Gas Phase H/D Exchange of Protonated Arginine Monomers and Dimers

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An electrospray ionization−fast flow technique has been employed to study the gas-phase H/D exchange reactions of protonated monomers and dimers of L-arginine with ND_3 and CH_3OD. Experimental results include mass spectra, semilogarithmic decay plots of reactant ions and relative abundances of the various cations undergoing consecutive H/D exchanges as a function of the flow rates (or concentrations) of the neutral deuterating reagents. Optimum apparent and site-specific rate constants are deduced by simulated fits based on solutions of simultaneous first-order differential equations. We find that the protonated monomer, which is known to be non-zwitterionic in the gas phase, exchanges efficiently with ND_3 a maximum of 4 hydrogens out of the 8 labile ones. The bulk of the reactivity of ND_3 with the protonated monomer is centered at a single site. The data are interpreted by ammonia stabilizing the zwitterion structure of the protonated arginine monomer by forming a salt-bridge complex, $C(NH_2)_2^+-NH-CH_2-CH_2-CH_2-CH(NH_2)-COO^-$-$NH_4^+$. Ammonia/arginine·$H^+$ complexes are indeed observed in the flow tube experiment upon increasing the flow tube pressure through an increased flow of the helium carrier gas. The protonated dimer has been calculated previously to be more stable in the salt-bridge or ion−zwitterion form than in the simple protonated or ion−molecule form. We find that the dimer exchanges with ND_3 all of its 15 labile hydrogen atoms. A "relay" exchange mechanism can explain the results for the zwitterionic dimer since there is a carboxylate group onto which the proton can be transferred. This allows exchange of all the guanidino hydrogens that are not exchanged in the monomer. Some experimental results are explained by assuming the coexistence of the two isomeric protonated dimer structures: the ion−zwitterion form and the ion−molecule form. These results include bimodal deuterium distributions in the mass spectra and two equal site-specific reactivities that are ascribed to two equivalent carboxyl groups of the ion−molecule isomer form of the dimer.

Introduction

Amino acids are known to exist as zwitterions in solution. However, in the gas phase even arginine, the most basic acid, exists in the nonionic neutral configuration.[1] Calculations for the monomer show[2] that the neutral conformer is energetically slightly more stable than the zwitterionic form. At the same time, density functional theory (DFT) calculations on the protonated dimer of arginine[3] and on other charged aggregates[4] have demonstrated a reversed stability pattern with the salt-bridge or ion−zwitterion form being more stable than the simple protonated or ion−molecule form. Protonated arginine clusters can be easily generated in the gas phase by electrospray ionization (ESI).[5] The dissociation kinetics of proton-bound dimers of several amino acids were investigated with blackbody infrared radiative dissociation (BIRD), and the binding energy of the dimer of arginine was found to be the highest. This was taken as experimental evidence that protonated dimers of arginine are bound by a salt bridge in the gas phase.[6]

Protonated arginine monomer, with a guanidino group in the side chain (see Chart 1), might be expected to exchange 8 labile hydrogens. However, it was previously observed to exchange only poorly with ND_3, with a maximum of 4 exchangeable hydrogens.[7] Protonated arginine dimer (Chart 2) has 15 labile hydrogens. We are reporting here on results concerning H/D exchange of gas-phase protonated arginine monomers and dimers with ND_3 and CH_3OD, respectively. The experimental setup we are using is a unique electrospray ionization/fast flow apparatus, described previously.[8] Protonated arginine monomers and dimers are introduced through an electrospray source that is directly connected to the flow reactor. Neutral ND_3 or CH_3-OD is introduced into the flow tube through a ring inlet, and helium that is used as a buffer gas enters the flow tube at the upstream end. Arginine dimer will be demonstrated to undergo more efficient H/D exchange than the monomer. The results

CHART 1: The Protonated Arginine Monomer

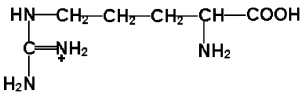
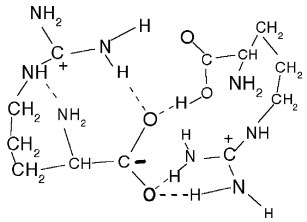


CHART 2: The Protonated Arginine Dimer; Schematic Salt-Bridge Structure (Adapted from a More Accurate Ball-and-Stick Structure Given in Reference 3)[a]



[a] The protonated arginine molecule on the right is the one given in Chart 1 and has 8 labile hydrogens. The neutral arginine molecule on the left is zwitterionic and has 7 labile hydrogen atoms.

