The Reactivity of Gaseous Ions of Bradykinin and Its Analogues with Hydro- and Deuteroiodic Acid

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Received May 10, 1999

Abstract: The kinetics of attachment of hydroiodic acid to gaseous protonated bradykinin, des-Arg^1-bradykinin, des-Arg^9-bradykinin, and their respective methyl esters are reported. Rate constants range from 9.3×10^{-11} cm^3-s^{-1} for attachment of hydroiodic acid to the $(M + H)^+$ ion of bradykinin to $\ll 3 \times 10^{-12}$ cm^3-s^{-1} for a slow-reacting component of a population of $(M + H)^+$ ions derived from des-Arg^9-bradykinin. In the cases of the $(M + 2H)^{2+}$ ions from bradykinin and the $(M + H)^+$ ions from des-Arg^9-bradykinin, the rate data could not be fit with a single rate constant, indicating the presence of at least two non-interconverting ion populations. For the $(M + 2H)^{2+}$ ions, it was demonstrated that the two reacting structures could be induced to interconvert upon gentle activation. The attachment sites are the most basic neutral sites of the molecule, viz., arginine and the N-terminus in the case of bradykinin and its analogues. A simple picture is proposed to estimate the rate constant for hydroiodic acid attachment to a fully exposed neutral basic site. The picture is based upon the assumption that a significant degree of proton transfer from hydroiodic acid to the attachment site occurs and therefore estimates that the capture radius is the distance at which the endoergicity of the proton-transfer reaction is just compensated for by the Coulomb attraction of the ion pair. Storage of the ions in the presence of deuterioiodic acid (DI) showed evidence for hydrogen/deuterium exchange. Two competing mechanisms can lead to H/D exchange, one of which involves initial DI attachment to a neutral basic site. However, experimental evidence suggests that the gas phase H/D exchange reactions result primarily from a "relay-type" mechanism proposed for similar systems reacting with D_2O. These results provide important new information to facilitate the use of hydroiodic acid attachment kinetics and H/D exchange kinetics using DI as chemical probes of three-dimensional gaseous polypeptide ion structure.

Introduction

Since the advent of electrospray[1] and matrix-assisted laser desorption,[2] it has become possible to study charged peptides and proteins in the gas phase. Dissociation methods have been applied to such ions for the purpose of deriving primary structure information.[3] Indeed, unimolecular dissociation of polypeptide ions has become central to peptide sequencing and protein identification by mass spectrometry.[4] The higher order, or three-dimensional structures of polypeptide and protein ions are also of interest from several standpoints. For example, an area of ongoing discussion is the relationship between polypeptide structure in the condensed phase and in the gas phase. The study of naked and partially solvated gas-phase ions might serve to highlight the role of solvation in influencing peptide and protein structures in solution.[5] Aside from the relationship between gas-phase and condensed-phase structures, however, gas-phase structures are clearly important in influencing the chemical reactivity of gaseous ions. For example, there is increasing evidence that intramolecular interactions influence the unimolecular dissociation chemistry of polypeptide ions.[6]

There are currently several approaches under development for studying the three-dimensional structures of ions in the gas phase. A newly developed and promising approach involves direct structure determination by electron diffraction of ions trapped in a quadrupole ion trap.[7] Those that are directly related to the overall geometric shape of the ion include the measure-

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10.1021/ja991553g CCC: $18.00
Published on Web 09/08/1999

ment of ion mobilities,[8] collision cross sections,[9] and the imaging of surface damage resulting from the impact of high-energy ions.[10] Most ion mobility measurements have been performed on small- and medium-sized proteins and have shown that multiple gas-phase conformations can be resolved.[8b,f,9] In several cases, modestly sized, singly or multiply charged peptides have been the subject of study.[8e,h−j] For example, Wyttenbach and co-workers showed that singly protonated, singly sodiated, and doubly sodiated ions of bradykinin have structures that have indiscernible collision cross sections.[8e] Counterman et al., through high-resolution ion mobility measurements, showed that the cross sections of the singly and doubly protonated bradykinin change by only 1 Å^2.[8i] Thus, the absolute sizes of the bradykinin ions do not differ appreciably, despite the fact that calculations indicate that a variety of ion conformations are accessible under the prevailing conditions. In this same study, multiple structural conformations of the singly protonated bradykinin ion were observed, whereas the mobility of the doubly protonated bradykinin ion indicated only one conformation.

Information regarding the surface reactivity of ions, which can be influenced by the three-dimensional structure of the ion, can be obtained via ion/molecule reaction chemistry.[11] So-called chemical probes include, for example, proton transfer from multiply charged polypeptide ions to strong neutral bases,[12] and hydrogen/deuterium (H/D) exchange.[13] Gas-phase H/D exchange kinetics can reveal the presence of more than one structural conformation of a protein, provided there are differences in the number of exposed exchangeable sites, and in some isolated cases these structures have been loosely correlated with solution-phase structures.[14] These reactions are, presumably, controlled by the localized charge site of the peptide or protein ions.[15] In contrast, hydroiodic acid (HI) has been observed to react with peptide and protein ions via attachment to available neutral basic sites.[16] Also, evidence has been presented that HI attachment kinetics can be used to distinguish secondary structures of gas-phase protein ions,[17] in analogy with the measurement of H/D exchange kinetics. However, since the reactive sites associated with HI attachment and most H/D exchange reactions are expected to differ, the measurement of the kinetics of HI attachment might be expected to constitute a chemical probe that complements H/D exchange and proton transfer.

Bradykinin (BK, *RPPGFSPFR*) is one of the most thoroughly studied gaseous polypeptide ions. Many properties of gas-phase BK ions, such as kinetic stability and distance between charge sites, have been inferred from the unimolecular dissociation of different charge states of BK and its analogues,[6a,18] e.g. des-Arg^n-bradykinin (n = 1 or 9). Likewise, gas-phase ion mobility[8e,i,j] and H/D exchange[13a,b] experiments have also used ions of BK as models. From the cross sections derived from ion mobility measurements, the three-dimensional structure of the singly and doubly protonated BK gaseous ion is proposed to have a folded, compact structure facilitated by intramolecular interactions between the basic and acidic sites in the ion. Thus, the information already known about the ions of BK and its analogues make them good candidate systems to evaluate the usefulness of HI reactivity as a chemical probe of gas-phase ion conformations.

In the present report, we show that the kinetics of HI attachment to protonated BK ions (and ions of various BK derivatives) can provide useful and new information concerning gas-phase ion structures. Rate constants for the attachment of HI in a quadrupole ion trap mass spectrometer were derived for ions of bradykinin, des-Arg^1-bradykinin, and des-Arg^9-bradykinin. The methyl ester derivatives of each ion were also studied to determine how the C-terminus affects attachment of HI through acid/base interactions. Non-linear kinetic behavior observed for the bradykinin $(M+2H)^{2+}$ ions, as well as the bradykinin methyl ester $(M+2H)^{2+}$ and des-Arg^9 bradykinin $(M+H)^+$ ions, suggests multiple reactive conformations, which have not previously been fully resolved by other gas-phase structural probes. Other interesting structural effects are also apparent in the des-Arg derivatives. Experiments have been conducted that indicate that HI attachment kinetics for these ions might be interpreted simply on the basis of the rate of collision of HI with reactive sites. Gas-phase H/D exchange experiments, achieved by storage of ions in the presence of deuterioiodic acid (DI), indicate that DI may constitute a viable

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alternative or complement to D_2O for H/D exchange studies. This work reveals important general insights into HI attachment to and H/D exchange (using DI) with gaseous polypeptide ions in addition to providing new information on BK ions.

Experimental Section

Bradykinin, des-Arg[1]-bradykinin, and des-Arg[9]-bradykinin were purchased from Sigma Chemicals (St. Louis, MO). The methyl esterification reaction[19] was performed on 50−100 nmol quantities. All reagents were HPLC grade or better, and water was purified to 18 MΩ with a Millipore (Bedford, MA) water purification system. For electrospray, concentrated stock solutions (700−1000 pmol/μL) of the compounds in 50:50 water/methanol were diluted to a concentration between 10 and 30 pmol/μL with 100% methanol. The concentration of the electrosprayed solution had no effect on the reaction kinetics of the gas-phase ions; e.g., the reaction rate for 10 pmol/μL bradykinin was the same as that for 100 pmol/μL. Also, the solvent composition had no effect on the kinetics of the gas-phase reaction (solvent ratios of 2:1, 1:1, 1:2, and 1:4 water/methanol gave results which were no different from those of 100% methanol solutions).

The instrument used for the bradykinin/HI reaction is a Finnigan MAT ion trap mass spectrometer (San Jose, CA) that has been modified for the study of externally generated ions. The external source and electrospray modifications have been described elsewhere.[20] The ion trap analyzer is pumped by a Varian (Walnut Creek, CA) Turbo-V 550-ICE MacroTorr high-corrosive applications turbomolecular pump, which is roughed by a Varian SD-301 mechanical pump.

The ion/molecule reactions were performed with a background pressure of 1 mTorr of He in the ion trap, maintained using a Granville-Phillips 203 (Boulder, CO) variable leak valve. The HI pressure was maintained and monitored throughout the experiments at 2×10^{-6}−1.2×10^{-5} Torr by sampling the vapor above a 57% w/w aqueous solution of HI (Aldrich, St. Louis, MO) through a separate Granville-Phillips 203 valve. At room temperature, this provides a mole fraction of 0.98 HI over the solution (0.02 H_2O) initially. The stainless steel surfaces of the valve used for the HI leak must be allowed to saturate with HI before the reaction product is observed in the mass spectrometer. An HI pressure of 8×10^{-6}−1×10^{-5} Torr must be maintained for ∼3−4 h upon first exposure to a new or newly cleaned valve. Also, if the valve has not been exposed to HI for an extended period, a saturation time is also required. All pressures reported have been corrected for differences in ionization potentials of various gaseous species (HI, DI, He) compared to the ion gauge calibration with N_2.[21] The base pressure of the instrument, i.e., before introduction of any gas, is $\sim 5 \times 10^{-7}$ Torr.

