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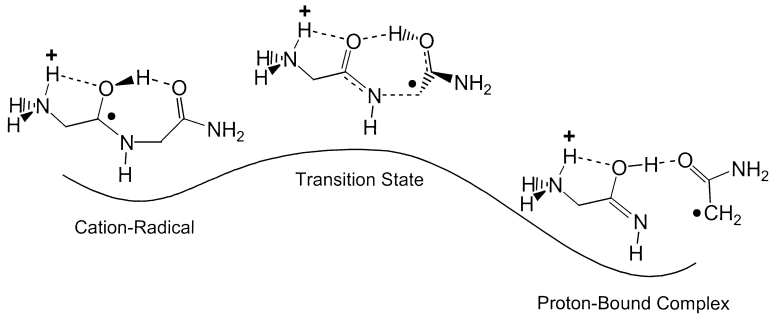

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N−C_α Bond Dissociation Energies and Kinetics in Amide and Peptide Radicals. Is the Dissociation a Non-ergodic Process?

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Abstract: Dissociations of aminoketyl radicals and cation radicals derived from β-alanine *N*-methylamide, *N*-acetyl-1,2-diaminoethane, N_α-acetyl lysine amide, and N_α-glycyl glycine amide are investigated by combined density functional theory and Møller−Plesset perturbational calculations with the goal of elucidating the mechanism of electron capture dissociation (ECD) of larger peptide and protein ions. The activation energies for dissociations of N−C bonds in aminoketyl radicals decrease in the series N−CH_3 > N−CH_2-CH_2NH_2 ≫ N−CH_2CONH_2 ≈ N−$CH(CONH_2)(CH_2)_4NH_2$. Transition state theory rate constants for dissociations of N−C_α bonds in aminoketyl radicals and cation-radicals indicate an extremely facile reaction that occurs with unimolecular rate constants $>10^5$ s^{-1} in species thermalized at 298 K in the gas phase. In neutral aminoketyl radicals the N−C_α bond cleavage results in fast dissociation. In contrast, N−C_α bond cleavage in aminoketyl cation-radicals results in isomerization to ion−molecule complexes that are held together by strong hydrogen bonds. The facile N−C_α bond dissociation in thermalized ions indicates that it is unnecessary to invoke the hypothesis of non-ergodic behavior for ECD intermediates.

Introduction

Homolytic bond dissociation energies of small molecules represent one of the fundamental sets of chemical thermodynamic data[1] which have also served as a testing ground for refining experimental and computational methods with the goal of improving accuracy. In contrast to stable molecules, for which extensive data are available, much less is known about bond dissociation energies in more complex systems of biological relevance. This is especially true for transient intermediates of biological reactions, such as radicals that are produced by radiative damage,[2] oxidative stress,[3] or other interactions with highly reactive species.[4] Recently, protein and peptide radical-cations have been generated transiently in the gas phase by electron capture reduction of multiply charged cations.[5] The cations are generated by electrospray ionization,[6] transferred to the vacuum system of a mass spectrometer, and trapped by electrostatic and magnetic fields in an ion-cyclotron resonance cell.[7] Upon exposure to thermal electrons, a fraction of ions are reduced to cation-radicals that in part dissociate by cleavage of the N−C_α bonds of the peptide backbone,[8] C_α−alkyl side chain bonds,[9] or cystine S−S bonds.[10]

A reaction mechanism has been suggested that involves initial electron capture by a charge site in the ion, typically a protonated Arg or Lys residue, or the terminal amino group. Reduction of the guanidinium or ammonium groups is thought to produce unstable radicals that dissociate by N−H bond cleavage. The departing hydrogen atom is transferred to or recaptured by a nearby amide carbonyl to form a transient aminoketyl radical. Because of the substantial exothermicity of electron capture by the peptide cation, the aminoketyl radical can be highly excited and dissociate by cleavage of the N−C_α bond to form an amine-terminus peptide fragment with an enolimine group (***c***-type ion series)[11] and a carboxylate-terminus peptide fragment that has an α-radical site (***z***-type ion series) (Scheme 1). This process, which has been termed electron capture dissociation (ECD), holds promise as an efficient method of peptide sequencing by mass spectrometry. In addition, it was suggested that the aminoketyl radical dissociation occurs before the reactant

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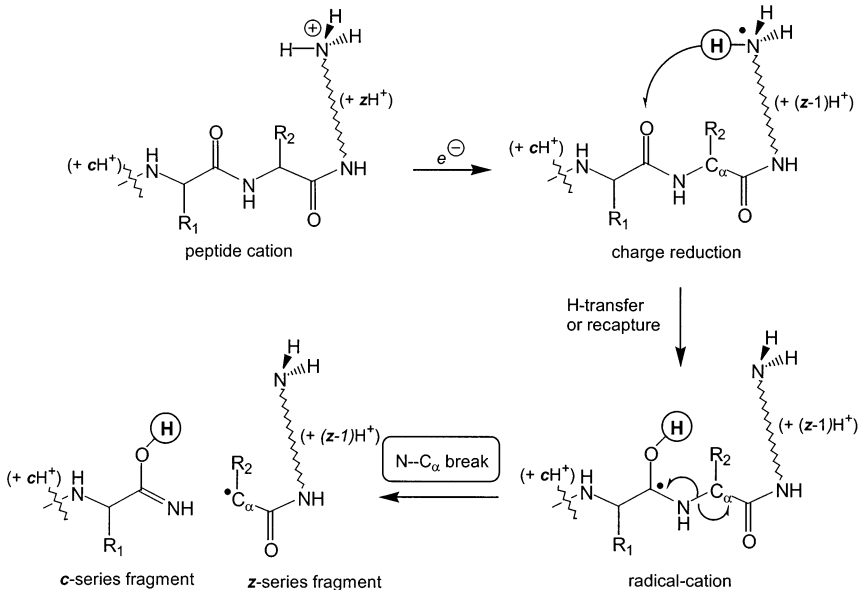
Scheme 1



undergoes internal redistribution of vibrational energy, and the dissociation was postulated to be non-ergodic.[5,12]

Interestingly, there are very few examples of non-ergodic dissociations of polyatomic gas-phase ions, the propen-2-ol/acetone system having being studied the most,[13] and the energetics and dynamics of its dissociations appear to be well understood. In contrast, virtually nothing is presently known about the energetics and kinetics of dissociations of gas-phase peptide and protein cation-radicals occurring in ECD. The non-ergodic hypothesis for peptide cation-radical dissociations was based in part on density functional theory calculations in a model aminoketyl radical corresponding to a hydrogen atom adduct to *N*-methylacetamide, where N−CH_3 bond dissociation was predicted to dominate at high internal energies.[10] However, recent experimental and high-level ab initio studies of aminoketyl radicals derived from formamide[14] and *N*-methylacetamide[15] found O−H bond cleavages to predominate. In particular, the branching ratio for O−H and N−C_α bond cleavage in the *N*-methylacetamide radical was predicted by RRKM calculations to increase at high excitations, in quantitative agreement with experiment.[15] These results have cast doubt on one of the premises for non-ergodic dissociations in larger systems; however, it was not clear whether the results from the small neutral radicals could be extrapolated to much larger peptide and protein cation-radicals.[15]

In a previous communication we have addressed the mechanism of H atom transfer and recapture in five model amide and peptide systems, e.g., β-alanine, β-alanine *N*-methylamide isomers, *N*-acetyl-1,2-diaminoethane, and N_α-glycylglycine amide, that resulted in aminoketyl radical formation.[16] A common feature of these systems was that H atom transfer from the ammonium group to the amide carbonyl oxygen was 60−80 kJ mol^{-1} exothermic and proceeded with rate constants ranging from 10^{10} to 10^{12} s^{-1} in both thermalized and isolated radicals. The H atom transfer was found to compete with N−H bond dissociation followed by recapture of a translationally hot H atom by an adjacent amide carbonyl. Thus, both mechanisms appear to be viable for the formation of aminoketyl radical intermediates in peptides.[16]

The present paper follows up on the previous study and addresses the energetics and kinetics of N−C_α bond dissociations in aminoketyl radicals derived from β-alanine *N*-methylamide, *N*-acetyl-1,2-diaminoethane, N_α-acetyl lysine amide, and N_α-glycylglycine amide. These model systems accommodate various types of N−C_α bonds and allow one to study the effects of noncovalent interactions by hydrogen bonds in the reactants, transition states, intermediates, and products.