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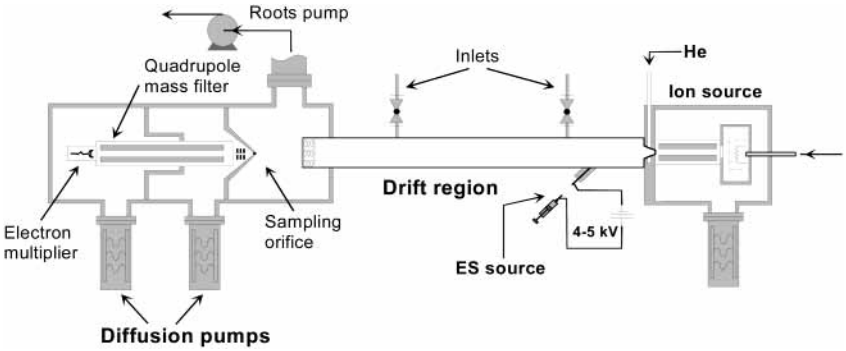

Figure 1. Schematic drawing of the experimental setup combining ES ionization with a fast flow technique. The ion source and quadrupole mass spectrometer of the SIFT part of the apparatus were not used in the present experiments.

will be discussed in light of the proposed structures for the protonated monomer and protonated dimer, respectively.

Experimental Section

The electrospray ionization/fast flow apparatus is shown schematically in Figure 1. It consists of a selected ion flow tube (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 has 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 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 near an electron impact ion source 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. Stainless steel tubes 15 cm in length and 0.05 cm i.d. are 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. A capillary tube of 0.05 cm i.d. introduces an air leak into the flow tube with a pressure of 0.07 Torr and a flow rate of 1.3 L/min (STP); these numbers have to be added to the helium pressure and helium flow rate when calculating rate constants. The experiments to be described were carried out at total flow velocities of ~6000−7000 cm s^{-1} leading to typical flow tube pressures of ~0.1−0.35 Torr and reaction times of several milliseconds.

Ions are electrosprayed ~10 mm through ambient air into the grounded capillary tube from a stainless steel syringe needle biased at 5 kV dc. Dilute solutions of arginine in a polar solvent are delivered to the electrospray needle at flow rates of 2−10 μL min^{-1} from a 1 mL syringe mounted on a model 100 KD Scientific Syringe Pump. The temperature of the capillary tube and of the flow tube does not exceed ~30 °C.

The L-arginine used in the experiments was purchased from Sigma/Aldrich (St. Louis, MO), with a stated minimum purity of 98.5%. A few experiments were carried out on 1-ethylguanidine sulfate from Sigma/Aldrich, with a stated minimum purity of 98%. ND_3 and CH_3OD were also from Sigma/Aldrich, with a stated isotopic purity that exceeds 99% at. % D. Stock solutions of water−methanol−acetic acid (50:50:0.1) were prepared for this series of experiments.

