Observation of Pronounced $b^{\bullet},y$ Cleavages in the Electron Capture Dissociation Mass Spectrometry of Polyamidoamine (PAMAM) Dendrimer Ions with Amide Functionalities

Sunyoung Lee
Department of Chemistry and Interdisciplinary Program of Integrated Biotechnology, Sogang University, Seoul, Republic of Korea

Sang Yun Han and Tae Geol Lee
Division of Chemical Metrology and Materials Evaluation, Korea Research Institute of Standards and Science, Daejeon, Republic of Korea

Gyusung Chung
Department of Chemistry, Konyang University, Chungnam, Republic of Korea

Duckhwan Lee and Han Bin Oh
Department of Chemistry and Interdisciplinary Program of Integrated Biotechnology, Sogang University, Seoul, Republic of Korea

We report the electron capture dissociation (ECD) mass spectrometry of the third generation polyamidoamine (PAMAM) dendrimer that contains amide functionalities. The dendrimer was chosen because it offers a unique opportunity to understand the ECD behavior of the amide functionality in a framework other than peptides/proteins. In this study, PAMAM ECD was found to exhibit a fragmentation pattern strikingly different from that of ordinary peptide/protein ECD. Specifically, ECD of multiply protonated PAMAM ions gave rise to significant $b^{\bullet},y$ cleavages as well as S,E dissociations but, unexpectedly, only minor $c,z^{\bullet}$ fragmentations are observed. In an effort to account for the unexpectedly different fragmentation pattern, a comparative ECD experiment on the poly(propylene imine) dendrimer in which the amide bond moiety is not available and density functional theory calculations (B3LYP/6-311 + G(d)) investigations on the model system of a charge-solvated single-repeat unit were carried out. On the basis of these results, we discuss here possible implications of intramolecular charge-solvation, energy barriers in dissociation reactions, and macromolecular properties of the dendritic molecule for understanding the reaction pathway of PAMAM ECD. (J Am Soc Mass Spectrom 2006, 17, 536–543) © 2006 American Society for Mass Spectrometry

Electron capture dissociation (ECD) offers a very powerful tandem mass spectrometry analysis of peptide/protein ions by exhibiting extensive backbone fragmentations [1–6]. In particular, ECD has made possible the detailed top-down characterizations of intact proteins [7, 8], especially useful for the analysis of post-translational modifications (PTMs) [8, 9]. Such a powerful potential of ECD applications has led to numerous experimental and theoretical investigations [1–5, 10–17].

In most ECD research, the focus has been mainly on the peptide/protein ions or its model compounds that contain amide functionalities. The amide bond is a central structural moiety in which main ECD processes are taking place, and its thermochemical and electronic properties such as relative proton affinities, electron-proton recombination energies, and the shape of potential energy surfaces have been suggested to play a critical role in determining the ECD pathways. However, so far, ECD of other molecular systems with the amide functionality has been studied only rarely [6],

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Address reprint requests to Dr. H. B. Oh, Department of Chemistry and Interdisciplinary Program of Integrated Biotechnology, Sogang University, Sinsudong 1, Mapogu, Seoul 121-742, Republic of Korea. E-mail: hanbinoh@sogang.ac.kr

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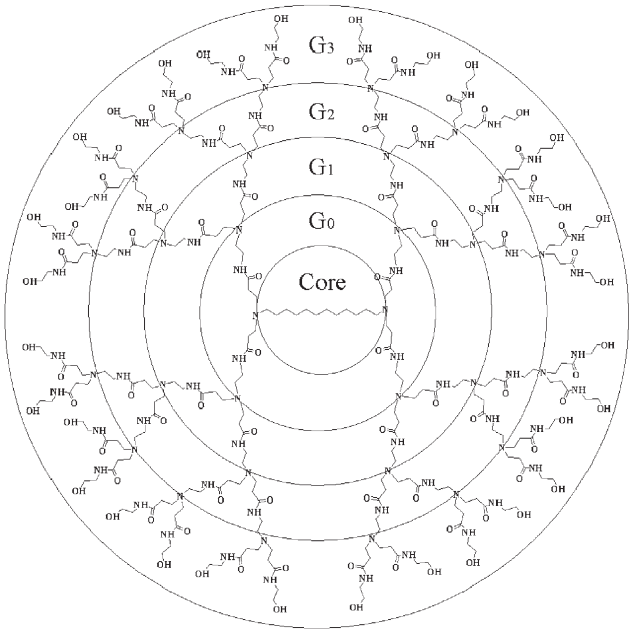


Scheme 1. Molecular structure for the third generation PAMAM (Polyamidoamine)-amidoethanol dendrimer with a 1,12 diaminododecane core and hydroxy termini: circles denote the generation number to which each polymer repeat unit belongs.

despite its apparent importance for understanding the ECD processes. Thus, here we have investigated the polyamidoamine (PAMAM) dendrimer (Scheme 1) as a model system containing the amide functionality in a framework other than that of peptide/proteins. The present PAMAM ECD is expected to provide an opportunity to study different aspects of the ECD process, which could not be revealed in the ECD of ordinary peptides/proteins.

The third generation PAMAM dendrimer examined in this study has a 1,12 diaminododecane core and amidoethanol surface end-groups (see Scheme 1) [18–20]. In addition to the availability of the amide bond, this synthetic polymer has properties suitable for the present experiments; for example, good monodispersity for the production of highly abundant molecular ions in electrospray ionization (ESI). In contrast to peptide/proteins, this dendritic molecule does not contain a flexible basic sidechain that is a likely site for protonation but instead, basic tertiary amines are located along the skeleton of dendritic branches. Such a structural difference provides an interesting feature from which to learn more about the general aspects of the ECD processes.

