Gas-Phase H/D Exchange Reactions of Polyamine Complexes: $(M + H)^+$, $(M + \text{alkali metal}^+)$, and $(M + 2H)^{2+}$

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Gas-phase hydrogen/deuterium exchange reactions between noncovalent polyamine complexes and D_2O, CH_3OD, or ND_3 are undertaken in a quadrupole ion trap mass spectrometer. Structural features of the protonated polyamines can be differentiated by the rates and overall extent of exchange, specifically the presence of propylene units and/or a cyclic structure noticeably decreases exchange compared to the exchange observed for acyclic polyamines with only ethylene bridges between amino groups. Significant differences are observed for singly protonated vs. doubly protonated complexes, where the doubly protonated complexes undergo more efficient exchange at a higher rate than the analogous singly protonated complexes. Molecular modeling calculations suggest that more diffuse conformations may exist for the higher charge states, thus facilitating H/D exchange. In addition, H/D exchange reactions between the alkali metal cationized complexes and ND_3 are nearly quenched, compared to the significant exchange seen for singly protonated complexes. A conformational change or the loss of a low energy reaction pathway may explain the limited exchange reactions seen when a bulky cation replaces a proton in the complex. (J Am Soc Mass Spectrom 2000, 11, 711–721)

Gas-phase hydrogen/deuterium (H/D) exchange reactions can be effectively monitored by mass spectrometry to provide information on the gas-phase conformations of ions and the locations of functional groups containing active hydrogens [1–6]. In conjunction with the emergence of softer ionization techniques, such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), H/D exchange is being used to probe the higher order gas-phase structures of biomolecules, such as amino acids, peptides, proteins, and drugs [7–13]. The extent of deuterium incorporation depends on the accessibility of hydrogens in the analyte structure, whether the available hydrogens are involved in hydrogen bonds with nearby (in space) basic groups, and the differences in gas-phase basicities between each basic site and the deuterated reagent.

Noncovalent interactions, including hydrogen bonding, can play a significant role in the conformations of molecules, such as the tertiary structures of proteins and the quaternary structures of enzymes and substrates. Multiple hydrogen bond formation between the deuterated reagent and the analyte is proposed to be involved in one mechanism by which hydrogen/deuterium exchange occurs for multifunctional compounds [4]. As a result, H/D exchange has the potential to be a powerful tool to study complexes possessing intramolecular hydrogen bonds or other noncovalent bonds to determine how they affect both the number and rates of exchanges. Currently however, there have only been a few studies involving hydrogen/deuterium exchange of noncovalently bound complexes [14–18]. The H/D exchange reactions of proton-bound dimers of amino acids and peptides were compared to the reactions of the corresponding protonated monomers in one study, and the overall exchange level was found to increase for the dimers [14]. An increase was also found in the overall exchange level of vancomycin group antibiotics when paired with their peptide substrates in work reported by Heck and co-workers [15]. On the other hand, the overall rates of exchange were found to decrease when amino acids and dipeptides were complexed with saccharides in recent work from the Lebrilla group [16]. One common feature of all of these studies is that the noncovalently bound complexes have more labile hydrogens than the monomers alone. This fact can make the interpretation of the results more complex and make it difficult to deconvolute the effects solely due to the structure of the complex from those due to formation or disruption of hydrogen bonds in the noncovalent complex, thus giving different accessibilities and neighbors to the hydrogen atoms involved. Work done by Cotter and co-workers circumvented this problem by examining protonated and sodium cation-

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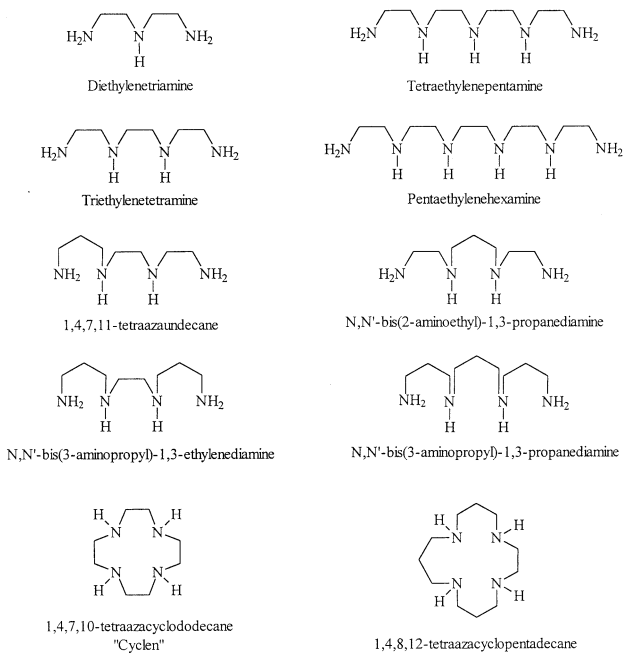

Figure 1. Structures of compounds.

ized peptide ions [17]. They found that hydrogen/deuterium exchange of the sodium cationized peptide was nearly quenched for gramicidin S and several smaller peptides. More recent work done by the Marshall group focused on the hydrogen/deuterium exchange reactions of bradykinin in various protonated and sodium cationized forms in an effort to probe for zwitterionic structures [18]. In their case the $(M + Na^+)$ ion reacted with D_2O 1000 times faster than the $(M + H)^+$ ion, and the $(M + 2H)^{2+}$ ion reacted about 10 times faster than the $(M + H)^+$ ion.

The goal of the present study is to compare the H/D exchange reactions of protonated and alkali metal cationized polyamines, with an emphasis on evaluating the factors that affect exchange, such as ligand flexibility, size, basicity, and location of basic groups. The compounds studied are well-characterized, highly basic, multifunctional ligands (analogs of crown ethers and glymes) (Figure 1). These compounds form a variety of complexes via electrospray ionization, including singly protonated, doubly protonated, and alkali metal cationized (Li^+, Na^+, and K^+) species. The extent of exchange upon reaction with ND_3, CH_3OD, or D_2O was monitored in a quadrupole ion trap mass spectrometer. Comparison of reagent reactivity and kinetic data were obtained. Molecular modeling calculations were also undertaken to provide insight into the structures of the protonated and metal cationized complexes during H/D exchange.

Experimental

All experiments were performed with a Finnigan quadrupole ion trap mass spectrometer with an electrospray interface modeled after the Oak Ridge National Laboratory design [19]. The ion trap was operated in the mass selective instability mode and the ITD electronics were modified to allow axial modulation using stored waveform inverse Fourier transform (SWIFT). All solutions were made in either methanol or acetonitrile at polyamine concentrations ranging from 1×10^{-4} to 3×10^{-3} M. To facilitate formation of the alkali metal complexes, alkali metal chlorides were added at a ligand to metal ratio of 1:2. Addition of the metal salts also enhanced formation of doubly protonated species. Water was added to enhance solubility in some cases. A Harvard syringe pump delivered the solutions at 3.5 μL/min to the stainless steel electrospray needle, which was held at 3.8 kV. The base pressure of the ion trap was 6×10^{-5} torr in the ESI mode. No helium buffer gas was used.