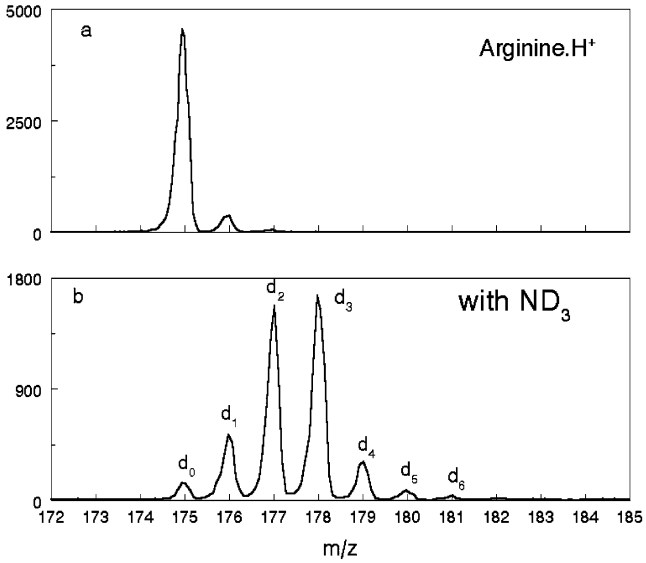

Figure 2. Mass spectra (ion counts as a function of the mass-to-charge ratio, in Thomson units) obtained by the detector quad for (a) the protonated monomer of arginine and (b) the protonated monomer following H/D exchange with ND_3 at a flow rate of 5.3×10^{18} molecules/s and a reaction time of 11.7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

Results and Discussion

The protonated monomer and dimer of L-arginine, L-arginine·H^+ and (L-arginine)$_2H^+$, were produced by electrospray ionization and were injected into the flow tube. We reacted these ions in the flow tube with either ND_3 or CH_3OD and monitored the incorporation of deuterium as a function of flow rate of the deuterating reagent at a constant reaction time. All of the experiments were carried out under thermal, room-temperature conditions. A few experiments were carried out on the protonated monomer and dimer of 1-ethylguanidine.

Mass Spectra. *(a) Arginine·H^+.* Mass spectra of the protonated monomer of arginine without ND_3 flow and with ND_3 flow, respectively, are presented in Figure 2. When protonated arginine was reacted under the highest ND_3 flows employed, $\sim 5.3 \times 10^{18}$ molecules/s, we observed, as was reported previously,[7] four hydrogen exchanges (the fourth being considerably weaker than the third) and a fifth and sixth very weak ones (see Figure 2). The calculated ND_3 concentration in this experiment is 1.75×10^{13} molecules/cc, and the reaction time, 11.7 ms. In contrast with these results, we have observed very efficient exchange of all the five labile hydrogens of protonated

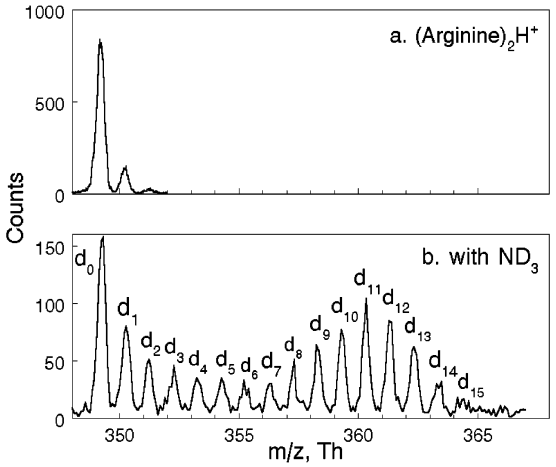

Figure 3. Mass spectra of the protonated dimer of arginine, (a) without ND_3 and (b) with ND_3 flow; reaction time, 11.4 ms; ND_3 flow, $4.4_6 \times 10^{18}$ molecules/s; ND_3 concentration, $1.4_4 \times 10^{13}$ molecules/cc.

L-serine at considerably lower ND_3 concentrations of 1.5×10^{12} molecules/cc.[8g] This is entirely expected in view of the very high proton affinity of arginine, PA = 251.2 kcal/mol, compared to that of serine, PA = 218.6 kcal/mol.[9] Ammonia with a proton affinity PA = 204 kcal/mol can undergo complexation and partial proton transfer within the complex with protonated serine. This is required for the proton shuttle to induce H/D exchange. However, the process is much more endothermic in the case of arginine and prevents efficient H/D exchange of the protonated arginine monomer.[7] It has become clear however[7] that the proton affinity difference between the amino acid and the deuterating agent, ΔPA, is only one of the factors that influence H/D kinetics. The difference in PA between two functional groups in the molecule influences the kinetics most directly. An alternative mechanism is possible for arginine making the site-specific exchange of a single labile hydrogen very efficient (see below).

