After this correction, the average number of water molecules lost from the EC process ranges from 10.3 to 10.1 for Mg to Ba, respectively, and corresponds to average internal energy depositions of 4.5 to 4.4 eV, respectively. From the distribution of products observed, we conclude that the average width of the energy deposition corresponds to less than ±1 water molecule or less than ±10 kcal/mol (0.4 eV). The number of water molecules lost via ECD of $Ca(H_2O)_{30}^{2+}$ is indistinguishable from that for $Ca(H_2O)_{32}^{2+}$, suggesting that neither the water binding nor the recombination energy changes significantly for clusters of this size.

Electron recombination energies correspond to the adiabatic ionization energies of the singly charged clusters formed by EC. Adiabatic ionization energies for clusters this large have not been reported, but values for $Mg(H_2O)_n^+$, $n = 1-19$, have;[12] these values decrease with increasing cluster size and asymptotically approach a value between 4.0 and 4.8 eV (MP2) or between 5.1 and 5.5 eV (BLYP) for the larger clusters. In order to obtain a rough estimate of these values for larger calcium clusters, vertical recombination energies were calculated for smaller cluster of $Ca(H_2O)_n^{2+}$ and for $Mg(H_2O)_n^{2+}$, and these values were found to rapidly converge to within 0.3 eV by $n = 4$.[13] This indicates that recombination energies for even larger clusters of these two metal ions should be similar. Calculations for $Ca(H_2O)_{29}^{2+}$ resulted in a value of 4.5 eV, but this value must be considered only a very rough estimate. A more detailed theoretical analysis is needed. By comparison to our experimental results for even larger Ca clusters that indicate an average and maximum internal energy deposition due to just EC of ~4.4 and ~4.8–5.2 eV, respectively, we conclude that the majority of the recombination energy is converted into internal energy of these precursor ions.

These results indicate that ions in an ECD experiment are not significantly activated by inelastic non-capture collisions with electrons when low electron kinetic energies are used. These results also clearly demonstrate that the vast majority of the recombination energy resulting from electron capture is converted into internal energy of the reduced precursor ions, indicating that the dissociation of these ions is statistical. Future studies on ECD of extensively hydrated ions of small organic molecules, peptides, and proteins will likely provide additional insights into the ECD processes for these species.

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(13) Candidate low-energy structures were identified using Monte Carlo conformation searches with the MMFF94 force field and subsequently minimized at the B3LYP/LACVP**++ level; single-point energies were calculated for these structures with a single electron added and used to obtain a vertical electron attachment energy.

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