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Rate and extent of gas-phase hydrogen/deuterium exchange of bradykinins: evidence for peptide zwitterions in the gas phase

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Abstract

The gas-phase H/D exchange of bradykinin $[M + H]^+$, $[M + Na]^+$, $[M + 2H]^{2+}$, and $[M + H + Na]^{2+}$ ions; des-Arg^1-bradykinin, des-Arg^9-bradykinin, and bradykinin fragment 2-7 $[M + H]^+$ ions; and O-methylbradykinin $[M + H]^+$ and $[M + 2H]^{2+}$ ions with D_2O have been examined by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry at 9.4 T. The different peptides vary widely in exchange rate and extent of deuterium incorporation. H/D exchange was slowest and deuterium incorporation was least for bradykinin $[M + H]^+$, $[M + H + Na]^{2+}$ and bradykinin methyl ester $[M + 2H]^{2+}$ ions. In contrast, H/D exchange and extent of deuteration are higher for des-Arg^1-bradykinin, des-Arg^9-bradykinin, and bradykinin fragment 2-7 $[M + H]^+$ ions; and highest for bradykinin $[M + Na]^+$ and $[M + 2H]^{2+}$, and O-methylbradykinin $[M + H]^+$. Because the most likely site of protonation is the guanidino group of arginine, the above reactivity pattern strongly supports a zwitterion form for protonated gas-phase bradykinin. (Int J Mass Spectrom 182/183 (1999) 221–231) © 1999 Elsevier Science B.V.

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1. Introduction

Ben Freiser's primary research area was Fourier transform ion cyclotron resonance (FTICR) mass spectrometry. Especially during the 1980's, Ben was the unquestioned leader in development of techniques and applications of FTICR MS for ion-molecule chemistry (particularly transition metal ion chemistry), and the attention his research attracted was principally responsible for building and maintaining interest in the technique during that period. In this paper, we make use of one of Ben's more recent innovations, a simple and elegant pulsed gas introduction technique [1] that has opened up the use of FTICR MS for quantitative gas-phase ion-molecule reaction kinetics at high pressure. Following Ben Freiser's untimely death late in 1997, several projects were undertaken to recognize his contributions. In particular, this special issue is timed to coincide with a half-day symposium honoring Ben at the 1999 Pittsburgh Conference, March 7–12, 1999, in Orlando, FL.

Hydrogen/deuterium exchange techniques have proved useful in the investigation of solution-phase protein structure and conformation [2]. In particular,

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Dedicated to the memory of Ben Freiser to commemorate his many seminal contributions to mass spectrometry and gas phase ion chemistry.

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nuclear magnetic resonance (NMR) combined with H/D exchange has been particularly successful in exposing the structural dynamics of proteins [3–7]. With the introduction of matrix-assisted laser desorption ionization (MALDI) [8] and electrospray ionization (ESI) [9,10], large biomolecules can be ionized and introduced into the gas phase for analysis by mass spectrometry. The investigation of solution-phase structural dynamics by use of H/D exchange and mass spectrometry complements NMR [11–20].

An obvious question is whether or not solution-phase structural features of biomolecules are retained upon ionization and introduction into the gas phase. ESI is considered to be a particularly "soft" ionization method, so that the resultant gas-phase ion might be expected to retain at least some native solution-phase structural features. Gas-phase H/D exchange [21–24] and ion mobility mass spectrometry [25–29] have recently been used to test for conformation and structural integrity of gas-phase biomolecular ions.

Gas-phase H/D exchange and mass spectrometry have been used with great success to elucidate reaction mechanisms and structure of simple organic ions [30–32]. More recently, the method has made possible the resolution and quantitation of the conformational distribution of gas-phase biomolecular ions [33]. The conformational distributions for multiply protonated bovine ubiquitin and cytochrome *c* cations obtained by gas phase H/D exchange levels [22,24,34] and gas-phase ion-mobility mass spectrometry [25–27] reveal striking similarities. Although both techniques reflect the size and chemical reactivity of a gas-phase protein cation, neither method directly proves that solution-phase structural elements are preserved in the gas phase.