Calculations

Standard ab initio and density functional theory calculations were performed with the Gaussian 98 suite of programs.[17] Optimized geometries were obtained with density functional theory calculations using Becke's hybrid functional, B3LYP,[18] and the 6-31+G(d,p) or 6-31++G(d,p) basis sets. Harmonic frequencies were calculated to characterize stationary states as local minima (all real frequencies) and first-order saddle points (one imaginary frequency). Complete optimized geometries and uncorrected harmonic frequencies are available from the author upon request. Single-point energies were obtained with

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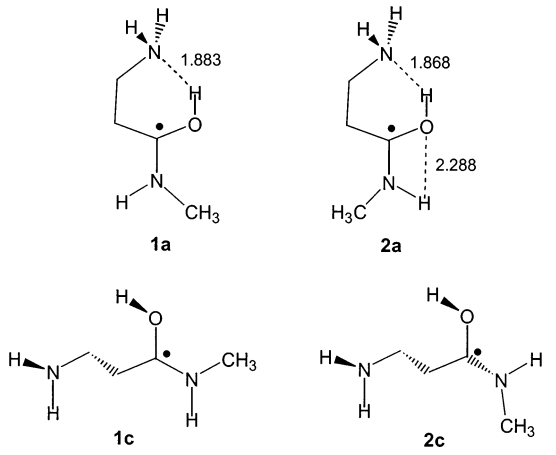
Chart 1



B3LYP and Moller−Plesset perturbational calculations[19] truncated at second order, MP2, with frozen core and valence electron only excitations. The basis sets in the single-point calculations were 6-311++G(2df,p) for β-alanine *N*-methylamide and *N*-acetyl-1,2-diaminoethane radicals and 6-311++G(2d,p) for N_α-acetyl lysine amide and N_α-glycylglycine amide radicals. The single-point energies from the B3LYP and spin-projected MP2 calculations were averaged and used to calculate B3-PMP2 relative energies that are discussed throughout. This empirical procedure[20] has been shown to result in efficient cancellation of errors inherent to the B3LYP and MP2 formalisms and to provide relative and activation energies that in accuracy compare favorably with high-level composite Gaussian 2 or Gaussian 2(MP2) calculations, as reported for several ion and radical systems.[21] For selected reactions we also performed single-point calculations with quadratic configuration interaction including single, double, and perturbational triple excitations,[22] QCISD(T)/6-31++G-(d,p). These energies were extended to the larger basis set using a linear energy relationship, E{QCISD(T)/6-311++G(2df,p)} ≈ E{QCISD(T)/6-31++G(d,p)} + E{MP2/6-311++G(2df,p)} − E{MP2/6-31++G-(d,p)}. Transition state theory (TST) calculations were performed using the standard formula. Rotational and vibrational partition functions for reactants and transition states that were used in TST calculations were obtained from B3LYP/6-31+G(d,p) moments of inertia and scaled harmonic frequencies, respectively.

Results

***β*-Alanine *N*-Methylamide Radicals.** Four aminoketyl radicals derived from β-alanine *N*-methyl amide were obtained as stable conformers (**1a**, **2a**, **1c**, and **2c**, Chart 1). Radicals **1a** and **2a** that have an intramolecular H-bond between the OH and amine groups and differ in the methyl group orientation are practically isoenergetic, while being 22−24 kJ mol^{-1} more stable than the open-chain isomers **1c** and **2c**, respectively.[16] The electronic structure of **1a**, **1c**, **2a**, and **2c** was elucidated by Mulliken population analysis, which showed that the unpaired electron occupied an antibonding π-orbital (29α), which was delocalized among the C (major), N, and O (minor) atoms of the aminoketyl group, and the out-of-plane hydrogen atoms of the CH_2 and CH_3 groups. The calculated atomic spin densities indicate predominant spin localization at the ketyl carbon atom, as shown for **1a** (Figure 1). The unpaired electron can be expected to destabilize bonds at atoms adjacent to the ketyl carbon, e.g., the O−H, N−H, N−CH_3, and C_α−C_β bonds in **1a**−**2c**. Of these, N−H bond dissociations require substantial activation energies, as calculated for other systems.[14,15] The other relevant bond dissociation (BDE) and transition state (TS) energies referring to 0 K and including zero-point corrections are summarized in Table 1. The BDEs show very low endothermicities for the loss of methyl (BDE = 15−30 kJ mol^{-1}) and somewhat more endothermic dissociations of the C_α−C_β bonds (BDE = 39−72 kJ mol^{-1}). However, the N−CH_3 bond dissociations must overcome substantial potential energy barriers in the pertinent TS (Table 1). These show that N−CH_3 bond

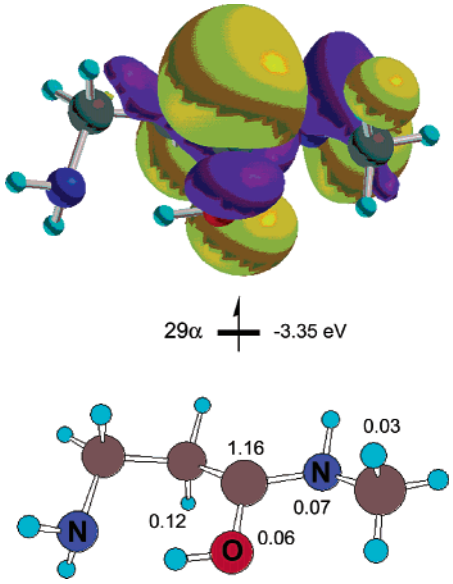


Figure 1. Atomic spin densities and singly occupied molecular orbital (29α) in **1a**.

Table 1. Bond Dissociation and Activation Energies in β-Alanine-*N*-methylamide Radicals and Cation-Radicals

	relative energy[a]	
species/reaction	B3LYP[b]	B3-PMP2[c]
1a → **1b** + CH_3•	34	30
→ **TS1ab**	91	95
→ **1e** + CH_2NH_2•	70	72
→ **TS1ae**	113	116
2a → **2b** + CH_3•	17	15
→ **TS2ab**	81	85
2c → **2d** + CH_3•	22	19
→ **TS2cd**	80	85
→ **2e** + CH_2NH_2•	37	39
→ **TS2ce**	102	104
1g^{+}• → **2g**$^{+}$•	2	3
1g^{+}• → **1h**$^{+}$ + CH_3•	50	47
→ **TS1gh**	97	102
2g^{+}• → **TS1gh**[d]	95	99

[a] In units of kJ mol^{-1} at 0 K. [b] Calculations with the 6-31++G(d,p) basis set. [c] From averaged B3LYP and PMP2 single-point energies calculated with the 6-311++G(2df,p) basis set. [d] Dissociations of **1g**$^{+}$• and **2g**$^{+}$• converge to the same transition state.