A simple ion/molecule reaction experiment consisted of four steps: (1) ion injection, (2) ion isolation, (3) ion/molecule reaction period, and (4) acquisition of mass spectrum. Ion injection was performed by gating in ions from the external electrospray source and trapping at a radio frequency (rf) potential of 800 V_{0-p}. The procedure for ion isolation of high mass-to-charge ratio ions has been given previously.[22] An ion isolation window of 5 *m/z* units was used to isolate the ions to ensure that there was no off-resonance power absorption.[23] The ion/molecule reaction kinetics were found to be insensitive to the rf potential applied to the ring electrode over a range of at least 500−1000 V_{0-p}. All data reported here were collected using an rf potential of 800 V_{0-p} during the ion/molecule reaction period. Acquisition of the mass spectrum was achieved by resonance ejection of the ions at $q_z = 0.908$.[24] Relatively low dipolar resonance ejection voltages (typically 1.5−2.0 V_{p-p}) were used to avoid dissociation of the relatively fragile $(M + nH + mHI)^{n+}$ ions. Collision-induced dissociation of the ion/molecule complex was achieved by applying a single-frequency resonance excitation to the ion of interest with $q_z = 0.2$. H/D exchange was performed by sampling the vapor above a 57% w/w solution of DI in D_2O (Aldrich, St. Louis, MO). The ions of interest were selected and stored in the presence of DI at a partial pressure of 1×10^{-5} Torr. To resolve the isotopes for each ion, the effective mass scan rate of the mass spectrometer (5555 amu-s^{-1}) was slowed typically by a factor of 5 for singly charged ions, 12 for doubly charged, and 20 for triply charged ions. The theoretical and experimental consequences of slowing the mass scanning rate of an ion trap mass spectrometer have been discussed in detail elsewhere.[25]

Since the only ions present in the mass spectrum after the ion/molecule reaction time elapsed were the $(M + nH)^{n+}$ and $(M + nH + HI)^{n+}$ (and, in some cases, $(M + nH + 2HI)^{n+}$) ions, the intensities (at peak apex) of these ions were normalized by dividing the intensity of each ion by the total ion intensity. Pseudo-first-order kinetics prevail because HI number density is constant and always much greater than the ion number density. To determine the reaction rate constants, the normalized reactant ion intensity was first plotted vs time and then fit to a multidimensional exponential decay of the form

$$I(t) = \sum_{i=1}^{n} P_i(e^{-k_i[HI]t}) \qquad (1)$$

where $n = 1$ or 2. For most of the ions studied, the reactant ion intensity as a function of time, $I(t)$, fit well to a single-term exponential decay, i.e., $n = 1$. A poor fit to a single-exponential term was produced by the depletion of the $(M + 2H)^{2+}$ bradykinin and methyl ester bradykinin ions, implicating two populations having different reaction rates (discussed below). The data presented in this report have been plotted as $-\ln(I/I_0)$ vs time to facilitate comparisons with linear and nonlinear pseudo-first-order kinetic behavior for some of the ion/molecule reactions.

Results

Bradykinin is a relatively small nonapeptide (*RPPGFSPFR*), with two basic arginine residues at opposite ends of the molecule. It is among the most studied of all small peptides in the gas phase, and there are several predictions as to the three-dimensional gas-phase structures of ions derived from this molecule from a variety of measurements and calculations.[6a,8e,26] Unlike most other ion/molecule reactions, the attachment reaction of HI with protonated BK proceeds through a mechanism that is dependent on the availability of free and unhindered basic sites in the ion.[16] Consequently, the BK $(M + nH)^{n+}$ ion, where $n = 1$ or 2, is reactive (see below), but when $n = 3$, the ion population was found to be unreactive, even at HI pressures as high as 2×10^{-5} Torr. This observation is expected from the fact that the three most basic sites in BK, viz. the two arginine residues and the N-terminus, are presumably protonated in the $(M + 3H)^{3+}$ ion and are, therefore, unavailable for reaction with HI. Thus, the kinetics of HI attachment to protonated peptides can be expected to provide information on the availability of neutral basic sites on the ion surface, but attachment of HI can provide no information for ions in which all of the most basic sites are protonated. However, as shown below, the H/D exchange properties of DI with the

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Table 1. Reaction Rate Constants for Bradykinin and Analogues with HI

peptide	sequence	k, cm^3-s^{-1} ($\times 10^{-11}$)
bradykinin $(M + H)^+$	***R***PPGFSPF***R***	9.3 ± 0.2^a
bradykinin $(M + H)^+$	***R***PPGFSPF***R***	1.04 ± 0.04^b
bradykinin methyl ester $(M + H)^+$	***R***PPGFSPF***R***-Me	6.9 ± 0.1^a
bradykinin methyl ester $(M + H)^+$	***R***PPGFSPF***R***-Me	0.8 ± 0.1^b
bradykinin $(M + 2H)^{2+}$	***R***PPGFSPF***R***	2.1 ± 0.1^c
bradykinin $(M + 2H)^{2+}$	***R***PPGFSPF***R***	0.18 ± 0.01^d
bradykinin methyl ester $(M + 2H)^{2+}$	***R***PPGFSPF***R***-Me	1.5 ± 0.1^c
bradykinin methyl ester $(M + 2H)^{2+}$	***R***PPGFSPF***R***-Me	0.12 ± 0.02^d
des-Arg^1-bradykinin $(M + H)^+$	PPGFSPF***R***	8.4 ± 0.2
des-Arg^1-bradykinin methyl ester $(M + H)^+$	PPGFSPF***R***-Me	4.0 ± 0.6
des-Arg^9-bradykinin $(M + H)^+$	***R***PPGFSPF	0.33 ± 0.02^e
des-Arg^9-bradykinin methyl ester $(M + H)^+$	***R***PPGFSPF-Me	1.58 ± 0.07

[a] Addition of first HI. [b] Addition of second HI. [c] Fast-reacting component. [d] Slow-reacting component. [e] Only ~12% of population reactive with measurable rate.

$(M + 3H)^{3+}$ ion illustrates that H/D exchange may provide information concerning fully protonated ion structures. The pseudo-first-order rate constants for the various ions used for this study are shown in Table 1, along with the corresponding sequence of each ion.

Bradykinin $(M + H)^+$. Much of the phenomenology associated with HI attachment to polypeptide ions is demonstrated by the attachment of HI to singly protonated bradykinin (BK $(M + H)^+$). As stated above, the reaction proceeds by saturating the available basic sites with HI molecules. Therefore, the $(M + H)^+$ ions (containing three basic sites, N-terminus and two arginine residues), having one basic site occupied by a proton, reacts with two HI molecules (Figure 1). Under idealized conditions, where all reactive sites in the molecule are equivalent, the rates for a consecutive reaction such as the following,

$$(M + H)^+ \xrightarrow{k_1} [(M + H)\text{- -HI}]^+ \xrightarrow{k_2} [(M + H)\text{- -2HI}]^+ \quad (2)$$

should show kinetics wherein the $(M + H)^+$ ion reaction rate constant is twice the rate constant of the $[(M + H)\text{- -HI}]^+$ ion, viz. $k_1 = 2k_2$. To determine both reaction rate constants simultaneously, the consecutive reaction shown above was modeled (Figure 2), both as ideal ($k_1 = 2k_2$, dotted lines) and then by varying the k_2 value (solid lines). The ideal model follows the depletion of the $(M + H)^+$ ion and the growth of the $[(M + H)\text{- -HI}]^+$ ion, providing an excellent fit with a rate constant of 9.3×10^{-11} cm^3-s^{-1}. However, the ideal model does not fit either the decay of the $[(M + H)\text{- -HI}]^+$ ion or the growth of the $[(M + H)\text{- -2HI}]^+$ ion. Clearly, the attachment of the second molecule of HI proceeds far more slowly than the attachment of the first molecule of HI. Excellent agreement with experimental data is obtained when the second rate constant (k_2) is replaced by a value that is approximately an order of magnitude lower than the first rate constant (k_1) (see Table 1).

When the $(M + H)^+$ ion of the bradykinin methyl ester (BK-Me) was studied, similar reaction profiles were obtained, and this reaction could be modeled in the same way. For this case, though, both rate constants (k_1 and k_2) are lower by approximately the same factor, 0.7, when compared to the respective values for non-methylated BK $(M + H)^+$.

Bradykinin $(M + 2H)^{2+}$. When the doubly protonated ions of BK and BK-Me are reacted with HI, the depletion of the $(M + 2H)^{2+}$ ion intensity does not follow linear pseudo-first-order kinetics (squares, Figure 3). Instead, the ion depletion data, $[-\ln(I/I_0)]$ vs time, are nonlinear during the first 400 ms and then become linear from that point until the $(M + 2H)^{2+}$ ions are completely depleted at much longer reaction times. The

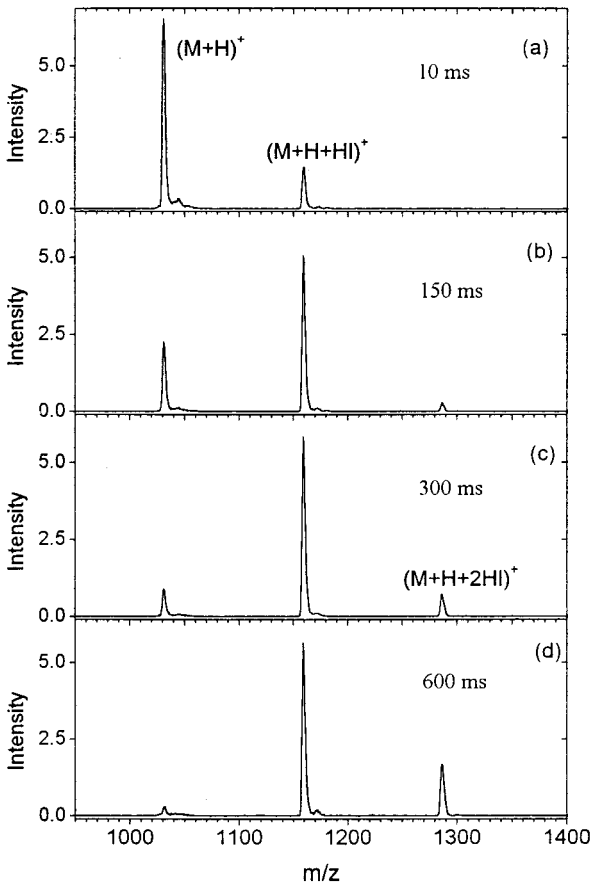

Figure 1. Mass spectra of ions obtained by isolating the BK $(M + H)^+$ ions and storing them in the ion trap for 10 (a), 150 (b), 300 (c), and 600 ms (d) with an HI pressure of 2.5×10^{-6} Torr.