The possibility that gas-phase peptide ions can exist as zwitterions, as in solution, has stimulated considerable debate. Locke and McIver demonstrated that thermally desorbed gas-phase amino acids react with ions of protonated references bases and deprotonated references acids in a manner consistent with a nonzwitterionic neutral amino acid [35]. From PM3 calculations, Campbell et al. suggested that $(Gly)_4$ and $(Gly)_5$ could form zwitterions [36]. They observed the same gas-phase H/D exchange reactivity for protonated methyl ester of $(Gly)_4$ (a nonzwitterion) and protonated $(Gly)_4$, suggesting that the two species are similar and, hence, that protonated $(Gly)_4$ is nonzwitterionic. Schnier et al. recently concluded that protonated bradykinin exists in the gas phase as a zwitterionic species [37], based on differences in activation energy for dissociation of singly- and doubly-protonated bradykinin and several of its analogs in blackbody infrared radiative dissociation (BIRD) measurements. Further attempts to verify the existence of zwitterionic species have met with little success. The research groups of Bowers and Clemmer compared the ion mobility derived collision cross sections for bradykinin $[M + H]^+$ and $[M + 2H]^{2+}$ (putative zwitterions) and $[M + Na]^+$ (potentially nonzwitterion); unfortunately, the ion mobilities for all three species were quite similar, so that it was not possible to settle the zwitterion question definitively [38,39]. Although gas-phase H/D exchange offers an attractive alternative approach, the only prior examination of bradykinin was by Campbell et al., who observed no gas-phase H/D exchange with ND_3 of protonated bradykinin produced by Cs^+ ion bombardment Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) at 7 T [40]. Along the same lines, Dookeran and Harrison found that Arg-Gly and Gly-Arg $[M + H]^+$ both exchange very slowly when reacted with ND_3 in the collision cell of a sector mass spectrometer [41].

In this paper, we present a critical comparison of the gas-phase H/D exchange of protonated bradykinin and several of its analogs with D_2O, designed to expose differences between zwitterionic and nonzwitterionic forms. The present experiments are greatly facilitated by our recently reported method for simultaneous trapping and detection of electrospray-ionized peptide mixtures at high pressure (1×10^{-5} Torr) and long trapping period (up to 1 h), in which deuterium uptake is observed directly by stored waveform inverse Fourier transform (SWIFT) [42,43] isolation of the monoisotopic species, followed by high field (9.4 T) high-resolution FTICR mass analysis [44]. That approach significantly increases sample

throughput and grants access to very small rate constants ($< 10^{-11}$ cm^3 mol^{-1} s^{-1}). We reacted putative zwitterions ($[M + H]^+$, $[M + Na]^+$, $[M + 2H]^{2+}$, and $[M + H + Na]^{2+}$ of bradykinin, $[M + H]^+$ ions of des-Arg^1-bradykinin, des-Arg^9-bradykinin, and bradykinin fragment 2-7), and putative nonzwitterions ($[M + H]^+$ and $[M + 2H]^+$ ions of O-methylbradykinin) with D_2O for reaction periods up to 1 h (i.e. $>$ 3 000 000 ion-neutral collisions). As explained below, our data expose dramatic differences in exchange rate and extent, and the differences are consistent with gas-phase zwitterion forms of all bradykinins containing an underivatized carboxyl terminus.

2. Experimental

2.1. Sample preparation

Bradykinin, des-Arg^1-bradykinin, des-Arg^9-bradykinin, and bradykinin fragment (2-7) were obtained from Sigma Chemical Company (St. Louis, MO); D_2O (99.9% D) was obtained from Cambridge Isotope Labs (Andover, MA) and acetyl chloride was obtained from Aldrich Chemical Company (Milwaukee, WI). All reagents were used without further purification. The methyl ester of bradykinin was prepared in situ by dissolving 1 mg of bradykinin in 1 mL of dry MeOH w/2% acetyl chloride. The solution was allowed to react for 6 h and then diluted in dry MeOH to a final concentration of 10 μM [45]. Peptide samples were prepared by dissolving each peptide in 50:50 MeOH:H_2O to a concentration of 1 mM followed by serial dilution in 50:50 MeOH:H_2O w/0.25% Acetic acid to a final concentration of 10 μM. Peptide mixtures were prepared similarly except that the final peptide concentrations were optimized to yield approximately equal relative abundance for each isolated parent ion [34].

2.2. Mass analysis

Experiments were performed with a previously described 9.4 T ESI FTICR mass spectrometer configured for external ion accumulation (Fig. 1) [46,47]. Peptide mixtures were prepared in 50:50 MeOH:H_2O with 0.25% acetic acid and infused into a tapered 50 μm i.d. fused silica micro-ESI needle [48,49] at a rate of 300 nL min^{-1} and a concentration of $\sim$ 10–50 μM for each analyte in the mixture. Typical ESI conditions were: needle voltage = 1.8 kV and heated capillary current = 3.5 A. Ions were accumulated in a linear octopole ion trap (operated at 1.5 MHz) for 5–10 s and then transferred to a 4″-Penning trap through a second octopole ion guide (operated at 1.5 MHz). ICR trapping voltage was set to 2 V, because higher trapping voltage resulted in ion loss (presumably because of radial magnetron expansion). The most abundant monoisotopic ions for each species under investigation were isolated from each solution by use of SWIFT mass-selective ion ejection. Immediately following isolation, the ions were cooled by a short pulse of He gas to 1×10^{-6} Torr. The monoisotopic parent ions were then allowed to react with D_2O pulsed into the vacuum chamber via a 3-way pulsed valve/leak valve combination described previously [1,44]. The partial pressure of D_2O during the pulse rose to 1×10^{-5} Torr within $\sim$2 s and remained stable throughout the course of the H/D exchange period. Ions were allowed to react with the D_2O for varying periods up to 1 h. The exchange reagent pulse was followed by a 6 min pumpdown during which the pressure fell rapidly to 2×10^{-7} Torr ($\sim$10 s) and achieved a final pressure of 8×10^{-8} Torr. The neutral pressure was measured with a Granville-Phillips (Boulder, CO) Model 274 ion gauge. The ions were then subjected to broadband frequency sweep excitation (50–300 kHz) and detection (300 kHz bandwidth and 64 kWord data). Typical initial base pressure for the instrument was 2×10^{-9} Torr. All experiments were controlled by an Odyssey data station (Finnigan Corp., Madison, WI). The time-domain ICR signal (single scan) was subjected to baseline correction followed by Hanning apodization and one zero fill before Fourier transformation and magnitude calculation.