Besides, the PAMAM dendrimer molecule has also been a subject of extensive medical research since its introduction as a regio-chemical mimicry of globular proteins by Tomalia et al. [19]. Its possible applications are in drug delivery, gene transfection, anthrax detection, cardiac marker diagnostic, and prevention of human immunodeficiency virus (HIV) [20]. Therefore, this ECD mass spectrometry (MS) study carries a practical importance as an illustrative example of the detailed structural analysis of dendritic polymers.

Experimental and Theory

Experiments were performed on a commercial 4.7 T Fourier transform mass spectrometer (FTMS: Ionspec Inc., Lake Forest, CA) equipped with ESI capabilities. A detailed description of the experimental setup can be found elsewhere [21]. In brief, 100 μM PAMAM (the third generation, monoisotopic mass = 7076.5 Da: Aldrich, Seoul, South Korea) and 50 μM poly(propylene) (the third generation, monoisotopic mass = 1685.7 Da, Aldrich) were prepared in 49:49:2 (vol:vol:vol) $CH_3OH/H_2O/CH_3COOH$ solution, and were infused directly through a fused silica capillary (i.d. = 100 μm) emitter at a flow rate of ~1 μl/min using a syringe pump (Harvard Apparatus 22, Holliston, MA).

ECD experiments were performed by exposing the molecular ions of interest, isolated by a single stored waveform inverse Fourier transform (SWIFT) waveform, to 1.0–4.5 s electron radiation. Electrons were generated on the surface of an indirectly heated dispenser cathode (Heatwave Labs, Watsonville, CA). ECD efficiency was found to be very sensitive to a subtle change in various parameters, such as electron beam time, electron kinetic energy, and the voltage applied to the cathode. Each of them was finely tuned to optimize ECD fragmentations, particularly with the electron kinetic energy being set to 0.45–0.51 eV. All the tandem mass spectrometry (MS/MS) spectra were averaged over 30–50 scans. The resulting MS/MS spectra were analyzed manually because of the unconventional character of the sample molecule.

Density functional theory calculations (DFT) were performed on the model systems of a single-repeat unit of the PAMAM dendrimer molecule and its corresponding radicals. The local minimum structures for the model ions were optimized with the B3LYP density functional method using the 6-311 + G(d) basis set, whereas the unrestricted B3LYP method with the same basis set was employed for the corresponding radicals. All calculations were performed with the program GAMESS [22].

Results

ECD Applications on 4+–8+ Dendrimer Molecular Ions

Multiply protonated molecular ions of PAMAM dendrimer were subjected to ECD, resulting in a total of 62 fragment ions see Supplementary Material section (which can be found in the electronic version of this article). Electrospray of the polymer in a standard ESI solution yielded the molecular-ion mass spectrum shown in Figure 1a, in which abundant peaks appear at *m/z* 787.3 (9+), 885.6 (8+), 1012.0 (7+), 1181.0 (6+), 1416.3 (5+), and 1770.2 (4+). The monoisotopic mass of the dendrimer was measured to be 7076.5 Da, in good agreement with the theoretical mass (2.4 ppm error). This spectrum includes a small amount of synthetic

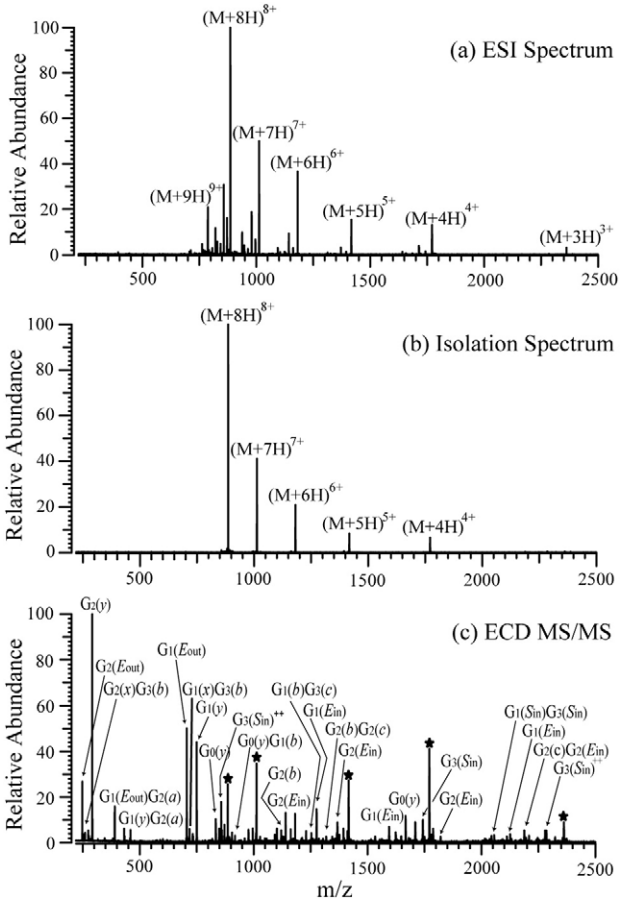


Figure 1. MS spectra of the 3rd generation PAMAM dendrimer obtained by (**a**) simple ESI; including 3+–9+ molecular ions, synthetic failures, and nozzle-skimmer fragment ions, (**b**) isolation of 4+–8+ molecular ions by a SWIFT waveform with multi-notches, and (**c**) subsequent ECD application to the isolated molecular ions (averaged over 30 scans): asterisks representing the dendrimer molecular ions.