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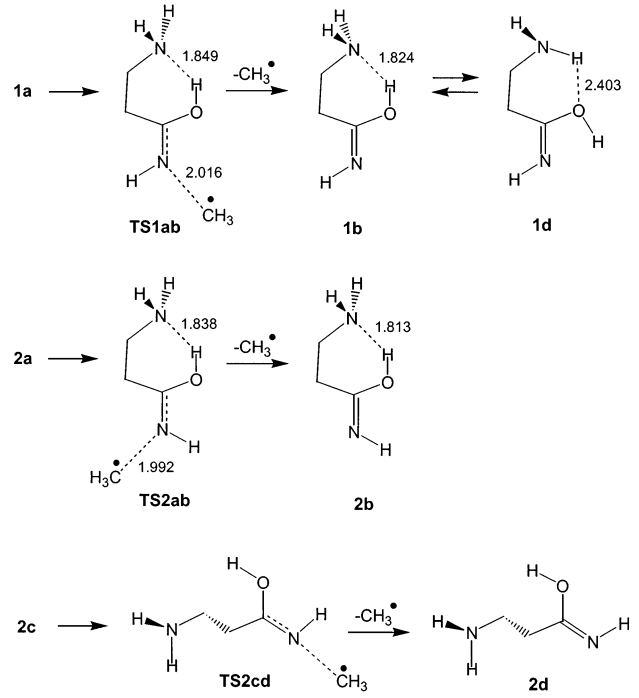
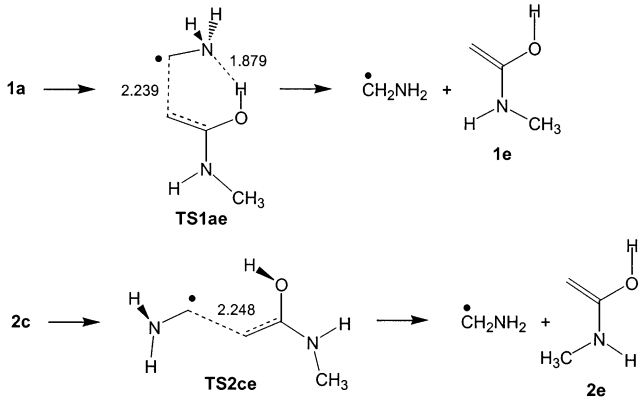
(22) Pople, J. A.; Head-Gordon, M.; Raghavachari, K. *J. Chem. Phys.* **1987**, *87*, 5968−5975.

Scheme 2



Scheme 3



dissociation is most favorable in **2a**, where it requires 85 kJ mol^{-1} in **TS2ab** to yield the enolimine **2b**, whereas the same dissociation in **1a** requires 95 kJ mol^{-1} in **TS1ab** (Scheme 2). An N−CH_3 bond dissociation in the open-chain radical **2c** proceeds through **TS2cd** to yield enolimine **2d** (Scheme 2) and requires 85 kJ mol^{-1} relative to **2c**, which is 105 kJ mol^{-1} relative to the most stable conformer **1a** (Table 1). It may be noted that the dissociation energetics in **1a** and **2a** are very similar to that in *N*-methylacetamide radicals, where E_{TS} = 85−95 kJ mol^{-1}.[15] This suggests that the hydrogen bond between the ketyl OH group and the amine group has a negligible effect on the N−CH_3 bond dissociation.

Dissociation of the C_α−C_β bond in **1a** and **2c** produces a CH_2NH_2 radical and enolimines **1e** and **2e**, respectively (Scheme 3) and requires E_{TS} = 116 and 104 kJ mol^{-1} above the respective reactants (Table 1). In summarizing the calculated BDE and TS energies, loss of CH_3 through **TS2ab** should be the preferred dissociation of radicals **1a**−**2c**.

***β*-Alanine *N*-Methylamide Cation-Radicals.** The effect of charging an adjacent group in *β*-alanine *N*-methylamide radicals was studied with cation-radicals **1g**$^{+\bullet}$ and **2g**$^{+\bullet}$ that can be thought of as protonated radicals **1a** and **2a** (Scheme 4). **1g**$^{+\bullet}$ and **2g**$^{+\bullet}$ are practically isoenergetic at the present level of theory and are predicted to exist in a ∼50/50 thermal equilibrium. The N−CH_3 bond dissociation in cation-radicals **1g**$^{+\bullet}$ and **2g**$^{+\bullet}$ yields a single protonated enolimine **1h**$^+$ (Scheme 4) and requires 47 and 44 kJ mol^{-1} from **1g**$^{+\bullet}$ and **2g**$^{+\bullet}$, respectively (Table 1). The effect of the adjacent positive charge manifests itself by a slightly increased barrier for cleavage of the N−CH_3 bond, which requires 102 kJ mol^{-1} from **1g**$^{+\bullet}$ to **TS1gh**. Interestingly, the N−CH_3 bond dissociations in **1g**$^{+\bullet}$ and **2g**$^{+\bullet}$ converge to the same transition state (**TS1gh**, Scheme 4) in which the departing methyl is perpendicular to the plane of the forming enolimine group.

***N*-Acetyl-1,2-diaminoethane Radicals and Cation-Radicals**. The properties of ketyl radicals derived from *N*-acetyl-1,2-diaminoethane were studied for two hydrogen-bonded conformers, **3a** and **3b** (Scheme 5), of which the former is 19 kJ mol^{-1} more stable (Table 2). Conformers **3a** and **3b** are separated by a small energy barrier for O−H bond rotation in **TS3ab**, E_{TS} = 22 kJ mol^{-1} relative to **3a** (Table 2). An N−CH_2 bond dissociation in **3a** is 27 kJ mol^{-1} endothermic (38 kJ mol^{-1} by QCISD(T)) and overcomes a 69 kJ mol^{-1} energy barrier in **TS3ac** to form acetamidenolimine (**3c**) and a $^\bullet CH_2CH_2NH_2$ radical. By comparison, dissociation of the O−H bond is 38 kJ mol^{-1} endothermic (41 kJ mol^{-1} by QCISD(T)) in **3a**. The loss of H proceeds by O−H bond rotation that isomerizes **3a** to **3b** and the latter then eliminates the H atom through **TS3bd**, which is 67 kJ mol^{-1} above **3b** and 86 kJ mol^{-1} above **3a**. The calculated TS energies and mechanisms strongly indicate that cleavage of the N−CH_2 bond should be the kinetically favored dissociation of radical **3a**.