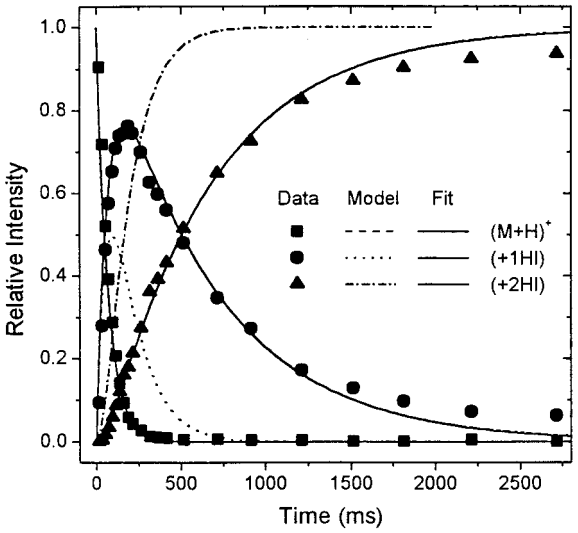

Figure 2. Normalized ion intensity profile for the ion/molecule reaction of bradykinin $(M + H)^+$ ion with HI at 2.5×10^{-6} Torr. The normalized ion intensities for the $(M + H)^+$ (■), $[(M + H)\text{- -HI}]^+$ (●), and $[(M + H)\text{- -2HI}]^+$ (▲) ions show the fast reaction for the first addition of HI, followed by a slower addition of the second HI molecule to the $[(M + H)\text{- -HI}]^+$ ion. The reaction data were modeled first as consecutive reactions involving equivalent reaction sites, i.e., $k_1 = 2k_2$ (broken lines). Then the second rate was varied to provide the best fit to the experimental data for addition of the second HI molecule ($k_1 = 8.9k_2$; solid lines), see Table 1 for determined rate constants.

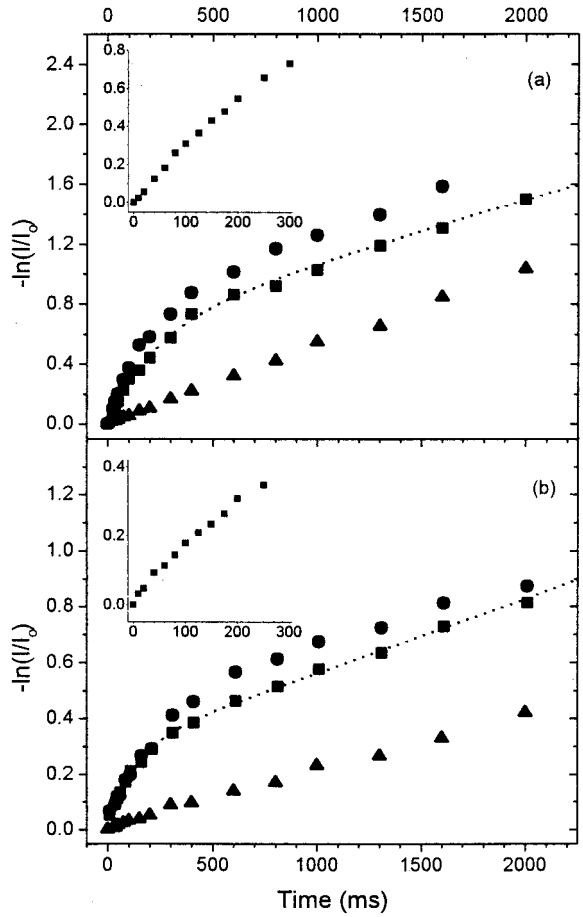

Figure 3. Normalized ion intensity profiles ($-\ln(I/I_o)$ vs reaction time) for BK (a) and BK-Me (b) $(M + 2H)^{2+}$ ions ($P_{HI} = 1 \times 10^{-5}$ Torr). The kinetic data for the ions obtained directly from electrospray (■) show that, at shorter reaction times, the data appear curved, while at longer times linear behavior is seen. The isolation of the slow-reacting species (▲) was achieved by reacting the mixture of two species and then reisolating the unreacted $(M + 2H)^{2+}$ ion and reacting for the time shown. An attempt to isolate the fast-reacting population yielded ions (●) that showed behavior similar to that of the initial electrospray population. Insets show the near-linear region in the kinetic data that is seen at short reaction times. The dual population exponential fit to the data is shown as the dotted line overlaying the experimental data obtained for the mixture.

nonlinear behavior exhibited by the $(M + 2H)^{2+}$ ion is analogous to the nonlinear H/D exchange behavior seen by Cassady and co-workers with ubiquitin $(M + 12H)^{12+}$, in which two gas-phase ion conformations (a "*fast-reacting*" and "*slow-reacting*" population) were proposed to explain nonlinearities in the kinetic data.[14a] Similarly, the data that follow are consistent with those of the initial BK $(M + 2H)^{2+}$ ion population, being comprised of non-interconverting (at room temperature) fast- and slow-reacting components.

The slow-reacting population was isolated in the ion trap mass spectrometer by exploiting the difference in reaction rates between the fast and slow components, and its reaction kinetics were determined in the absence of the fast-reacting population. This was achieved in the ion trap by four steps: (1) isolating the $(M + 2H)^{2+}$ ions, (2) reacting the isolated $(M + 2H)^{2+}$ ions for 400 ms, (3) then *re*isolating the remaining $(M + 2H)^{2+}$ ions, and finally (4) reacting the newly isolated ion population. In this way, the slower reacting species can be isolated because the fast-reacting population is depleted in step 2 and then ejected from the ion trap in step 3. The difference in reaction rates for the original $(M + 2H)^{2+}$ population and the *re*isolated, slow-reacting species is immediately evident when the normalized intensities (triangles, Figure 3) are compared to those of the normal population (squares). The reaction rate for the slow-reacting component, obtained by fitting the kinetic data to a dual rate expression, was the same as that obtained directly from the rate of loss of the isolated slow-reacting population. Figure 4a shows a spectrum resulting from the 400-ms reaction of isolated $(M + 2H)^{2+}$ ions formed via electrospray (i.e., the spectrum acquired after step 2), and Figure 4b is a spectrum obtained for a 400-ms reaction of the slow-reacting population (i.e., result obtained after step 4). The $(M + 2H)^{2+}$ ion intensity depletion for the slow reactant follows linear pseudo-first-order kinetics throughout the entire time frame of the experiment, and the reaction goes to completion by nearly 9 s. The slow-reacting conformation has a rate constant of 1.8×10^{-12} cm^3-s^{-1}, over an order of magnitude lower than that of the fast-reacting population.

The ion/molecule complex is relatively fragile, such that the $[(M + 2H)- -HI]^{2+}$ product ion can be easily converted back to $(M + 2H)^{2+}$ reactant without further fragmentation. Presumably, the isolation of the fast-reacting species could be achieved through exploiting differences in reaction rates and fragmenting the complex ions at specific times. An attempt to isolate the fast-reacting conformation involved five steps: (1) isolating the $(M + 2H)^{2+}$ ion, (2) reacting the $(M + 2H)^{2+}$ ions for 300 ms, (3) isolating the $[(M + 2H)- -HI]^{2+}$ complex ion, (4) fragmenting the $[(M + 2H)- -HI]^{2+}$ ion to $(M + 2H)^{2+}$ without causing dissociation of the $(M + 2H)^{2+}$ ion, and (5) reacting this $(M + 2H)^{2+}$ ion population. Since only a 300-ms reaction is used, the percentage of the slow-reacting species present as $[(M + 2H)- -HI]^{2+}$ at the end of step 2 is minimal, while the relative abundance of the fast-reacting population (present as the $[(M + 2H)- -HI]^{2+}$ product ion) is near maximum at this time. Then, the residual slow-reacting species is removed at step 3, leaving only the $[(M + 2H)- -HI]^{2+}$ product ions in the trap. By dissociating the $[(M + 2H)- -HI]^{2+}$ ions such that only the $(M + 2H)^{2+}$ ion is observed, an ion population containing primarily the fast-reacting population should be obtained in the form of $(M + 2H)^{2+}$ (step 4), and the kinetics for the reaction of this population could be obtained separately (i.e., in the absence of any slow-reacting species, so that mathematical deconvolution of two rates is not needed). However, when these steps are performed, the reaction kinetics (circles, Figure 3) roughly follow those of the original mixture of conformations that are obtained by ESI, with little enhancement of the fast-reacting population (~5−10%). Thus, to determine the reaction rate constants for the fast-reacting species, the entire data set was fit to a two-dimensional exponential decay ($n = 2$, eq 1), with the rate of the slow-reacting species held constant (since this value was determined independently). From the two-dimensional fit, the relative ion populations for the fast- and slow-reacting populations were found to be 45 ± 5% and 55 ± 5%, respectively.