(b) (Arginine)$_2$H$^+$. Mass spectra of the protonated dimer of arginine without ND_3 flow and with ND_3 flow, respectively, are presented in Figure 3. The spectrum in Figure 3b is for a ND_3 concentration of 1.44×10^{13} molecules/cc and a reaction time of 11.4 ms. Two striking results are evident:

(1) There is very efficient exchange of up to and including all the 15 labile hydrogens of the protonated dimer. In the flow tube experiment shown in Figure 3, the exchange maximizes at 11 deuterium atoms. ND_3 concentrations of $\sim 10^{14}$ molecules/cc maximize the mass spectra at 15 exchanges for a reaction time of ~12 ms. Independent experiments, carried out using Fourier transform ion cyclotron resonance (FTICR)[10] have indicated that on a time scale of 10−30 s with ND_3 at $\sim 10^{-6}$ Torr, the mass spectra for the protonated dimer maximize at 15 exchanges. In other words, contrary to the monomer, all the labile hydrogens undergo efficient exchange with ND_3.

Efficient hydrogen exchange is prevented in the monomer as well as in the dimer when the carboxyl and amino groups of arginine are absent. This point was checked by running experiments on ethyl guanidine. Both the protonated monomer and the protonated dimer of ethyl guanidine exchange a single labile hydrogen under the highest ND_3 flows employed, $\sim 5 \times 10^{18}$ molecules/s.

(2) Under the experimental conditions of Figure 3, the exchange distribution is bimodal. This means that there are two populations of the protonated dimer that exchange with ND_3 at very different rates. This can be ascribed to the two isomeric structures of the protonated dimer,[3] the salt-bridge or ion−zwitterion form on one hand and the ion−molecule form on the other. The salt-bridge structure has been calculated to be between 5.7 and 7.2 kcal/mol more stable than the ion−molecule form.[3] The relative stability of the ion−molecule isomer is such that it is not predicted to be present to any effective extent at room temperature. Furthermore, caution must be exercised in adopting the interpretation of the experimental data by the coexistence of the two isomeric structures. The reactant dimer ion is introduced with a stream of air into the flow tube and is not carried in as usual in a SIFT apparatus by virtue of the Venturi effect due to the helium carrier gas. This may cause inhomogeneous mixing of the neutral and ionic reagents if the helium pressure is not high enough. We have carried out a systematic study of the mass spectra as a function of ND_3 concentration, helium carrier gas flow rate, flow tube pressure, and reaction time. The bimodal distribution can be eliminated (see Figure 4) by increasing the ratio between the partial pressure of the helium carrier gas relative to the partial pressure of the air that is introduced via the leak from the ESI source. The H/D exchange that we do observe for the protonated dimer, Figure 4b, is ascribed solely to the more stable salt-bridge structure (see Chart 2).[3] A relatively high helium flow rate of ≥2.2 L/min was employed to avoid the observation of an unexchanged or slowly exchanging protonated dimer component in the distribution. No similar phenomena were observed in the case of exchange experiments carried out by us on the serine dimer under very similar conditions.[11] This strongly suggests that the bimodal distribution observed for the arginine dimer is not an artifact. By change of the instrumental parameters, the collision number distribution can be changed, so the differences can be eliminated or one type of ion (the ion−molecule form) can be forced to isomerize completely to the other (the salt-bridge structure).

