On the Relative Stability of Singly Protonated des-Arg[1]- and des-Arg[9]-Bradykinins†

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Normal-incidence surface-induced dissociation of singly protonated des-Arg[1]-bradykinin (PPGFSPFR) and des-Arg[9]-bradykinin (RPPGFSPF) has been studied using a specially designed Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS). We found that, with a reaction time of 1 s, the collision-energy-resolved fragmentation efficiency curve (FEC) for des-Arg[9]-bradykinin is shifted to lower energies by about 4 eV relative to the FEC for des-Arg[1]-bradykinin. Because the Arrhenius activation energies found by Williams and co-workers in BIRD experiments are 0.82 and 1.2 eV for des-Arg[1]- and des-Arg[9]-bradykinin, respectively, we expected to find the reverse order in our long-reaction-time FTICR-SID experiments. We rationalize the difference between our data and the thermal kinetics using Tolman's theorem to calculate threshold energies from the Arrhenius activation parameters. We found that, when the preexponential factor for reaction is very high ($>10^{17}$ s^{-1}) or very low ($<10^{10}$ s^{-1}), Tolman's correction becomes fairly large. In this case, the Arrhenius parameters are strongly correlated, and the activation energy depends on the reaction dynamics and temperature. This can result in the reversal of the order of the Arrhenius activation energies for different systems relative to the order of the threshold energies, meaning that conclusions about relative stabilities of large molecules should be based not on the thermal activation energies but rather on actual threshold energies, which can be calculated using Tolman's correction. The threshold energies extracted from the Arrhenius activation parameters are 1.15 and 1.24 eV for des-Arg[1]- and des-Arg[9]-bradykinin, respectively, assuming a temperature of 450 K. However, des-Arg[1]-bradykinin dissociates via a very tight transition state. Consequently, microcanonical rate−energy dependencies for these two peptides cross at very low internal energies and des-Arg[9]-bradykinin has a larger rate constant at internal energies sampled experimentally.

Introduction

Gas-phase fragmentation of singly and multiply protonated peptides is widely used for peptide sequencing and protein identification.[1] The most important structural information is extracted from the identification of sequence-specific fragments that originate from the backbone cleavage of peptides. Cleavage of peptide (amide) bonds results in formation of b and y ions if the charge remains on the N-terminus or C-terminus, respectively. Loss of small molecules such as CO, NH_3, and H_2O from the protonated peptide and its subsequent shorter chain fragments is commonly observed in tandem mass spectra. Fragmentation energetics and mechanisms of protonated peptides are usually described in the framework of the "mobile proton" model. According to this model, in the absence of strongly basic residues the proton in the protonated peptide is not localized but rather migrates along the peptide bond, thereby inducing cleavages at the various amide bonds.[2−4] However, when the protonated peptide contains a strongly basic residue, such as arginine (R) or lysine (K), the proton is sequestered at the basic site. In this case, the protonated peptide preferentially fragments via reaction pathways that do not require intramolecular proton transfer.

Fragmentation of large molecules is characterized by the dramatic decrease of decomposition rates with increasing number of vibrational degrees of freedom (DOF) in the activated molecule. The kinetic shift (KS) was originally defined as the excess internal energy required to produce detectable dissociation of a polyatomic ion on a typical mass spectrometric time scale of 10 μs.[5] However, large molecules can exhibit a substantial kinetic shift even several seconds after ion activation. Marzluff and Beauchamp have performed calculations on the unimolecular fragmentation of protonated glycine polymers,[6] concluding that, for the assumed dissociation energy of 2.5 eV for the 10-mer (DOF = 249), the kinetic shift is 21.5 eV for microsecond sampling times and 7.5 eV for a reaction time of 1 s. The effect is even more dramatic for the 50-mer, for which the calculated kinetic shifts are 107.5 and 43 eV for reaction times of 1 μs and 1 s, respectively. Consequently, conducting experiments on a long time scale provides a unique advantage for the fragmentation of large molecules because kinetic shifts are substantially reduced. Even for observation times of 1 s, the deposition of large amounts of internal energy is an important prerequisite for observing the fragmentation of large molecules.[7] This can be achieved using multiple-collision activation (MCA-CID), absorption of multiple photons in the IR or UV region,[8] or surface-induced dissociation (SID).[9]

This work presents a comparative SID study of the dissociation of the singly protonated bradykinin fragments des-Arg[1]-bradykinin (PPGFSPFR) and des-Arg[9]-bradykinin (RPPGFSPF), which differ only in the position of a basic arginine residue.