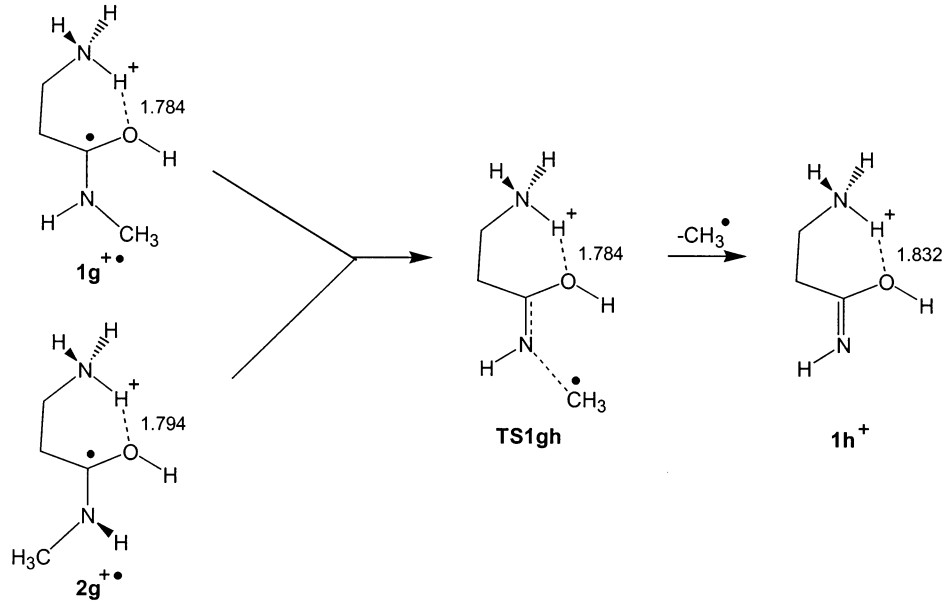
Protonation of **3a** forming cation-radical **3e**$^{+\bullet}$ has a dramatic effect on the mechanism of N−CH_2 bond dissociation in the latter. This bond cleavage proceeds through **TS3eh**, which is 66 kJ mol^{-1} above **3e**$^{+\bullet}$, but does not result in overall dissociation (Scheme 6). Instead, the incipient products, acetamide enolimine (**3f**) and the $^\bullet CH_2CH_2NH_3^+$ distonic ion (**3g**$^{+\bullet}$), remain engaged in an ion−molecule complex (**3h**$^{+\bullet}$), which is 32 kJ mol^{-1} more stable than **3e**$^{+\bullet}$. The thermochemical threshold for dissociation of **3h**$^{+\bullet}$ to **3f** and **3g**$^{+\bullet}$ is at 108 kJ mol^{-1}, which is 10 kJ mol^{-1} above **TS3eh**. These energies suggest that a fraction of **3e**$^{+\bullet}$ can isomerize to the more stable **3h**$^{+\bullet}$ without dissociation. In addition, the very stable complex **3h**$^{+\bullet}$ can function as a kinetic trap in dissociation of **3e**$^{+\bullet}$, having

Table 2. Bond Dissociation and Activation Energies in *N*-Acetyl-1,2-diaminoethane Radicals

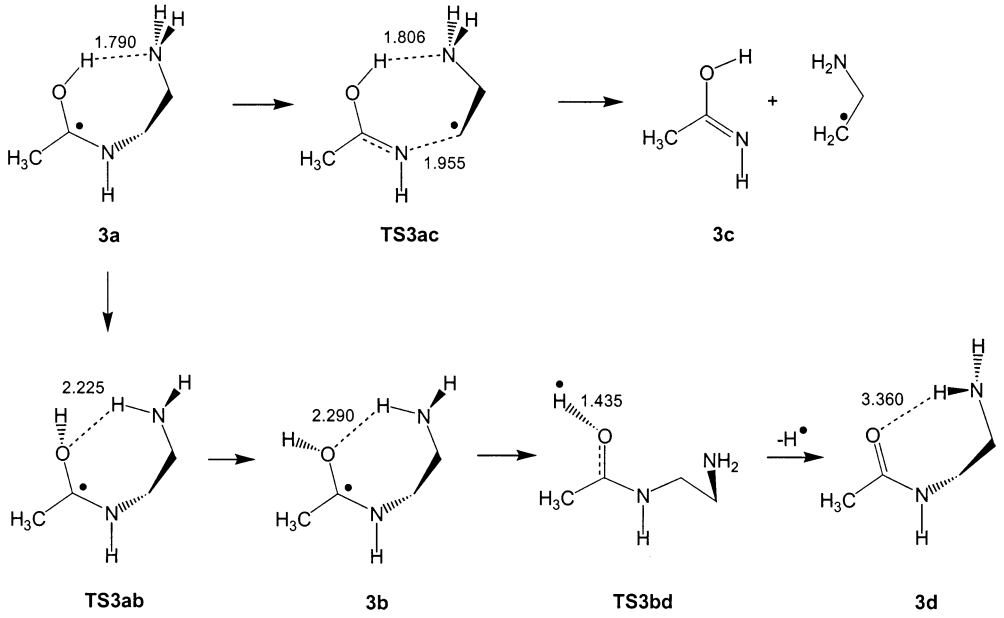
	relative energy[a]		
species/reaction	B3LYP[b]	B3-PMP2[c]	QCISD(T)[d]
3a → **3b**	21	19	
→ **TS3ab**	24	22	
→ **3c** + $^\bullet CH_2CH_2NH_2$	24	27	38
→ **TS3ac**	64	69	76
3b → **3d** + H$^\bullet$	38	19	
→ **TS3bd**	70	67	
3e$^{+\bullet}$ → **3f** + **3 g**$^{+\bullet}$	73	76	85
→ **3h**$^{+\bullet}$	−33	−32	
→ **TS3eh**	62	66	
→ **3i**$^+$ + H$^\bullet$	36	17	21
→ **TS3ei**	86	85	

[a] In units of kJ mol^{-1} at 0 K. [b] Calculations with the 6-31++G(d,p) basis set. [c] From averaged B3LYP and PMP2 single-point energies calculated with the 6-311++G(2df,p) basis set. [d] From effective QCISD(T)/6-311++G(2df,p) single-point energies.

Scheme 4



Scheme 5

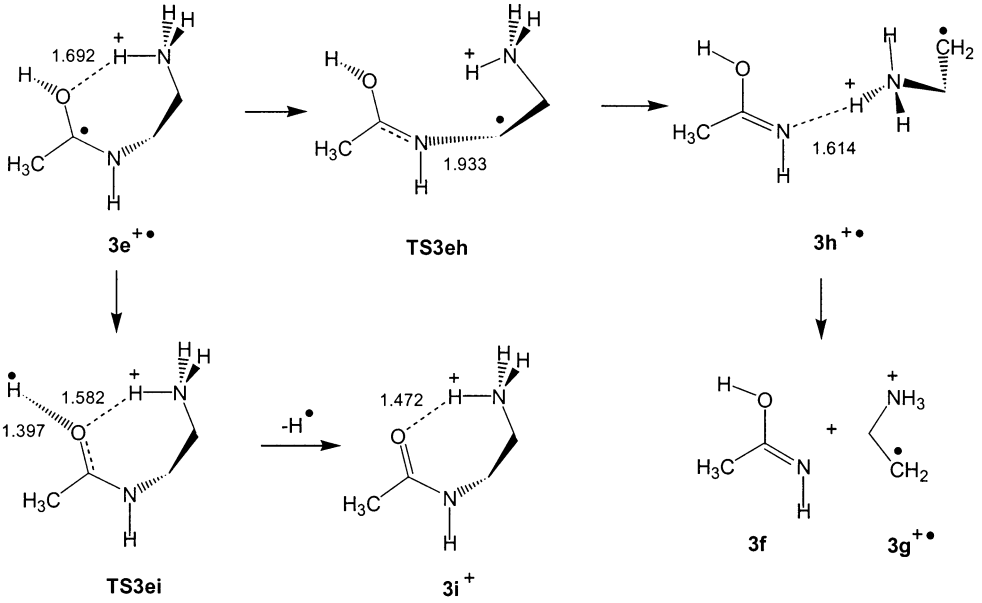


internal energies above the threshold for the formation of $CH_3C(OH)=NH$ and $^{\bullet}CH_2CH_2NH_3^+$. This model system indicates that ion−molecule complexes analogous to **3h**$^{+\bullet}$ may play an important role in ECD where substantial fractions of nondissociating cation-radicals are observed in the mass spectra. As a further note, O−H bond dissociation in **3e**$^{+\bullet}$ to form ion **3i**$^+$ is 17 kJ mol^{-1} endothermic and requires 85 kJ mol^{-1} in **TS3ei** (Scheme 6). Hence, the O−H bond dissociation energies are very similar in **3e**$^{+\bullet}$ and **3b** (Table 2) and appear not to be much affected by hydrogen bonding of the oxygen atom to the amine or ammonium groups.