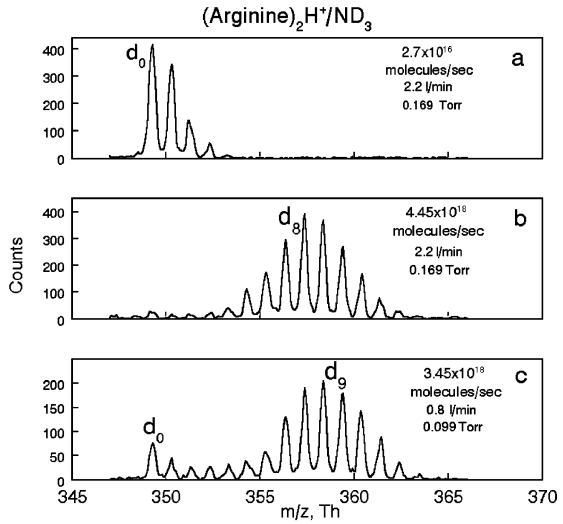

Figure 4. Mass spectra of the protonated dimer of arginine at the indicated ND_3 flows: (a) 2.7×10^{16}, (b) 4.45×10^{18}, and (c) 3.45×10^{18} molecules/s. The helium carrier gas flow rate, 2.2 L/min, and overall flow tube pressure, 0.169 Torr, are equal in the experiments of panels a and b, whereas the helium flow rate and pressure are 0.8 L/min and 0.099 Torr, respectively, in the experiment given in panel c.

Apparent, Site-Specific, and Overall Rates. The consecutive H/D exchange reactions observed for L-arginine·H$^+$ at relatively low ND_3 flow rates (up to $\sim 1.5 \times 10^{17}$ molecules/s) are presented in Figure 5. The undeuterated ion and its deuterated isotopomers each contribute a multiplet of isotopic peaks due to the natural abundances of the various carbon, nitrogen, oxygen, and hydrogen isotopes. Results of the type shown in

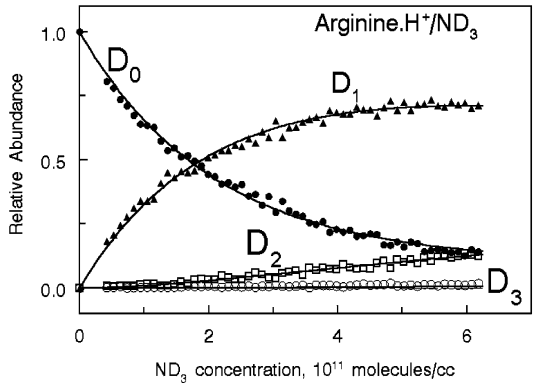

Figure 5. Relative abundance vs neutral ND_3 concentration in molecules/cc for the various indicated cations in the reaction of the protonated monomer of L-arginine with ND_3. The reaction time is $t = 12.2$ ms. The relative abundances given by the symbols are the isotopically deconvoluted experimental data for the reactant ion (●, D_0), singly H/D exchanged ion (▲, D_1), etc. The curves are the simulated fits for derivation of site-specific H/D exchange rate constants (see the text). The correlation factor R^2 is 0.998.

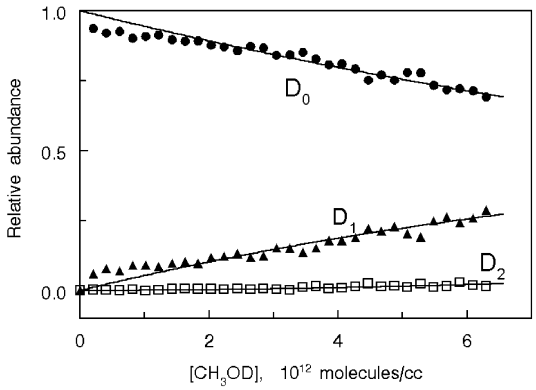

Figure 6. Relative abundance vs neutral CH_3OD concentration in molecules/cc for the various indicated cations in the reaction of the protonated monomer of L-arginine with CH_3OD. The reaction time is $t = 12.2$ ms. The curves are the simulated fits for derivation of site-specific H/D exchange rate constants. The correlation factor R^2 is 0.89.