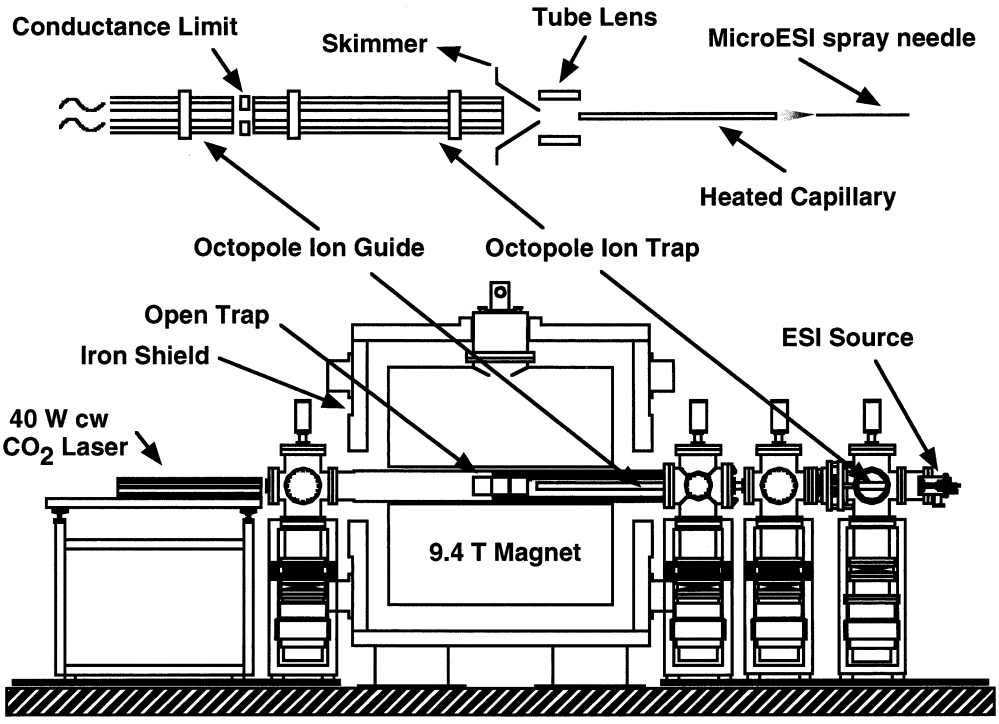

Fig. 1. Schematic diagram of the 9.4 tesla ESI FTICR mass spectrometer at the National High Magnetic Field Laboratory in Tallahassee, FL. The current configuration of the external electrospray ionization source and the octopole ion trap are depicted at the top of the figure.

3. Results and discussion

3.1. Gas-phase H/D exchange of bradykinin

The $[M + H]^+$, $[M + Na]^+$, $[M + 2H]^{2+}$, and $[M + H + Na]^{2+}$ ions of bradykinin were reacted with D_2O (1×10^{-5} Torr), and a mass spectrum was recorded after each of the various reaction periods.

$$\begin{array}{c} \mathbf{2\quad\quad 1\,1\,1\quad 1\,1\quad\quad 1} \\ \mathbf{NH_2\text{-}R\text{-}P\text{-}P\text{-}G\text{-}F\text{-}S\text{-}P\text{-}F\text{-}R\text{-}CO_2H} \\ \mathbf{4\quad\quad\quad 1\quad\quad 4} \end{array}$$

Bradykinin (17 exchangeable hydrogens)

Diagram 1.

Bradykinin is a nonapeptide in which the $[M + H]^+$, $[M + Na]^+$, $[M + 2H]^{2+}$, and $[M + H + Na]^{2+}$ ions each have 18, 17, 19, and 18 exchangeable hydrogens. Because the most abundant monoisotopic species in each
mass spectrum was isolated initially, deuterium incorporation could be observed directly, without the need to deconvolve with respect to the natural isotopic abundance distribution [44]. Fig. 2 displays deuterium incorporation time profiles for the $[M + H]^+$, $[M + Na]^+$, $[M + 2H]^{2+}$, and $[M + H + Na]^{2+}$ ions of bradykinin. Rate constant distributions were obtained from an in-house developed program [19]. In brief, the program uses the maximum entropy method (MEM) to solve for the rate constants for N parallel reactions from a plot of deuterium incorporation versus reaction period. For gas-phase H/D exchange, the N parallel reactions correspond to N hydrogens exchanging for deuterium. Fig. 3 shows a representative output of such a rate constant analysis. The output from MEM analysis is a distribution in which the area under each peak (normalized by the total number of