For the hydrogen/deuterium exchange experiments, one solution of interest was electrosprayed into the ion trap. A deuterated reagent, D_2O, methanol-O-*d*, or ammonia-d_3, was then admitted into the trap via a leak valve to a nominal pressure of $3–6 \times 10^{-4}$ torr. These pressures were not corrected for variations in ionization gauge sensitivities unless otherwise noted. The species of interest, either the $(M + H)^+$, $(M +$ alkali metal$^+)$, or $(M + 2H)^{2+}$ ions, were allowed to undergo reactions with the deuterated reagent for varying amounts of time while the extent of deuterium incorporation was monitored. Reactions were typically monitored for 0 to 8 s but were allowed to go up to 20 s for some of the kinetic plots. All reported rate constants are ±30% due to day-to-day variations in the base pressure and the true sample and reagent pressures relative to the pressures measured on the ionization gauge. In general, methanol was used as the solvent for all species undergoing reactions with deuterated ammonia. Results comparing the exchange of polyamines originating in acetonitrile or methanol solvents indicate that the solution solvent does not interfere with the exchange process to a noticeable extent, nor does it cause significant changes in the polyamine ion structures that the exchange behavior is altered. However, solutions were made in acetonitrile for species undergoing exchange with deuterated methanol in order to avoid "dilution" of the deuterated methanol admitted as the reagent gas to the ion trap. Ions were isolated by applying SWIFT waveforms to the endcap electrodes, created under LabView software (National Instruments Corp., Austin, TX) and transferred to an arbitrary waveform generator (Tektronix, Beaverton, OR) to eject all other ions from the trap [20].

All polyamines were obtained from Aldrich Chemical (Milwaukee, WI) and used without further purification. Ammonia-d_3 was purchased from Isotec (Miamisburg, OH), whereas methanol (CH_3OD) and deuterium oxide came from Cambridge Isotope Laboratories (Andover, MA). Reagent grade methanol and acetonitrile were acquired from EM Science (Gibbstown, NJ).

Molecular modeling experiments were undertaken

using the commercially available software package PC Spartan Pro (Wavefunction, Irvine, CA). For protonated and doubly protonated species, calculations were undertaken by first employing a conformational search using the molecular mechanics force field MMFF94, then geometry optimization was undertaken utilizing the semiempirical model PM3 on the lowest energy conformation. Because PM3 is not parameterized for alkali metals (no semiempirical method currently is), the alkali metal complexes were calculated similarly except that molecular mechanics was also utilized for geometry optimization. These calculations were performed on selected complexes in order to further examine the experimental trends in terms of ion conformations and hydrogen bonding.

Results and Discussion

Comparison of Exchange Reactions Involving $(M + H)^+$ Species

All of the polyamines examined formed abundant protonated species upon electrospray ionization. Given the high basicities of these compounds the extents and efficiencies of exchange were compared for three deuterated reagents to determine the best deuterating reagent for facilitating exchange. H/D exchange reactions were undertaken with diethylenetriamine and deuterated water, methanol-O-*d*, and ND_3, which have gas-phase basicities of 659.8, 724.7, and 818.8 kJ/mol, respectively [21]. The gas-phase basicities of the polyamines range from 966 ± 8 kJ/mol for diethylenetriamine to 1021 ± 8 kJ/mol for pentaethylenehexamine and 1,4,8,12-tetraazacyclopentadecane, the most basic polyamines [22]. One set of results is illustrated in Figure 2 for diethylenetriamine, the least basic polyamine. After 2 s of reaction time with each deuterated reagent at 5×10^{-4} torr (after correction for ionization gauge sensitivities [23]), exchange of up to all six labile hydrogens has taken place (peaks at *m/z* 110 were verified as noise and were unrelated to the exchange peaks). However, there is a clear difference in the extent of exchange, as shown by the relative differences in intensities of the deuterium-exchanged ions and the unexchanged precursor ion. Clearly, deuterated ammonia promoted the most extensive and most efficient exchange, and thus was used as the deuterating reagent for the rest of the studies of the monoprotonated polyamines. Deuterated methanol was chosen for the reactions of the doubly protonated polyamines for reasons described below.

The exchange results for the $(M + H)^+$ species reacting with deuterated ammonia are given in Table 1 along with the gas-phase basicities for each ligand. Only three of the polyamines exchanged the maximum number of hydrogens, whereas most exchanged one to five fewer than the maximum. No clear correlation between the maximum number of hydrogens exchanged (compared to the maximum number of labile hydrogens available for each polyamine) and basicity or any structural factor could be determined.

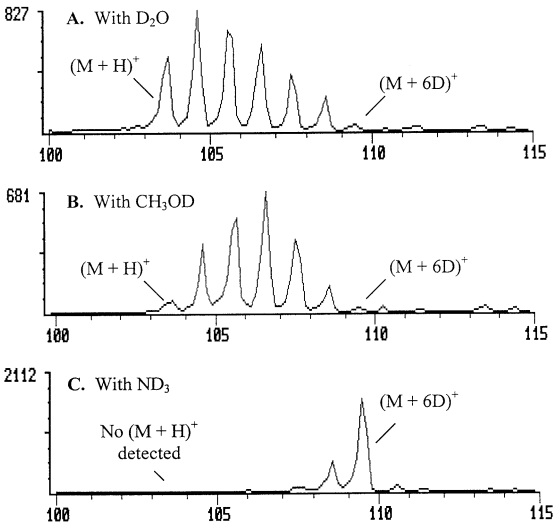

Figure 2. H/D exchange of diethylenetriamine after 2 s reacting with **(A)** D_2O, **(B)** CH_3OD, or **(C)** ND_3 at 5×10^{-4} torr. Pressures were corrected for ionization gauge sensitivities [23].

A more rigorous approach to evaluating hydrogen/deuterium exchange involves examination of kinetic parameters. Kinetic plots were obtained for the polyamines to correlate the structural features with relative rate constants. Intensity vs. time plots were constructed for the reactions of the polyamines with ND_3 ($4–5 \times 10^{-4}$ torr), and curve fits were generated from the KinFit program obtained from Dearden's group [24]. Examples of the resulting plots are shown in Figure 3 for protonated diethylenetriamine (Figure 3A) and cyclen (Figure 3B). As shown, the reaction between diethylenetriamine and ND_3 is nearly complete after only 2 s, whereas the unexchanged precursor, $(M + H)^+$, of cyclen is still visible after 20 s of reaction time and products corresponding to the incorporation of four and five deuteriums are visible but not quantifiable (<6% relative intensity).