Figure 2 were deconvoluted isotopically as described previously,[8d,e] and it is these isotopically deconvoluted data that are presented in Figure 5 as a function of ND_3 concentration. Isotopically deconvoluted experimental data can be compared with simulated data to extract rate constants. Simulations are carried out to deduce apparent and site-specific rate constants. Simulation of the kinetic data by solution of the associated set of coupled differential equations yields a set of apparent rate constants. An algorithm based on the Modelmaker program of Cherwell Scientific solves a set of independent simultaneous differential equations for a suggested reaction mechanism by the Runge−Kutta method and is the way for extracting site-specific rate constants that we have adopted.[8d,e] The smooth curves in Figure 5 are the simulated fits for derivation of site-specific rate constants from the isotopically deconvoluted experimental data. Similar deconvoluted data and their simulated fits for the reaction of protonated arginine and of protonated arginine dimer with CH_3OD are presented in Figures 6 and 7, respectively. The rate constants deduced are summarized in Table 1. We were unsuccessful in getting a simulated fit to the 15 consecutive H/D exchanges observed for the reaction of ND_3 with the protonated dimer, 11 of which are shown in Figure 8.

Overall disappearance rate constants were deduced from semilogarithmic decay plots of the reactant ion (see for example Figure 9). Table 1 summarizes all the rate constants obtained.

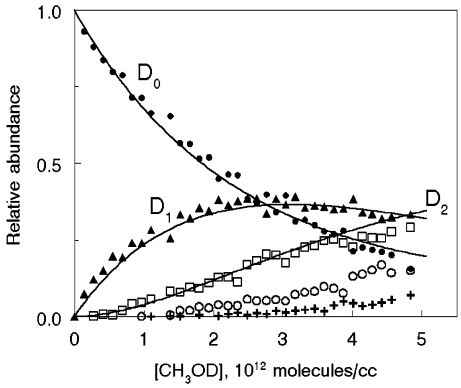

Figure 7. Relative abundance vs neutral CH_3OD concentration in molecules/cc for the various indicated cations in the reaction of the protonated dimer of L-arginine with CH_3OD; D_2 (□); D_3 (○); D_4 (+). The reaction time is $t = 12.8$ ms. The curves are the simulated fits for derivation of site-specific H/D exchange rate constants. The correlation factor R^2 is 0.84.

TABLE 1: Apparent, Site-Specific, and Overall H/D Exchange Rate Constants (cm^3 $molecule^{-1}$ s^{-1}) for Arginine·H^+ (Monomer) and (Arginine)$_2H^+$ (Dimer) with CH_3OD and ND_3

	$k_{apparent}$	$k_{site-specific}$	$\Sigma k_{site-specific}$	$k_{semilog\ plot}$
		CH_3OD		
monomer	2.2×10^{-11}	6.0×10^{-12}	9.0×10^{-12}	9.2×10^{-12}
	8.9×10^{-12}	2.4×10^{-12}		
	4.9×10^{-12}	4×10^{-13}		
		2×10^{-13}		
dimer	5.4×10^{-11}	2.5×10^{-11}	5.6×10^{-11}	$(4.1 \pm 0.7) \times 10^{-11}$
	4.1×10^{-11}	2.5×10^{-11}		
	2.0×10^{-11}	5.8×10^{-12}		
		ND_3		
monomer	1.0×10^{-9}	2.0×10^{-9}	2.1×10^{-9}	2.1×10^{-9} [a]
	1.4×10^{-10}	1.1×10^{-10}		
	1.2×10^{-10}	6.3×10^{-11}		6.4×10^{-10} [a]
	1.5×10^{-11}	6.0×10^{-12}		
dimer	1.8×10^{-10}	7.0×10^{-11}	1.8×10^{-10}	$(1.25 \pm 0.4) \times 10^{-10}$
	1.4×10^{-10}	7.0×10^{-11}		
	1.5×10^{-10}	2.2×10^{-11}		
	1.6×10^{-10}	1.8×10^{-11}		

[a] The lower value is the average value of 19 experiments; the higher value is for limiting low flow rates of ND_3; see the text.

The sums of the site-specific rate constants for each of the reaction systems studied are in fair agreement with the respective overall rate constants deduced from the corresponding semilogarithmic decay plots. The decay plots for the monomer reaction with ND_3 were slightly curved, giving higher rate constants for the lowest ND_3 flow rates employed. These were the flows that were necessarily employed in the simulations. Table 1 contains in addition the average value of the overall rate constant for the monomer reaction deduced from semilogarithmic decays over a wide range of ND_3 flow rates.