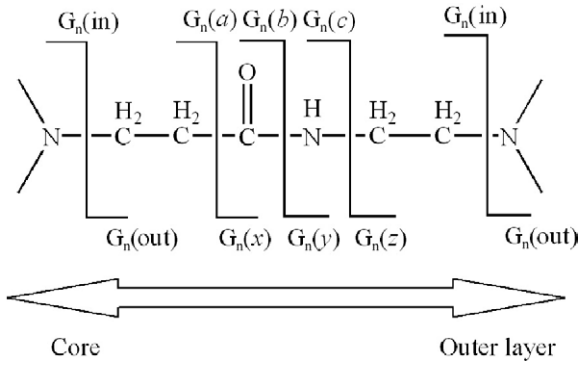
failures as well as fragment ions generated presumably by nozzle-skimmer collisions. For ECD MS, those unwanted ions were eliminated using a single broadband SWIFT waveform with multiple notches (Figure 1b), and then only the molecular ions with 4+ to 8+ charges were subjected together to electron radiation of low kinetic energy. Figure 1c displays the resulting ECD MS/MS spectrum for the PAMAM dendrimer ions.

Nomenclature and Assignment of PAMAM Dendrimer ECD Fragment Ions

ECD fragment ions in Figure 1c were assigned using newly devised notations. To facilitate prompt recognition, a new set of notations was carefully designed so that they follow closely the conventional nomenclature for peptide/protein fragment ions [23]. As displayed in Scheme 2, the notations are given in the form of $G_n(\boldsymbol{l})$, where the subscript n in G_n refers to the "generation number" to which the cleavage site belongs (see below) and $\boldsymbol{l}$ denotes the type of fragmentations; for example, $\boldsymbol{a}^{\bullet}$, $\boldsymbol{b}^{\bullet}$, $\boldsymbol{c}$, $\boldsymbol{x}$, $\boldsymbol{y}$, $\boldsymbol{z}^{\bullet}$, $\boldsymbol{S}_{\text{in or out}}$, and $\boldsymbol{E}_{\text{in or out}}$. The notations of $\boldsymbol{a}^{\bullet}/\boldsymbol{x}$, $\boldsymbol{b}^{\bullet}/\boldsymbol{y}$, and $\boldsymbol{c}/\boldsymbol{z}^{\bullet}$ are given in the exactly same manner as those for peptide ions, since the structural identity around the amide (peptide) bond is almost identical in both cases. ***S*** and ***E*** notations employed here stand for the start and end of each repeat unit, respectively.

Low ECD Efficiency for PAMAM Dendrimer Cations

ECD efficiency for the PAMAM dendrimer ions was found to be rather low when judged by the number of observed fragment ion peaks, compared to that of proteins with similar masses. Thus, in this work, a longer electron beam (1.0–4.5 s) was employed; in comparison, ≪1 s electron beam was reported to be used for bovine ubiquitin with a slightly larger mass (8.6 kDa). This low ECD efficiency for the PAMAM dendrimer ions can be attributed to the following. First, an excellent symmetry in the dendritic manifold restricts the possible cleavage sites only up to 21 (at maximum) under the assumption of a single ECD cleavage. The observed 62 fragment ions should, therefore, include multiple charge states of a fragment ion and doubly fragmented ions (approximately 23 ions). Application of a shorter electron beam resulted in a smaller number of fragment ions that were derived mostly from a single ECD cleavage. However, the general trends in fragmentation behavior observed in both cases did not exhibit a significant discrepancy. From the practical standpoint, this feasible controllability of the extent of fragmentation through the length of the electron beam provides a useful analytical tool that enables the easy localization of possible encapsulated groups of interest in dendritic polymers. Second, compact conformations of a symmetric dendrimer ion lowered the ECD efficiency via lower proton charge density. It has been generally accepted that ECD efficiency decreases as a function of z^3 as the charge state (z) becomes lower [2]. As shown in Figure 1b, ESI of the PAMAM dendrimer yielded notably lower charge states, i.e., ranging from 3+ to 9+ only, probably due to



Scheme 2. Notations for representing the fragmentation sites along the backbone of PAMAM dendrimer single-repeat unit.

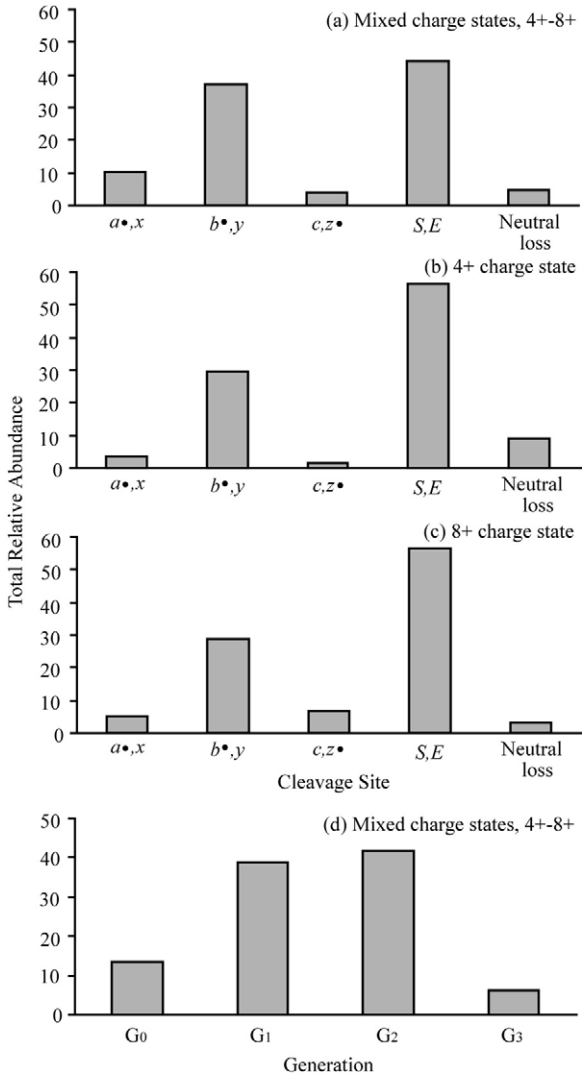


Figure 2. Histograms representing the results of the analysis based on the type of ECD fragment ions for (**a**) mixed charge states from 4+ to 8+, (**b**) 4+ charge state only, and (**c**) 8+ charge state only. (**d**): The analysis based on the generations in which fragmentation occurs.

its compact hyperbranched structure; in contrast, ubiquitin ions in a flexible single-strand form are reported to generally produce 6+ to 13+ molecular ions under similar ESI conditions [24, 25].