The relative rate constants [normalized to k_1 of *N*,*N*′-bis(3-aminopropyl)-1,3-ethylenediamine] obtained from the curve fits are shown in Table 2. The exchange factors shown in the last column will be discussed below. In order to examine trends among the polyamines and not necessarily to examine the behavior of individual exchange sites, only the first rate constants (k_1) were compared. The calculated first rate constants of the polyamines are very informative and can be correlated with the structural characteristics of the polyamines. The polyamines can be divided into two general groups. One group, including tetraethylenepentamine, triethylenetetramine, and diethylenetriamine, has high rate constants (i.e., greater than 5.0). The second group, which includes 1,4,8,12-tetraazacyclopentadecane, *N*,*N*′-bis(3-aminopropyl)-1,3-propanediamine, *N*,*N*′-bis(3-aminopropyl)-1,3-ethylenediamine, and cyclen, has low rate constants (i.e., less than or

Table 1. H/D exchange results and gas-phase basicities for protonated polyamines reacting with ND_3[a]

Compound	Gas-phase basicity[b] (kJ/mol)	Number of labile hydrogens (including ionizing proton)	Observed number of hydrogens exchanged with ND_3
Pentaethylenehexamine	1021	9	5
1,4,8,12-Tetraazacyclopentadecane	1021	5	2
N,*N*′-Bis(3-aminopropyl)-1,3-propanediamine	1012	7	2
Tetraethylenepentamine	1012	8	7
N,*N*′-Bis(3-aminopropyl)-1,3-ethylenediamine	1004	7	3
1,4,7,10-Tetraazacyclododecane (cyclen)	1004	5	3
1,4,7,11-Tetraazaundecane	1004	7	7
N,*N*′-Bis(2-aminoethyl)-1,3-propanediamine	1000	7	4
Triethylenetetramine	987	7	7
Diethylenetriamine	966	6	6

[a]Reactions were carried out with ND_3 at 4–5 $\times$ 10^{-4} torr for 8 s.
[b]Gas-phase basicity values taken from *J. Am. Soc. Mass Spectrom.* **1998,** *9*, 1043.

equal to 1.0). Three compounds, pentaethylenehexamine, *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine, and 1,4,7,11-tetraazaundecane, are borderline (k_1 values of 1.8 to 2.6). The compounds in the group with high rate constants are all acyclic and contain only ethylene bridges between the amino groups. These structural features allow the compounds to be flexible in the gas phase, but the two-carbon bridges between nitrogens may not allow formation of optimal intramolecular hydrogen bonding. Interaction with a deuterating reagent could promote formation of additional and stronger intermolecular hydrogen bonds between the polyamine and the reagent, thereby facilitating H/D exchange. This is supported by molecular modeling calculations of protonated triethylenetetramine as illustrated in Figure 4. The geometry optimized structure of protonated triethylenetetramine is shown in Figure 4A. The ionizing proton is bound most strongly to the secondary amine, and an intramolecular hydrogen bond is formed between the ionizing proton on N2 and the terminal amine, N4, with a calculated angle of 167°. Optimal hydrogen bond formation occurs when the angle is 180°, i.e., when the hydrogen is directly in line with both donor atoms. Interaction with ND_3 disrupts the intramolecular hydrogen bond in favor of two intermolecular hydrogen bonds between protonated triethylenetetramine and ND_3 as shown in Figure 4B. In this structure the ionizing proton on N2 is hydrogen bonded to the ND_3 nitrogen with a near optimal angle of 178°. A second hydrogen bond is formed between a deuterium on ND_3 and the terminal amine nitrogen N4 and has a calculated angle of 174°. The formation of these more optimal hydrogen bonds allows the ND_3 molecule to simultaneously interact with two functional groups of triethylenetetramine and thus promote more facile H/D exchange. Similar hydrogen bonding patterns account for the high rate constants calculated for protonated diethylenetriamine and tetraethylenepentamine.

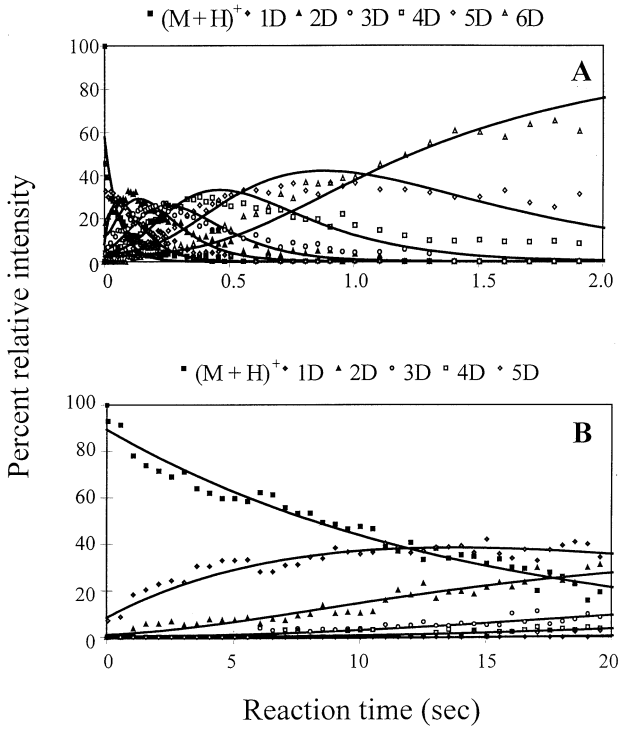

Figure 3. Kinetic plots of hydrogen/deuterium exchange of **(A)** (diethylenetriamine + H)$^+$ and **(B)** (cyclen + H)$^+$ reacting with ND_3. The points represent experimental intensities and the lines represent the corresponding curve fits produced by KinFit [24].

The group of polyamines with low rate constants includes 1,4,8,12-tetraazacyclopentadecane, *N*,*N*′-bis (3-aminopropyl)-1,3-propanediamine, *N*,*N*′-bis(3-aminopropyl)-1,3-ethylenediamine, and cyclen. These compounds either are cyclic (1,4,8,12-tetraazacyclopentadecane and cyclen) or are acyclic but contain at least one propylene bridge between amino groups [*N*,*N*′-bis(3-aminopropyl)-1,3-propanediamine and *N*,*N*′-bis(3-aminopropyl)-1,3-ethylenediamine]. The two cyclic compounds, cyclen and 1,4,8,12-tetraazacyclopentadecane, have two of the lowest rate constants. The relative rigidities of their structures compared to the acyclic compounds may be one reason they undergo less extensive exchange. The amino groups in the cyclic

Table 2. Relative rate constants[a] for H/D exchange of protonated polyamines reacting with ND_3