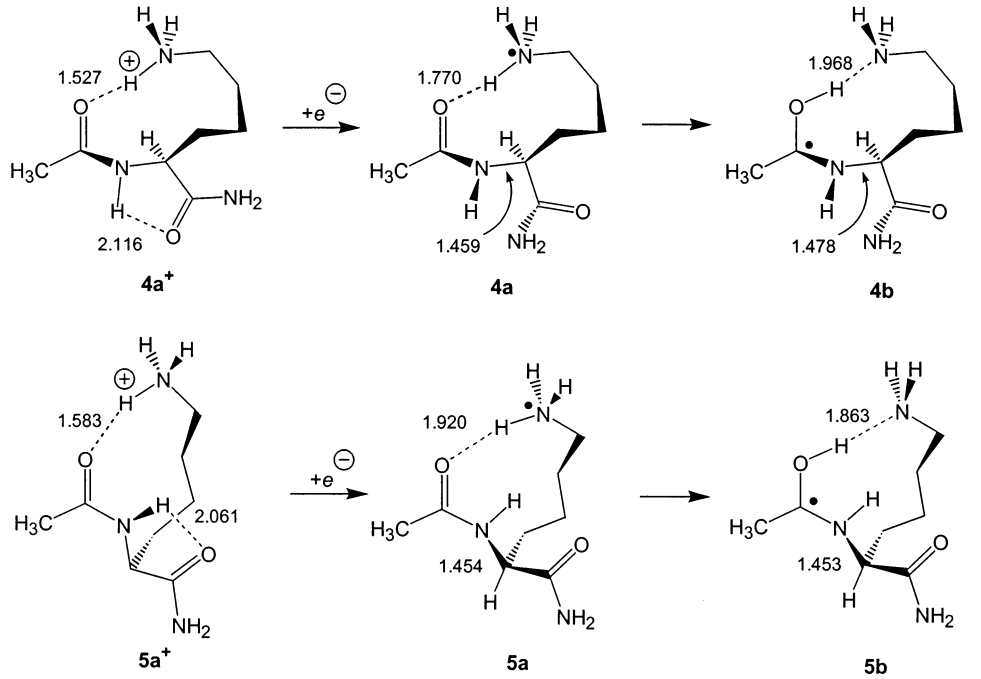
***N*$_\alpha$-Acetyl Lysine Amide Radicals.** Radicals derived from *N*$_\alpha$-acetyl lysine amide represent models for H atom transfer from a remote donor ammonium group to the acceptor carbonyl oxygen, followed by dissociation of the tertiary N−C$_\alpha$H bond. The structures of the *N*$_\alpha$-acetyl lysine-derived ϵ-ammonium and ketyl radicals are sketched in Schemes 7 and 8. Although there are a multitude of side-chain conformers of the *trans*- and *cis*-amide and aminoketyl radicals, only those arising by electron capture of the most stable internally solvated cations **4a**$^+$ and **5a**$^+$ were of interest here. Ammonium radicals **4a** and **5a** have weak hydrogen bonds between the respective ammonium proton and the carbonyl oxygen atom, as indicated by the C=O···H distances, which are 1.770 and 1.920 Å in the *trans*- and *cis*-amide, respectively, and are substantially longer than those in the corresponding ammonium cations (Scheme 7). The *trans*-ammonium radical **4a** is 35 kJ mol^{-1} more stable than the *cis*-isomer **5a** (Table 3).

Hydrogen transfer from the lysine ϵ-ammonium group onto the amide carbonyl is 63−90 kJ mol^{-1} exothermic depending on the amide bond configuration in the reactant. Note that the ketyl radicals **4b** and **5b** differ by only 7 kJ mol^{-1} in favor of the *trans*-isomer **4b**. N−C$_\alpha$H bond dissociations in **4b** and **5b** are slightly exothermic, making the ketyl radicals metastable with respect to the dissociation products, which are the 6-aminohexanamide-2-yl radical **4c** and acetamide enolimines

Scheme 6



Scheme 7



3c and **5c** (Scheme 8). The transition states for the N−C$_\alpha$ bond dissociation in **4b** and **5b** were found to occur at similar N−C$_\alpha$ bond lengths, 1.812 and 1.828 Å in **TS4bc** and **TS5bc**, respectively, but showed different TS energies. Interestingly, dissociation of the more stable conformer **4b** requires only 42 kJ mol^{-1} in **TS4bc**, indicating a very facile reaction. Dissociation of the *cis*-conformer **5b** requires 51 kJ mol^{-1} in **TS5bc**.

N*$_\alpha$-Glycylglycine Amide Radicals.** Four isomers, **6a**, **6b**, **6c**, and **6d**, were found as local energy minima for *N*$_\alpha$-glycylglycine ketyl radicals. These isomers are close in energy and are likely to readily interconvert by rotations about the single N−C bonds. Conformers **6a**, **6b**, and **6c** can dissociate by simple bond cleavage to produce the respective glycine enolimine isomers **6e**−**g** and α-acetamide radical **7a** (Scheme 9). The N−C$_\alpha$ bond dissociations proceed through distinct TS (**TS6ae**, **TS6bf**, and **TS6cg**, Scheme 9) that differ in energy (Table 4). The most favorable of these is the N−C$_\alpha$ bond cleavage through **TS6ae**, which requires only 37 kJ mol^{-1} above **6a** and is overall nearly thermoneutral ($\Delta H_{rxn,0} = -1$ kJ mol^{-1}, Table 4). In contrast, simple N−C$_\alpha$ bond cleavage in **6d** to yield acetamide enol **7b** as a ***z-type fragment and a glycine amidyl radical, $H_2NCH_2CONH^\bullet$, as a ***c***-type fragment is predicted not to occur. Radical $H_2NCH_2CONH^\bullet$ is unbound and dissociates spontaneously to $^\bullet CH_2NH_2$ and HNCO (Scheme 10). The overall reaction enthalpy for this dissociation, $\Delta H_{rxn,0} = 84$ kJ mol^{-1}, is prohibitively high, and the system prefers to dissociate through alternative pathways. The most favorable one involves a proton transfer[23] between the ketyl and carbonyl groups, which is coupled with cleavage of the N−C$_\alpha$ bond to yield glycine enolimine isomer **6g** and radical **7a** as the respective ***c***- and

(23) The calculated atomic charge densities indicate substantial positive charge on the migrating hydrogen atom, which is therefore denoted as a proton.