The inability to cleanly separate and isolate the fast-reacting species can be explained in conjunction with results obtained by collisional activation of the slow-reacting species. If the slow-reacting species is isolated, as discussed above, and then activated with a relatively low resonance excitation amplitude so as to avoid fragmentation of the $(M + 2H)^{2+}$ ion, the reaction kinetics of the postactivation population mimic those of the original mixture of fast- and slow-reacting ions obtained directly from electrospray. This is achieved by a six-step experiment: (1) isolation of the $(M + 2H)^{2+}$ ion, (2) reaction for 400 ms, (3) *re*isolation of the $(M + 2H)^{2+}$ ion, (4) reaction for an

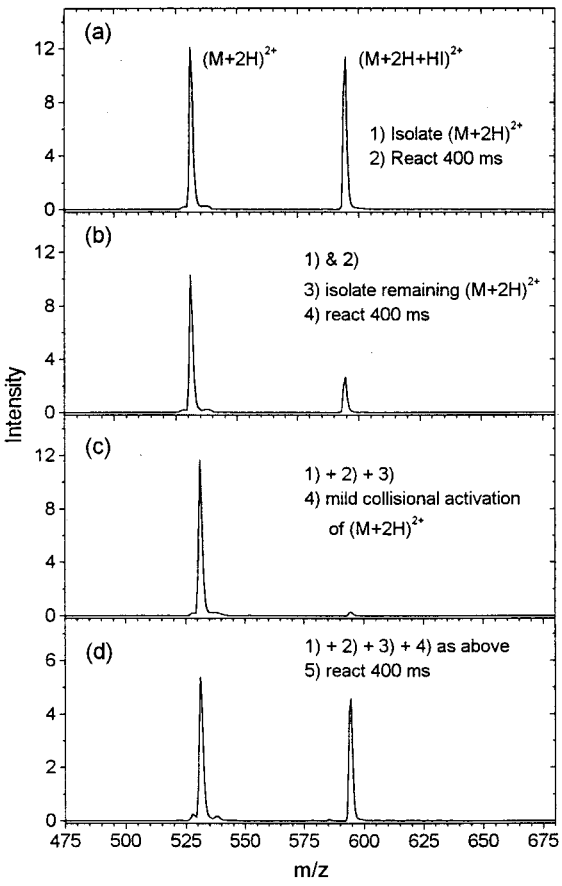

Figure 4. Isolation, comparison, and conversion of the slow-reacting population of bradykinin $(M + 2H)^{2+}$ at HI pressure of 9×10^{-6}. (a) A typical mass spectrum obtained after reacting the initial electrospray ion population for 400 ms. (b) The resulting spectrum when the $(M + 2H)^{2+}$ ion seen in (a) is reisolated and then subjected to another 400-ms reaction, showing the decreased reaction rate. (c) The collisional activation of the $(M + 2H)^{2+}$ ion after a 400-ms reaction and reisolation steps, showing no fragmentation of the ion during activation. (d) The result of storage of the interconverted ion population of (c) for 400 ms.

additional 400 ms to ensure that the slow-reacting species is isolated cleanly, (5) removal of the additional 400-ms reaction in step 4, and activation of the $(M + 2H)^{2+}$ ion by resonant single-frequency excitation ($q_z = 0.2$; amplitude = 60 mV; duration = 5 ms), followed by (6) reaction of the $(M + 2H)^{2+}$ ion. The spectra obtained for steps 1, 4, 5 and 6 are shown in Figure 4a−d, respectively, with the final reaction (step 6) set at 400 ms. When the $(M + 2H)^{2+}$ ions of the slow-reacting species (Figure 4b) are activated (Figure 4c), the reaction rate of the resulting population (Figure 4d) is identical to that of the mixture (Figure 4a) that is obtained during normal electrospray ionization; i.e., the low-energy activation of the slow-reacting $(M + 2H)^{2+}$ ion leads to isomerization, yielding a mixture of the two reactive populations (fast and slow).

The results just described probably account for the inability to form an ion population enriched in the fast-reacting component by dissociation of the $[(M + 2H)- -HI]^{2+}$ ion. Collisional activation of the noncovalent $[(M + 2H)- -HI]^{2+}$ complex ion apparently provides sufficient energy to allow for isomerization of the $(M + 2H)^{2+}$ ions. It also follows that the two ion populations, giving rise to the different reaction rate constants, will not be differentiable through low-energy dissociation methods. Dissociation of the slow-reacting $(M + 2H)^{2+}$ population by resonant excitation yielded a product ion spectrum that was indistinguishable from that derived from the mixture of

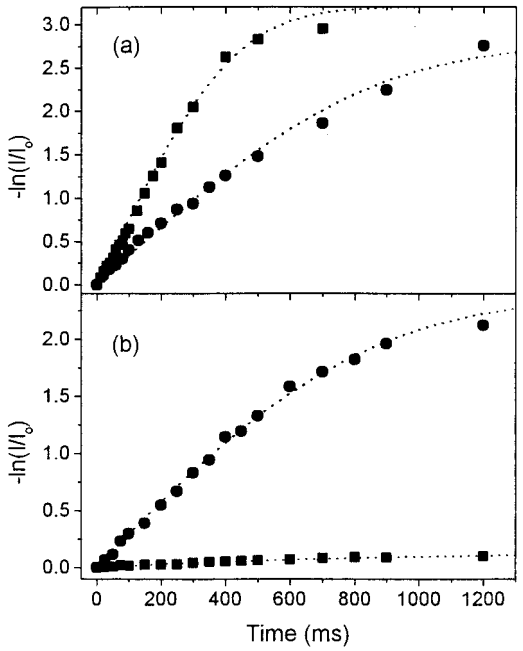

Figure 5. Kinetic data for the ion/molecule reaction of des-Arg^{1,9}-BK and methyl esters with HI. (a) des-Arg^1-BK (■) and des-Arg^1 BK-Me (●) $(M + H)^+$ ions at an HI pressure of 2.5×10^{-6} Torr. (b) des-Arg^9-bradykinin (■) and its methyl ester (●) $(M + H)^+$ ions at an HI pressure of 8.7×10^{-6}. The respective exponential fits are shown as dotted lines overlaying each set of data.

$(M + 2H)^{2+}$ ions. In addition, experiments showed that the conversion of the slow-reacting conformation could be effected during the isolation step, through off-resonance power absorption. Thus, special care was taken during isolation steps, and wide *m/z* windows were used to isolate the ions in this study.

Two reactive populations with relative differences in rate constants similar to those of the non-methylated BK $(M + 2H)^{2+}$ ions were also present in the BK-Me $(M + 2H)^{2+}$ ions at approximately the same ratios (Figure 3b). Upon methylation, the rate constants for the fast- and slow-reacting populations (found at approximately the same relative abundance) were both lowered by approximately a factor of 0.7. This is the same factor observed for the $(M + H)^+$ BK and BK-Me ions. Thus, for bradykinin $(M + H)^+$ and $(M + 2H)^{2+}$, methylation of the C-terminus has the same general effect on the rate constants for the addition of HI.

des-Arg-Bradykinin Derivatives $(M + H)^+$. To investigate the possible mechanisms giving rise to the different reactive species found in the BK $(M + H)^+$ and $(M + 2H)^{2+}$ ions, four chemically modified bradykinin derivatives were studied. Along with the methyl esters discussed above, the $(M + H)^+$ ions of des-Arg^1-bradykinin (PPGFSPFR) and des-Arg^9-bradykinin (RPPGFSPF) and their methyl esters were also studied. The reaction rates and overall kinetics for the $(M + H)^+$ ions of these four compounds are dramatically different (see Table 1). The des-Arg^1-BK $(M + H)^+$, which presumably reacts through the free N-terminal proline (assuming protonation at the Arg^9 position), has a rate constant that is much greater than those found for the BK $(M + 2H)^{2+}$ bradykinin ion. In rough accordance with the methyl esters discussed above, the rate constant decreases by ~50% upon methylation of the des-Arg^1-bradykinin. However, unlike the $(M + 2H)^{2+}$ ion of bradykinin, the kinetics (Figure 5a) for the addition of HI to des-Arg^1-bradykinin and the methyl ester derivative are linear until the $(M + H)^+$ ion is depleted (<5% intensity), indicating only one reactive gas-phase population.

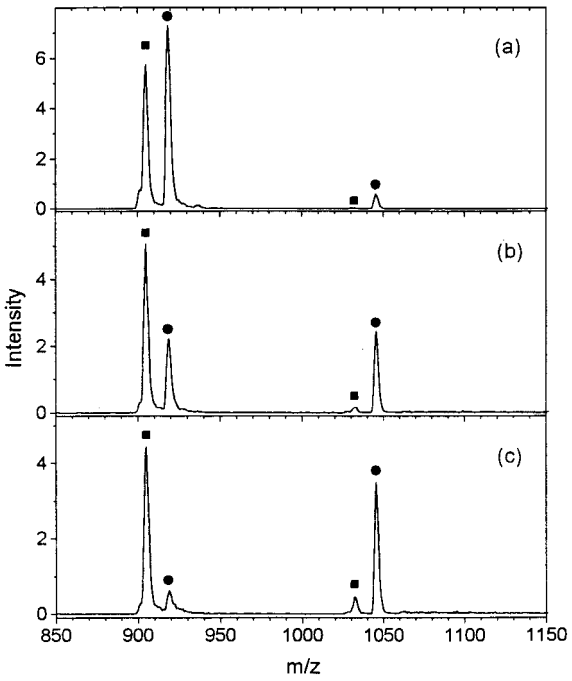

Figure 6. Simultaneous reaction of des-Arg^9-BK $(M + H)^+$ (■) and its methyl ester (●) with HI at reaction times 10 (a), 60 (b), and 310 ms (c).

The des-Arg^9-BK ion and its methyl ester show reactivity that contrasts sharply with those of the BK and des-Arg^1-BK ions. Only 12% of the $(M + H)^+$ ion population of des-Arg^9-BK was reactive, and the rate constant for that population was one of the lowest for the bradykinin family (3.3×10^{-12} cm^3-s^{-1}). However, the $(M + H)^+$ methyl ester derivative of des-Arg^9 had the same rate constant as the fast-reacting population of the BK-Me $(M + 2H)^{2+}$ ions (1.5×10^{-11} cm^3-s^{-1}). The mass spectra from simultaneous reactions of des-Arg^9-BK $(M + H)^+$ and its methyl ester (Figure 6) demonstrate the unreactive nature of the des-Arg^9-BK and the reactive nature of the des-Arg^9-BK-Me. Of all of the ions in the BK family described here, the only case in which methylation of the C-terminus increased the reaction rate was with the des-Arg^9-BK system.