Compound	k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8	Exchange factor[b]
Pentaethylenehexamine	2.6	2.3	2.1	2.0	1.5	NM	NM	NM	5
1,4,8,12-Tetraazacyclopentadecane	0.12	NM	NM	NM	NM	NA	NA	NA	0.2
N,*N*′-Bis(3-aminopropyl)-1,3-propanediamine	0.49	NM	NM	NM	NM	NM	NM	NA	0.4
Tetraethylenepentamine	8.0	6.8	5.4	4.3	3.3	2.0	1.6	1.2	30
N,*N*′-Bis(3-aminopropyl)-1,3-ethylenediamine	1.0	0.90	0.90	NM	NM	NM	NM	NA	1
1,4,7,10-Tetraazacyclododecane (cyclen)	0.72	0.64	0.49	NM	NM	NA	NA	NA	0.8
1,4,7,11-Tetraazaundecane	2.6	2.6	2.2	1.6	1.2	1.2	0.82	NA	5
N,*N*′-Bis(2-aminoethyl)-1,3-propanediamine	1.8	1.6	1.7	1.5	1.6	NM	NM	NA	3
Triethylenetetramine	33	30	28	22	14	6.0	2.6	NA	∞[c]
Diethylenetriamine	180	130	85	68	34	14	NA	NA	∞[c]

[a]Rate constants calculated from the KinFit program [24] and normalized to *N*,*N*′-bis(3-aminopropyl)-1,3-ethylenediamine. NM indicates the signal intensity could not be reliably measured for exchange of the *n*th hydrogen (<15%). NA indicates the *n*th hydrogen was not exchangeable. Reactions were carried out with ND_3 at 4–5 × 10^{-4} torr. All values are ±30%.
[b]Exchange factors calculated as the sum of the peak heights of all deuterium-exchanged ions divided by the final peak height of the unexchanged precursor ion. These factors provide only a qualitative indication of the efficiency of H/D exchange and they are a function of the extent of reaction. Reactions were carried out with ND_3 at 4–5 × 10^{-4} torr for 8 s.
[c]Because of the absence of any detectable precursor ion after the reaction time, the exchange factors for diethylenetriamine and triethylenetetramine are infinitely large.

structures are more restricted in their ability to accommodate optimal or multiple hydrogen bond formation to the deuterating agent. Because H/D exchange is believed to occur by a hydrogen bonded intermediate, anything that would hamper the formation of that intermediate would therefore impede exchange as well. The geometry optimized structure for (cyclen + H^+ + ND_3) is shown in Figure 4C. One intermolecular hydrogen bond is formed between one amino proton (on N4) and ND_3; however, that places the deuterating reagent above the ring and off to one side. The relative rigidity of the ring structure, in addition to an intramolecular cross-ring hydrogen bond between N2 and N4, makes it difficult for the other amino groups in the ring to interact with the ammonia molecule, thus slowing the exchange process. In addition, ND_3 has been shown to undergo multiple exchanges in a single collision event [25]. Because both cyclic polyamines have only a single hydrogen available for exchange at each amine site, multiple collision events are required for multiple exchanges, thus leading to less efficient H/D exchange with ND_3.

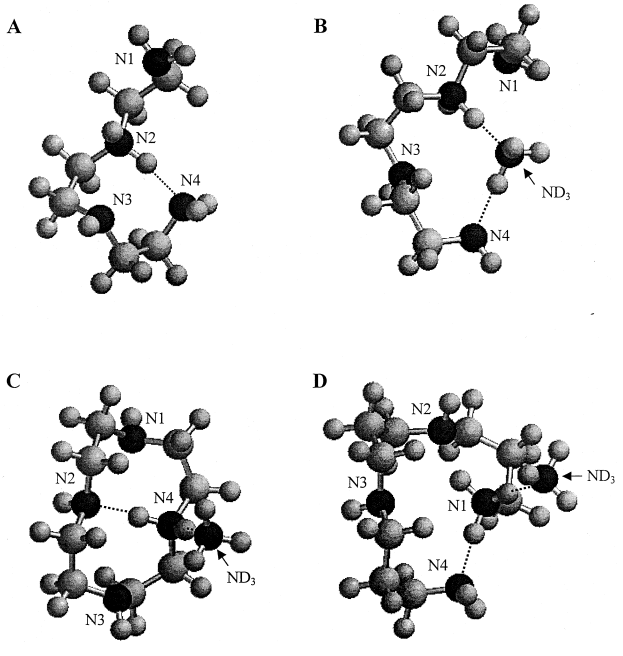

Figure 4. Geometry optimized structures for **(A)** protonated triethylenetetramine, **(B)** protonated "triethylenetetramine reacting with ND_3" prior to (C) **(C)** protonated cyclen reacting with ND_3, and **(D)** protonated *N*,*N*′-bis(3-aminopropyl)-1,3-propanediamine reacting with ND_3. Hydrogen bonds are indicated with dashed lines. Atom key: small white = hydrogen, medium white = carbon, medium black = nitrogen (numbered).

Because three-carbon bridges offer more flexibility than two-carbon bridges, the polyamines with propylene units can form more optimal *intramolecular* hydrogen bonds and thus are less likely to interact fully with the deuterating reagent via *intermolecular* bonds. Figure 4D shows the geometry optimized structure of protonated *N*,*N*′-bis(3-aminopropyl)-1,3-propanediamine reacting with ND_3. Because of the formation of a strong intramolecular hydrogen bond between the terminal amino groups N1 and N4 (with an angle of 174°), protonation is favored on a primary amine (N1). ND_3 then forms only a single intermolecular hydrogen bond with another hydrogen on N1. This results in the ND_3 molecule being placed above the "ring" and off to the side, thus restricting access to multiple active hydrogen sites and reducing exchange.

Three polyamines are borderline between the high and low rate constant groups. Pentaethylenehexamine is an acyclic polyamine with only ethylene bridges but has a comparatively low first rate constant with regard to the other analogous acyclic compounds. Similarly, 1,4,7,11-tetraazaundecane and *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine each have one propylene bridge but have relatively high rate constants compared to other polyamines with propylene bridges. Interestingly,

k_1 of 1,4,7,11-tetraazaundecane is almost one-third greater than that of its isomer, *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine, providing key evidence that the position of a propylene bridge within a structure also exerts a notable influence on exchange. These cases can be understood by examining the trends within the groups and the overall basicities of the compounds. First, concerning pentaethylenehexamine relative to the acyclic compounds with only ethylene bridges, the rate constants are largest for the smallest compounds (>10 for diethylenetriamine and triethylenetetramine) and decrease for the larger compounds (8.0 for tetraethylenepentamine and 2.6 for pentaethylenehexamine). As the size of the polyamines increases, the overall flexibility of the compounds increases along with the likelihood of forming more optimal intramolecular hydrogen bonds. This could result in a reduction in the ability of the polyamine to bind the deuterating reagent strongly, or the interaction of ammonia-d_3 could be localized to a single amino group and thus result in lower overall exchange. In addition, pentaethylenehexamine is the most basic polyamine studied, and the difference in basicity between it and ammonia is around 200 kJ/mol. In the absence of favorable interactions between multiple active sites in the polyamine and ammonia, the efficiency of exchange is decreased due to the large basicity difference. Thus, for the series of ethylene-bridged polyamines, the overall flexibility and basicity correlate strongly with the H/D exchange rate constants.