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Fragmentation of singly protonated bradykinin fragments has been previously studied using blackbody infrared radiative dissociation (BIRD)[10] and SID.[11] The latter experiments were performed using a tandem-quadrupole mass spectrometer on the microsecond time scale. It was found that both ions require approximately the same SID collision energy (78.8 eV for des-Arg[1]-bradykinin and 78.3 eV for des-Arg[9]-bradykinin) to obtain 50% fragmentation of the precursor ion. In contrast, BIRD studies indicate that Arrhenius activation parameters for these two ions are quite different. In particular, activation energies of 0.82 and 1.2 eV and preexponential factors of 10^7 and 10^{12} s^{-1} were found for des-Arg[1]- and des-Arg[9]-bradykinin, respectively. Obviously, fragmentation of des-Arg[1]-bradykinin requires lower energy; further it proceeds via a very tight transition state (TS), whereas fragmentation of des-Arg[9]-bradykinin is more energetically demanding but kinetically more favorable. From this result, the degree of overlap between the SID fragmentation efficiency curves on a microsecond time scale can provide an indication that the microcanonical rate−energy dependencies for these two ions cross at higher internal energies.

In our laboratory, we have implemented SID on a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) with an observation time ranging from several milliseconds to many seconds. Consequently, much of the kinetic shift associated with unimolecular fragmentation of large molecules is eliminated in our studies. From the above discussion, we expected that the collision energy-resolved fragmentation efficiency curve (FEC) for the precursor ion of des-Arg[1]-bradykinin would be shifted to lower collision energies relative to the FEC for des-Arg[9]-bradykinin, because it has a lower dissociation threshold. Surprisingly, our results indicate just the opposite. Specifically, for a reaction time of 1 s, the FEC of des-Arg[1]-bradykinin is shifted by about 4 eV to higher collision energies as compared to that of des-Arg[9]-bradykinin. It is shown here that the apparent contradiction between the BIRD experiments and our results can be rationalized using Tolman's theorem.[12]

Experimental Section

Mass Spectrometry. The custom-built 6-T FT-ICR mass spectrometer employed in this work is described elsewhere.[13] Briefly, protonated peptides are formed in an external electrospray source. High transmission of ions from atmospheric pressure into the vacuum system is achieved using an electrodynamic ion funnel constructed following the design developed by Smith and co-workers at Pacific Northwest National Laboratory (PNNL).[14] After the ion funnel, ions undergo collisional relaxation in a collisional quadrupole, followed by mass selection using a commercial Extrel quadrupole and accumulation in a third radio-frequency-only quadrupole, typically for 0.3 s. The pressure in the accumulation quadrupole was maintained at ca. 2×10^{-3} Torr to assist ion trapping and enable efficient collisional relaxation of ions.

The SID surface is introduced to the rear trapping plate of the ICR cell using a custom probe and vacuum-lock system. A self-assembled monolayer (SAM) of fluorinated alkanethiol (FC_{12}) on a Au(111) single crystal (Monocrystals, Richmond Heights, OH) was used as the SID target. The gold crystal was cleaned in a UV cleaner for about 15 min prior to SAM deposition. The SAMs were prepared by immersing the gold crystal in a 1 mM ethanol solution of the FC_{12} alkanethiol [CF_3-$(CF_2)_9C_2H_4SH$] for at least 24 h. Extra layers of the SAMs were removed by thorough rinsing of the surface with ethanol.

Ions from the electrospray interface are extracted into an electrostatic ion guide, transferred through the ICR cell, and collided with a surface along the surface normal. The collision energy is varied by changing the dc offset applied to the ICR cell. We have shown that this approach eliminates defocusing of the ion beam by the ion transfer optics as a function of ion kinetic energy.[13] As a result, the ion current on the surface is independent of collision energy. During ion−surface impact, the front trapping plate of the ICR cell and the excite/detect electrodes are kept at the same potential, whereas the potential on the surface and the rear trapping plate is higher by 2 V. Scattered ions are trapped by raising the potential on both trapping plates by 15 V above the potential on the excite/detect electrodes. After a predefined reaction delay, ions are excited using a broadband chirp excitation and detected using a very sensitive preamplifier constructed by the Instrument Development Laboratory at PNNL. The experiments are controlled by a Midas data station.[15]