H/D Exchange. To explore plausible mechanisms underlying the attachment of HI to neutral basic sites in the protonated bradykinin and analogue ions, gas-phase H/D exchange reactions were performed using DI. Regardless of HI/DI attachment rates, all ions were reactive toward gas-phase H/D exchange at some nominal rate. However, the H/D exchange reactions of ions with high HI/DI attachment rates (e.g., BK $(M + H)^+$, des-Arg^1-BK $(M + H)^+$, BK $(M + 2H)^{2+}$) could not be monitored over extended periods due to the formation of the DI attachment product. Under high-resolution conditions, the attachment product ion dissociated during the resonant ejection mass analysis period. Since all ions, except the BK $(M + 3H)^{3+}$, show attachment to some extent, the kinetics of H/D exchange using DI can be followed for only relatively short reaction times. The exchange rates found here should not be assumed to characterize the entire ion population because the populations of ions monitored during the exchange are only those that do not react (attach HI) within a given reaction time. In addition, the full extent of exchange could not be determined in many cases, primarily due to loss of ions resulting from conversion to attachment product.

The slow-reacting or nonreactive ions (with respect to attachment), however, can be used to follow the extent of H/D exchange and show an interesting trend toward H/D exchange with gaseous DI. Figure 7 illustrates the extent of H/D exchange during a 1.8-s storage period in 1×10^{-5} Torr of DI for three selected ions that are either negligibly reactive or unreactive toward attachment. The slowest reacting ion with regard to HI or DI attachment, des-Arg^9-BK $(M + H)^+$ (Figure 7a), also shows the slowest H/D exchange rate ($\sim$0.14 s^{-1}) when compared to the other bradykinin and analogue ions. However, this ion exchanged 8 out of a possible 13 exchangeable hydrogens during a 1-min storage period. The BK $(M + 2H)^{2+}$ ions exchange at a slightly higher rate ($\sim$0.57 s^{-1}) than the des-Arg^9-BK $(M + H)^+$ ions. However, the overall extent of exchange could not be followed to completion due to the conversion to attachment product. The doubly protonated ion exchanged at least 6 hydrogens (19 exchangeable) before signal degradation at 2.5 s of ion storage at 1×10^{-5} Torr of DI.

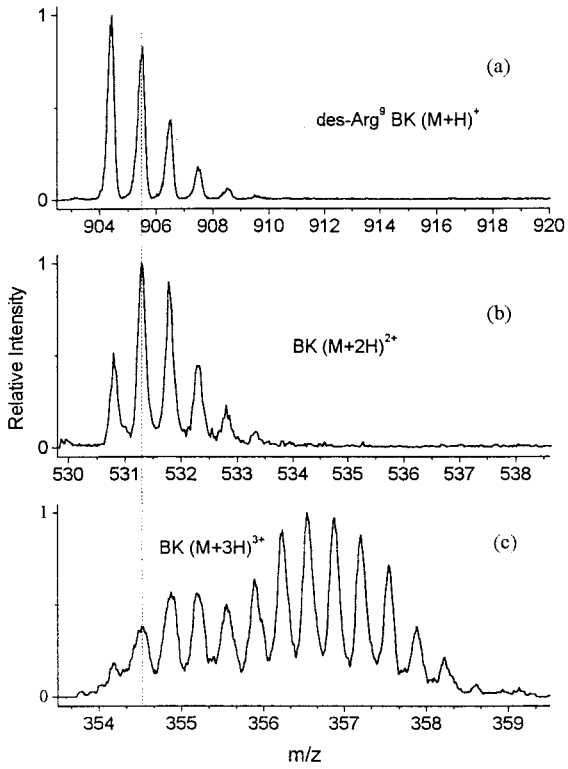

Figure 7. Gas-phase H/D exchange of (a) des-Arg^9-BK $(M + H)^+$, (b) BK $(M + 2H)^{2+}$, and (c) $(M + 3H)^{3+}$ occurring during 1.8-s storage of ions in the presence of 1×10^{-5} Torr of DI. The dotted line indicates the monoisotopic ion for each of the structures i.e., all ^{12}C, ^{1}H, ^{16}O, ^{14}N. The initial isotope ratios of each ion were checked before the introduction of DI into the vacuum system and found to match calculated ratios.

While the BK $(M + 3H)^{3+}$ ion shows no attachment product during storage of ions in pressures as high as 2×10^{-5} Torr of HI or DI, the H/D exchange rates for this ion are the fastest among the bradykinin analogues. This ion exchanges 11 hydrogens (20 exchangeable) during a 1.8-s storage time in the presence of DI. In addition, as can be seen in Figure 7c, the exchange distributions clearly show that this ion population is comprised of at least two distinct components. The slow-reacting population exchanges at a rate of $\sim$1.1 s^{-1} and the fast-reacting population at a rate of $\sim$5.6 s^{-1}.

Discussion

General Reaction Properties. The reaction rates for the attachment of HI to the ions of this study are substantially lower than the ion/molecule collision frequency. The fastest reacting ion, singly protonated bradykinin, was reacted at an HI pressure

of 2.5×10^{-6} Torr. At this pressure, the collision frequency for a single bradykinin ion colliding with an HI molecule is ~60 s^{-1}, based on the cross section of BK determined from the two independent studies of Counterman et al.[8i] and Wyttenbach et al.[8e] The measured reaction rate at 2.5×10^{-6} Torr was 7.4 s^{-1}. Thus, approximately one in eight collisions results in a product ion being formed. In contrast, the slowest reacting ion, the BK-Me $(M + 2H)^{2+}$, was found to have a reaction rate 3 orders of magnitude lower than its corresponding collision frequency, assuming the same cross section. Clearly, these reactions are far from unit efficient, based on the hard-sphere collision picture. They are also less than unit efficient based on polarization theory.[27] That is, point charge/polarization models that are usually used to determine collision rates for relatively small ions colliding with neutrals also predict collision rates that significantly exceed the HI attachment rates observed here. For example, based on polarization theory, the rate constant calculated for singly protonated bradykinin colliding with HI at 300 K is 5.97×10^{-10} cm^3-s^{-1}.[28] This would lead to a collision rate of 48 s^{-1} at an HI pressure of 2.5×10^{-6} Torr. This rate constant is at least 6 times greater than any rate constant reported here.

In principle, both structural and energetic factors can give rise to the observation of less-than-unit reaction efficiency in the attachment of HI to polypeptide ions when either of the models for defining the collision rate just mentioned is used. The following discussion relates the observations and reasoning used to interpret the HI attachment kinetics reported in this study. Structural factors that might affect the inherent reactivity of a site include (i) the extent to which a reactive site might be physically blocked from attack by HI, essentially a steric effect, and (ii) local chemical interactions, such as intramolecular proton binding and acid−base (e.g., "salt bridge") interactions. The latter effect is categorized as a structural effect because the extent to which such intramolecular interactions can occur is governed by the composition of the polypeptide and the structural motifs that it can assume. The mechanism by which localized chemical interactions can affect HI attachment kinetics, however, can be either through affecting the capture cross section or by affecting the stability of the collision complex (see below). Even in the absence of intramolecular interactions, HI attachment kinetics might be expected to vary, depending upon the identity of the attachment site, viz. N-terminus, arginine, lysine, or histidine, as a result of differing binding energetics. The role of binding energetics can be understood within the context of the simplified energy diagram of Figure 8 and the following kinetic scheme:

$$(M + nH)^{n+} + HI \underset{k_{-c}}{\overset{k_c}{\rightleftharpoons}} [(M + nH)\text{- -HI}]^{n+*} \underset{k_{-s}[bath]}{\overset{k_s[bath]}{\rightleftharpoons}} [(M + nH)\text{- -HI}]^{n+} \quad (3)$$

The capture rate, associated with rate constant k_c, is defined here as the rate at which a molecule of HI collides with a reactive site. The HI binding strength can influence k_c insofar as the long-range attractive part of the potential (see Figure 8) is related to the well depth. The reverse reaction, the rate of which is represented by k_{-c}, is the unimolecular dissociation of the excited collision complex to yield the reactants. The rates, k_s[bath] and k_{-s}[bath], represent the collisional cooling[29] and excitation rates of the collision complex and the product, respectively, where [bath] is the number density of the bath gas (He, HI, air). Activation and deactivation rates associated with radiative absorption and emission are omitted here for simplicity. Applying the steady-state approximation to $[(M + nH)^{n+}\text{- -HI}]^*$ yields a loss rate of the reactant ion of

$$\frac{-d[(M + nH)^{n+}]}{dt} = k_c[HI] - k_{-c}\left(\frac{k_c[HI] + k_{-s}[bath][(M + nH)\text{- -HI}]^{n+}}{k_{-c} + k_s[bath]}\right) \quad (4)$$

The k_{-s}[bath]$[(M + nH)\text{- -HI}]^{n+}$ term appears due to the fact that HI attachment is a reversible process under these experimental conditions. It is important to recognize that, in principle, the equilibrium, $(M + nH)^{n+} + HI \leftrightarrows [(M + nH)\text{- -HI}]^{n+}$, can be achieved. However, for the systems studied here at room temperature, no evidence for the back reaction was noted. That is, when $[(M + nH)\text{- -HI}]^{n+}$ product ions were built up over long reaction periods, such that very little further change in the spectrum was observed, and then isolated and stored for variable periods of time, no fragmentation of the $[(M + nH)\text{- -HI}]^{n+}$ ions occurred. This observation suggests that the collisional activation rate (k_{-s}[bath]) under normal ion storage conditions is sufficiently small for the bradykinin (and analogue) ions studied here that it can be neglected. Under these circumstances, the rate equation simplifies to

$$\frac{-d[(M + nH)^{n+}]}{dt} = k_c[HI] - k_{-c}\left(\frac{k_c[HI]}{k_{-c} + k_s[bath]}\right) = \frac{(k_s[bath])(k_c[HI])}{k_{-c} + k_s[bath]} \quad (5)$$

Two limiting cases are when $k_{-c} \gg k_s$[bath] and when $k_{-c} \ll k_s$[bath]. In the former case, the attachment rate approaches zero,

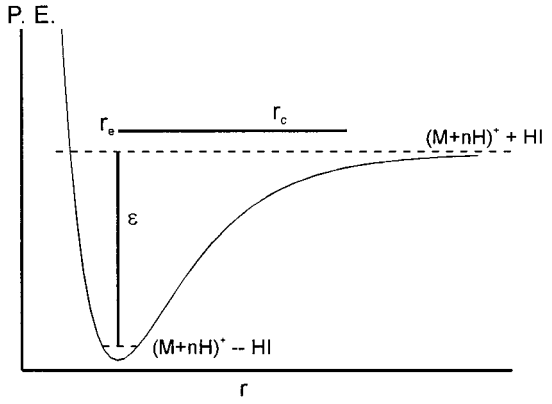

Figure 8. Simplified potential energy diagram for the attachment of HI to protonated bradykinin ions. The well depth (ϵ) is related to the HI binding strength, i.e., ΔG_{acid}(HI) − GB(base). The value of the capture radius (r_c) is related to the binding strength by eq 7. (See text for discussion.)