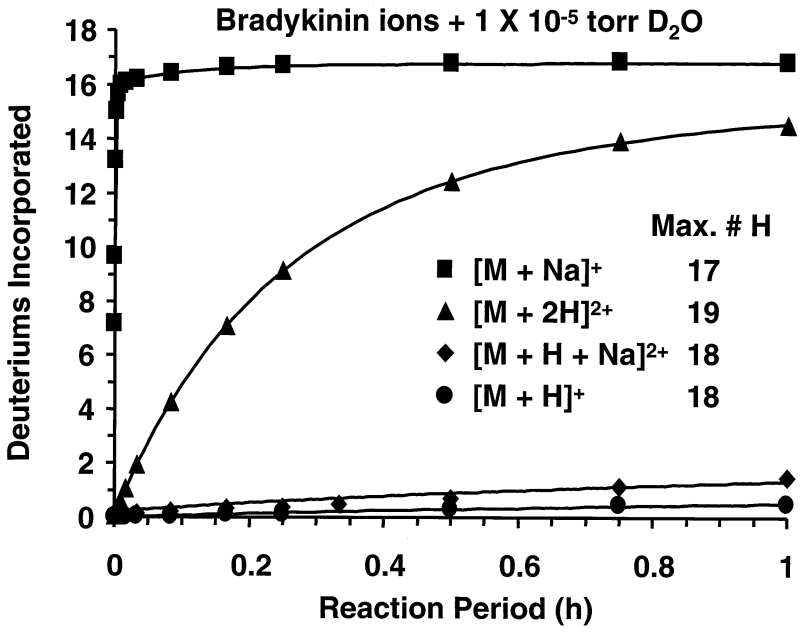

Fig. 2. Deuterium incorporation vs reaction period plots for the bradykinin $[M + H]^+$, $[M + Na]^+$, $[M + 2H]^{2+}$, and $[M + H + Na]^{2+}$ ions. Each curve drawn through the data points was obtained by fitting the rate constants according to the MEM method described in the text.

exchangeable hydrogens) corresponds to the number of rate constants within the range of values represented by that peak. If the exchange rate is too fast or slow to be measured, maximum entropy reports equal (flat) probability extending toward positive or negative infinity. In the following discussion, the MEM threshold for "fast" exchange is $> 10^4$ h^{-1} and for "slow" exchange is < 1 h^{-1}.

It is clear from Figs. 2 and 3 that, of the two singly-charged bradykinin ions, the $[M + Na]^+$ ion reacts more than 1000 times faster than the $[M + H]^+$ ion. In contrast, for the doubly charged ions, the $[M + 2H]^{2+}$ ion reacts at least 10 times faster than the $[M + H + Na]^{2+}$ ion. The $[M + H]^+$ and $[M + H + Na]^{2+}$ ions contain an identical number of exchangeable hydrogens (18) and both exchange at a rate too slow to measure under these conditions. Bradykinin $[M + Na]^+$ attains complete incorporation of all 17 exchangeable hydrogens within 10 min, whereas bradykinin $[M + 2H]^{2+}$ exhibits two distinguishable distributions of rate constants (one of which is quite low in relative abundance).

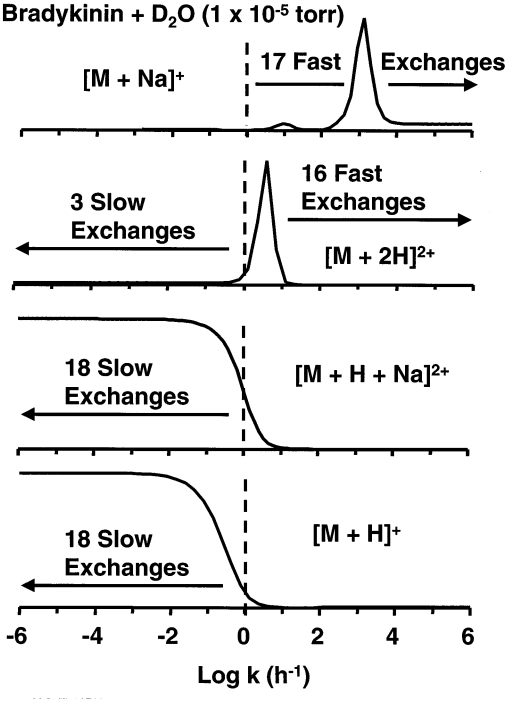

Fig. 3. The MEM-derived H/D exchange rate constant distribution for bradykinin $[M + H]^+$, $[M + Na]^+$, $[M + 2H]^{2+}$, and $[M + H + Na]^{2+}$ ions.

