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A review of gas-phase H/D exchange experiments: the protonated arginine dimer and bradykinin nonapeptide systems

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Dedicated to Professor Alan G. Marshall on the occasion of his 60th birthday, in honor and admiration of his outstanding contributions to the advancement of science, particularly the revolutionary discovery and development of FT-ICR, and with special thanks for his friendliness and hospitality.

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Abstract

Some recent gas-phase H/D exchange experiments are discussed. These experiments are carried out using an electrospray ionization-fast flow tube technique. Some of the goals are: (1) deducing site-specific rate constants; (2) learning about conformers and isomers of biomolecules; (3) understanding H/D exchange mechanisms. The experimental technique, and its advantages and disadvantages are reviewed, by comparison with other methods such as FT-ICR. The different methods of analyzing the data are described including a newly developed algorithm for extracting site-specific rate constants. The examples of bradykinin (singly and doubly protonated) and the proton bound dimer of arginine are discussed in some detail. Both systems are known to exist as gas-phase salt-bridge structures in which two arginines are protonated and a carboxyl group is deprotonated yielding an ion–zwitterion configuration: $+ - +$. Some internal inconsistencies between H/D exchange results for ND_3 with the two systems are pointed out.

Keywords: Amino acid clusters; Peptides; H/D exchange; ESI; Flow tube; FT-ICR; Conformers; Site-specific rate constants

1. Introduction

We have chosen the topic of gas-phase H/D exchange experiments for this issue in honor of Alan G. Marshall for several reasons. Professor Marshall pioneered the area of gas-phase H/D exchange using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). The FT-ICR group at Florida State University headed by Alan Marshall has studied quite large biomolecules [1]. He and Marshall have also developed a special kinetic algorithm for determining site-specific rate constants [2]. At the Hebrew University of Jerusalem, we have demonstrated that an electrospray ion source combined to a flow tube reactor can be employed to study ion–molecule reactions of protonated biomolecules including H/D exchange [3]. This led to a collaborative effort between our own group and that of Professor Marshall in the form of a joint US–Israel Binational Science Foundation (BSF) research project on the gas-phase ion chemistry of small biomolecules. This collaboration has resulted in a recent joint publication [4].

There has been an increasing interest in recent years in anhydrous protein and peptide ions [5]. Evidence has been presented [1(a)] that conformational properties of biomolecules in solution are preserved in some cases during the process of electrospray ionization (ESI) that is used in mass spectrometry (MS) to introduce these biomolecules into the gas phase. Conformational changes in proteins were probed by hydrogen-exchange electrospray-ionization mass spectrometry (ESI-MS) [1(a),6]. The generally held idea has been that compact structures protect some labile hydrogen atoms from H/D exchange in the gas phase. As in the case of liquid solutions the reduction in amide hydrogen exchange rates in proteins in the gas phase is attributed to certain hydrogens such as those buried in the hydrophobic core, having little access to the exchange reagent, or hydrogens participating in hydrogen bonding such as those found in α-helices or β-sheets [7]. As a result, open conformers are expected to reach higher levels of exchange than compact ones. It has also been demonstrated [7] that ESI-MS plays a major role

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in studies of non-covalent complexes of proteins. H/D exchange by MS has complemented NMR to give insight into the populations and structures of folding intermediates [8]. Human recombinant [C22A] FK506-bonding protein amide exchange rates from mass spectrometry were found to match and extend those from NMR [1(b)]. ESI was combined with FT-ICR spectrometry and correlations were drawn between specific H/D exchange levels observed in the gas phase and conformations that have been characterized in solution [9]. H/D exchange was studied for shape-resolved cytochrome *c* conformers pre-selected through their drift velocities in gas phase ion mobility experiments [10].

The proper interpretation of H/D exchange experiments requires a detailed knowledge of the reaction mechanisms involved. A large number of studies of H/D exchange between protonated peptides and deuterated solvent molecules were performed with this end in mind and several alternative mechanisms have been proposed [11]. ND_3 was found to be the most efficient reagent studied for promoting H/D exchange. Other reagents studied include D_2O, CH_3OD, CD_3OD, DI, and CD_3CO_2D.