(27) (a) Langevin, P. M. *Ann. Chim. Phys.* **1905**, *5*, 245. (b) Eyring, H.; Hirschfelder, J. O.; Taylor, H. S. *J. Chem. Phys.* **1936**, *29*, 294. (c) Gioumousis, G.; Stevenson, D. P. *J. Chem. Phys.* **1958**, *29*, 294. (d) Su, T.; Bowers, M. T. *Gas-Phase Ion Chemistry*; Academic Press: New York, 1979; Vol. 1. (e) Su, T.; Bowers, M. T. *Int. J. Mass Spectrom. Ion Phys.* **1973**, *12*, 347. (f) Chesnavich, W. J.; Su, T.; Bowers, M. T. *J. Chem. Phys.* **1980**, *72*, 2641. (g) Su, T.; Bowers, M. T. *J. Chem. Phys.* **1982**, *76*, 5183.

(28) The rate constant was calculated using the rate theory of Su and Bowers (see ref 27g) The polarizability of HI was determined according to the method of Miller and Savchik (Miller, K. J.; Savchik, J. A. *J. Am. Chem. Soc.* **1979**, *101*, 7206). The dipole moment was taken from McClellan: McClellan, A. L. *Tables of Experimental Dipole Moments*, 2nd ed.; W. H. Freeman: San Francisco, 1963.

(29) Goeringer, D. E.; McLuckey, S. A. *Int. J. Mass Spectrom.* **1998**, *177*, 163.

and in the latter case the attachment rate is determined by k_c-[HI]. The collisional cooling rate is expected to be roughly equivalent for the suite of ions studied here. The differences in overall attachment rates are therefore expected to arise from either differences in the respective k_c[HI] values, differences in k_{-c} values, or a combination of both.

The lifetimes of the initially formed $[(M + nH)\text{- -HI}]^{n+}*$ species are expected to be related to the number of degrees of freedom of the complexes and the binding strengths. The differences in numbers of degrees of freedom of the ions investigated in this study are relatively small, and it is not expected that ion size is an important factor in giving rise to differences in the kinetics associated with, for example, ions of methylated and non-methylated bradykinin. Significant differences in kinetics associated with attachment to sites of greater or lesser binding strength, on the other hand, may play a role. The expected trend is to observe longer complex lifetimes (lower values of k_{-c} relative to the cooling rate) as the binding strength increases due to an increase in the density of available states in the complex. The binding strength of HI to a basic site is expected to increase as the difference between the gas-phase acidity (ΔG_{acid}) of HI (309.3 kcal/mol) and the gas-phase basicity (GB) of the attachment site decreases. The lower the energy requirement for proton transfer from HI to the basic site, the greater the ionic nature of the binding is expected to be. This process is facilitated by the Coulomb attraction associated with the ion-pair formation. Gas-phase rotational spectroscopy measurements of gaseous NH_4Cl[30] ($\Delta G_{acid}(HCl) - GB(NH_3) = 129$ kcal/mol) and NH_4I[31] ($\Delta G_{acid}(HI) - GB(NH_3) = 110$ kcal/mol) indicated that these species have relatively little ionic character and can be considered to be largely hydrogen-bonded. Rotational spectroscopy of gaseous $(CH_3)_3NHCl$[32] ($\Delta G_{acid}(HCl) - GB((CH_3)_3N) = 114$ kcal/mol), on the other hand, indicated that there is a significant degree of proton transfer associated with this species. Likewise, gas-phase NMR studies[33] have indicated that the $(CH_3)_3NHBr$ system ($\Delta G_{acid}(HBr) - GB((CH_3)_3N) = 99$ kcal/mol) can be considered to be an ion pair. For a given base (B), the maximum difference ($\Delta G_{acid} - GB$) that allows for a significant degree of proton transfer from different HX species (where X = Cl, Br, I) is expected to increase in the order HCl > HBr > HI, due to differences in BH^+-X^- bond length. However, the general trend for increasing likelihood of ionic bonding with decreasing values of $\Delta G_{acid}(HX) - GB(B)$ is expected with each acid. Thus, considering the HX acid trends and the experimental results achieved for gas-phase reactions between HX and amine bases, $\Delta G_{acid}(HI) - GB(B)$ values below ~100 kcal/mol should result in an ion-pair complex. While few measurements have been made that probe the nature of binding of HI to gaseous bases, the relatively low $\Delta G_{acid}(HI) - GB(B)$ values for arginine, lysine, and histidine (73.5, 88.5, and 88.5 kcal/mol, respectively)[34] suggest a high likelihood that the binding associated with HI attachment to polypeptide ions has a significant degree of ionic character. The higher propensity for HI attachment to polypeptide ions than that of either HBr or HCl[16a] might even suggest that the degree of ionic character associated with HI attachment is greater than those for the other HX species.

It is instructive to consider the limiting case whereby k_{-c} is assumed to be sufficiently small that $k_s(bath) \gg k_{-c}$, such that k_c is rate limiting. In this case, the rate expression simplifies to

$$\frac{-d[(M + nH)^{n+}]}{dt} = k_c[HI] \qquad (6)$$

Experimental support for this picture was obtained by measuring the attachment rate of HI to the $(M + H)^+$ and $(M + 2H)^{2+}$ ions of bradykinin with and without the helium bath gas. These experiments were conducted with total background pressure of 1 mTorr (9.7×10^{-4} Torr of He, 2.0×10^{-5} Torr of atmospheric gases from the vacuum/atmosphere interface, 1.4×10^{-5} Torr of HI) in one case and 3.4×10^{-5} Torr (HI and atmosphere only) in the other. The latter experiment yielded attachment rate constants of 1.2×10^{-11} and 1.5×10^{-12} cm^3-s^{-1} for the fast- and slow-reacting populations of BK $(M + 2H)^{2+}$, respectively, and similar relative abundances of each population. In the case of BK $(M + H)^+$, the "low-pressure" rate constants for the attachment of the first and second molecules of HI were 5.6×10^{-11} and 8.3×10^{-12} cm^3-s^{-1}, respectively. Thus, when the background pressure was lowered by a factor 18 (total neutral pressure), the rate constants for the attachment of HI were lowered by a factor of less than 2 for all ions studied. A reduction in rate of about 40%, as observed for the fast-reacting BK $(M + 2H)^{2+}$ ion and for the addition of the first molecule of HI to BK $(M + H)^+$, with an 18-fold decrease in bath gas pressure, suggests that $k_s[bath] \approx 25\ k_{-c}$ at 1 mTorr. This conclusion is reached by determining the ratio of $k_{-c}/k_s[bath]$ from eq 5 at the high- and low-pressure attachment rates.

Since k_c[HI] appears to be rate determining in these experiments, it is important to consider the role that HI binding strength might play in determining k_c[HI]. Such a role would lead to differences in binding rates to "unblocked" basic sites depending upon the identity of the site (i.e., arginine, lysine, histidine, N-terminus). It has already been pointed out that neither a hard-sphere collision picture, where the entire collision cross section of the ion is considered, nor an ion−induced dipole picture is particularly useful in yielding a maximum k_c[HI] value. Both models overestimate the attachment rates by at least a factor of 6. A simple and more useful picture that might be used in estimating maximum k_c[HI] attachment rates is one that considers the maximum distance of approach necessary to make probable proton transfer from HI to the basic site. The simplest version of this picture sets the capture radius as that distance in which the Coulomb attraction associated with an ion pair equals the difference in $\Delta G_{acid}(HI)$ and GB(base), viz.:

$$\frac{e^2}{r_c} = \Delta G_{acid}(HI) - GB(base) \qquad (7)$$

In other words, this picture determines the distance of approach necessary for Coulomb attraction to overcome the endoergicity of the proton-transfer reaction. The capture radius (r_c) values for arginine, lysine, and histidine are 4.5, 3.8, and 3.8 Å, respectively, yielding cross section values of 63 $Å^2$ for arginine and 45 $Å^2$ for lysine and histidine. For ions of mass comparable to those of bradykinin reacting with HI at 300 K, these cross sections translate to reaction rate constants of 9.3×10^{-11} and 6.6×10^{-11} cm^3-s^{-1}, respectively. The value of 9.3×10^{-11} cm^3-s^{-1} is slightly greater than the highest rate constant thus far observed for an arginine-containing peptide when the

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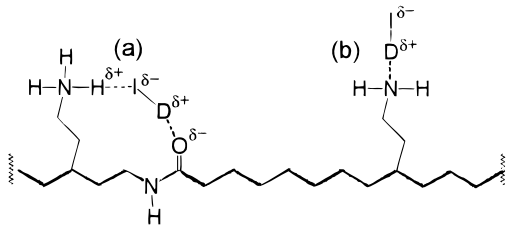

Figure 9. Illustration of two possible competing mechanisms for H/D exchange. The exact structure of individual amino acids and the peptide have been omitted for simplification.

measured rate is corrected for the number of available reactive sites. For example, singly protonated bradykinin adds the first molecule of HI at an overall rate constant of 9.3×10^{-11} cm^3-s^{-1}. This overall rate constant, however, must be corrected for the rate constant for attachment at the second available site (i.e., 1.04×10^{-11} cm^3-s^{-1}). This simple picture is considered a more useful model for determining the reaction efficiency for HI attachment than either the hard-sphere model that considers the cross section of the entire ion or a polarization model.