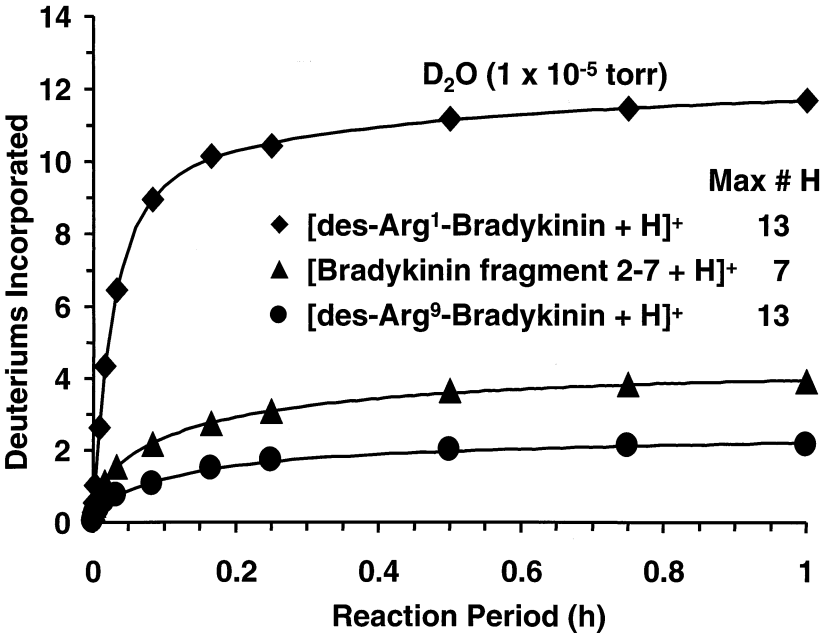

Fig. 4. Deuterium incorporation vs reaction period plots for the $[M + H]^+$, ions of des-Arg^1-bradykinin, des-Arg^9-bradykinin, and bradykinin fragment 2-7. Each curve drawn through the data points was obtained by fitting the rate constants according to the MEM method described in the text.

3.2. Gas-phase H/D exchange of des-Arg^1-bradykinin, des-Arg^9-bradykinin, and bradykinin Fragment 2-7

The effect of removal of one or both of the terminal arginines was investigated by performing gas phase H/D exchange of the $[M + H]^+$ ions of des-Arg^1-bradykinin, des-Arg^9-bradykinin, and bradykinin fragment 2-7 with D_2O (1×10^{-7} Torr). The deuterium incorporation versus time plots along with the MEM-derived rate constant distributions are shown in Figs. 4 and 5. Within the prescribed one hour reaction period, the $[M + H]^+$ ions of des-Arg^1-bradykinin (13 exchangeable hydrogens), des-Arg^9-bradykinin (13 exchangeable hydrogens), and bradykinin fragment 2-7 (7 exchangeable hydrogens) exchanged 12, 4, and 2 of their exchangeable hydrogens for deuterium (Fig. 4). From the rate constant distributions in Fig. 5 is clear that while the level of the deuterium incorporation is very different for the three species they all possess a distribution of fast

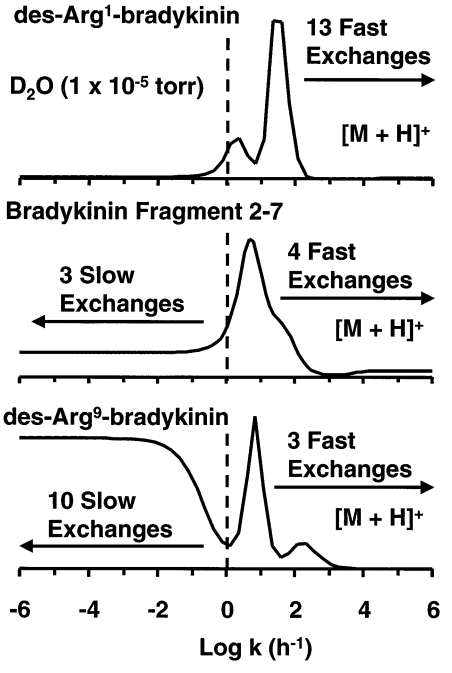

Fig. 5. The MEM derived H/D exchange rate constant distribution for the $[M + H]^+$ ions of des-Arg^1-bradykinin, des-Arg^9-bradykinin, and bradykinin fragment 2-7.