Pronounced $b^{\bullet}/y$ Cleavages

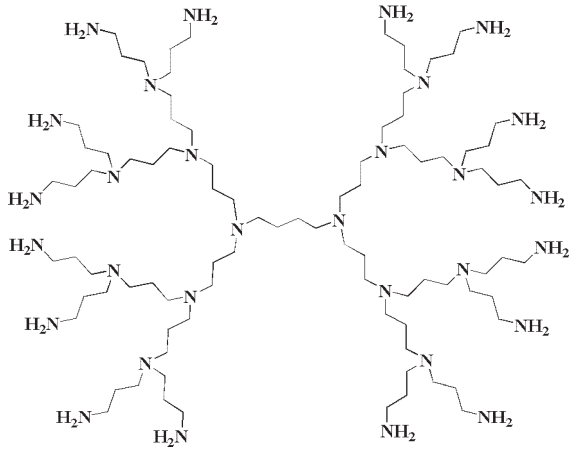
Analysis of the assigned fragment ions was performed on the basis of the type of fragmentations that occurred. In categorizing the fragment ions into the cleavage types as shown in Figure 2, $a^{\bullet}/x$, $b^{\bullet}/y$, and $c/z^{\bullet}$ are grouped as complementary pairs. Treating $a^{\bullet}$ ions separately from $b^{\bullet}/y$ ions is unusual, since $a^{\bullet}$ ions are generally derived from the consecutive dissociation of $b^{\bullet}$ fragment ions in peptide/protein ECD. Here, however, it is considered together with x ions as a pair, because both $b^{\bullet}$ and x ions, which are known not to appear in protein ECD, are observed explicitly in a non-negligible amount.

Interestingly, the analysis reveals that the S/E type of fragmentations is most abundant, being responsible for 44% of total fragmentations, and is followed by $b^{\bullet}/y$ cleavages (37%) (see Figure 2a). The $c/z^{\bullet}$ cleavages are found only as a minor channel (4%). It is notable that $b^{\bullet}/y$ cleavages are greatly pronounced, despite the fact that $b^{\bullet}$ fragments were not generally observed in protein ECD experiments. To ensure that abundant $b^{\bullet}/y$ ions should not result from the blackbody irradiation from the hot surface of a heated dispenser cathode (≥1000 °C, according to the data provided by the supplier, Heatwave Labs), a simple test was performed by exposing the PAMAM molecular ions to a long blackbody irradiation from the hot cathode surface, for example, 20 s, but it did not produce any fragmentation.

To further examine the unusual ECD pattern, sustained-off resonance irradiation collisionally activated dissociation (SORI-CAD) experiments were also carried out for the PAMAM cations isolated at a single charge state. At both low and high excitation energies, SORI-CAD mostly produced S/E fragment ions, in contrast to the PAMAM ECD in which a significant amount of $b^{\bullet}/y$ products were found. In particular, at high excitation energy, where the SORI-CAD resulted in extensive multiple cleavages, no $b^{\bullet}/y$ product ion was found assuming single or double cleavages. With low excitation energy, $b^{\bullet}/y$ fragment ions was not still observed, though most of fragment peaks could be successfully explained under the single cleavage assumption. Therefore, the PAMAM ECD, in which all fragments were formed by single or double fragmentations and abundant $b^{\bullet}/y$ fragments were found (37%), is unlikely to involve a simple consequence of energy deposition through charge recombination.

In peptide/protein ECD, $c/z^{\bullet}$ cleavages are, in general, responsible for 80–90% of total fragmentations and $a^{\bullet}/y$ ions are accountable for less than 20% of the total fragment ions [1, 2, 4]. Although b fragmentations are often observed in a non-negligible amount in the special cases of "in-beam" or "plasma" ECD experiments [3, 8, 26], in regular ECD experiments, $b^{\bullet}$ ions are observed only rarely. Even in the special cases of "activated-ion" ECD mentioned above, they are due, presumably, to CAD process occurring either in the nozzle-skimmer region or during the ion trapping, rather than to the ECD process itself. Therefore, the pronounced generation of $b^{\bullet}/y$ fragments in the ECD MS for PAMAM dendrimer cations, particularly when isolated by a SWIFT procedure, can be considered quite unique.

A series of ECD experiments were also performed on the dendrimer cations isolated at a single-charge state, i.e., at a respective charge state from 4+ to 8+. Fragmentation behaviors found at individual charge states, for example, Figure 2b for 4+ only and (Figure 2c) for 8+ only, were all similar to those for mixed charge states (Figure 2a). These analyses clearly indicate that some intriguing ECD mechanism responsible for the



Scheme 3. Molecular structure of the 3rd generation poly(propylene imine) dendrimer with a 1,4-diaminobutane core.

pronounced occurrence of $b^{\bullet}/y$ fragments is working consistently over the entire charge states of the PAMAM precursors and, thus, over their accompanying global conformations that are associated closely with pH (in this case, the number of protons) [27].

A further analysis of the fragment ions was carried out based on the generations where the ECD fragmentations took place. The results displayed in Figure 2d show that less ECD fragmentations occur in the outermost generation. Considering the fact that the outer generation carries more possible ECD cleavage sites (i.e., amide functionalities), the actual fragmentation efficiency for G_3 would be much less than appears in Figure 2d. This is in distinct contrast to the case of protein ECDs, where a terminal region, either N terminus or C terminus, is more prone to ECD cleavage.