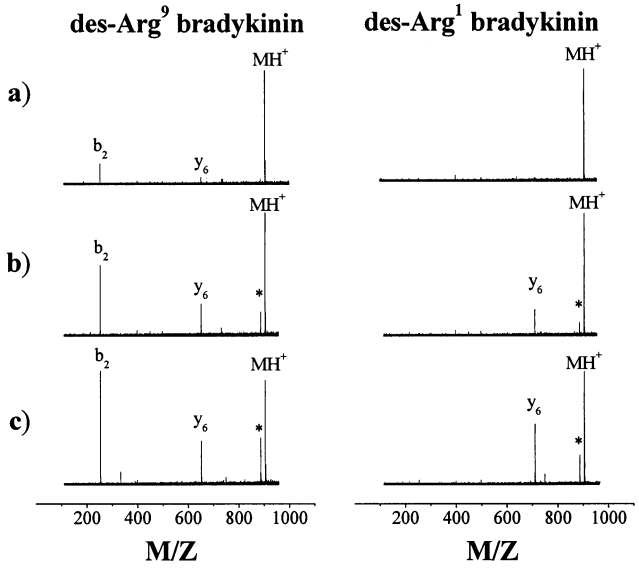

Figure 1. SID mass spectra of des-Arg[1]- (right) and des-Arg[9]-bradykinin (left) at collision energies of (a) 19, (b) 23, and (c) 26 eV. The peak corresponding to the loss of water from the parent ion is denoted by the asterisk.

Modeling. Vibrational frequencies of protonated precursor ions were estimated using AM1 calculations within the Insight II package (Biosym Technologies, San Diego, CA). Because RRKM calculations are sensitive not to the details of vibrational frequencies of the precursor ion and transition states but rather to the relative changes in frequencies along the reaction coordinate, a more elaborate modeling of vibrational frequencies was deemed unnecessary. Vibrational frequencies of the transition state were estimated by removing one frequency (C−N stretch) and varying 15 frequencies in the range 300−1500 cm^{-1} to reproduce a selected preexponential factor.

Results and Discussion

Collision energy-resolved SID spectra of des-Arg[1]- and des-Arg[9]-bradykinins at 19, 23, and 26 eV are shown in Figure 1. The spectra are very similar to BIRD spectra reported by Williams and co-workers.[10] The major fragments obtained at these energies correspond to the loss of water for both peptides and the formation of y_6 ion for des-Arg[1]-bradykinin and b_2/y_6 complementary ions for des-Arg[9]-bradykinin. It is clearly seen from the spectra that under the same experimental conditions, the fragmentation of des-Arg[9]-bradykinin outpaces the fragmentation of des-Arg[1]-bradykinin.

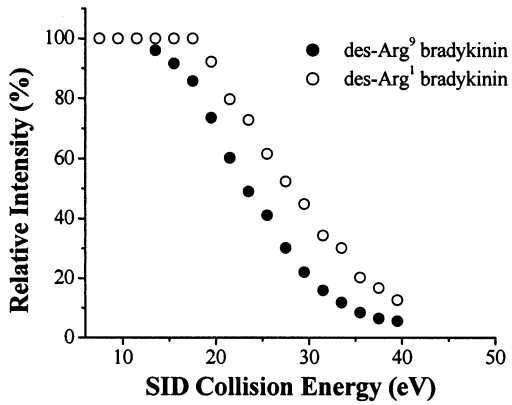

Figure 2. Fragmentation efficiency curves for des-Arg^1- (open circles) and des-Arg^9-bradykinin (solid circles) representing the relative amount of the surviving precursor ion as a function of collision energy.

The relative intensity of the parent ion as a function of SID collision energy is plotted in Figure 2. We refer to these curves as collision-energy-resolved fragmentation efficiency curves (FECs). The SID collision energy corresponding to 50% fragmentation ($E_{50\%}$) at a 1-s reaction delay is 23.5 ± 1.0 eV for des-Arg^9-bradykinin and 27.5 ± 1.0 eV for des-Arg^1-bradykinin. It follows that fragmentation of des-Arg^1-bradykinin at a 1-s reaction delay requires the collision energy to be 4 eV higher than fragmentation of des-Arg^9-bradykinin. As mentioned earlier, the $E_{50\%}$ values obtained on the microsecond time scale of a tandem-quadrupole mass spectrometer are the same for des-Arg^1-bradykinin (78.8 eV) and des-Arg^9-bradykinin (78.3 eV) within the experimental uncertainty.[11]

Our experiments differ from the tandem-quadrupole experiments in two aspects. First, in the latter experiments, ions strike the surface at 45°, whereas we investigate normal-incidence collisions. However, it has been reported that energy-transfer efficiency is largely independent of the incident angle.[16,17] Second, a substantially longer reaction time is sampled in FT-ICR experiments (1 s) as compared to the 10 μs sampled in tandem-quadrupole experiments. Because of the strongly reduced kinetic shift, much lower collision energies are required to observe fragmentation on a long time scale.