ESI-MS has been known [12] to produce multiply protonated peptides and proteins. The occurrence of 'fractional charging' by which cluster ions of amino acids are formed with more than one solute molecule per charge was discovered as well [13]. Large multimers were observed in the ESI-MS of peptides [14]. The formation of non-covalent supramolecular assemblies plays a critical role in biological systems. The discovery of multimers led to a series of studies of the clustering of the natural amino acids [15,16], the formation of peptide aggregates [17], and the complexation and clustering of proteins [18] under ESI-MS. A fascinating discovery has been [16] that serine undergoes a chiroselective self-directed oligomerization to form a singly protonated octamer. Collision-induced dissociation that was performed led to the suggestion that the protonated octamer is composed of four hydrogen-bonded dimers, stabilized by further extensive hydrogen bonding. Density functional calculations supported this model. Several other groups have been involved in the experimental and theoretical study of the octamer of serine and larger serine clusters, modeling of octamers comprising zwitterionic amino acid units [19] and discussions of the relevance of the data to the origin of life [20]. Several alternative structures of the protonated octamer have been considered [19,20].

Amino acids are known to exist as zwitterions in solution. In the gas phase, however, even arginine (Arg or R), the most basic amino acid, exists in the non-ionic neutral configuration [21]. Calculations for the monomer show [22] that the neutral conformer is energetically slightly more stable than the zwitterionic form. At the same time DFT calculations on the proton-bound dimer of arginine [23] have demonstrated a reversed stability pattern with the salt-bridge or ion–zwitterion form found to be more stable than the simple protonated or ion–molecule form.

In this paper, we will present a short summary of the recent work done at The Hebrew University on gas-phase H/D exchange of small biomolecules with special emphasis on the topics of clusters of amino acids and ion–zwitterion structures. The specific examples that will be covered include the protonated dimer of arginine, $(\text{arginine})_2H^+$, $(2R+H)^+$, and the diprotonated nonapeptide bradykinin, $(BK + 2H)^{2+}$, or $(\text{Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg} + 2H)^{2+}$. A major goal of our recent H/D exchange research was the derivation of site-specific rate constants. Our own work will be discussed in the light of known literature data.

2. Experimental

The electrospray ionization/fast flow apparatus is shown schematically in Fig. 1. It has been described in detail previously [3,24–32]. It consists of a SIFT apparatus that we constructed several years ago and modified to work with an electrospray (ES) source connected directly to the flow tube. The apparatus is made of a flow reactor that is 123 cm in length and an inner diameter of 74 mm. A neutral reagent is introduced into the flow tube through either one of two ring inlets. Tylan mass flow controllers define the flow rate of the neutral reactant into the flow tube. The quadrupole mass analyzer (652601 ABB EXTREL) is housed in a differentially pumped chamber that is separated from the flow tube

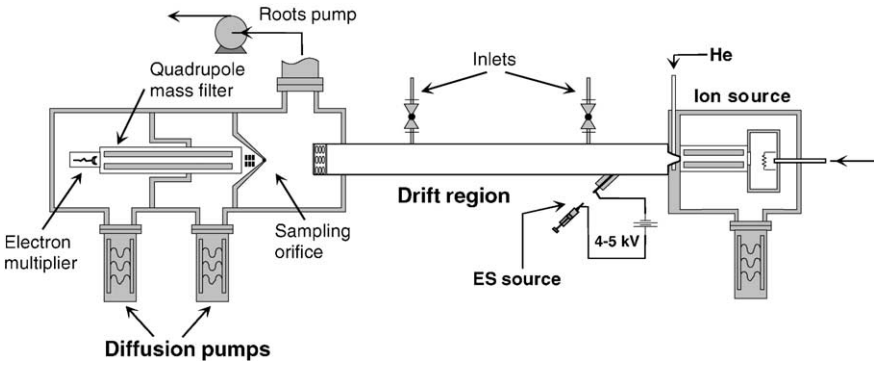

Fig. 1. Schematic drawing of the experimental setup combining electrospray (ES) ionization with a fast flow technique. The ion source and quadrupole mass spectrometer of the SIFT part of the apparatus are not used in the present series of experiments.

by a nose cone (NC) skimmer with a 1.0 mm sampling orifice. A small NC voltage is used for focusing ions into the analysis quadrupole. Helium buffer gas enters the flow tube at the upstream end through another Tylan flow controller. It is pumped through the tube by a Roots blower.

The electrospray ion source was designed as follows. A capillary tube serves as the interface between the electrospray and the helium flow reactor. A stainless-steel tube 15 cm in length and 0.05 cm i.d. is employed. The entire assembly is inserted into the flow tube at a distance of ∼96 cm from the sampling orifice, 135° to the direction of the helium flow, through an 'O'-ring type vacuum fitting. The capillary introduces an air leak into the flow tube with a pressure that has been remeasured recently of 0.065 Torr and a flow rate of 1 l/min (STP); these numbers have to be added to the helium pressure and helium flow rate when calculating rate constants. The experiments are carried out at total flow velocities of ∼6000–7000 cm/s leading to typical flow tube pressures of ∼0.1–0.35 Torr and reaction times of several milliseconds.