The DI experiments were conducted as a complementary experiment to the pressure study described above in order to determine the relative importance of the process represented by rate constant k_{-c}. If H/D exchange were to proceed exclusively via attachment of DI to a neutral basic site, followed by the loss of HI, then the observation of H/D exchange would indicate that k_{-c} cannot be neglected for interpreting the rate of HI or DI attachment. All of the ions subjected to reaction with DI in this study were observed to undergo H/D exchange. This observation would appear to be in contradiction with the results obtained at different background gas pressures. However, the BK $(M + 3H)^{3+}$ ion, which is unreactive toward HI attachment, showed both the fastest and most extensive degree of H/D exchange of all of the ions studied. This observation suggests that H/D exchange with DI may very well proceed largely via a mechanism that does not require attachment of DI to a neutral basic site. To account for H/D exchange involving D_2O and protonated polypeptides, Campbell et al.[15a] proposed a "relay-type" mechanism, which is mediated by the charge site. The gas-phase basicity of water (GB = 157.7 kcal/mol) is too low to result in H/D exchange with protonated peptides through the typical proton-bound dimer intermediate.[34] Wyttenbach and Bowers have recently reported D_2O H/D exchange simulations with protonated BK[15b] that support the mechanism proposed by Campbell et al. involving the interaction of D_2O with the charged site and nearby basic sites, such as amide oxygens. The GB of HI (143.7 kcal/mol) is lower than that of water. Therefore, it is unlikely that the observed H/D exchange with DI occurs via a proton-bound dimer intermediate. However, DI can engage in a "relay-type" mechanism, in analogy with that proposed for D_2O. In fact, when this mechanism is considered, DI H/D exchange might be expected to proceed at a faster rate than D_2O H/D exchange because of the greater ΔG_{acid} of DI versus that of D_2O. In the few cases for which H/D exchange rates have been measured for DI and D_2O with the same nominal species, rates of exchange with DI appear to be significantly greater. For example, Freitas and Marshall found electrospray-generated BK $(M + 2H)^{2+}$ to undergo H/D exchange with D_2O at a rate of 1.1×10^{-3} s^{-1} at 1×10^{-5} Torr of H_2O. In our experiments with DI at the same pressure, BK $(M + 2H)^{2+}$ H/D exchanges at a rate of $\sim$0.57 s^{-1} until the $(M + 2H)^{2+}$ ion population is depleted by the DI attachment reaction.

Figure 9 illustrates the two mechanisms discussed here by which DI H/D exchange can occur. The figure represents a polypeptide with two basic residues in which the residue at site a is protonated and the residue at site b is not protonated. The relay mechanism is illustrated at site a, where the iodide atom interacts with the charge site. This interaction facilitates protonation of a nearby basic site, such as an amide oxygen. When this occurs, neutral HI can be liberated probably with a subsequent deuteron transfer back to the basic residue. At site b, H/D exchange occurs after attachment of DI to the neutral basic residue and H/D scrambling associated with the subsequent loss of HI. While the latter mechanism cannot be precluded from making a contribution to the H/D exchange for the ions with available neutral basic sites, it is probably not important for the BK $(M + 3H)^{3+}$ ions. Furthermore, the background gas pressure experiment suggests that loss of HI or DI from the DI attached species is probably not a major process. On the other hand, since all of the ions are at least singly protonated, the relay-type mechanism for H/D exchange can be expected to contribute, and perhaps dominate, for all of the ions studied here.

Methylated versus Non-Methylated Ions. Conversion of bradykinin and its analogues (des-Arg-bradykinins) to the corresponding methyl esters was done to determine the role of the C-terminus in affecting HI attachment kinetics. Schnier et al.[6a] have implicated the existence of a salt bridge structure in bradykinin ions on the basis of blackbody infrared dissociation of methylated and non-methylated species. While the dissociation studies and the attachment studies reported here tend to sample ions of different temperatures and are therefore not directly comparable, it is worthwhile considering the effect a salt bridge might have on HI attachment rates. We have observed that the majority of ions that have been converted to the methyl ester tend to react somewhat more slowly (a factor of 2 or less) than the non-methylated ion. This is the case with bradykinin $(M + H)^+$ for both addition of the first and second molecules of HI, for both of the reactive populations noted for bradykinin $(M + 2H)^{2+}$, and for the ions derived from des-Arg^1-bradykinin $(M + H)^+$. The exceptional system is that of des-Arg^9-bradykinin in which methylation resulted in a dramatic increase in HI attachment rate.

It might be expected that a salt bridge would inhibit HI attachment at the basic site participating in the interaction since it is nominally protonated, e.g., $-NH_3^+$- -$^-$OOC$-$. However, it is conceivable that the carboxylic acid moiety could stabilize the attachment of HI to the basic site via hydrogen bonding, e.g., $-NH_3^{\delta+}$- -I$^{\delta-}$- -H$^{\delta+}$- -$^{\delta-}$OOC$-$. Such stabilization present in the native (non-methylated) ions would not be expected to *increase* the rate of attachment if the interaction of the carboxylic acid moiety with the attached HI molecule takes place *after* the initial capture, unless interaction with the carboxylic acid group is necessary to stabilize the HI attachment product (i.e., if k_{-c} is significant in the absence of stabilization). An alternative scenario, which is more consistent with the evidence that k_{-c} is small, is that the HI molecule inserts into a preexisting salt bridge structure. The mechanism by which a salt bridge presents a modestly larger capture cross section than the neutral basic site cannot be determined from these results. However, the degree of charge separation produced by a salt bridge could increase the contribution of polarization on the incoming HI molecule, which would then add to the long-range attraction of the potential.

Evidence supporting the stabilization of HI attachment by the carboxy terminus follows from comparison of dissociation rates derived from methylated and non-methylated ions under identical collisional activation conditions. For example, the HI

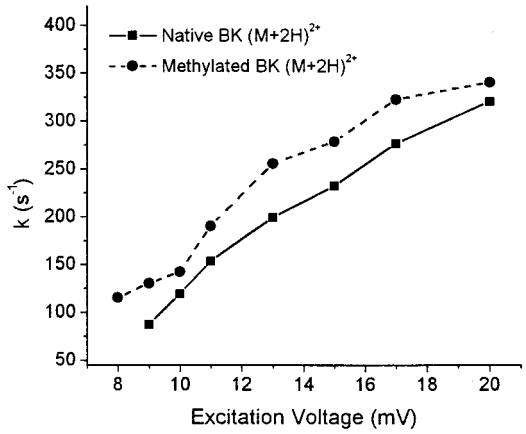

Figure 10. Dissociation rates for the doubly protonated ions of bradykinin and its methyl ester.

attachment products from methylated and non-methylated doubly protonated bradykinin ions were fragmented over a range of identical single-frequency (resonant) excitation conditions, with the result that the dissociation rates for the methyl ester derivative were consistently larger than those for the non-methylated $[(M + 2H)- -HI]^{2+}$ ion (Figure 10). This observation is consistent with the C-terminus stabilizing the interaction because dissociation rates in the quadrupole ion trap collisional activation experiment are related to ion stability.[35]

The body of observations described here appear to be consistent with there being a significant degree of salt-bridging in the bradykinin ions, both singly and doubly protonated, and the des-Arg^1-bradykinin $(M + H)^+$ ion. A salt bridge interaction is not necessarily precluded in the des-Arg^9-bradykinin system, but methylation results in a much different effect on HI attachment kinetics, which probably reflects a significant change in ion structure as a result of methylation.

Bradykinin $(M + H)^+$. Singly protonated bradykinin is perhaps the most studied of the bradykinin ions and their analogues. Wyttenbach et al. showed that this ion tends to adopt a relatively compact shape, which is consistent with a high degree of intramolecular charge solvation by the carbonyl oxygens in the polypeptide chain.[8e] Schnier et al. presented evidence for the involvement of salt-bridging based on dissociation studies, as mentioned above.[6a] Freitas et al. have recently reported that the $(M + H)^+$ ion of bradykinin derived from electrospray undergoes H/D exchange with D_2O at a very low rate,[13a,13b] whereas Wyttenbach et al. observed much faster H/D exchange kinetics with D_2O[15b] when the $(M + H)^+$ ions were formed by matrix-assisted laser desorption. The latter results were modeled on the basis of the recently proposed mechanism for H/D exchange with D_2O.[15] Both studies were carried out using Fourier transform ion cyclotron resonance instrumentation. The different observations derived from the two studies might result from possible differences in the temperature at which the reactions were affected and/or might also highlight the importance of ion preparation in determining the results of experiments with chemical probes of higher order structure. The $(M + 2H)^{2+}$ ion reacting with HI, as discussed below, can also be used to make this point.

Hydroiodic attachment kinetics indicate that there is a single reactive population, as evidenced by linear pseudo-first-order kinetics for both the attachment of the first and second molecules of HI. The second molecule of HI attaches much more slowly than does the first. This can be accounted for by the lowered gas-phase basicity of the N-terminus, relative to arginine. That is, the first molecule of HI can attach to an arginine, which, based on the behavior of the methylated species, might be involved in a salt bridge, and the second molecule must then attach to the N-terminus. The lower basicity of the N-terminus, relative to that of arginine, would be expected to lead to a lower attachment rate. However, the N-terminus may also be involved in solvating charge, which might also affect reactivity. Interestingly, the rate for attachment of the second molecule of HI is affected by methylation of the carboxy terminus in proportion to the rate for attachment of the first molecule of HI. This observation suggests that there is also a significant degree of interaction with the carboxy terminus at the site of attachment of the second HI molecule as well.