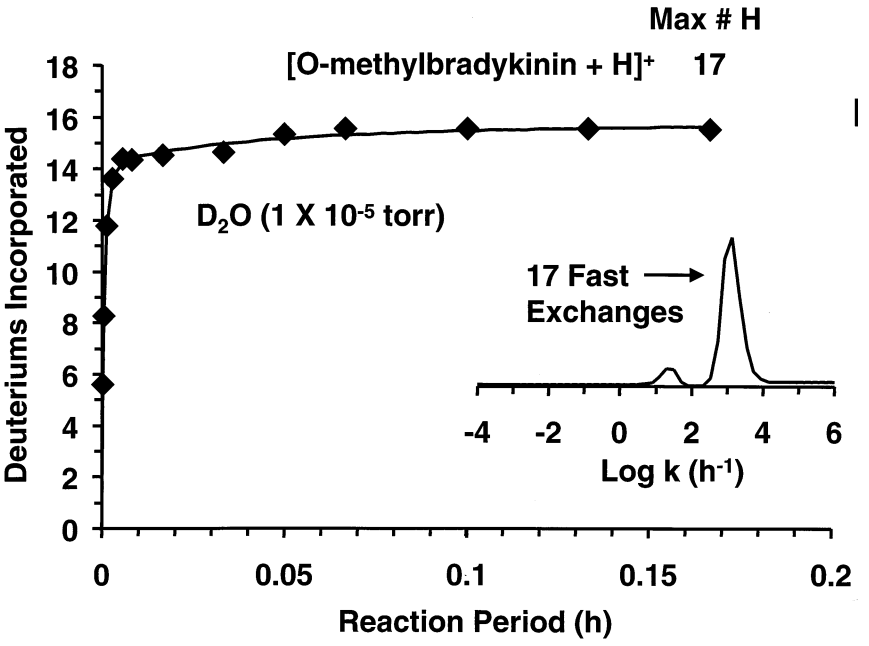

Fig. 6. Deuterium incorporation vs reaction period plot for O-methylbradykinin $[M + H]^+$ ions. The curve drawn through the data points was obtained by fitting the rate constants according to the MEM method described in the text. Inset: MEM derived H/D exchange rate constant distribution for O-methylbradykinin $[M + H]^+$ ions.

exchanging rate constants. However, the most striking observation was manifested in the large difference in the exchange rates of des-Arg^1-bradykinin and des-Arg^9-bradykinin. The location of the arginine group has a strong influence on the rate of exchange. In addition, the removal of either arginine group greatly enhances the rate of exchange for the protonated bradykinin.

3.3. Gas-phase H/D exchange of O-methylbradykinin

To test for the existence of a zwitterionic species, we performed H/D exchange of bradykinin methyl ester $[M + H]^+$ and $[M + 2H]^{2+}$ ions with D_2O (1×10^{-5} Torr). The O-methylbradykinin $[M + 2H]^{2+}$ ion was the most abundant species in the ESI FTICR mass spectrum, along with minor amounts of unreacted bradykinin observed as bradykinin $[M + 2H]^{2+}$ and $[M + H]^+$ ions. No O-methylbradykinin $[M + H]^+$ ions were observed even upon dilution to reduce the solvent acid concentration below 0.25%. However, O-methylbradykinin $[M + H]^+$ ions were formed upon SWIFT isolation of the $[M + 2H]^{2+}$ ion of O-methylbradykinin, presumably because of proton stripping of translationally excited ions during the SWIFT isolation. The O-methylbradykinin $[M + H]^+$ ions formed in this manner were isolated and cooled by a short pulse of He gas and their deuterium incorporation and rate constant distribution determined (Fig. 6). The $[M + H]^+$ ion of O-methylbradykinin exchanges all 17 of its exchangeable hydrogens very rapidly with D_2O. But because of its low initial relative abundance, no signal could be observed after 10 min of gas phase H/D exchange with D_2O. In contrast, the $[M + 2H]^{2+}$ did not undergo any appreciable exchange with D_2O even after a reaction period of one hour. In fact, of all the electrospray-ionized bradykinins examined here, the $[M + 2H]^{2+}$ ion of bradykinin exchanged the least with D_2O (Fig. 7).

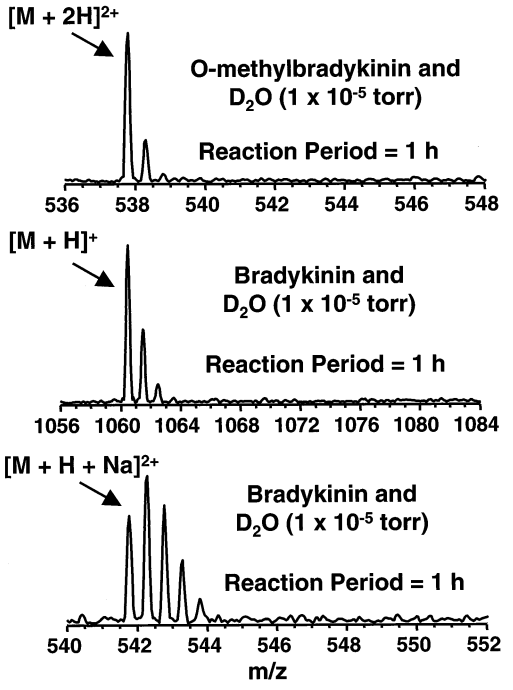

Fig. 7. ESI FTICR mass spectra following 1 h of gas-phase H/D exchange of bradykinin $[M + 2H]^{2+}$ (top), bradykinin $[M + H]^{+}$ (middle), and bradykinin $[M + H + Na]^{2+}$ (bottom) with D_2O (1×10^{-5} Torr).