The most serious discrepancy between the 10-μs and 1-s SID experiments is that the relative energy requirements for the fragmentation of the bradykinin fragments are different. If des-Arg^9-bradykinin had a lower dissociation threshold resulting in the shift of its FEC at a 1-s reaction delay toward lower energies and a tighter transition state than des-Arg^1-bradykinin, this discrepancy could be rationalized. This would cause the microcanonical rate−energy dependencies for des-Arg^9-bradykinin and des-Arg^1-bradykinin to cross at the higher internal energies required for the measurement of the FEC at less than 10-μs observation times. However, this surmise disagrees with the Arrhenius parameters obtained from BIRD experiments, namely, $E_a = 0.82$ eV, $A = 10^7$ s^{-1} for des-Arg^1-bradykinin and $E_a = 1.2$ eV, $A = 10^{12}$ s^{-1} for des-Arg^9-bradykinin.

Although the ensemble of ions activated by ion−surface impact is characterized by a distribution of internal energies, its behavior is better described by examining the dissociation of a microcanonical ensemble rather than a thermal distribution. The Arrhenius activation energy (E_a) can be converted into the dissociation threshold (E_0) using Tolman's theorem[12]

$$E_a = E_0 + \langle E' \rangle - \langle E \rangle \quad (1)$$

where $\langle E' \rangle$ and $\langle E \rangle$ are the average energy of the transition state and the average energy of all molecules, respectively.

TABLE 1: Calculated Correction Factors and Arrhenius Activation Energies for the Threshold Energy $E_0 = 1$ eV

	$T = 450$ K		$T = 1000$ K	
log(A)	$\langle E' \rangle - \langle E \rangle$ (eV)	E_a (eV)	$\langle E' \rangle - \langle E \rangle$ (eV)	E_a (eV)
7	−0.333	0.667	−0.465	0.535
9	−0.201	0.799	−0.289	0.711
11	−0.089	0.911	−0.137	0.863
13	0.007	1.007	−0.008	0.992
15	0.091	1.091	0.101	1.101
17	0.163	1.163	0.194	1.194
19	0.224	1.224	0.272	1.272
21	0.277	1.277	0.341	1.341

The activation entropy ($\Delta S^{\ddagger}$) can be calculated from the preexponential factor (A) using the expression of the absolute reaction rate theory

$$A = e\frac{k_B T}{h}\exp\left(\frac{\Delta S^{\ddagger}}{R}\right) \quad (2)$$

where k_B and h are Boltzmann's and Plank's constants, respectively; R is the molar gas constant; and T is the temperature. In most unimolecular gas-phase studies, the conversion between A and $\Delta S^{\ddagger}$ is performed at 1000 K. A reaction proceeding via a tight transition state is characterized by a negative activation entropy and a low preexponential factor.

Because the average energy and the activation entropy are additive and we are interested in the difference in these parameters between the reactant and the transition state, only transition modes that change in the course of the reaction were considered in our calculations. We used a frequency of 1000 cm^{-1} as the reaction coordinate and 15 frequencies as the transition modes. The transition frequencies were varied in the range 200−2000 cm^{-1}. This provided enough flexibility to model preexponential factors from 10^6 to 10^{21} s^{-1}. The average energy of each vibrational mode, $\langle E_i \rangle$, is given by the standard expression

$$\langle E_i \rangle = \frac{h\nu_i}{e^{h\nu_i/k_B T} - 1} \quad (3)$$

where ν_i is the vibrational frequency.

It follows from eq 1 that the Arrhenius activation energy is the sum of the threshold energy and the correction factor $\langle E' \rangle - \langle E \rangle$. We used the above formalism to calculate the correction factor for a transition state (TS) of varying degree of tightness. The results of our calculations are summarized in Table 1 and Figure 3. Calculations were performed at two temperatures: 450 K, which is the average temperature used in the BIRD experiments on bradykinin fragments, and 1000 K. The Arrhenius activation energies shown in Table 1 and Figure 3 were calculated from eq 1 assuming that the threshold energy is 1 eV.