1,4,7,11-Tetraazaundecane has one terminal three-carbon bridge yet has a k_1 of 2.6, which is almost one-third larger than any other polyamine with at least one propylene unit. Within the subset of acyclic polyamines with propylene units, the rate constants decrease as more propylene groups are added, starting at 1.8 for one propyl group [*N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine], decreasing to 1.0 for two propyl groups [*N*,*N*′-bis(3-aminopropyl)-1,3-ethylenediamine], and decreasing still to 0.49 for three propyl groups [*N*,*N*′-bis(3-aminopropyl)-1,3-propanediamine]. The flexibility of the polyamines increases with increasing numbers of propyl groups and thus the ability to form strong intramolecular hydrogen bonds, as illustrated for *N*,*N*′-bis(3-aminopropyl)-1,3-propanediamine in Figure 4D. As shown, this capacity for forming strong intramolecular bonds limits the interaction between the polyamine and deuterated ammonia. In fact, molecular modeling suggests that while protonation on a primary amine is favored for the polyamines with three and two propyl groups in order to form a stabilizing ring structure, protonation on a secondary amine is just as favorable for the polyamines with only one propyl group. In addition, as the basicity of the polyamines decreases, the difference in basicity between a polyamine and deuterated ammonia also decreases, thus increasing the efficiency of exchange. Therefore, the two least basic compounds with only one propyl bridge have the highest rate constants of the subset of compounds with propyl bridges.

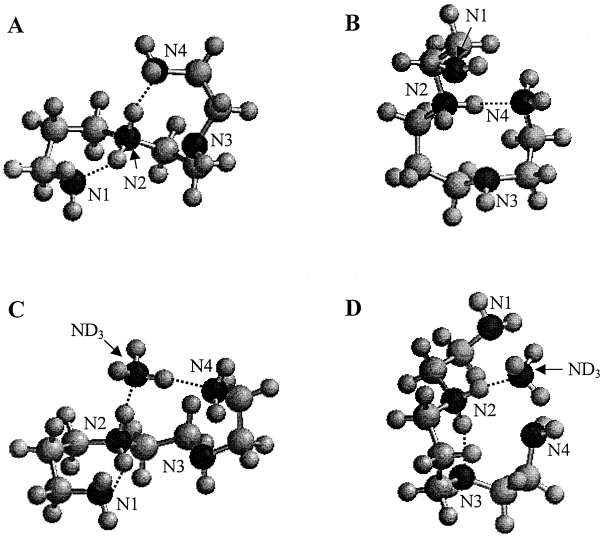

Figure 5. Geometry optimized structures for protonated **(A)** 1,4,7,11-tetraazaundecane and **(B)** *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine and protonated **(C)** 1,4,7,11-tetraazaundecane and **(D)** *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine reacting with ND_3. Hydrogen bonds are indicated with dashed lines. Atom key: small white = hydrogen, medium white = carbon, medium black = nitrogen (numbered).

The difference in first rate constants for the two isomers, 1,4,7,11-tetraazaundecane and *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine, stems from the position of the propylene bridge. 1,4,7,11-Tetraazaundecane is slightly more basic than *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine [22], and it undergoes a higher extent of hydrogen/deuterium exchange with deuterated ammonia. The geometry optimized structures for protonated 1,4,7,11-tetraazaundecane and *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine are shown in Figure 5A, B, with the ionizing proton on the secondary amine (N2) in both cases. Both ions have an intramolecular hydrogen bond between the ionizing proton and the farthest terminal amine, N4. The propyl group in the middle of the molecule allows the hydrogen bond to have a more optimal angle, 176°, in the case of *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine as opposed to 167° for 1,4,7,11-tetraazaundecane (bond angles and distances shown in Table 3). Because 1,4,7,11-tetraazaundecane has the three-carbon bridge between N1 and N2, an additional hydrogen bond can form between those two amino groups. Although it has a less than optimal angle, 144°, it serves as another stabilizing interaction for the protonated polyamine. The calculated structures for protonated 1,4,7,11-tetraazaundecane and *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine reacting with ND_3 are shown in Figure 5C, D. In the case of *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine, ND_3 interacts solely with the ionizing proton on N2. Interestingly, a new intramolecular hydrogen bond is formed between the two amino groups separated by the center three-carbon

Table 3. Calculated bond distances and angles for protonated 1,4,7,11-tetraazaundecane and *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine structures shown in Figure 5

Structure		Bond distance (Å)	Bond angle (°)
(A) (1,4,7,11-tetraazaundecane + H)$^+$			
	N1–H–N2	1.81	144
	N2–H–N4	1.77	167
(B) [*N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine + H]$^+$			
	N2–H–N4	1.76	176
(C) (1,4,7,11-tetraazaundecane + H + ND_3)$^+$			
	N1–H–N2	1.81	144
	N2–H–N_{ND3}	1.72	170
	N_{ND3}–D–N4	1.84	175
(D) [*N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine + H + ND_3]$^+$			
	N_{ND3}–H–N2	1.76	171
	N2–H–N3	1.81	144

bridge, N2 and N3, analogous to what was seen for protonated 1,4,7,11-tetraazaundecane. This structure causes the two terminal amino groups to be too far away to simultaneously interact with the deuterated ammonia molecule. In contrast, ND_3 inserts itself in between the hydrogen bond between N2 and N4 in 1,4,7,11-tetraazaundecane (Figure 5C). This allows the deuterating agent to simultaneously interact with two amino groups with active hydrogens. The original intramolecular hydrogen bond between N1 and N2 appears unperturbed by the addition of ND_3. These additional stabilizing intermolecular hydrogen bonds formed between the deuterating agent and 1,4,7,11-tetraazaundecane as a result of the position of the propyl group enhance the hydrogen/deuterium exchange reactions and explain the higher extent of exchange observed for 1,4,7,11-tetraazaundecane compared to its structural isomer *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine.

Comparison of Exchange Reactions Involving (M + Alkali Metal$^+$) Species

Hydrogen/deuterium exchange was also undertaken for those polyamines that formed (M + alkali metal$^+$) complexes with Li^+, Na^+, and K^+ [diethylenetriamine, *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine, *N*,*N*′-bis(3-aminopropyl)-1,3-ethylenediamine, cyclen, tetraethylenepentamine, and pentaethylenehexamine]. An example of spectra observed for these experiments is given in Figure 6 for a solution of cyclen with sodium and potassium. Figure 6A shows the electrospray spectrum of the solution in the absence of a deuterating reagent, whereas the spectrum in Figure 6B shows the same solution after 3 s of reaction time with ND_3 present in the vacuum chamber. This spectrum clearly indicates that, under identical conditions, exchange with the singly protonated analyte is faster and more efficient than exchange with either the sodium or potassium cationized complex.