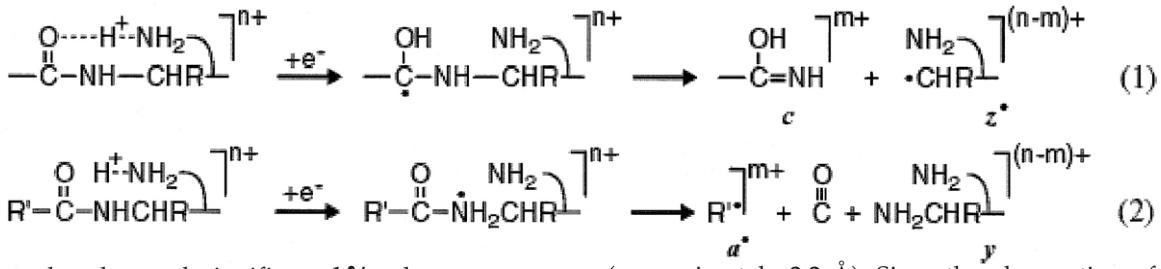
Evidence for Intramolecular Solvation of a Quaternary Amine

In view of the gas-phase proton affinity, the tertiary amines in the dendrimer are the most favorable sites for protonation [28], [for example, gas-phase proton affinity values for $(CH_3CH_2)_3N$, $(CH_3CH_2)_2NH$, $(CH_3CH_2)NH_2$, and $(CH_3CH_2)_2CO$ are 981.8, 952.0, 912.0, and 888.5 kJ/mol, respectively] and, thus, are likely to serve as primary electron capture sites. The observation of abundant **S**/**E** ions may indicate that the *N*-ether bond around the tertiary amine is very susceptible to ECD cleavage. However, another ECD experiment performed on the third generation poly(propylene imine) dendrimer with a 1,4-diaminobutane core (see Scheme 3) reveals that the tertiary amines themselves in this dendrimer are not intrinsically susceptible to ECD backbone fragmentation. Unlike the PAMAM dendrimer, this dendrimer does not contain an amide (peptide) structural unit, but does have tertiary amines connected repeatedly by propylene units. ECD for this dendrimer was expected to take place mainly on the tertiary amines, to yield ***S*** and ***E*** products, as in the PAMAM ECD. However, electron capture by the 4+ dendrimer ions did not give any noticeable fragmentation, but instead simply reduced the 4+ charge to 3+ or 2+ (spectrum not shown) [21]. A close inspection of the exact masses of the 3+ and 2+ reduced molecular ions shows that the reduction is merely due to single or two hydrogen atom losses upon electron capture by the proton(s) [29, 30]. In general, in the case of protein ECD, H-atom loss is observed as only a minor channel.

Accordingly, there should be some reason for the PAMAM dendrimer to undergo backbone dissociation upon electron capture, which otherwise would exhibit the simple H-atom loss as observed in the poly(propylene imine) dendrimer experiment. As a matter of fact, a major structural difference between the two dendrimers lies in the fact that only the PAMAM dendrimer contains amide bond moieties in its backbone. With respect to the role of the amide bond in the ECD mechanism, many recent investigations have suggested the intramolecular charge-solvation (proton sharing) model [10, 11, 15–17]. According to this model, a proton on the tertiary amine is hydrogen-bonded to the amide functionality (possibly either the carbonyl oxygen atom or the amide nitrogen atom) to constitute an ECD precursor state favorable for consecutive H-atom transfer upon neutralization by electron capture. Hydrogen-bonding is expected to play a certain role in stabilizing the energetic H-atom to avoid simply losing a H-atom as found in the poly(propylene imine) dendrimer experiments, but to proceed to backbone fragmentations. This is in line with a recently postulated gas-phase protein structural model "in which protonated functionalities (e.g., the H_3N^+ group of a protonated lysine in bovine ubiquitin 13+ ion) are hydrogen-bonded (shared), presumably to the backbone carbonyl group" [10].