Scheme 8

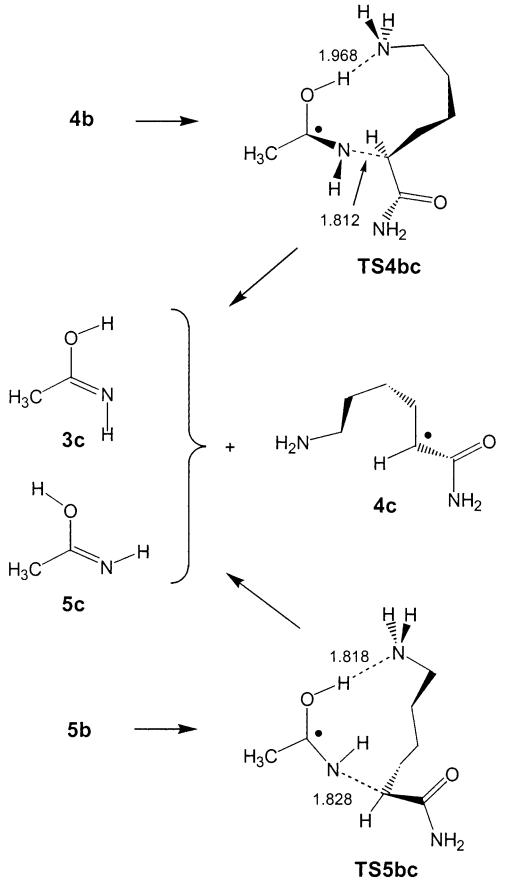


Table 3. Bond Dissociation and Activation Energies in N_α-Acetyl Lysine Amide Radicals

species/reaction	relative energy[a] B3LYP[b]	B3-PMP2[c]
4a$^+$ → **5a**$^+$	28	22
4a → **5a**	40	35
4b → **5b**	9	7
4a → **4b**	−75	−90
5a → **5b**	−44	−63
4b → **3c** + **4c**	−18	−5
4b → **TS4bc**	33	42
5b → **5c** + **4c**	−27	−12
5b → **TS5bc**	45	51

[a] In units of kJ mol^{-1} at 0 K. [b] Calculations with the 6-31++G(d,p) basis set. [c] From averaged B3LYP and PMP2 single-point energies with the 6-311+G(2d,p) basis set.

z-type fragments (Scheme 10). Although this dissociation is mildly endothermic, $\Delta H_{rxn,0} = 20$ kJ mol^{-1}, the very low TS energy for this pathway, $E_{TS} = 42$ kJ mol^{-1} relative to **6d**, should make it competitive with the N−C_α bond cleavage in the Gly-2 ketyl radical **6a**.

***N*$_\alpha$-Glycylglycine Amide Cation-Radicals**. An interesting proton shuffling is calculated to accompany the dissociation of cation-radicals derived from Gly-Gly-NH_2. A single isomer (**6h**$^{+\bullet}$) with an internal (Gly-2) ketyl group was found as a local energy minimum, whereas an isomer with the terminal (Gly-1) ketyl radical group isomerized spontaneously to **6h**$^{+\bullet}$ upon geometry optimization. Cation-radical **6h**$^{+\bullet}$ shows strong hydrogen bonding of the OH proton which is chelated between the amide carbonyl oxygens, and another H-bond between the ammonium group and the Gly-2 ketyl oxygen. Upon stretching

Table 4. Bond Dissociation and Activation Energies in N_α-Gly-Gly-NH_2 Radicals and Cation-Radicals

species/reaction	relative energy[a] B3LYP[b]	B3-PMP2[c]
6a → **6b**	−9	−8
→ **6c**	4	2
→ **6d**	−6	−9
→ **6e** + **7a**	−3	−1
→ **TS6ae**	32	37
→ Gly-Gly-NH_2 + H$^\bullet$	65	48
6b → **6f** + **7a**	7	11
→ **TS6bf**	68	71
6c → **6g** + **7a**	6	9
→ **TS6cg**	39	46
6d → **6g** + **7a**	16	20
→ **TS6dg**	36	42
→ $^\bullet CH_2NH_2$ + HN=C=O + **7b**	89	84
6h$^{+\bullet}$ → **6j**$^+$ + **7a**	60	67
→ **6l**$^+$ + **7a**	83	89
→ **TS6hi**	33	40
→ **6i**$^{+\bullet}$	−13	−8
→ **6f** + **7b**$^{+\bullet}$	120	121
→ **6k**$^+$ + **7b**	196	202

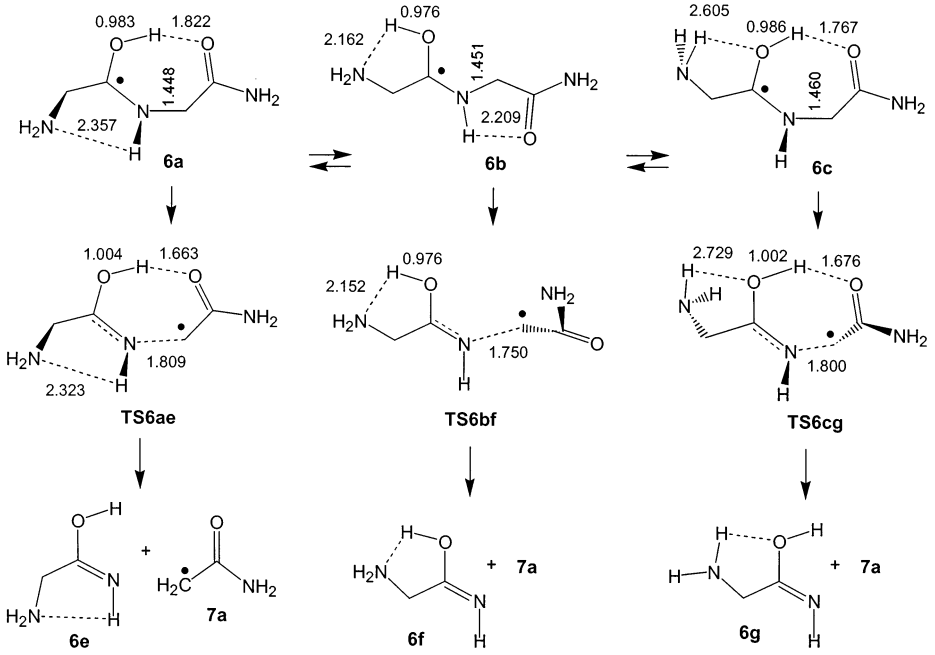
[a] In units of kJ mol^{-1} at 0 K. [b] Calculations with the 6-31++G(d,p) basis set. [c] From averaged B3LYP and PMP2 single-point energies with the 6-311++G(2d,p) basis set.

the N−C_α bond in **6h**$^{+\bullet}$, the Gly-2 ketyl proton migrates to the Gly-1 carbonyl in **TS6hi** while remaining H-bonded to the Gly-2 oxygen atom. The latter develops a strong H-bond to one of the ammonium protons in **TS6hi** at an H···O distance of 1.637 Å (Scheme 11). Upon further stretching the N−C_α bond in **TS6hi**, the ketyl proton migrates back to the Gly-2 oxygen atom, and the reacting cation-radical rearranges to an ion−molecule complex (**6i**$^{+\bullet}$) which is 8 kJ mol^{-1} *more stable* than **6h**$^{+\bullet}$. Dissociation of **6i**$^{+\bullet}$ to protonated acetamide enolimine (**6j**$^+$) and radical **7a** requires 75 kJ mol^{-1} and proceeds as a continuously endothermic process without an additional energy barrier.[24] The reason for the ping-pong hydrogen transfer accompanying the N−C_α bond cleavage in **6h**$^{+\bullet}$ is most likely due to the reaction energetics, as bond dissociations yielding either **6f** and **7b**$^{+\bullet}$ or **6k**$^+$ and **7b** as the respective ***c***- and ***z***-type fragments (Scheme 11) are highly endothermic, requiring 121 and 202 kJ mol^{-1}, respectively, at their thermochemical thresholds relative to **6h**$^{+\bullet}$, to be compared to 67 kJ mol^{-1} for the competing channel forming **6j**$^+$ (Table 4).