Ions are electrosprayed ∼5 mm through ambient air into the grounded capillary tube from a non-conductive capillary made of fused silica tubing i.d. 50 μm biased at 5–6.5 kV dc. The contact between the power supply and the solution is provided by a metallic union some distance from the spray capillary tip. A large series resistor (∼10 GΩ) was placed between the power supply and the contact to the ESI to increase stability [33]. Dilute solutions of the biomolecule under study in a polar solvent are delivered to the electrospray needle at flow rates of 1–5.5 μl/min from a 1 ml syringe mounted on a model 100 KD Scientific Syringe Pump.

From the point of view of gas-phase H/D exchange the instrument has several experimental drawbacks compared to the FT-ICR [1]. The first is the relatively poor mass resolution of the quadrupole mass filter. For example, singly protonated arginine ions with and without reaction with ND_3 demonstrate isotopic multiplets that are separated by whole units of mass-to-charge ratio, *m/z* (Fig. 2a and b), that are easily resolved [30]. However, doubly protonated bradykinin ions that have isotopic multiplets separated by 0.5 *m/z* units are already only partially resolved (Fig. 2c) [25]. Furthermore, monoisotopic ions can be isolated for H/D exchange in the FT-ICR by stored waveform inverse Fourier transform (SWIFT) excitation [4] whereas the flow-tube experimental data need to be deconvoluted isotopically [25]. An additional drawback is that it is rather difficult to observe in the flow tube many consecutive H/D exchanges. We have carried out simplified calculations of collision numbers. In the case of $(BK+2H)^{2+}$, we adopted the collision cross section from ion mobility experiments and assumed an average thermal velocity. The calculation indicates that an ion undergoes about 1600 collisions with the deuterating reagent in the FT-ICR experiment for a time $t = 100$ s at a pressure of 5×10^{-7} Torr. In order to get the same number of collisions over a period of 13 ms in the flow tube experiment a deuterating reagent flow velocity of $\sim 35 \times 10^{18}$ molecules/s is required. The pressure in the FT-ICR experiment can be

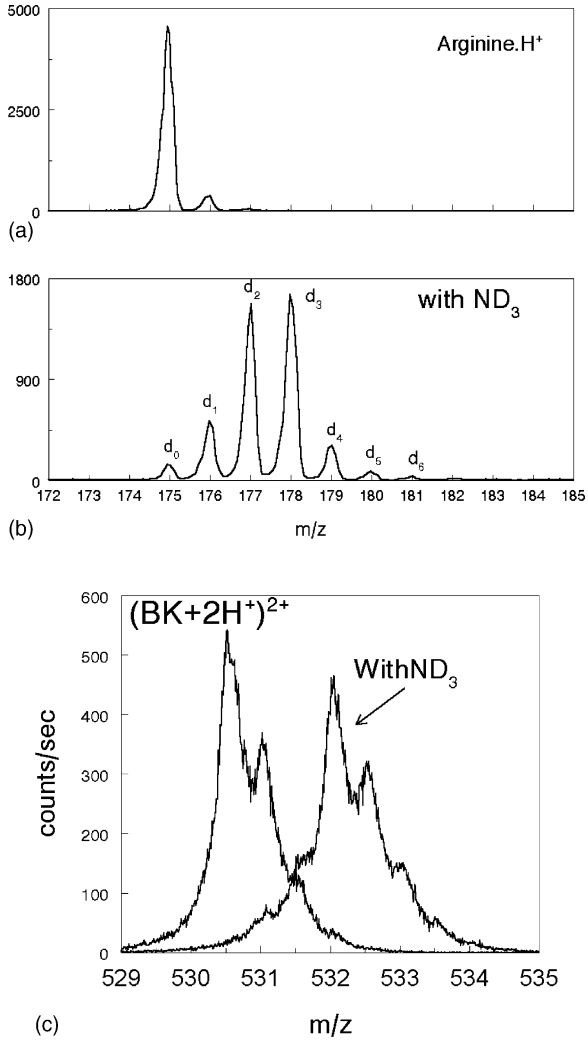

Fig. 2. Mass spectra (ion counts as a function of the mass-to-charge ratio, in Thomson units) obtained by the quadrupole detector for (a) the protonated monomer of arginine, (b) the protonated monomer following H/D exchange with ND_3 at a flow rate of 5.3×10^{18} molecules/s and a reaction time of 11.7 ms, and (c) for the reactant ion of the doubly protonated bradykinin and for the product following partial deuterium exchange by ND_3. The spectra are uncorrected for ^{13}C or ^{15}N isotopic contributions.

increased and the reaction time can be extended. However, the maximum available flow rate of the deuterating reagent that determines its concentration in our flow tube experiment is limited; furthermore the tube length and the carrier gas velocity limit the reaction time.