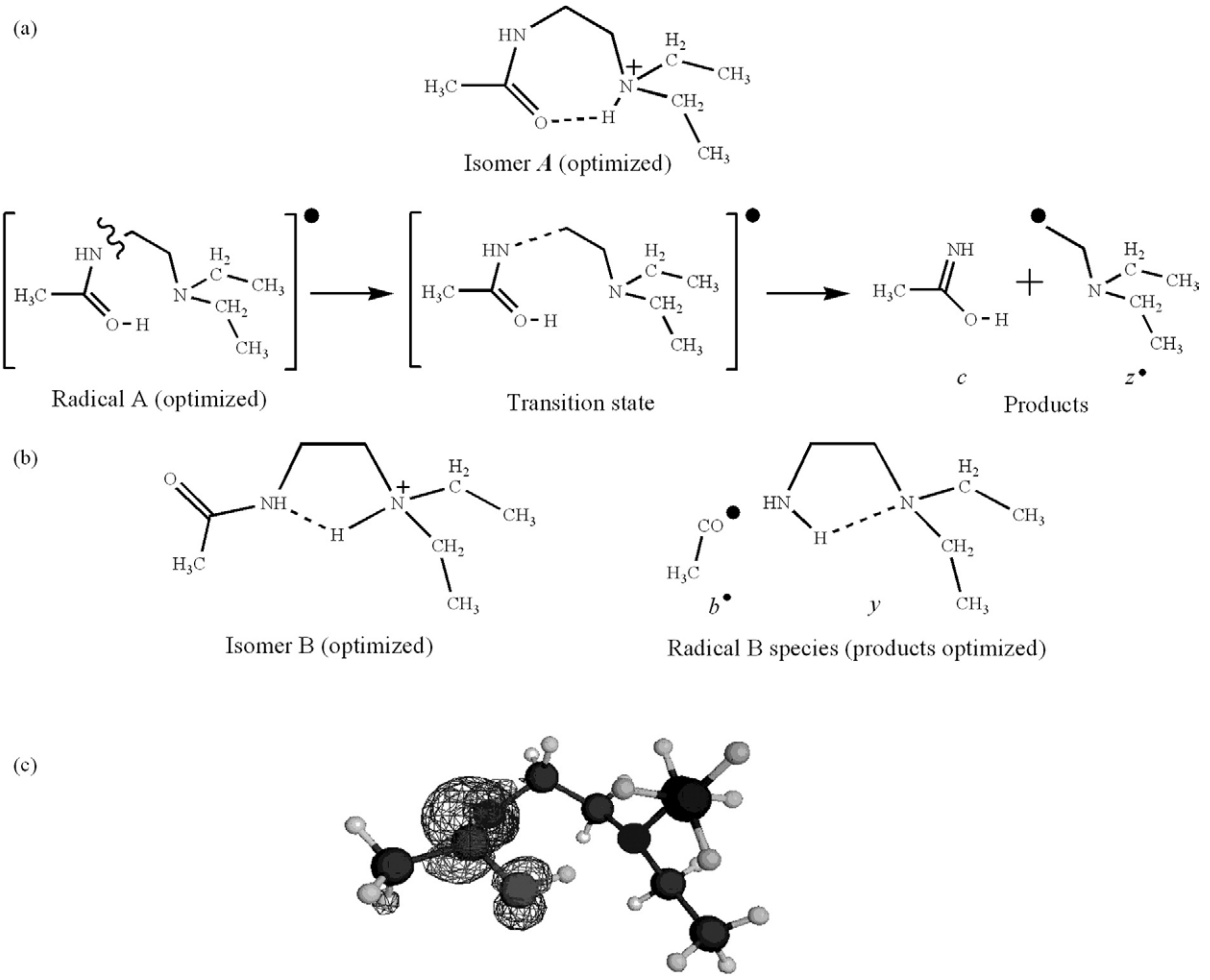
Discussion

As shown above, PAMAM dendrimer ECD exhibited the fragmentation patterns abnormal in terms of the cleavage sites, e.g., pronounced $b^{\bullet}/y$ cleavages. Then a question arises as to what causes this unexpected ECD behavior. We have sought mechanistic reasoning using DFT calculations on the single-repeat unit of the polymer as a model system at the theory level of B3LYP/6-311 + G(d). We chose the two plausible precursor structures shown in Scheme 4a and b (isomer ***A*** and ***B***, respectively), since these reflect well the general features of the protein ECD mechanism given in eqs 1 and 2 [1, 2]. Model isomer ***A*** and ***B*** in Scheme 4 represent the structures in which a proton on the quaternary amine is hydrogen-bonded to the carbonyl oxygen atom and to the amide nitrogen atom, respectively, thus forming a 7-membered and a 5-membered ring.



From the observed significant $b^{\bullet}/y$ cleavages, one can suggest that the isomer *B* type configuration is much more available in the precursor state than the isomer *A* type; as eqs 1 and 2 reveal, O—H bonding and N—H bonding are generally known to lead to $c/z^{\bullet}$ and $a^{\bullet}/y$ (or $b^{\bullet}/y$) fragmentations, respectively. However, the calculations reveal that isomer *A* is more stable in energy by about 15.2 kcal/mol. This is probably due to stronger hydrogen-bonding interactions in isomer *A*. This is clearly supported by the finding that the bond length for C = O—H-N in isomer *A* (approximately 1.8 Å) is short than that of (O = C)HN—H-N in isomer *B* (approximately 2.2 Å). Since the observation of abundant $b^{\bullet}/y$ fragment ions cannot be explained by simply considering precursor-ion populations, we extended our investigation to the ground-state potential energy surfaces of the corresponding radical species (radical *A* and *B*), using unrestricted B3LYP/6-311 + G(d).

For radical *A*, geometry optimization led to an H-atom transferred form. A transition-state for the subsequent dissociation of this optimized radical *A* into *c* and $z^{\bullet}$ fragments was further examined and the calculations predicted the energy barrier of about 18.0 kcal/mol (Scheme 4a). From these calculated



Scheme 4. Schematic molecular representations for (**a**) geometry-optimized isomer *A* (upper) and the $c/z^{\bullet}$ dissociation channel for radical *A* (lower); (**b**) optimized isomer *B* (left) and radical *B* species (right) that are fragmented from an incipient radical species (see the main text). A 3D diagram for the singly occupied molecular orbital (SOMO) of radical *A* is displayed in (**c**); electron density was found mainly on the carbonyl carbon, consistent with eq 1.

results, one can speculate that electron capture by the protonated site of isomer ***A*** may prompt an H atom-transfer within the hydrogen-bond framework in radical ***A***. Then, further dissociation into ***c*** and $z^{\bullet}$ products can proceed along the reaction path that has the energy barrier of 18 kcal/mol, provided that the generated radical has sufficient internal energy to overcome the barrier. However, according to the experimental observations, this reaction path does not seem to be active enough to lead to abundant $c/z^{\bullet}$ products. The reason for this might be found in the high-energy barrier, but it is still not clear whether it is high enough to retard the dissociation into $c/z^{\bullet}$ products, considering the possible vibrational excitations upon the Franck-Condon transition from the ionic to the radical states. For example, the estimate of the Franck-Condon excitation for the C(O) − NH − CH_3 + $CH_3NH_3^+$ system was about 17 kcal/mol [5].

