

# Gas-Phase Reactions of Bradykinin Ions with Hydroiodic and Deuterioiodic Acid

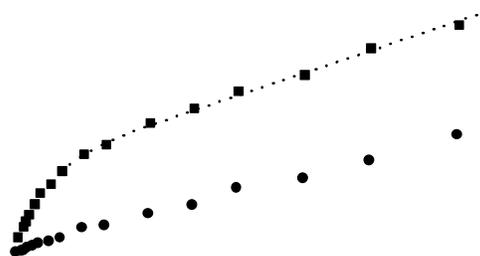
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Gas phase reactions between HI or DI and selected bradykinin (and analog) ions were performed in a quadrupole ion trap mass spectrometer. The bradykinin (BK, *RPPGFSPFR*) ions were generated by electrospray ionization, mass selected and then stored in the presence of HI or DI for various reaction times before mass analysis of reactant and product ions. The suite of ions studied included, BK (M+H)<sup>+</sup>, BK (M+2H)<sup>2+</sup>, des-Arg<sup>n</sup> BK (M+H)<sup>+</sup> (n=1 and 9) and the O-methylester (BK-Me) of each. 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.<sup>1</sup> Consequently, the BK (M+nH)<sup>n+</sup> ion, where n is 1 or 2, is reactive, but when n = 3, the ion population was found to be unreactive even at HI pressures as high as 2 x 10<sup>-5</sup> 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)<sup>3+</sup> 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 (M+3H)<sup>3+</sup> ion illustrates that H/D exchange may provide information concerning fully protonated ion structures.

**HI and DI Attachment** Under the experimental conditions used in these studies pseudofirst-order kinetics prevail, and the reaction rate constants can be derived from the depletion of the reactant ions intensity as a function of time. The attachment rate constants ranged from 9.3 x 10<sup>-11</sup> cm<sup>-3</sup>-s<sup>-1</sup> for attachment of HI to BK (M+H)<sup>+</sup> to << 1 x 10<sup>-12</sup> for HI attachment to a slow-reacting population of des-Arg<sup>9</sup> BK (M+H)<sup>+</sup> ions. Accumulation and subsequent storage of the HI addition product ions for extended periods showed no evidence for the reverse reaction (dissociation), thus, ions in this study did not approach equilibrium. Methylation of the bradykinin and analogs typically resulted in a 30 – 50% decrease in attachment rates, except des-Arg<sup>9</sup>. In the case of des-Arg<sup>9</sup> BK (M+H)<sup>+</sup>, ~90% of the population is unreactive towards attachment, and the remaining population reacts extremely slow, but upon methylation, the ion population reacts with a single rate comparable other BK ions.

When the doubly protonated ions of BK and BK-Me are reacted with HI, the depletion of the (M+2H)<sup>2+</sup> ion intensity does not follow linear pseudofirst-order kinetics (squares, Figure 1). Instead, the ion depletion data, [-ln(I/I<sub>0</sub>)] vs. time, is non-linear during the first 400 ms and then becomes linear from that point until the (M+2H)<sup>2+</sup> ions are completely depleted at much longer reaction times. The data is consistent with the initial BK (M+2H)<sup>2+</sup>



ion population being comprised of non-interconverting (at room temperature) fast- and slow-reacting components. By exploiting the difference in reaction rate constants for the two populations, isolation of the slow-reacting population was achieved through a second isolation of the  $(M+2H)^{2+}$  ion, after the fast-reacting population was depleted during a 400 ms reaction. Thus, the depletion of the slow-reacting population (circles, Figure 1) could be monitored separately, and its rate constant determined independently. Mild collisional activation of the slow-reacting ion population (excitation of ion below the dissociation threshold) caused the slow-reacting population to convert to a population of ions with similar reactivity to those obtained directly from electrospray (i.e. a mixture of fast- and slow-reacting ions).

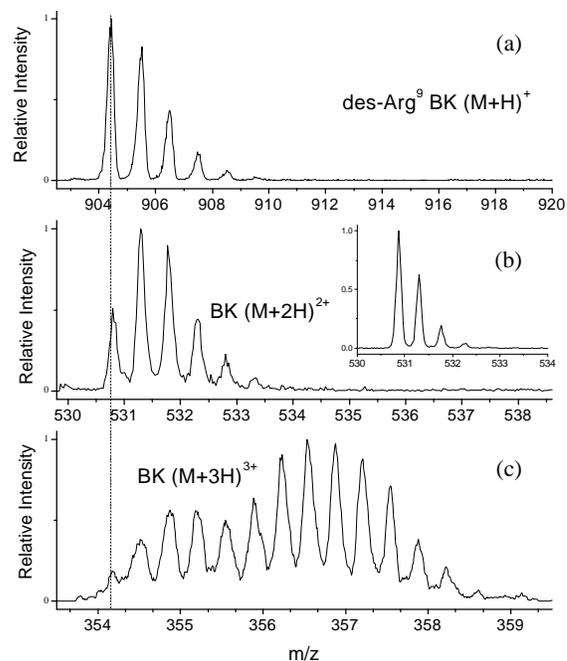
**DI H/D Exchange** Regardless of attachment rates, all ions were reactive towards gas phase H/D exchange with DI at some nominal rate. However, the H/D exchange reactions of ions with high HI/DI attachment rates (e.g. BK  $(M+H)^+$ , des-Arg<sup>1</sup> BK  $(M+H)^+$ , BK  $(M+2H)^{2+}$ ) could not be monitored over extended periods due to the formation of the DI attachment product. The slow- or non-reactive ions (with respect to attachment), however, can be used to follow the extent of H/D exchange (Figure 2). The slowest reacting ion with regard to HI or DI attachment, des-Arg<sup>9</sup> BK  $(M+H)^+$  (Fig. 2a), also shows the slowest H/D exchange rate ( $\sim 0.14 \text{ s}^{-1}$ ) when compared to the other bradykinin and analog ions. However, this ion exchanged 8 out of a possible 13 exchangeable hydrogens during a 1 min. storage period ( $P_{\text{DI}} = 1 \times 10^{-5}$  Torr). The BK  $(M+2H)^{2+}$  ions (Figure 2b) exchange at a slightly higher rate ( $\sim 0.57 \text{ s}^{-1}$ ) than the des-Arg<sup>9</sup> 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 seconds of storage. While the BK  $(M+3H)^{3+}$  ion shows no evidence of attachment, the H/D exchange rates for this ion are the fastest among the bradykinin analogs. This ion exchanges 11 hydrogens during a 1.8 s storage time in the presence of DI. In addition, as can be seen in Figure 2c, 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  $\sim 1.1 \text{ s}^{-1}$  and the fast-reacting population at a rate of  $\sim 5.6 \text{ s}^{-1}$  under these conditions.

This work and recent results for HI attachment to ubiquitin ions<sup>2</sup> 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. Since the attachment reaction inherently involves the interaction of two neutral species (HI and basic site), it is expected to be able to provide, at least in some cases, information that complements that derived from physical and other chemical probes, which are largely charge-site mediated.

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<sup>1</sup>(a) Stephenson, J. L.; McLuckey, S. A. *J. Am. Chem. Soc.* **1997**, *119*, 1688. (b) Stephenson, J. L.; McLuckey, S. A. *Anal. Chem.* **1997**, *69*, 281.

<sup>2</sup> Stephenson, J. L.; Schaaff, T. G.; McLuckey, S. A. *J. Am. Soc. Mass Spectrom.* In press.



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