On the other hand, it is well known that flow tube experiments furnish truly thermal conditions. Also, since the concentration of the neutral is quite accurately known, second order rate constants are deduced experimentally. It is very difficult to know the exact pressure of the neutral deuterating reagent in the FT-ICR experiment. As a result, the pseudo-first-order rate constants deduced for H/D exchange reactions are often not converted to second order rate constants. Working on the same system (the arginine dimer) using FT-ICR as well as the fast flow technique [4] has

demonstrated the right conditions for the flow-tube system to get the same overall degree of H/D exchange as under FT-ICR. Working on the same system has also provided a cross check of the rate constants and an indirect measure of the ion source pressure in the FT-ICR experiment [4].

3. Data analysis

The undeuterated ion and its deuterated isotopomers each contribute a multiplet of overlapping isotopic peaks due to the natural abundances of the various carbon, nitrogen, oxygen, and hydrogen isotopes (see Fig. 2). The first step in the analysis of the flow tube mass spectral data is the isotopic deconvolution [25]. This step is not necessary for FT-ICR experiments as noted earlier, but can be applied when the relative abundance of single isobars to be isolated through SWIFT is very low [34]. Let the abundances of the various hydrogen/deuterium isotopomers be given by m, $m+1$, $m+2, \ldots, m+n$. If the peak shape of the multiplet given by the undeuterated isotopomer (e.g., arginine·H^+; Fig. 2a) is $f(m)$, then the peak shapes of the other hydrogen/deuterium isotopomers will be $f(m+1)$, $f(m+2), \ldots, f(m+n)$. These multiplets will be identical with the undeuterated multiplet but displaced 1, 2, and n mass units (or, in the case of doubly charged ions, Fig. 2c, 0.5, 1, and $n/2$ units). The overall peaks as measured will be the sum of these multiplets multiplied by their abundances, that is [25], $\sum(m+n)f(m+n)$, where the summation is over n. The function $f(m)$ may be established experimentally from runs at zero flow rate, e.g., Fig. 2a for arginine·H^+ and Fig. 2c—multiplet labeled $(BK+2H^+)^{2+}$—for doubly protonated bradykinin. The proportions of the deuterated isotopomers contributing to the products of the reaction may be deduced by combining these distributions in different proportions to give the minimum sum of squares deviation from the experimental results [25]. An alternative approach is the maximum entropy method (MEM) applied by Marshall and coworkers [34] to FT-ICR data.

Once the spectra have been deconvoluted, one obtains a set of curves for consecutive deuterium exchanges, as a function of flow rate of the deuterating agent in the flow tube or, in the case of FT-ICR, as a function of reaction time. Simulation of the kinetic data by solution of the associated set of coupled differential equations yields a set of apparent rate constants, however it is the set of site-specific rate constants that are of interest [2,11(g,j,k)]. The first algorithm that we have adopted [25,26,28,30], based on a Modelmaker program of Cherwell Scientific, solves a set of independent simultaneous differential equations for a suggested reaction mechanism by the Runge–Kutta method. Lebrilla and coworkers have used a similar approach [11(g)]. If one sets up a sequence of reactions both parallel and sequential, the program will predict the concentrations of the various intermediates and products if given the appropriate rate constants. Conversely, given experimental data, it will determine the optimum rate constants by one of the standard non-linear regression methods. He and Marshall [2] have used a weighted quasi-Newton algorithm for function minimization and a variable-order, variable-step Adams algorithm for ordinary differential equations to solve site-specific rate constants. The methods were shown to be internally consistent [25] because the rate constants that emerged for the glycine/D_2O system were similar.

Green and Lebrilla [11(k)] have pointed out that these treatments are computationally intensive to the point where they become impractical for more than about 15 exchanges. We found indeed that the approach broke down when we tried to apply it to the arginine dimer problem; we were unsuccessful in getting a simulated fit to the 15 consecutive exchanges observed for the reaction of ND_3 with the protonated dimer [30].