As for the radical ***B*** species, however, geometry optimization did not lead to a stable intact molecule, but instead proceeded immediately to fragmentation, resulting in $b^{\bullet}/y$ type of products (Scheme 4b). This suggests that for this dissociation reaction there exists no significant barrier. The subsequent dissociation of $b^{\bullet}$ into $a^{\bullet}$ and **CO** was also calculated and an energy barrier of about 20 kcal/mol was predicted. These results can be interpreted as follows. In contrast to radical ***A***, incipient radical ***B*** species newly formed upon electron capture by isomer ***B*** may immediately undergo a barrierless dissociation into fragments of the $b^{\bullet}/y$ kind. Consecutive dissociation of $b^{\bullet}$ into $a^{\bullet}$ and **CO** can be hampered by the existing energy barrier of 20 kcal/mol. Seemingly, the possible barrierless, thus immediate upon electron capture, transition into $b^{\bullet}/y$ products in the ECD of isomer ***B*** may very well account for the observed pronounced production of $b^{\bullet}/y$ products in the PAMAM dendrimer ECD.

Although the density functional method has, in general, been successfully used to describe chemical reactions on the ground-state potential energy surface in a qualitative way, it may not give a reliable absolute value for the energy barrier of a transition-state. For example, Zubarev made a theoretical attempt to explain the abundant $c,z^{\bullet}$ type of cleavages in ECD, using a model system of C(O) − NH − CH_3 + $CH_3NH_3^+$ at the theory level of B3LYP/cc-pVTZ [5]. But in that study, it was found difficult to suggest a plausible explanation because of the high-energy barrier (about 30 kcal/mol) calculated for N—C_α bond dissociation, which is the opposite of our case. Besides, as suggested in the amide superbase model described by Syrstad and Turecek., the inclusion of the excited-state potentials is evidently needed to better describe ECD reactions [15], which is in fact very expensive and beyond our present computational resources. Another fact to consider is that we employed only the single-repeat moiety of the PAMAM dendrimer as a model system for this theoretical study. This model moiety is thought to reflect some essential aspects of ECD reactions as an actual reaction site. Nevertheless, it should be considered that the macromolecular properties of the dendrimer may play some role in the real ECD process of this PAMAM molecular ion.

Another interesting finding we need to consider comes from a recent ECD study of the peptides with isoaspartic acid. This amino acid is an isomer of aspartic acid with C_β incorporated into the backbone [31]. In the study, a unique cleavage of the C_α—C_β backbone bond upon electron capture was observed. If we increase the bond length of the backbone by one methylene unit from the α-amino acid type, it then becomes similar to this PAMAM case. Thus, the ECD of the β-amino acid type of a repeat unit may have an implication on the observed pronounced $b^{\bullet}/y$ cleavages in the present PAMAM ECD study.

Conclusions

The ECD fragmentation behavior for the third generation PAMAM dendritic polymer was investigated in this study. This polymer was chosen in an attempt to shed light on the ECD behavior of the amide functionality using a molecular framework other than peptides or proteins. The ECD of the PAMAM dendrimer produced a significant amount of $b^{\bullet}/y$ fragment ions, as well as abundant ***S***, ***E*** ions that were derived from *N*-ether bond cleavages. However, the $c,z^{\bullet}$ type of cleavages, which were generally produced as a major channel in most previous peptide/protein ECD studies, were found as only a minor channel in the PAMAM ECD. To account for this unexpected observation of pronounced $b^{\bullet}/y$ fragment ions, we further examined the ECD of the poly(propylene imine) dendrimer for comparison, and we carried out DFT calculations on the model systems of single-repeat unit moieties. We discussed the possibility that intramolecular charge-solvation, reaction barriers, and macromolecular properties of the whole molecule may be in play in determining the fate of the ECD processes.

Acknowledgments

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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.* **1998,** *120,* 3265–3266.
2. Zubarev, R. A.; Horn, D. M.; Fridriksson, E. K.; Kelleher, N. L.; Kruger, N. A.; Lewis, M. A.; Carpenter, B. K.; McLafferty, F. W. Electron Capture Dissociation for Structural Characterization of Multiply Charged Protein Cations. *Anal. Chem.* **2000,** *72,* 563–573.
3. Sze, S. K.; Ge, Y.; Oh, H.-B.; McLafferty, F. W. Top-Down Mas Spectrometry of a 29-kDa Protein for Characterization of Any Posttranslational Modification to Within One Residue. *Proc. Natl. Acad. Sci. U.S.A.* **2002,** *99,* 1774–1779.