Bradykinin $(M + 2H)^{2+}$. Doubly protonated bradykinin shows, perhaps, the most interesting phenomenology in that there are clearly at least two non-interconverting reactive populations that are present in roughly equal abundance at room temperature. Furthermore, the barrier to isomerization between the two is lower than the barriers for dissociation of the $(M + 2H)^{2+}$ ion. For this reason, it would be difficult to distinguish the two reactive species based on relatively slow ion activation methods, such as ion trap collisional activation. In addition, due to the collisional cooling inherent in quadrupole ion traps, the preferential formation of one population over the other could not be affected by ionization conditions (i.e., collisional activation in the atmospheric interface). Figure 11 shows a qualitative energy diagram that is consistent with the set of observations made for the $(M + 2H)^{2+}$ ions and the following discussion. As this diagram indicates, by exciting one of the conformers above the barrier for isomerization and subsequently allowing the excited ion population to cool, a mixture of reactive structures is formed. Ion mobility measurements have not shown there to be resolvable differences in sizes of $(M + 2H)^{2+}$.[8i]

The "fast"-reacting component shows an attachment rate that is roughly a factor of 2 higher than the rate for attachment of the second molecule of HI to the $(M + H)^+$ ion. If the two ionizing protons in the $(M + 2H)^{2+}$ ion are assumed to be associated with the arginine residues, the HI molecule is then expected to attach to the N-terminus, as is the second molecule of HI to attach to the $(M + H)^+$ ion. It might be concluded that the N-terminus of the fast-reacting component of the $(M + 2H)^{2+}$ ion is somewhat more exposed to attack by HI than the N-terminus of the $(M + H + HI)^+$ ion. However, it is not clear at this point what role the total charge on the ion might play in affecting k_c[HI] via polarization. Therefore, care should be taken in drawing conclusions from comparisons of relatively small differences in rates for ions of different charge state. However, it is probably much safer to conclude that the reactive site in the slow-reacting component is significantly less exposed than that in the fast-reacting component. In both cases, however, the carboxy terminus plays a subtle role in affecting the HI attachment kinetics, as also observed in the singly protonated ions.

des-Arg-Bradykinin Ions. The des-Arg^9- and des-Arg^1-bradykinin ions show dramatically different behavior, which is consistent with both blackbody infrared dissociation data[6a] and recent H/D exchange results.[13a,b] In the latter work, it was observed that H/D exchange with D_2O was much faster with the des-Arg^1 bradykinin ion than with the des-Arg^9-bradykinin ion. No studies were reported for the methyl esters of these

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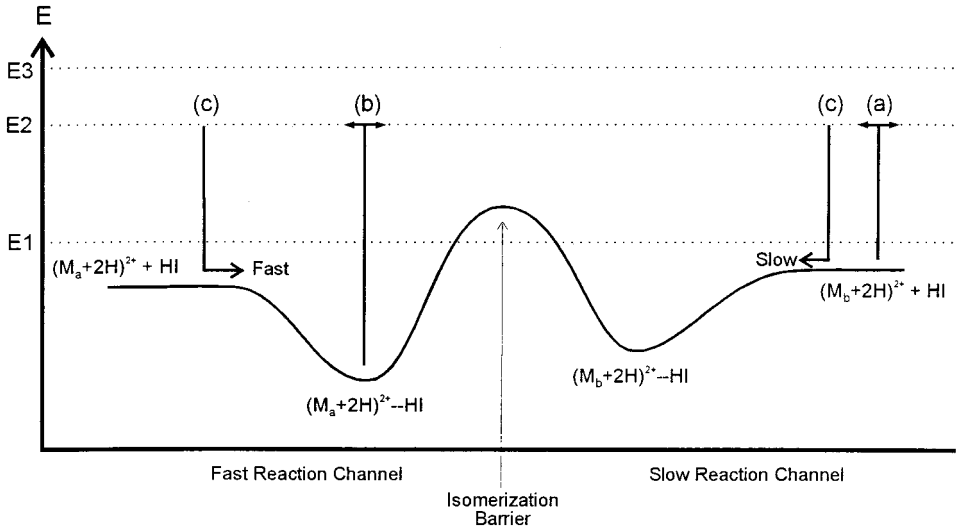

Figure 11. Simplified potential energy diagram providing a qualitative illustration of the interconversion of the BK $(M + 2H)^{2+}$ fast- and slow-reacting populations. (a) Collisional activation of the isolated slow-reacting population from the ion at room temperature (E1) to an energy above the isomerization barrier but below the dissociation barrier (E3). This ion is subsequently collisionally cooled by the He bath gas (c) into the original two reactive populations. (b) Dissociation of the $[(M + 2H)$- -$HI]^{2+}$ complex in the attempt to isolate the fast-reacting population. Once the energy of the ion population is raised above the isomerization barrier, both populations are generated upon collisional cooling in the bath gas (c).

ions. In analogy, HI attachment to protonated des-Arg^1-bradykinin is relatively fast, reacting with a rate constant of 8.4×10^{-11} cm^3-s^{-1}, whereas only a small population of the protonated des-Arg^9-bradykinin ($12 \pm 3\%$) reacts ($k = 3.3 \times 10^{-12}$ cm^3-s^{-1}), and the majority of the ions react at least an order of magnitude more slowly.

The attachment rate constant for des-Arg^1-bradykinin is almost as large as that for the $(M + H)^+$ ion of bradykinin and is significantly higher than those for the attachment of the second molecule of HI to the $(M + H)^+$ ion of bradykinin and for the attachment of HI to the $(M + 2H)^{2+}$ ion of bradykinin. In the latter cases, we have assumed that attachment most likely occurs at the N-terminus. If it is assumed that the ionizing proton on des-Arg^1-bradykinin is associated with the arginine residue, then the magnitude of the attachment rate constant would appear to be relatively high for a normal N-terminus. However, proline is the N-terminal amino acid of des-Arg^1-bradykinin, and it is reasonable to expect that the secondary nitrogen of proline has a higher gas-phase basicity than the primary amine present at the N-terminus of other amino acids. Measurements of HI attachment rates to collision-induced dissociation products derived from polypeptides have also indicated that y-type fragments with an N-terminal proline show markedly higher attachment rates than other y-type fragments,[36] which is also consistent with the N-terminal proline being more reactive toward HI attachment. The decrease in attachment rate constant upon methyl esterification is consistent with the behavior of the bradykinin ions.

The $(M + H)^+$ ion population of des-Arg^9-bradykinin showed a minor slowly reactive population and a major essentially unreactive population. This behavior suggests that the reactive basic site in this molecule is not exposed to attack by HI. The slow rate of H/D exchange involving both DI and D_2O[13a,b] is also consistent with a structure in which the exchangeable hydrogens have limited availability. The carboxy terminus appears to play a significant role in affecting the structure of this ion because methylation yields a population of ions that react with a single rate constant and one that is comparable to those for the "fast"-reacting doubly protonated bradykinin ions and for the attachment of the second molecule of HI to singly protonated bradykinin. The value of the rate constant is similar to those where attachment is deemed to take place at an N-terminus with a primary amino group, as is present in BK $(M + 2H)^{2+}$. Although this system shows methyl esterification to increase the HI attachment rate, unlike the other systems described in this study, it cannot be concluded that a salt bridge structure is absent in the non-methylated ion. Rather, it can only be concluded that the potential attachment sites in the non-methylated ion are relatively unexposed and that methyl esterification results in a change in structure that makes an attachment site available for attack.

(36) Schaaff, T. G.; Stephenson, J. L.; McLuckey, S. A. Unpublished results.

Conclusions

The kinetics of attachment of hydroiodic acid to ions derived from bradykinin and related systems have been measured. Experiments were conducted that indicated that none of the ions in this study approached equilibrium. While neither the hard-sphere nor the polarization models appear to approximate the maximum possible HI attachment rate, a simple approach is proposed to establish the maximum HI attachment rate that is based upon the largest distance of approach that allows for proton transfer from HI to the basic attachment site. This approach assumes that HI attachment is an irreversible process under the conditions used here. The attachment rates measured with and without helium bath gas are consistent with this assumption.

Methyl esterification of the ions indicated that the carboxy terminus can play a role in determining the HI attachment rate. In most cases, methyl esterification of the carboxy terminus resulted in a modest decrease in attachment rate. Collisional activation experiments show that the HI-attached species with the carboxy terminus is more stable than that with the methyl-esterified C-terminus. This observation supports the speculation that a molecule of HI can insert into a salt bridge interaction, which is not present in the methyl-esterified species. In the case of the $(M + H)^+$ ions of des-Arg^9-bradykinin, methyl esterification increased the HI attachment rate. This observation is attributed to a significant change in the three-dimensional structure of this ion that results in the exposure of a reactive site to attack by HI.

Several ions, such as doubly protonated bradykinin, doubly protonated methyl-esterified bradykinin, and singly protonated des-Arg^9-bradykinin, exhibited nonlinear pseudo-first-order kinetics for the attachment of HI. Good fits to the data could only be made using two rate constants. These results suggest that there are two non-interconverting ion structures within the ion populations isolated for study. In the case of doubly protonated bradykinin, it was shown that these ion populations could interconvert at energies lower than those necessary for dissociation. Likewise, H/D exchange using DI also shows two reactive populations for the BK $(M + 3H)^{3+}$ ions. To date, no other chemical or physical probes of gas-phase polypeptide ion structure have indicated the coexistence of isomeric structures of bradykinin $(M + 2H)^{2+}$ or $(M + 3H)^{3+}$ ions at comparable abundance.

This work illustrates the potential role that measurement of HI attachment kinetics and H/D exchange using DI can play in addressing issues of polypeptide three-dimensional structure in the gas phase. It is expected to be able to provide, at least in some cases, information that complements that derived from other physical and chemical probes. The other chemical probes, such as measurement of proton-transfer kinetics and measurement of the kinetics of H/D exchange with neutral species such as D_2O, CD_3OD, etc., are largely charge-site mediated. While charge may play an indirect role in the rates of HI attachment to polypeptide ions, the reaction inherently involves the interaction of two neutral species. The information that might be obtained via H/D exchange measurements with DI versus D_2O is the subject of further study.

Acknowledgment. This research was sponsored by the National Institutes of Health under Grant GM45372. Oak Ridge National Laboratory is managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under Contract No. DC-AC05-96OR22464. The authors acknowledge Dr. Paul D. Schnier for many helpful discussions. T.G.S. acknowledges support through appointments to the Oak Ridge National Laboratory Postdoctoral Research Associates Program, administered jointly by the Oak Ridge Institute for Science and Education and Oak Ridge National Laboratory.

JA991553G