For all compounds studied, a dramatic difference is observed between the exchange of singly protonated versus alkali metal cationized complexes. A kinetic plot for the exchange of (diethylenetriamine + K^+) with ND_3 was undertaken (data not shown). Compared to the exchange of (diethylenetriamine + H)$^+$ shown in Figure 3A, the exchange is shut down for the potassium cationized complex. Although it appears as though some exchange may be occurring, the relative intensity of the "1D" ion is never greater than 14%, and this is possibly due to incomplete ejection of the ^{13}C isotope peak rather than hydrogen/deuterium exchange. Regardless, a relative rate constant (k_1) of 0.051 was generated using the relatively poor fit obtained, indicating the k_1 for exchange of (M + K^+) is at least 3600 times lower than k_1 for exchange of (M + H)$^+$. These results are similar for the six polyamines examined with Li^+, Na^+, and K^+, and they are independent of the identity of the alkali metal ion.

One explanation for the extreme differences in reactivity involves the thermodynamics of the reactions. Hydrogen/deuterium exchange between an ion and deuterated ammonia has been proposed to proceed via proton transfer from the ion to ND_3 to form ND_3H^+,

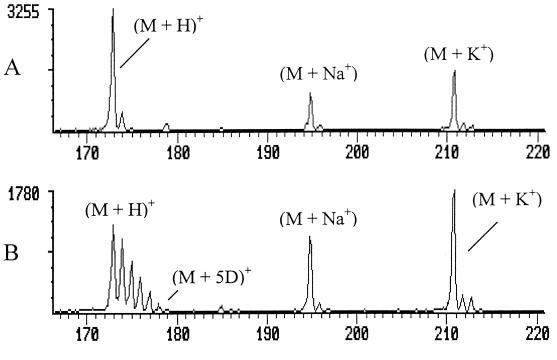

Figure 6. Hydrogen/deuterium exchange of cyclen (M + H)$^+$, (M + Na^+), and (M + K^+) ions. **(A)** Electrospray spectrum of cyclen and KCl solution with no ND_3 admitted. A significant sodium adduct is also present. **(B)** After 3 s of reaction time with ND_3 at 5×10^{-4} torr, there is exchange of all five active hydrogens from the (M + H)$^+$ ion but little exchange with either of the alkali metal adducts.

which is solvated by the neutral molecule [27–29]. The final step is a transfer of D^+ from the protonated ammonia back to the neutral molecule. Complexation of an alkali metal ion, which is less charge dense relative to a proton, results in less incipient positive charge localization on each of the amine groups, meaning that the active hydrogens are less acidic than those of the analogous protonated complexes. Consequently, the hydrogens should be less labile for exchange and the proton transfer mechanism leading to exchange may be thermodynamically unfavorable. For example, molecular modeling indicates there is about a 250 kJ/mol difference in the energies required to deprotonate $(cyclen + H)^+$ as opposed to $(cyclen + Na^+)$, with $(cyclen + Na^+)$ having the larger energy barrier. This large energy difference could explain the inefficient H/D exchange of the alkali metal cationized polyamines.

Another factor that may contribute to the lack of H/D exchange for the alkali metal cationized complexes stems from the gas-phase structures of the complexes. The gas-phase structures of the singly protonated and sodium cationized complexes are quite different, and that is reflected in the observed differences in exchange. The sodium ion is significantly bigger than the proton [26] and may be situated above the ring of cyclen if it cannot fit inside the cavity without causing undue strain. This could cause significant steric hindrance for the approach of a deuterated molecule on one side of the ring, and thus result in a slower rate of exchange. In contrast, the presence of the smaller proton on the $(cyclen + H)^+$ species should allow ND_3 easier access to the active hydrogens of cyclen.

Molecular modeling calculations were undertaken in order to further elucidate the structural differences in the complexes. Utilizing a molecular mechanics conformational search and the resulting lowest energy conformations, structures of $(cyclen + H + ND_3)^+$ and $(cyclen + Na^+ + ND_3)$ were compared. Examples of the resulting optimized structures are shown in Figure 7. In both cases the optimal placement of the ionizing proton or sodium cation was essentially in the center of the cyclen ring. This is reasonable because the lone pair electrons on the four nitrogen atoms of cyclen are polarized by the ionizing proton or sodium ion and help stabilize the resulting positive charge. However, the differences in structures are clearly illustrated (Figure 7). A "pinched in" structure results for monoprotonated cyclen because a cross-ring intramolecular hydrogen bond is formed. Thus two of the amino groups are in relatively close proximity. In contrast, the amino groups in sodium cationized cyclen are all spread apart and roughly equidistant from the sodium cation. A molecule of ND_3 interacting with monoprotonated cyclen is forced off to the side but interacts directly with the protonated ring nitrogen. The energy minimized structure of ND_3 interacting with sodium cationized cyclen shows deuterated ammonia interacting directly with the sodium ion and being relatively far away from the amino groups in the ring. This inhibits hydrogen/deuterium exchange in two ways. First, the deuterated ammonia is physically too far away from the amine nitrogens to interact to any great extent. Second, this structure suggests that the deuterating agent is more strongly attracted to the sodium ion regardless, and that even if it were closer to an active hydrogen exchange may not be a favored reaction.

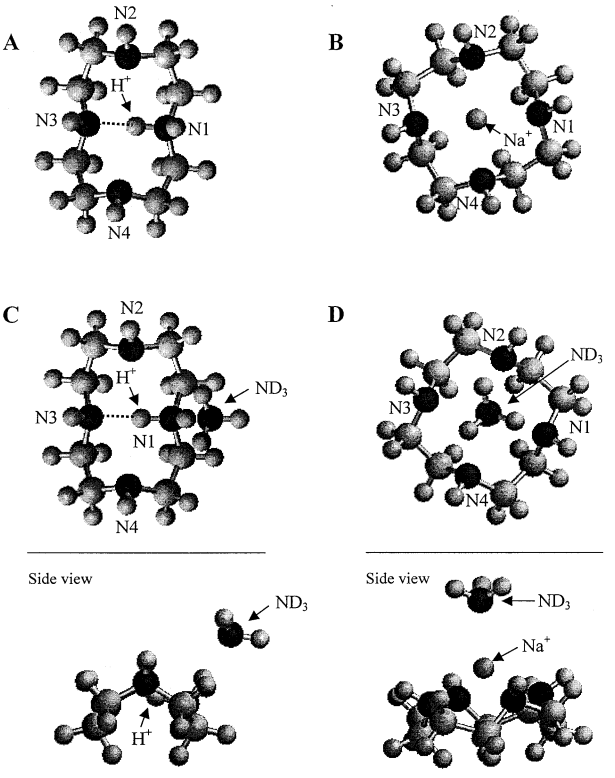

Figure 7. Geometry optimized structures for **(A)** protonated and **(B)** sodium cationized cyclen and **(C)** protonated and **(D)** sodium cationized cyclen reacting with ND_3. Both top and side views are shown for **(C)** and **(D)**. Hydrogen bonds are indicated with dashed lines. Atom key: small white = hydrogen, medium white = carbon, medium grey = sodium, medium black = nitrogen (numbered).