Several results transpire:

(1) The reaction rate constants with CH_3OD are considerably lower than for ND_3. This is as expected in view of the considerably lower proton affinity of CH_3OD (PA = 180.3 kcal/mol)[9] compared to that of ND_3 (PA = 204 kcal/mol). The higher gas-phase basicity of ammonia leads to stronger bonding in the collision complex and an expected longer lifetime of the complex. Such a longer lifetime can contribute to the observed high exchange rates with ammonia. Additional factors that need to be taken into account might be the number of deuterium atoms that can be exchanged per collision and the different steric factors for ND_3 and CH_3OD. The exchange mechanisms for CH_3OD on one hand and ND_3 on the other might be different.

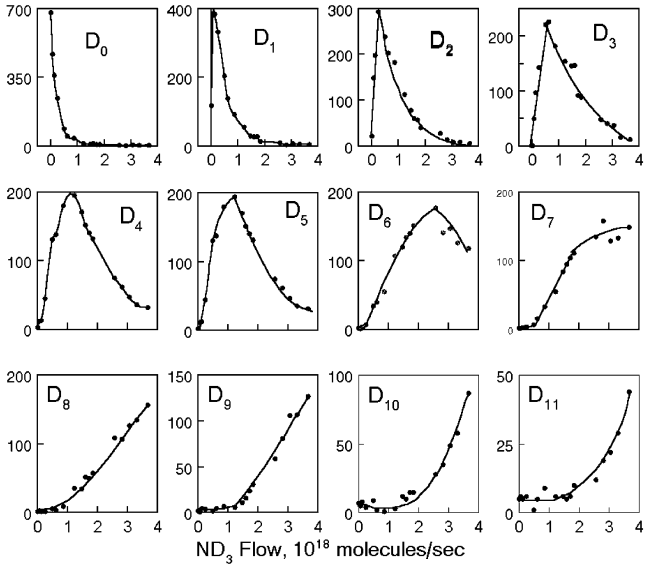

Figure 8. Ion counts vs neutral ND_3 flow rate (in molecules/s) for the various indicated cations in the reaction of the protonated dimer of L-arginine with ND_3; helium flow, 3.5 L/min; pressure, 0.234 Torr; reaction time, 12.4 ms; 1×10^{18} ND_3 molecules/s corresponds to 3.53×10^{12} ND_3 molecules/cc. The curves are not simulated fits but serve to lead the eye.

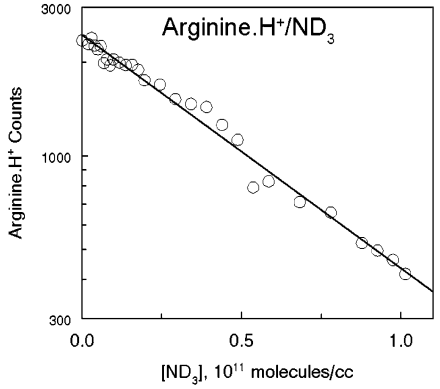

Figure 9. Semilogarithmic plot of the decay of primary ions as a function of the neutral concentration for the reaction of the protonated monomer of L-arginine with ND_3; $t = 12$ ms.

ND_3 is basic enough to gain a proton and be solvated by most amino acids and peptides, whereas CH_3OD is incapable of deprotonating these ions and is postulated to exchange via a "relay mechanism", in which the reagent simultaneously gains a D while losing an H.[12,13] As noted earlier, even ammonia has a hard time deprotonating protonated arginine. Nevertheless, the overall rate constants for ammonia are 1 or 2 orders of magnitude higher than for methanol and the fraction of labile hydrogens that undergo exchange is considerably higher, particularly for the dimer.