A new algorithm has been developed for extracting site-specific rate constants for H/D exchange and has successfully been applied to the arginine dimer problem [4]. Consider a species (amino acid, peptide or cluster) with N replaceable hydrogens. Each molecule is represented by an N bit binary number, where 1 represents deuterium and 0 represents hydrogen. In the H/D exchange system, it is assumed that each of these protons is replaced independently in a first order process with rate constants $k_1, k_2, \ldots, k_n, \ldots, k_N$.

The probability $P_{n,t}$ that the nth hydrogen will have been replaced by deuterium after time t is given by:

$$P(\mathrm{D})_{n,t} = 1 - \exp(-k_n t)$$

The probability that the nth hydrogen will not have been replaced is then:

$$P(\mathrm{H})_{n,t} = \exp(-k_n t)$$

The overall probability of any structure occurring is the product of the individual probabilities, $P(\mathrm{D})_{n,t}$ and $P(\mathrm{H})_{n,t}$ selected according to whether the digit in the corresponding binary number is 1 or 0.

For example if $N = 5$, the probability P_t of the structure core-HHDDH (binary equivalent 00110) is given by:

$$\begin{aligned} P_t &= P(\mathrm{H})_{1,t} \times P(\mathrm{H})_{2,t} \times P(\mathrm{D})_{3,t} \times P(\mathrm{D})_{4,t} \times P(\mathrm{H})_{5,t} \\ &= \exp(-k_1 t) \times \exp(-k_2 t) \times (1 - \exp(-k_3 t)) \\ &\quad \times (1 - \exp(-k_4 t)) \times \exp(-k_5 t) \end{aligned}$$

Summation of the digits of the binary number gives the number of deuteriums in the intermediate, and the probabilities of structures containing the same numbers of deuteriums can be summed to give an overall probability for that number of replacements.

The experimental profiles for the abundance of species containing 0, 1, 2, …, n, …, N deuterium atoms after various reaction periods (for the FT-ICR experiment) or flow rates (for the flow-tube experiment) are then normalized and compared with the above calculation of the predicted probabilities at the same flow rates. Fitting the experimental

curves by a theoretical model can be done by minimization of the distance between the theoretical and experimental profiles. The algorithm minimizes the mutual entropy or the Kullback–Leibler (KL) [35] information divergence between the observed concentrations and the model.

The algorithm can treat the data for a single conformer. Extensions of the present computer code and/or new algorithms need to be developed for cases where two or more conformers are present.

4. Bradykinin

Several studies of gas-phase H/D exchange of singly, doubly, and triply protonated bradykinin (BK) and of several BK derivatives have appeared in the past [1(c),11(a,o–q),36]. Experiments were carried out at the relatively low pressures of FTICRs and ion traps (10^{-7}–10^{-5} Torr) as well as in the fast flow experiment [25] at relatively high pressures (0.1–0.4 Torr, comprised mainly of the helium carrier gas). Intermediate pressures of a few mTorr were used more recently in a linear ion trap (LIT) [37].

Singly protonated BK ions do not undergo H/D exchange in FTICR experiments with either ND_3 [11(a)] or D_2O [1(c)]. It has been assumed that the high proton affinity of the end arginine groups and the compact shape of the zwitterionic structure prevent the exchange from taking place. A model was developed [11(o)] describing the deuterium uptake of an ensemble of $(BK + H)^+$ gas-phase ions which are exposed to D_2O vapor. However the earlier reports on H/D exchange with D_2O [11(o),38] are now known to be due to quadrupolar axialization in the FTICR experiment to keep the ions centered in the cell. This axialization converted magnetron motion into cyclotron motion and heated the ions enough to produce H/D exchange that would not have occurred at room temperature (A.G. Marshall, private communication, August 2000). Intermediate pressures provided new evidence for H/D exchange due to an increased number of collisions in the trap [37]; however, the singly protonated ion is considerably less reactive than the doubly and triply protonated BK.