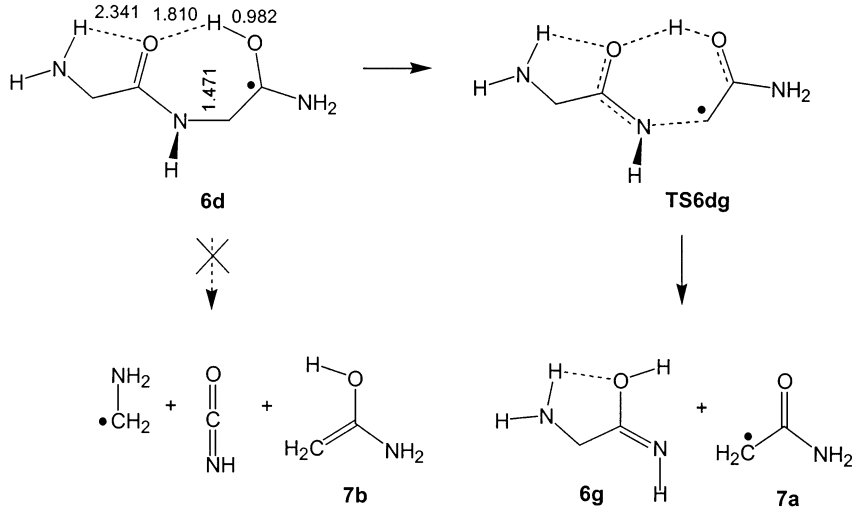
Discussion of Dissociation and Transition State Energies. The calculated dissociation and transition state energies show that in neutral ketyl radicals the N−C_α bond dissociation energies depend on the nature of the leaving radical group. The BDEs decrease in the series N−CH_3 > N−$CH_2CH_2NH_2$ > N−CH_2CONH_2 > N−$CH(CONH_2)(CH_2)_4NH_2$, which inversely correlates with the radical stabilization energies.[25] This suggests that the stability of the radical product is the determining factor. The BDEs in conformational isomers show only small differences depending on the relative stabilities of the reactant and product conformations. For example, the *cis*-amide radical **2a** has a lower BDE than the *trans*-amide radical **1a**, due to the lower stability of the former. Likewise, the N−C_α bond dissociation in the *cis*-amide radical **5b** is 7 kJ mol^{-1} more

(24) This followed from an examination by B3LYP/6-31++G(d,p) calculations of the potential energy surface along the N−C_α coordinate.
(25) (a) Leroy, G.; Sana, M.; Wilante, C. *THEOCHEM* **1991**, *74*, 37−45. (b) Leroy, G.; Sana, M.; Wilante, C. *THEOCHEM* **1991**, *80*, 303−328.

Scheme 9



Scheme 10



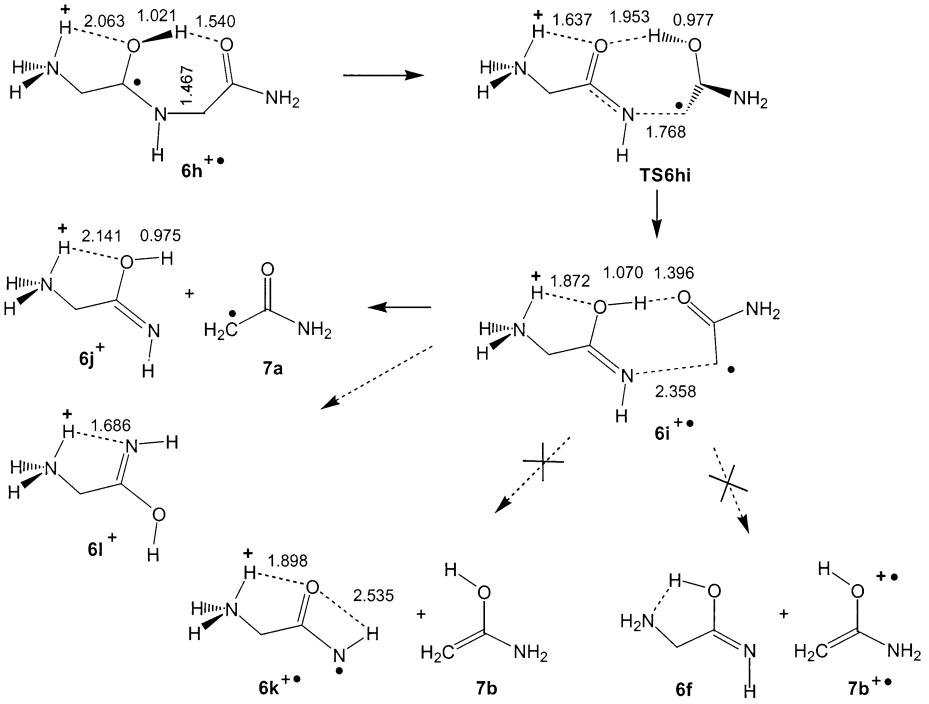
exothermic than in the *trans*-amide radical **4b**, again due to different reactant relative energies.

The TS energies for the N−C_α bond cleavage follow a trend that is similar to that for the BDEs in that the weaker bonds require lower E_{TS}, for example, E_{TS}(N−CH_3) > E_{TS}(N−CH_2-CH_2NH_2) > E_{TS}(N−CH_2CONH_2) ≈ E_{TS}[N−CH($CONH_2$)$(CH_2)_4$-NH_2]. This indicates that radical site stabilization by the leaving group affects the TS energy. These effects are qualitatively understandable from the TS electronic structures that show ∼50% of total spin density at the C_α atom of the ***z***-type moiety in the transition state, so that it develops a substantial radical character in the course of N−C_α bond dissociation. Conformational effects on the TS energies are relatively small. In **1a**, **2a**, and **2c** the TS energies are closely similar, so that the most stable conformer **1a** has the highest activation energy for loss of methyl. In the lysine radical conformers **4b** and **5b**, it is the less stable **5b** that has the higher activation energy for N−C_α bond cleavage (Table 3). This implies that the nonbonding interactions that destabilize **5b** against **4b** are slightly amplified in the TS of the dissociation.

A substantially different situation is encountered with the dissociations of cation radicals $\mathbf{3e}^{+\bullet}$ and $\mathbf{6h}^{+\bullet}$. Dissociation of the N−C_α bonds in the cation-radicals in general requires activation energies that are only slightly higher than those in neutral radicals, which applies to $\mathbf{1g}^{+\bullet}$ and $\mathbf{2g}^{+\bullet}$ as well. However, the ***z***-type fragments containing polar groups are tightly bound to the enolimine ***c***-type fragments by hydrogen bonds, so that the resulting *H-bonded complexes are lower in energy than the reactants*, and the overall isomerization is exothermic. The proton location in the complexes depends on the topical proton affinities of the constituent molecules. In $\mathbf{3e}^{+\bullet}$, the 2-aminoethyl radical is the more basic moiety and is protonated. In $\mathbf{6h}^{+\bullet}$, the glycine enolimine is more basic than the acetamide radical and retains the proton in $\mathbf{6j}^{+}$. A further dissociation of the complexes mandates breaking the hydrogen bond, which requires substantially greater energies than the N−C_α bond cleavage in the aminoketyl radical-cations. Hence, *breaking the hydrogen bond, not the N−C_α bond cleavage, represents the rate-determining step*.