Examination of the results presented in Table 1 leads to several important conclusions. The correction factor $\langle E' \rangle - \langle E \rangle$ strongly depends on the value of the preexponential factor. A low preexponential factor (tight TS) corresponds to a large negative correction factor, whereas the correction factor becomes positive when the preexponential factor is large. This is a direct consequence of the relative change in the spacing between vibrational levels of the reactant and the TS as a function of the degree of tightness/looseness of the transition state. For reactions proceeding via a tight TS, the spacing between the vibrational levels in the TS is increased relative to that in the reactant molecule. This results in a lower average energy of

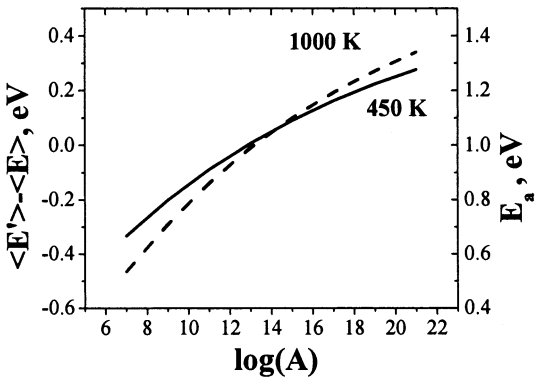

Figure 3. Semilogarithmic plot of Tolman's correction factor, $\langle E' \rangle - \langle E \rangle$ (left axis), and the resulting Arrhenius activation energy, E_a (right axis), as a function of the preexponential factor for the threshold energy of 1 eV.

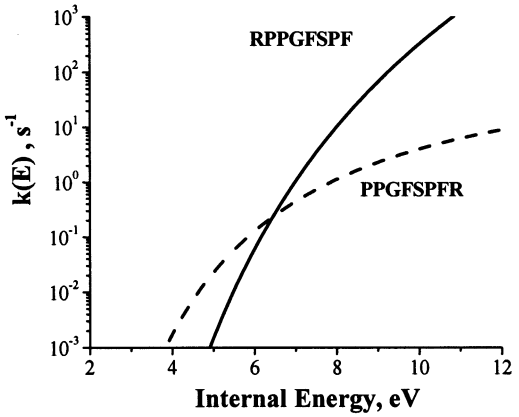

Figure 4. Microcanonical rate−energy dependencies for des-Arg[1]-bradykinin (dashed line), $E_0 = 1.15$ eV and $\Delta S^{\ddagger} = -29.4$ eu, and des-Arg[9]-bradykinin (solid line), $E_0 = 1.24$ eV and $\Delta S^{\ddagger} = -6.4$ eu.

the TS and negative correction factor. However, when the spacing in the TS decreases, at some point, the average energy of the TS exceeds the average energy of the reactant, and the correction factor becomes positive. Preexponential factors for unimolecular reactions of relatively small molecules are usually in the range $10^{10}-10^{16}$ s^{-1}. For these reactions, the correction factor is quite small. However, recent studies on the thermal fragmentation of peptides and proteins indicate that unimolecular dissociation of these large floppy molecules can be associated with substantial entropy changes, likely attributed to the folding/unfolding of biomolecules in the course of reaction. The preexponential factor for thermal decomposition of biomolecules can be as low as 10^5 s^{-1} and as high as 10^{38} s^{-1}.[10,18] For these extreme values, the correction factor between the Arrhenius activation energy and the threshold energy can become quite significant. Moreover, the correction factor depends on temperature. It follows that the Arrhenius activation energy is a strong function of temperature for extreme values of preexponential factors.

Consider, for example, the bradykinin fragments studied in this work. The Arrhenius activation energies are 0.82 and 1.2 eV for des-Arg[1]-bradykinin and des-Arg[9]-bradykinin. The corresponding threshold energies calculated for 450 K are 1.15 and 1.24 eV, and those calculated for 1000 K are 1.28 and 1.27 eV. Note that the relative positions of the threshold energies have changed. Clearly, activation entropy is an important factor in the decomposition of complex ions, and conclusions about the relative stability of large molecules should not be based on the thermal activation energies alone. For very large or very small preexponential factors, the Arrhenius activation parameters become cross-correlated and cannot be used for such comparison. Table 1 shows that, for the same threshold energy, the Arrhenius activation energy changes from 0.67 to 1.28 eV as the preexponential factor changes from 10^7 to 10^{21} s^{-1}. The extraction of threshold energies from Arrhenius activation parameters and the comparison of threshold energies rather than activation energies is therefore a very important issue for large molecules.