3.4. Evidence for peptide zwitterions in the gas phase

It is universally accepted that amino acids and proteins exist as zwitterions in aqueous solution. However, it is worth noting that glycine does *not* behave as a zwitterion in the gas phase, provided that glycine is introduced into the gas phase as a *neutral* molecule [35]. Of course, a proton added to a neutral gas-phase glycine molecule will attach to the terminal amine to generate a singly-charged (nonzwitter) cation. However, glycine electrosprayed from aqueous solution presumably begins as a zwitterion; during the electrospray ionization process the proton adds to the carboxyl terminus to yield the same singly-charged (nonzwitter) gas-phase cation. For larger peptides/proteins the proton need not necessarily attach to the carboxyl terminus but may attach to another basic site within the molecule, as for a peptide such as bradykinin in which the solution-phase zwitterion contains three sites more basic than the carboxylate anion: the guanidino group on each of the two arginines (gas-phase basicity, $\Delta G_b^{\circ}(\mathrm{Arg}) = 245.2$ kcal mol^{-1}) and the *N* terminus.

Schnier et al. have previously inferred that protonated bradykinin does form a zwitterion based on the observed difference in the activation energy for blackbody infrared dissociation (BIRD) of bradykinin $[M + H]^{+}$ and O-methylbradykinin $[M + H]^{+}$ [37]. Ion mobility collision cross sections [38] proved inconclusive in determining whether bradykinin $[M + H]^{+}$ exists as a zwitterion. Recently, we have been able to resolve multiple peptide conformations that exhibit similar collision-cross sections based on their reactivity toward H/D exchange [44]; therefore, the present experiments afford a direct test for the presence of peptide zwitterions, based on comparison of H/D exchange rates for a peptide and its O-methylated derivative.

Of all the naturally occurring amino acids, arginine has the largest gas-phase basicity [33]. Furthermore, there is strong evidence to suggest that positive charge is localized on the arginine residues in protonated peptides. Dongre et al. examined the surface-induced collisional dissociation (SID) fragmentation efficiencies of singly- and doubly-protonated des-Arg-bradykinins [50]. They proposed that for the doubly-protonated species, the first charge is localized on the arginine and the second is located on a less basic group which, under high-energy collision-induced dissociation conditions, can be “mobile” throughout the peptide. The addition of a second arginine residue resulted in an increase in the dissociation threshold energy, suggesting that the second charge was also localized on the second arginine. That study provided the first evidence for a bradykinin zwitterion. The singly charged species may in fact be present as a gas-phase zwitterion in which the *N* terminus and arginine residue are both protonated. A second proton would be expected to bind to the acidic carboxyl anion giving rise to a decrease in SID threshold energy. If des-Arg-bradykinin $[M + H]^{+}$ ions are zwitterionic in the gas phase then bradykinin $[M + 2H]^{2+}$, which possesses an additional protonated ar-

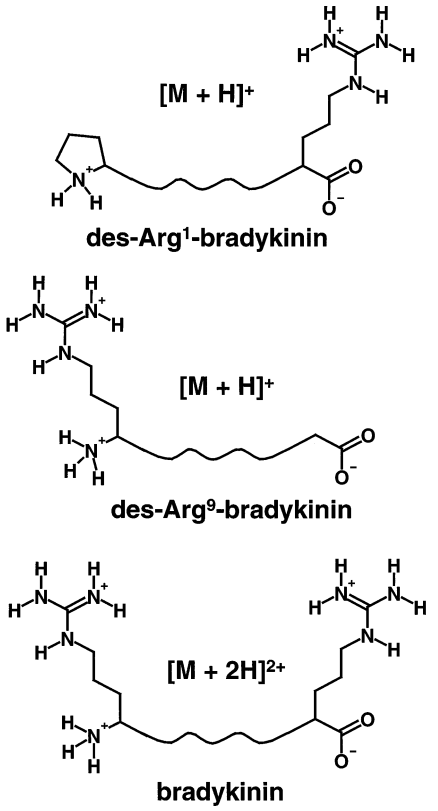

Fig. 8. Structures for des-Arg^1-bradykinin $[M + H]^+$ (top), des-Arg^9-bradykinin $[M + H]^+$ (middle), and bradykinin $[M + 2H]^{2+}$ (bottom). The arginine residues are protonated in each case.