There are 19 exchangeable hydrogens in $(BK + 2H)^{2+}$—3 at the N-terminus, 10 guanidine (arg^1, arg^9), 1 hydroxy (ser^6), and 5 amide hydrogens. The amine end group is protonated in addition to the two arginines in the zwitterionic di-protonated BK (see Fig. 8 of Ref. [1(c)]) leading to three equivalent labile hydrogens at the N-terminus not available in the singly protonated BK. Doubly protonated BK, $(BK + 2H)^{2+}$, is known to undergo exchange in FTICR experiments with CH_3OD [11(q)] and with D_2O [1(c)]. Three hydrogens were exchanged with CH_3OD with similar site-specific rate constants [11(q)] whereas 16 fast exchanges and 3 slow ones were observed for D_2O over an extended period of 1 h at a relatively high pressure of 1×10^{-5} Torr [1(c)]. We reacted $(BK + 2H)^{2+}$ in the flow tube with either ND_3 or CH_3OD and monitored the incorporation of deuterium as a function

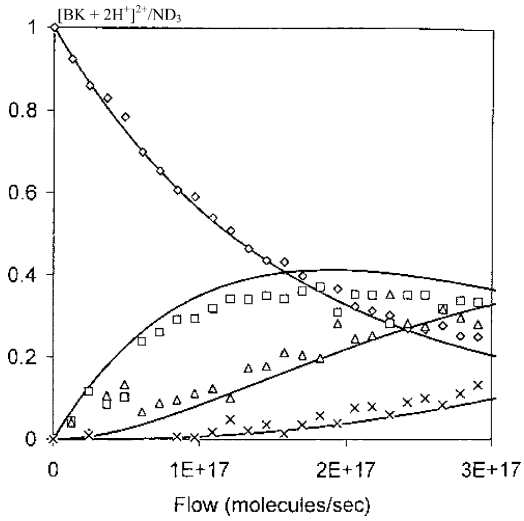

Fig. 3. Relative abundance vs. flow rate of the neutral deuterating reagent for various cations in the reaction of $[BK + 2H^+]^{2+}$ with ND_3. Symbols are experimental with, ◇, □, Δ, and × representing species in which zero, one, two, and three hydrogens have been replaced by deuterium, respectively. The curves are the simulated fits for derivation of site-specific rate constants.

of flow rate of the deuterating reagent at a constant reaction time [25]. The exchange of three labile hydrogens was clearly observed for CH_3OD in agreement with Green and Lebrilla [11(q)]. These are proposed to be the three equivalent labile hydrogens at the N-terminus. Three labile hydrogens of BK are also observed to exchange in the flow tube experiment with ND_3 at relatively low flow rates (Fig. 3; [25]). There is evidence for a minor contribution from an additional exchange of a fourth labile hydrogen. The rate and extent of gas-phase H/D exchange of BKs furnished evidence for the existence of zwitterions (i.e., salt-bridge structures) in the gas phase [1(c),25]. The C-terminus is deprotonated in the suggested salt-bridge structure (Fig. 8 of Ref. [1(c)]). The rate data for $(BK + 2H)^{2+}$ indicated the presence of at least two non-interconverting ion populations [11(p)]. At least three conformers of the $(BK + 2H)^{2+}$ ions were observed [37] in the reaction with 5.7 mTorr CD_3OD (with an added nitrogen base pressure of 1.7 mTorr) over a trapping period range of 0–80 s.

Contrary to the case of GLY_2H^+ [3(a)], for which we were able to observe the formation of collisionally stabilized complexes with NH_3, analogous complexes of doubly protonated BK with ammonia could not be collisionally stabilized [25]. A different mechanism for H/D exchange may be operative, leading to a large number of exchanges [37] but at relatively slow rates so that the small number of collisions in the flow tube experiments we have performed so far [25] was not enough to observe these consecutive reactions. The more efficient exchange observed for des-Arg^9-bradykinin (dA^9BK) [25] is accompanied by formation of collisionally stabilized complexes between doubly protonated dA^9BK and ND_3 at a He carrier gas pressure of about 0.2 Torr. Com-

Table 1
Apparent, site-specific, and overall H/D exchange rate constants (cm^3/molecule s) for $(BK + 2H^+)^{2+}$

$k_{apparent}$	$k_{site\text{-}specific}$	$\sum k_{site\text{-}specific}$	$k_{semilog\ plot}$
CH_3OD			
5.0×10^{-11}	1.6×10^{-11}	4.8×10^{-11}	4.3×10^{-11}
3.3×10^{-11}	1.6×10^{-11}		
1.9×10^{-11}	1.6×10^{-11}		
ND_3			
1.44×10^{-9}	9.2×10^{-10}	1.53×10^{-9}	1.31×10^{-9}
7.1×10^{-10}	3.0×10^{-10}		
3.7×10^{-10}	2.9×10^{-10}		
	1.8×10^{-11}		

plexation is apparently made possible in this case in view of the less compact structure of dA^9BK compared to BK itself and takes place via hydrogen-bonded intermediates that promote H/D exchange.