Because BIRD data for the bradykinin fragments were obtained at 450 K, we can establish the following dissociation parameters: $E_0 = 1.15$ eV and $\Delta S^{\ddagger} = -29.4$ eu for des-Arg[1]-bradykinin and $E_0 = 1.24$ eV and $\Delta S^{\ddagger} = -6.4$ eu for des-Arg[9]-bradykinin. Microcanonical rate−energy dependencies calculated with these parameters for the bradykinin fragments are shown in Figure 4. Consistent with lower threshold energy, the rate constant for des-Arg[1]-bradykinin (PPGFSPFR) is larger than that for des-Arg[9]-bradykinin (RPPGFSPF) at low internal energies. However, because of the significant difference in the activation entropies, the rate−energy curves cross at a rate constant of 0.2 s^{-1}, and des-Arg[9]-bradykinin has a larger rate constant at all internal energies above the crossing-point energy of 6.4 eV. It should be noted that both ions exhibit a large kinetic shift even on a time scale of seconds. However, at longer reaction times, radiative relaxation efficiently competes with dissociation. The so-called "intrinsic" kinetic shift is defined as the amount of energy required to make the dissociation rate equal to one-tenth of the radiative rate.[19,20] Preliminary modeling of the time-resolved SID data obtained in our laboratory indicates that radiative rates for large peptides are on the order of 10 s^{-1}.[21] It follows that the internal energy required to achieve dissociation rate constant of 1 s^{-1} will define the intrinsic kinetic shift for these molecules.

The internal energy required to reach the rate constant of 1 s^{-1} (reaction time of 1 s) is 7.9 eV for des-Arg[1]-bradykinin and 6.9 eV for des-Arg[9]-bradykinin. Clearly, at this reaction time, des-Arg[1]-bradykinin requires 1 eV more energy to fragment than does des-Arg[9]-bradykinin. This result is in agreement with our experimental observation that the fragmentation efficiency curve of des-Arg[1]-bradykinin is shifted to higher collision energy. The observed 4-eV shift in collision energy is consistent with 25% efficiency of the kinetic-to-internal energy transfer, a typical value for SID on fluorinated SAM surfaces.

Although our results agree well with those of thermal experiments, they disagree with SID results obtained on a microsecond time scale, which indicate the same energetic requirements for the fragmentation of the bradykinin fragments. From Figure 4, it is clear that, on a microsecond time scale, SID fragmentation efficiency curves should diverge much more than they do in our experiments conducted at a 1-s reaction time. We believe that this discrepancy is evidence for a change in the mechanism of ion−surface interaction at the high collision energies sampled in the tandem-quadrupole experiments. Further research will address this issue in detail.

Conclusions

Studies of the surface-induced dissociation of peptides in FT-ICR MS provide a unique advantage over other instrument configurations because the long observation time substantially reduces the kinetic shift, enabling fragmentation to be observed at lower collision energies. In this study, we found that bradykinin fragments exhibit a substantial kinetic shift even at a 1-s reaction delay. Although the fragmentation threshold for

des-Arg^1-bradykinin is lower than that for des-Arg^9-bradykinin, its collision-energy-resolved fragmentation efficiency curve is shifted toward higher collision energies. We explained this result by extracting threshold parameters from the Arrhenius activation parameters obtained in BIRD experiments and calculating microcanonical rate constants using the RRKM formalism. Although des-Arg^9-bradykinin has a slightly higher threshold energy its rate constant increases faster with the internal energy than the rate constant calculated for des-Arg^1-bradykinin. Consequently, rate−energy dependencies for these two systems cross at 6.4 eV and rapidly diverge at higher internal energies. We found that a dissociation rate constant of 1 s^{-1} is reached at 6.9 eV internal energy for des-Arg^9-bradykinin and 7.9 eV for des-Arg^1-bradykinin, in good agreement with our experimental results.

We found that the threshold energies for des-Arg^1- and des-Arg^9-bradykinin differ by only 0.1 eV, whereas the difference between Arrhenius activation energies is 0.38 eV. Our analysis of activation parameters using Tolman's theorem demonstrates that, when the preexponential factor for reaction is very high or very low (as has been found for some large biomolecular ions), the Arrhenius activation parameters become strongly cross-correlated, and Tolman's correction factor used in eq 1 becomes very large. This can result in the reversal of the order of Arrhenius activation energies for different systems relative to the order of the threshold energies.

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