Comparison of Exchange Reactions Involving $(M + 2H)^{2+}$ Species

The five polyamines which formed intense doubly protonated ions via electrospray ionization either when sprayed alone or with the addition of an alkali metal salt (LiCl, NaCl, or KCl) are 1,4,8,12-tetraazacyclopentadecane, *N,N'*-bis(3-aminopropyl)-1,3-propanediamine, tetraethylenepentamine, 1,4,7,11-tetraazaundecane, and triethylenetetramine. Figure 8 contains example spectra for exchange reactions between deuterated methanol and singly versus doubly protonated triethylenetetramine. The singly protonated ion undergoes five exchanges while $(M + H)^+$ remains the dominant peak after 1 s reaction time. The doubly protonated peak undergoes seven exchanges and after the

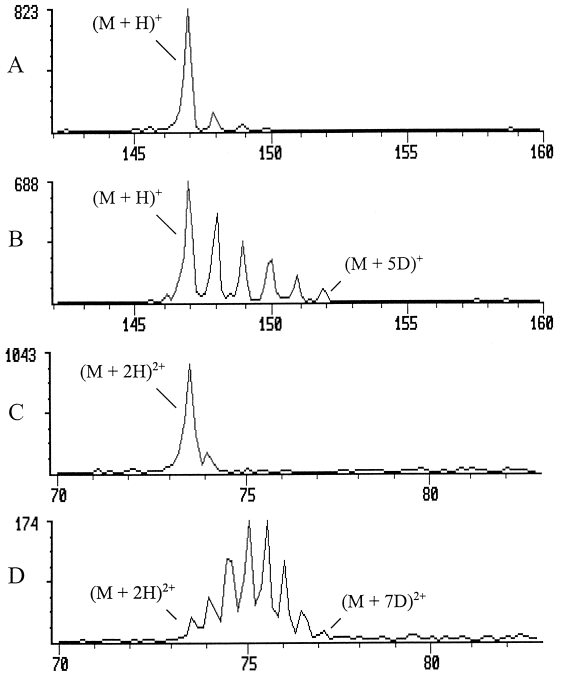

Figure 8. Hydrogen/deuterium exchange of triethylenetetramine $(M + H)^+$ and $(M + 2H)^{2+}$ ions. **(A)** Close-up of the electrosprayed $(M + H)^+$ ion with no CH_3OD admitted. **(B)** After 1 s of reaction time with CH_3OD at 4×10^{-4} torr, there is exchange of five active hydrogens. **(C)** Close-up of the $(M + 2H)^{2+}$ ion from the same solution on the same day, with no CH_3OD added. **(D)** After 1 s of reaction time with CH_3OD at 4×10^{-4} torr, there is exchange of seven active hydrogens.

same amount of reaction time $(M + 3D)^{2+}$ and $(M + 4D)^{2+}$ are the most abundant ions.

Methanol-O-*d* was chosen as the exchange reagent for the doubly protonated species because in most cases when ammonia-d_3 was used, the signal intensity for the doubly protonated ion decreased dramatically, due to deprotonation of the doubly protonated ion by ND_3. Although the second proton affinities for these compounds are not known, they are most certainly lower than their first proton affinities, and it is likely that a strong base such as ammonia could preferentially abstract a proton instead of exchange a deuterium for a proton. However, ions of *m/z* lower than 40 were typically ejected from the trap under the conditions used in this study, and thus the formation of ND_3H^+ ions of *m/z* 21 could not be monitored. In any case, the results with methanol-O-*d* clearly indicate greater exchange for all of the doubly protonated species compared to the singly protonated species.

A rigorous kinetic analysis was not performed for the doubly protonated polyamines for several reasons. First, the doubly protonated ions undergo facile deprotonation and when any deuterating reagent was added, even to $\sim 1 \times 10^{-4}$ torr, the signal for the doubly protonated ion would decrease dramatically, often by a factor of 10 or more. In most cases the ion signal that remained would be sufficient for a single time point in the kinetic study, but the overall signal quality was poor over a 10 or 20 s time range. In addition, the reactions of the doubly protonated polyamine ions were faster and more efficient than those of the singly protonated ions. The reactions proceeded to a substantial degree before any formal reaction time was allowed between the ions and deuterated methanol, thus resulting in kinetic curves that could not be accurately fit. This factor stems from the series of intrinsic timing delays in the ion trap scanning program, causing a period of about 500 ms in which the ions are stored irrespective of the formal reaction time. Finally, any attempt to completely isolate the $(M + 2H)^{2+}$ ion resulted in ejection of the ion from the ion trap instead of isolation. A more stable position for the ions in their pseudopotential wells could not be found such that complete isolation was possible without concurrent ion ejection.

Table 4. H/D exchange factors for polyamine complexes reacting with CH_3OD[a]

Compound	Exchange factors for $(M + H)^+$	Exchange factors for $(M + 2H)^{2+}$
1,4,8,12-Tetraazacyclopentadecane	1	7
N,*N*′-Bis(3-aminopropyl)-1,3-propanediamine	3	12
Tetraethylenepentamine	3	61
1,4,7,11-Tetraazaundecane	2	31
Triethylenetetramine	4	19

[a]Exchange factors calculated as the sum of the peak heights of all deuterium-exchanged ions divided by the final peak height of the unexchanged precursor ion. These factors provide only a qualitative indication of the efficiency of H/D exchange and they are a function of the extent of reaction. Reactions were carried out with CH_3OD at 3–4 $\times$ 10^{-4} torr for no more than 3 s. For a given polyamine, the $(M + H)^+$ and $(M + 2H)^{2+}$ experiments were carried out under identical conditions. All values ± 30%.