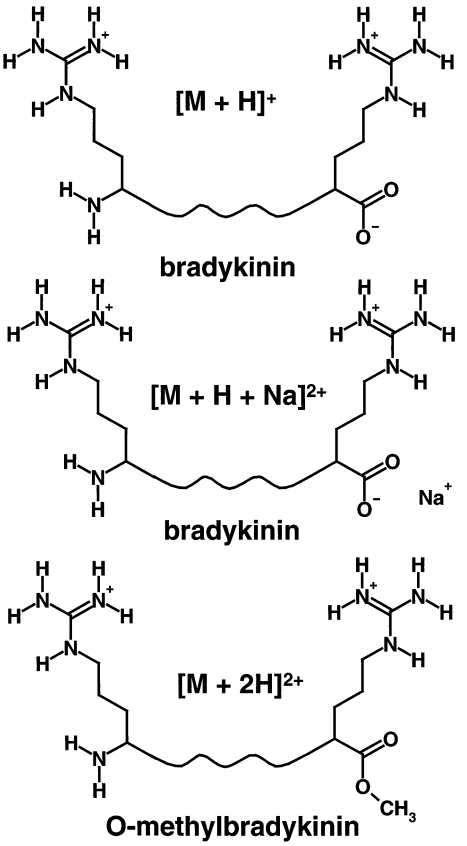

Fig. 9. Structures for bradykinin $[M + H]^+$ (top), $[M + H + Na]^{2+}$ (middle), and O-methylbradykinin $[M + 2H]^{2+}$ (bottom). Both arginines are protonated in each case.

ginine group, should also have similar reactivity (Fig. 8). Those species do possess some similarity in reactivity with D_2O, but the difference in location of the arginine residue generates enough difference in reactivity to prevent a conclusive interpretation. A more reasonable comparison of the reactivity between putative zwitterionic and nonzwitterionic species is therefore provided by comparing H/D exchange for protonated bradykinin and its doubly protonated methyl ester.

For protonated bradykinin the localization of charge on the arginine would suggest that the protonated species may exist as either a zwitterion in which both arginines are protonated or a species in which only one arginine and the carboxyl group are protonated. However, for O-methylbradykinin, formation of a zwitterion is blocked at the C terminal carboxylic acid by the presence of the methyl group; thus, for O-methylbradykinin $[M + 2H]^{2+}$ ion, the charge must be located on the arginines. Because O-methylbradykinin $[M + 2H]^{2+}$ reacts with a rate and extent very similar to that for bradykinin $[M + H]^+$, we infer that the two species are structurally similar. Although the two species may indeed both be present as zwitterions, they would not necessarily have similar conformations. In fact, from the present data, the conformations of these species cannot be determined. Calculations have shown that protonated bradykinin adopts a salt-bridged conformation whereas the doubly charge O-methylbradykinin might be elongated to reduce Coulomb repulsion. Finally, bradykinin [M +

$H + Na]^{2+}$, although not necessarily zwitterionic, should also exhibit similar structure (i.e. localization of protons on its two arginines), based on its H/D exchange reactivity. The species shown in Fig. 9 represent zwitterionic species of bradykinin and its methyl ester in which the charge is localized on the arginines.

3.5. From solution to the gas phase

The extent to which solution-phase peptide and/or protein structure is retained upon removal of solvent to yield a gas phase structure is a matter currently in great debate. It is generally acknowledged that solution phase conditions can profoundly influence the observed mass spectrum. Katta and Chait have shown by solution phase H/D exchange and ESI mass spectrometry that the native and denatured forms of bovine ubiquitin in solution produce quite different mass spectra [51]. But the issue still remains as to what happens to a peptide/protein once the solvent interactions are removed.

Ion-mobility mass spectrometry and gas-phase H/D exchange offer complementary insight into this issue. In a recent study on the H/D exchange of the protein, ubiquitin, we observed multiple gas-phase conformations [34] correlating closely with independently measured ion mobility collision cross sections for different conformations of the same protein [27]. Also, detailed comparisons have also been undertaken on the gas-phase conformations of cytochrome *c* [22–26]. The present study provides strong evidence for the existence of gas-phase zwitterions that originate in solution.

4. Conclusions

The present gas-phase H/D exchange experiments combined with ion dissociation experiments strongly suggest that singly- and possibly doubly-charged bradykinin behaves as a zwitterion in the gas phase. For example, we observe very similar H/D exchange rate and extent for O-methylbradykinin $[M + 2H]^{2+}$ (in which both arginines are protonated) and bradykinin $[M + H]^{+}$ (in which both arginines are protonated only if the ion is zwitterionic). Moreover, bradykinin $[M + H]^{+}$ and $[M + H + Na]^{2+}$, along with the $[M + 2H]^{2+}$ ion of O-methylbradykinin all exhibit similar reactivity with D_2O, suggesting similar structures in which both arginines are protonated. Thus, bradykinin $[M + H]^{+}$ ion must exist as a zwitterion. From the nature of the gas-phase H/D exchange technique, it is difficult to infer what conformation any of these species may adopt. The conformations of these species remains to be investigated further by molecular orbital calculations and also ion-mobility mass spectrometry.

The present results further demonstrate the usefulness of gas-phase H/D exchange techniques for understanding peptide and protein gas-phase structure and conformation. Supplementation by other gas-phase techniques (ion-mobility mass spectrometry, collision-induced dissociation, surface-induced dissociation, BIRD) as well as molecular modeling and solution-phase NMR, promises to extend our understanding of the effect of the removal of solvent on protein structure.

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