4. Cooper, H. J.; HÏkansson, K.; Marshall, A. G. The Role of Electron Capture Dissociation in Biomolecular Analysis. *Mass Spectrom. Rev.* **2005,** *24,* 201–222.
5. Zubarev, R. A. Reactions of Polypeptide Ions with Electrons in the Gas Phase. *Mass Spectrom. Rev.* **2003,** *22,* 57–77.
6. Koster, S.; Duursma, M. C.; Boon, J. J.; Heeren, R. M. A.; Ingemann, S.; van Benthem, R. A. T. M.; de Koster, C. G. Electron Capture Dissociation and Collisionally Activated Dissociation Mass Spectrometry of Doubly Charged Hyperbranched Polyesteramides. *J. Am. Soc. Mass Spectrom.* **2003,** *14,* 332–341.
7. Zhai, H. L.; Dorrestein, P. C.; Chattejee, A.; Begley, T. P.; McLafferty, F. W. Simultaneous Kinetic Characterization of Multiple Protein Forms by Top Down Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2005,** *16,* 1052–1059.
8. Sze, S. K.; Ge, Y.; Oh, H.-B.; McLafferty, F. W. Plasma Electron Capture Dissociation for the Characterization of Large Proteins by Top Down Mass Spectrometry. *Anal. Chem.* **2003,** *75,* 1599–1603.
9. Häkansson, K.; Cooper, H. J.; Emmett, M. R.; Costello, C. E.; Marshall, A. G.; Nilsson, C. L. ECD and IRMPD MS/MS of an N-Glycosylated Tryptic Peptide to Yield Complementary Sequence Information. *Anal. Chem.* **2001,** *73,* 4530–4536.
10. Breuker, K.; Oh, H.-B.; 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.* **2004,** *101,* 14011–14016.
11. Leymarie, N.; Costell, C. E.; O'Connor, P. B. Electron Capture Dissociation Initiates a Free Radical Reaction Cascade. *J. Am. Chem. Soc.* **2003,** *125,* 8949–8958.
12. Sawicka, A.; Skurski, P.; Hudgins, R. R.; Simons, J. Model Calculations Relevant to Disulfide Bond Cleavage via Electron Capture Influenced by Positively Charged Groups. *J. Phys. Chem. B* **2003,** *107,* 13505–13511.
13. Turecek, F.; Syrstad, E. A. Mechanisms and Energetics of Intramolecular Hydrogen Transfer in Amide and Peptide Radicals and Cation Radicals. *J. Am. Chem. Soc.* **2003,** *125,* 3353–3369.
14. Turecek, F. N–C Bond Dissociation Energies and Kinetics in Amide and Peptide Radicals. Is the Dissociation a Nonergodic Process? *J. Am. Chem. Soc.* **2003**, 125, 5954–5963
15. Syrstad, E. A.; Turecek, F. Toward a General Mechanism of Electron Capture Dissociation. *J. Am. Soc. Mass Spectrom.* **2005,** *16,* 208–224.
16. O'Connor, P. B.; Leymarie, N. M.; Costello, C. E. Electron Capture Dissociation of Post-Translationally Modified Peptides. *Proceedings of 50th ASMS Conference on Mass Spectrometry and Allied Topics*; Orlando, FL, June 2002.
17. Mihalca, R.; Kleinnijenhuis, A. J.; McDonnel, L. A.; Heck, A. J. R.; Heeren, R. M. A. Electron Capture Dissociation at Low Temperatures Reveals Selective Dissociations. *J. Am. Soc. Mass Spectrom.* **2004,** *15,* 1869–1872.
18. He, M.; McLuckey, S. A. Tandem Mass Spectrometry of Half-Generation PAMAM Dendrimer Anions. *Rapid Commun. Mass Spectrom.* **2004,** *18,* 960–972.
19. Tomalia, D. A.; Kaplan, D. A.; Kruper, W. J. Jr.; Cheng, R. C.; Tomlinson, I. A.; Fazio, M. J.; Edwards, D. S. U.S. Patent 5 728 461, **1994**.
20. Boas, U.; Heegaard, P. M. H. Dendrimers in Drug Research. *Chem. Soc. Rev.* **2004,** *33,* 43–63 and references therein.
21. Han, S. Y.; Lee, S. Y.; Oh, H.-B.Comparable Electron Capture Efficiencies for Various Protonated Sites on the 3rd Generation Poly(propylene imine) Dendrimers: Probed by SORI-CAD and Electron Capture Dissociation Mass Spectrometry (ECD MS). *Bull. Korean Chem. Soc.* **2005,** *26,* 740–746.
22. Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, K.; Matsunaga, N.; Nguyen, K. A.; Su, S.; Windus, T. L.; Dupuis, M.; Montgomery, J. A. General Atomic and Molecular Electronic Structure System. *J. Comput. Chem.* **1993,** *14,* 1347–1363.
23. Roepstorff, P.; Fohlman, J. Proposal for a Common Nomenclature for Sequence Ions in Mass Spectra of Peptides. *Biomed. Mass Spectrom.* **1984,** *11,* 601.
24. Breuker, K.; Oh, H.-B.; Horn, D. M.; Cerda, B.; McLafferty, F. W. Detailed Unfolding and Folding of Gaseous Ubiquitin Ions Characterized by Electron Capture Dissociation. *J. Am. Chem. Soc.* **2002,** *124,* 6407–6420.
25. Oh, H.-B.; Breuker, K.; Sze, S. K.; Ge, Y.; Carpenter, B. K.; McLafferty, F. W. Secondary and Tertiary Structures of Gaseous Protein Ions Characterized by ECD Mass Spectrometry and Photofragment Spectroscopy. *Proc. Natl. Acad. Sci. U.S.A.* **2002,** *99,* 15863–15868.
26. Horn, D. M.; Ge, Y.; McLafferty, F. W. Activated Ion Electron Capture Dissociation for Mass Spectral Sequencing of Larger (42 kDa) Proteins. *Anal. Chem.* **2000,** *72,* 4778–4784.
27. Lee, I.; Athey, B. D.; Wetzel, A. W.; Meixner, W.; Baker, J. R., Jr. Structural Molecular Dynamics Studies on Polyamidoamine Dendrimers for a Therapeutic Application: Effects of pH and Ggeneration. *Macromolecules* **2002,** *35,* 4510–4520.
28. NIST Web site at http://webbook.nist.gov/chemistry/form-ser.html.
29. Breuker, K.; Oh, H.-B.; Cerda, B. A.; Horn, D. M.; McLafferty, F. W.Hydrogen Atom Loss in Electron-Capture Dissociation: A Fourier Transform-Ion Cyclotron Resonance Study with Single Isotopomeric Ubiquitin Ions. *Eur. J. Mass Spectrom.* **2002,** *8*, 177–180.
30. Yao, C.; Turecek, F. Hypervalent Ammonium Radicals. Competitive N——C and N—H Bond Dissociations in Methyl Ammonium and Ethyl Ammonium. *Phys. Chem. Chem. Phys.* **2005,** *7,* 912–920.
31. Cournoyer, J. J.; Pittman, J. L.; Ivleva, V. B.; Fallows, E.; Waskell, L.; Costello, C. E.; O'Connor P. B. Deamidation: Differentiation of Aspartyl from Isoaspartyl Products in Peptides by Electron Capture Dissociation. *Protein Sci.* **2005,** *14,* 452–463.