Table 1 summarizes the flow tube rate data for BK from Ref. [25]. The sum of the site-specific rate constants is observed to be nearly equal to the rate constant derived from a semilogarithmic plot of the pseudo-first-order decay of the unexchanged reactant ion (Table 1). This fact and the quality of the fit (Fig. 3) are good indicators for the reliability of the derived site-specific rate constants.

5. The proton bound dimer of arginine

The protonated monomer of arginine (R), that is known to be non-zwitterionic in the gas phase, exchanges efficiently with ND_3 a maximum of four hydrogens out of the eight labile ones [11(l),30]. Derivation of site-specific rate constants for this system [30] demonstrated that the bulk of the reactivity of ND_3 with the protonated monomer is centered at a single site. This has a site-specific rate constant, $k_{site\text{-}specific} = 2.0 \times 10^{-9}$ cm^3/molecule s whereas the sum over all the site-specific rate constants is $\sum k_{site\text{-}specific} = 2.1 \times 10^{-9}$ cm^3/molecule s. Ammonia/R·H^+ complexes were observed in the flow tube experiment. The exchange data were interpreted [30] by ammonia stabilizing the zwitterion structure of the protonated arginine monomer by forming a salt-bridge complex, $C(NH_2)_2^+$-NH-CH_2-CH_2-CH_2-CH(NH_2)-COO^--NH_4^+. We found that the proton bound dimer of arginine exchanges with ND_3 all of its 15 labile hydrogen atoms. A "relay" mechanism was suggested to explain the results [30] for the salt-bridge ion–zwitterionic structure of the dimer, $C(NH_2)_2^+$-NH-CH_2-CH_2-CH_2-CH(NH_2)-COO^--$C(NH_2)_2^+$-NH-CH_2-CH_2-CH_2-CH(NH_2)-COOH [23] since there is a carboxylate group onto which the proton can be transferred. This allows the exchange of all the guanidino hydrogens that are not exchanged in the monomer.

Bimodal deuterium distributions were observed under some conditions in the mass spectra of exchanging populations of the arginine dimer indicating, as in the case of $(BK + 2H)^{2+}$ [11(p),37], the presence of two non-interconverting ion populations. An example is shown in Fig. 4 These data were explained by assuming the coexistence of the two isomeric protonated dimer structures: the ion–zwitterion form and the ion–molecule form [30].

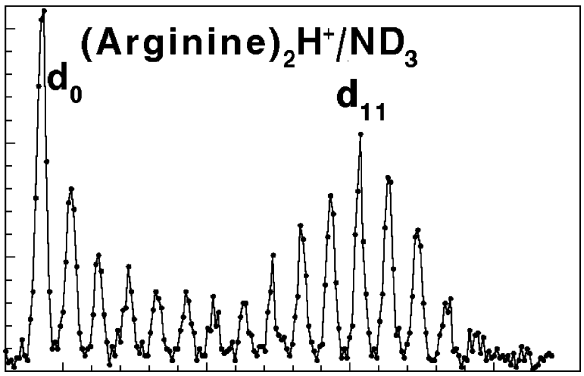

Fig. 4. Mass spectra of the protonated dimer of arginine with ND_3 flow; reaction time is 11.4 ms; ND_3 flow: 4.46×10^{18} molecules/s; ND_3 concentration: 1.44×10^{13} molecules/cm^3.

Sets of profiles for consecutive deuterium exchanges as a function of the ND_3 flow rate were obtained (Fig. 5). We have applied the newly developed algorithm for extracting site-specific rate constants for H/D exchange to the data. A single high site-specific rate constant, $\sim 2 \times 10^{-11}$ cm^3/molecule s, and 14 low ones, $\leq 6 \times 10^{-12}$ cm^3/molecule s, are in agreement with the gas-phase ion–zwitterion salt-bridge structure of $(2R + H)^+$, that has a single carboxyl hydrogen, 4 amine hydrogens and 10 guanidino hydrogens. The single relatively fast rate is ascribed to the lone carboxyl hydrogen. Thus, whereas arginine monomer is neutral, the protonated dimer is zwitterionic [4,23].