The fact that dissociation energies for single hydrogen bonds

Scheme 11



on the order of 67−76 kJ mol^{-1} are obtained for relatively small systems suggests that very stable complexes should exist in larger peptide cation-radicals, where bonding can be mediated by several H-bonds. Hence, the extraordinary stability of peptide cation-radicals formed by electron capture can be ascribed to strong H-bonds that do not allow the ***c***-type and ***z***-type fragments to separate in the gas phase. This interpretation is consistent with recent experimental data on ECD of gas-phase protein ions where additional excitation by heating or collisional activation was necessary to achieve slow dissociation of partially neutralized cations.[26] It should also be noted that the attractive forces due to multiple hydrogen bonding are to some extent counterbalanced by Coulombic repulsion in multiply charged protein ions in the gas phase.

Dissociation Kinetics. The mechanism suggested in the present work is further corroborated by the kinetics of N−C$_\alpha$ bond dissociations in aminoketyl radicals and cation-radicals. The rate constants depend on the reaction conditions that determine the energy transfer between the reactants and environment. For isolated ions, the rate constants can be treated by the RRKM model, while under thermal conditions, the unimolecular rate constants can be expressed by transition state theory (TST) and plotted as a function of temperature. Figure 2 shows the log *k* values calculated by TST using the B3-PMP2 activation energies. The N−C$_\alpha$ bond dissociations in **4b**, **6a**, and **6d** that produce α-amino acid radicals are fast so that $k > 10^5$ s^{-1} at 298 K.

When extrapolating these calculated reaction rate constants to dissociations of reduced-charge ions produced by ECD, one has to consider the ion internal energy and experimental conditions that determine the reaction time scale. Precursor ions are typically trapped for seconds in an ion cyclotron resonance cell prior to electron capture. During this time, the ions exchange

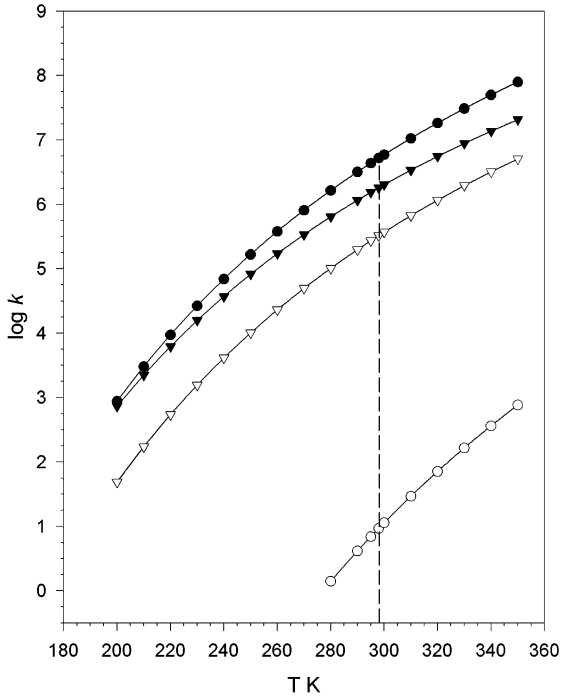


Figure 2. Thermal unimolecular rate constants for N−C$_\alpha$ bond dissociations in **3a** (○), **4b** (●), **6a** (▼), and **6d** (▽).

energy with the cell walls by radiative heat transfer,[27] so that they can have near thermal internal energies, which scale with both the number of internal degrees of freedom and cell temperature.[28] For example, a doubly charged (phenylalanine)$_{10}$ ion consisting of $N = 205$ atoms is estimated to have approximately $0.18 \times (3N - 6)RT = 273$ kJ mol^{-1} internal

(26) (a) Horn, D. M.; Ge, Y.; McLafferty, F. W. *Anal. Chem.* **2000**, *72*, 4778−4784. (b) Horn, D. M.; Breuker, K.; Frank, A. J.; McLafferty, F. W. *J. Am. Chem. Soc.* **2001**, *123*, 9792−9799.

(27) (a) Dunbar, R. C.; McMahon, T. B. *Science* (Washington, D.C.) **1998**, *279*, 194−197. (b) Ryzhov, V.; Dumbar, R. C. *Int. J. Mass Spectrom. Ion Processes* **1997**, *167/168*, 627−635. (c) Ryzhov, V.; Yang, Y.-C.; Klippenstein, S. J.; Dunbar, R. C. *J. Phys. Chem. A* **1998**, *102*, 8865−8870.

(28) Tureček, F. *Org. Mass Spectrom.* **1991**, *26*, 1074−1081.

energy at 300 K.[28] Electron capture proceeds through a series of Rydberg states in which the recombination energy (RE) can be either converted nonradiatively into vibrational energy of the reduced-charge ion or lost by photon emission. The recombination energy of a peptide ion forming an ammonium radical in its ground electronic state depends on the precursor ion charge state; for example, a doubly charged ion is estimated to have RE $\approx 3.3 + 0.8 = 4.1$ eV or 396 kJ mol^{-1}.[16] Hence, full internal conversion of recombination energy would cause a substantial increase of temperature in a medium-size reduced-charge ion (e.g., for $N = 205$) and will result in accelerated dissociation. With much larger ($N > 2000$) multiply charged protein ions studied by ECD, the energy gain by RE is proportionately less important when compared with the precursor ion thermal energy. However, because the aminoketyl N$-$C$_\alpha$ bonds are extremely weak, they will rapidly dissociate even in thermal reduced-charge ions on a time scale that is orders of magnitude shorter than the observation time in ECD measurements. The energy to thermalize the trapped reduced-charge ions and drive their dissociation can be provided by radiative heating by which large ions attain thermal equilibrium on a time scale of <1 s.[29] From the comparison of the rates for radiative heating[30] and N$-$C$_\alpha$ bond dissociation, it appears that the former is the rate-determining process.

It is important to note that the TST rate constants depend only weakly on the number of degrees of freedom in the reacting system through the corresponding ratio of partition functions of the transition state and the reactant, $Q^{\ddagger}/Q_r$, and are dominated by the energy-dependent exponential term. This implies that the results from the current model systems are practically size-independent and can be extrapolated to larger peptide and protein radicals, as long as the activation energies remain similar. The previous interpretation of ECD dissociation mechanisms postulated fast non-ergodic dissociation of N$-$C$_\alpha$ bonds due to a large excitation energy provided by electron capture by ammonium groups and exothermic rearrangement to ketyl radicals.[5,8] It was presumed that at such high excitations N$-$C$_\alpha$ bond dissociation occurs with rates that are faster than intramolecular vibrational relaxation. The present calculations suggest that, unless supported by evidence, the non-ergodic hypothesis is unnecessary. At reaction times observed in ECD studies, N$-$C$_\alpha$ bond cleavage does not require such excitation and is complete even in thermal peptide aminoketyl radicals.

Conclusions

This computational study shows that N$-$C$_\alpha$ bond cleavage is extremely facile in model peptide ketyl radicals and cation-radicals. In the absence of hydrogen bonding, peptide ketyl radicals dissociate with rate constants $> 10^5$ s^{-1} at 298 K. The rate-determining step in cation-radical dissociations is cleavage of hydrogen bonds that hold together the ***c***- and ***z***-type fragments in stable ion-molecule complexes.

Acknowledgment. This work is dedicated to Professor Fred W. McLafferty on the occasion of his 80th birthday. Support of this work by the National Science Foundation (Grants CHE-0090930 and CHE-9808182) is gratefully acknowledged. Thanks are due to Dr. Philip Mayer and Erik Syrstad for helpful discussions.

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