(2) The bulk of the reactivity of ND_3 with the protonated monomer seems to be centered at a single site (see Table 1). The structure of the protonated monomer (Chart 1) demonstrates two possible alternative sites. A single labile hydrogen that is a conceivable candidate for exchange is situated at the NH group of the guanidino group. However, guanidine itself exchanges rather poorly with ND_3[7] as does also ethyl guanidine (present data). The carboxyl hydrogen can be exchanged in an indirect mechanism for example via a protonated amine group.[7] The protonated group of the monomer is the very basic side-chain guanidino group (Chart 1), and exchanging the carboxyl would require migration of the proton to the carboxyl via the intermediacy of ND_3H^+ or through space, both of which are not very plausible. The rather high rate constant is surprising. There is a near equality between the experimental rate constant $\Sigma k_{\text{site-specific}} = 2.1 \times 10^{-9}$ cm^3 molecule^{-1} s^{-1} (Table 1) and the calculated ion−polar molecule-capture rate constant based on the parametrized expression of Su and Chesnavich,[14] $k_{\text{cap}} = 2.14 \times 10^{-9}$ cm^3 molecule^{-1} s^{-1}. ES may produce metastable zwitterionic ions, which are stabilized via proton transfer catalyzed by the ND_3 molecule. An alternative explanation for the findings is that ammonia stabilizes the zwitterion structure of arginine by forming a salt-bridge complex, $C(NH_2)_2^+-NH-CH_2-CH_2-CH_2-CH(NH_2)-COO^-NH_4^+$, by analogy to the betaine/ammonia complex.[8c,15] This means that the very effective first site-specific H/D exchange of the protonated arginine monomer by ammonia involves the carboxyl hydrogen. Ammonia/arginine·H^+ complexes are indeed observed in the flow tube experiment upon increasing the flow tube pressure through an increased flow of the helium carrier gas.

(3) The protonated arginine dimer displays two equal site-specific rate constants in its reaction with either CH_3OD or ND_3 (Table 1). These are not easily identified in the salt-bridge structure (Chart 2). Rather, the alternative ion−molecule dimer isomer,[3] in which the protonated arginine monomer (Chart 1) is solvated by a neutral arginine molecule, could provide two such equivalent sites in the form of the two carboxyl groups. There are two possible explanations for this conclusion: (a) The actual energy difference between the two isomers, the salt-bridge and the ion−molecule structures, is smaller than it was originally calculated to be by Strittmatter and Williams.[3] (b) Even if this is not the case, complexation with methanol or ammonia might stabilize the ion−molecule isomer.

(4) As noted earlier, contrary to the monomer all the labile hydrogens of the dimer undergo exchange with ND_3. However, the overall rate constant is lower for the dimer than it is for the monomer (Table 1). Efficient H/D exchange is usually ascribed to open structures.[8] The calculated dimer structures of arginine are rather compact (ref 3 and Chart 2). This may be the reason for the relatively low rate constant compared to the monomer. Also, in the neutral dimer the arginines are arranged in a head to tail fashion with the guanidinium group of one molecule interacting with the carboxylate of the neighboring arginine.[16] This head to tail arrangement is still seen in the protonated dimer (Chart 2). The carboxyl group that was free in the monomer to interact with the ND_3 reagent can no longer do so. On the other hand, there must be some other mechanism allowing proton mobility and access of the ND_3 to all the exchangeable hydrogens of the dimer. The relay exchange mechanism should be considered for ND_3 in this case. In the case of the monomer, there is no other group to transfer the proton from the guanidyl, so protons on the guanidyl group remain unexchanged. However, in the case of the zwitterionic dimer there is a carboxylate group onto which the proton can be transferred. After this step, one of the guanidyl groups becomes deprotonated, so it can be an acceptor for the next step, and in this way those protons can be exchanged as well. The H/D exchange data demonstrate that a very pronounced change in structure occurs on going from the monomer to the dimer: the change from a protonated neutral arginine for the monomer to a charged zwitterionic species stabilized by a salt bridge for the dimer.

Future studies involving simulations of the present data for the protonated dimer/ND_3 reaction and extraction of site-specific rate constants for all the labile hydrogens will hopefully shed more light on the consecutive order of H/D exchange for different sites reacting at different rates.

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References and Notes

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