There is a remaining apparent internal inconsistency between the bradykinin and arginine data. Whereas the protonated R dimer, whose stable conformer has the ion–zwitterion structure, undergoes exchange with ND_3 of all of its 15 labile hydrogens, the singly protonated BK ion, that has similarly a salt-bridge, ion–zwitterion conformation as its most stable structure [23], does not [11(a)]. It has been pointed out by Beauchamp and coworkers [11(a)] that the so-called 'onium' ion H/D exchange mechanism, by which ammonia forms an ammonium complex with a positively charged center that is then stabilized by solvation, would require that the favorable salt-bridge be disrupted. However, the so-called 'relay' mechanism [11(a)], that has been suggested for the exchange mechanism of the protonated R dimer with ND_3 [30], can also operate for the salt-bridge conformer of BK, since there is a carboxylate group onto which the proton can be transferred. As a result, all the guanidino hydrogens should be exchangeable in protonated BK, albeit at a slow rate of $\leq 5 \times 10^{-12}$ cm^3/molecule s. This mechanism may be the reason why H/D exchange has been observed for protonated BK with D_2O, under conditions allowing many successive collisions to take place [37].

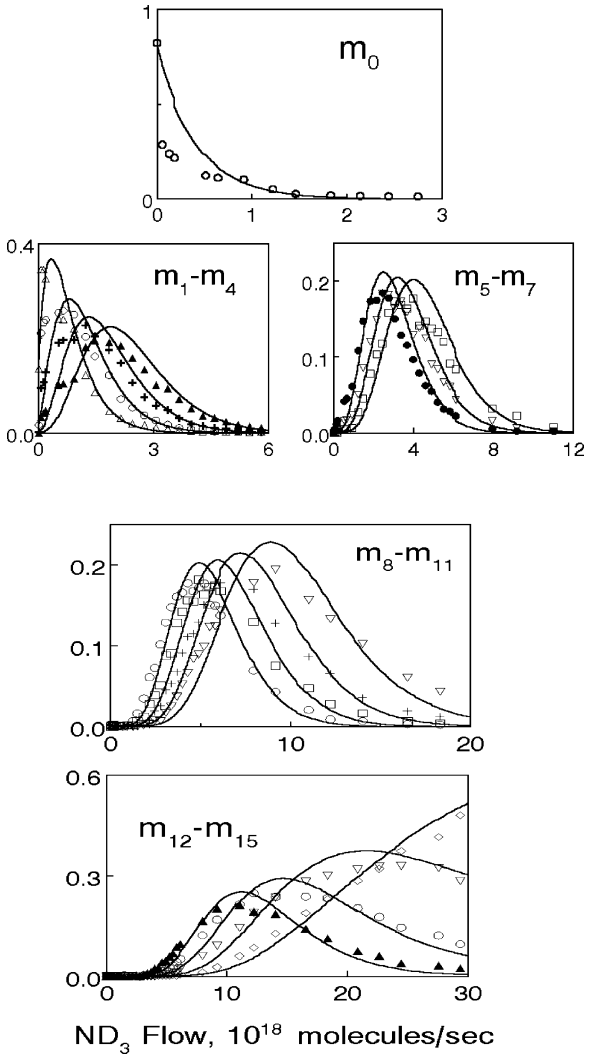

Fig. 5. Relative abundance vs. neutral flow rate in molecules/s for the various indicated cations for the 15 consecutive exchanges in the reaction of the protonated arginine dimer, $(2R + H)^+$, with ND_3. The reaction time is 12.24 ms; helium carrier gas flow: 4.9 l/min; flow tube pressure: 0.286 Torr. The points are experimental; the continuous curves are the results of the simulation based on the algorithm that minimizes the KL divergence. The results are presented as m_0, m_1, etc. because the ions contain contributions from ^{13}C, ^{15}N, and other natural isotopes in addition to the H and D isotopes. Convolutions with the natural isotopic abundance and the experimental peak shape were carried out for comparison of the simulated data with experiments.

The prevailing H/D exchange mechanisms need to be understood better. For example, do the intricate differences between $(BK + H)^+$ and $(2R + H)^+$ play a role in the H/D exchange? The former is a nonapeptide with seven intermediate amino acids covalently binding the two end R groups whereas the latter is an electrostatically and hydrogen-bonded cluster of the two arginines that has an additional carboxyl group that is absent in the peptide. The diprotonated $(BK + 2H)^{2+}$ has the additional protonated amine group present in the zwitterion structure that is absent in $(BK + H)^+$. That raises its reactivity considerably but at the specified protonated amine site. The protonated amine group may interfere with the relay mechanism suggested above, by shielding the carboxylate group from the deuterating reagent.

In the long run, it would be advantageous to learn about the contribution of the different coexisting conformers of $(BK + 2H)^{2+}$ and/or $(2R + H)^+$ mentioned earlier to site-specific rate constants. The algorithm that has been applied to the arginine dimer problem [4] treated experimental data that indicated the presence of a single conformer.

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