However, as shown by the example in Figure 8, single time point data could be collected relatively easily and reproducibly for both the $(M + H)^+$ and $(M + 2H)^{2+}$ ions for the five polyamines studied. Thus exchange of the singly and doubly protonated ions could be directly compared to each other. In order to simplify the process of determining the extent and efficiency of exchange, "exchange factors" were calculated as the sum of the peak heights of all deuterium-exchanged ions divided by the final peak height of the unexchanged precursor ion. A larger exchange factor corresponds to a more extensive incorporation of deuterium. For the example shown in Figure 8, exchange factors were calculated as 4 for $(M + H)^+$ and 19 for $(M + 2H)^{2+}$. The resulting exchange factors are shown in Table 4. In each case, the exchange was much more efficient for the doubly protonated ions compared to the singly protonated ions. Note that the exchange factors are "snapshots" of the entire exchange reaction taken at one point in time. In addition, because all of the hydrogens present in the polyamines are attached to amine nitrogens (i.e., are in similar environments), and because these exchange factors are being used to com-

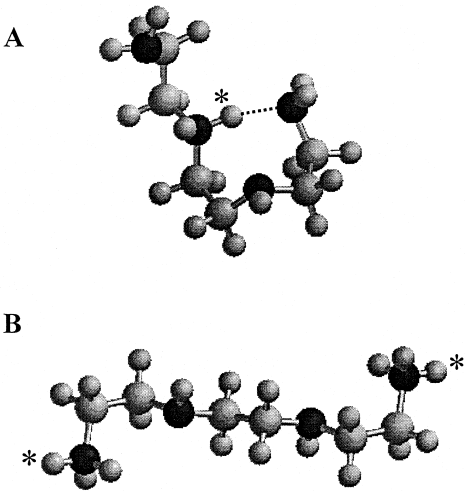

Figure 9. Geometry optimized structures of **(A)** singly protonated and **(B)** doubly protonated triethylenetetramine. Ionizing protons are indicated with asterisks. Hydrogen bonds are indicated with dashed lines. Atom key: small white = hydrogen, medium white = carbon, medium black = nitrogen.

pare two charge states of a single compound, the use of exchange factors in this instance where a full kinetic analysis is difficult or impossible is warranted. In fact, exchange factors correlate remarkably well with the k_1 values obtained for the singly protonated polyamines using ND_3 (see Table 2), and thus the exchange factors represent an alternate way to screen the H/D exchange reactivities of protonated species. Nevertheless, rate constants should be determined whenever possible because they provide information about the relative efficiencies of the sequential exchange reactions.

The differences in exchange factors between the singly protonated and doubly protonated polyamines are quite dramatic, with the doubly protonated ions having exchange factors that are greater by at least an order of magnitude. This is in good agreement with the general trend of higher reactivity for multiply charged ions [30]. In addition to the obvious changes imparted on the polyamine ion (increased charge density and an extra site for H/D exchange), a second proton also affects the conformation of the ion. Electrostatic effects most likely direct the complex to become more spread out or diffuse. Molecular modeling calculations support this assumption (Figure 9). The lowest energy doubly protonated structure calculated for triethylenetetramine has one proton on each primary amine and has the two charged amino groups separated from each other by 9.4 Å (shown in Figure 9B). The lowest energy singly protonated structure for triethylenetetramine is rather compact and is shown in Figure 9A. The proton is on a secondary amino group and the farther terminal amine folds over to stabilize the charge by hydrogen bonding. The two primary amino groups are calculated to be only 4.6 Å apart in this structure. These differences in the structures of the monoprotonated and doubly protonated complexes suggest that the more diffuse doubly protonated complex undergoes more rapid exchange than the more compact singly protonated complex because the active hydrogens of the former are not tied up in intramolecular hydrogen bonds and they are associated with charge-rich centers. Similar results were found in recent work from the Clemmer group [11]. Compact and diffuse conformations of cytochrome *c* were studied via both ion-mobility and hydrogen/deuterium exchange studies. They found that the compact conformations of the +8 to +10 charge states exchanged an average of 46 protons, whereas the diffuse conformations of the same charge states exchanged an average of 63 protons. These trends were interpreted as signifying that the more diffuse structures facilitated exchange because the hydrogens are less protected. They found no dependence on charge state for the actual numbers of protons exchanged, but they did note that as the charge state increased, the rate constants for exchange increased [11].

Cassady obtained somewhat contradictory results in a study of the +3 versus +4 charge states of three synthetic peptides, each containing four basic lysine residues with eight glycine residues in different primary sequences [12]. For two of the peptides, the +3 charge states were more compact than the +4 charge states and subsequently underwent more hydrogen/deuterium exchange at a higher rate. For the third peptide, the +3 and +4 charge states had similar compact structures and underwent similar exchange, but the +4 charge state had a greater rate of exchange [12]. Thus for two different charge states with a similar conformation, the more highly charged species would undergo exchange at a higher rate. However, for charge states with different conformations, the more compact conformations generally underwent more exchange. Cassady's interpretation was that the less highly charged (+3), more compact peptide ions underwent more extensive exchange because they better facilitated the formation of hydrogen-bonded intermediates involving the deuterated reagent. The reason for the discrepancy between Cassady's work and the present work is not clear. It may be that in some cases, conformation plays a larger role in the interaction with the exchange reagent. In peptides for example, the charged basic groups may be much farther away from other basic groups and much more dependent on tertiary structure to get close enough together to interact. In smaller compounds such as the polyamines, the higher charge states may be more reactive regardless of conformation or in combination with the more diffuse conformation, which allows better access to the labile hydrogens.

Conclusions

The combination of hydrogen/deuterium exchange and molecular modeling allowed the investigation of noncovalently bound multidentate polyamine complexes in the gas phase. Large differences in the reactivities of

singly protonated, doubly protonated, and alkali metal cationized polyamine complexes were observed. The efficiencies of the H/D exchange reactions for the protonated polyamines were well-correlated with the formation of inter- vs. intramolecular hydrogen bonds, as influenced by the flexibility of the polyamine. For example, the presence and position of a propylene bridge relative to an ethylene bridge reduces the rates of H/D exchange because of formation of stronger intramolecular hydrogen bonds. The exchange reactions between alkali metal cationized polyamines and ND_3 were effectively quenched. This can be explained either by the loss of a low energy exchange pathway between the alkali metal cationized species and the reagent gas or by a structural change that was induced by the presence of a bulkier cation as compared to a proton. The latter explanation could result in loss of the ability to form and/or stabilize the (M + alkali metal$^+$ + ND_3) intermediate, whereas the former may result in a kinetically unfavorable reaction mechanism.

In contrast, doubly protonated polyamine ions underwent exchange of more hydrogens and at greater rates than the singly protonated polyamine ions. The doubly protonated polyamine ions have a higher charge density than their monoprotonated counterparts, allowing more efficient and rapid exchange. Additionally, calculations suggest that the doubly charged ligands may exist in more diffuse conformations, thus allowing access to more hydrogens that are not involved in intramolecular hydrogen bonds and further facilitating exchange. These studies of model noncovalently bound systems can be used to further understand the reactions and conformations of larger systems, such as peptides and proteins, which are known to be involved in intramolecular hydrogen bonding and form many types of metal complexes in the gas phase.

Acknowledgments

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