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Published on Web 10/18/2003

The Effect of the Initial Water of Hydration on the Energetics, Structures, and H/D Exchange Mechanism of a Family of Pentapeptides: An Experimental and Theoretical Study

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Abstract: A series of gas-phase experiments and extensive theoretical modeling was done on the family of singly protonated peptides AARAA, Ac-AARAA, and AARAA-OMe. $(AARAA)H^+$ underwent extensive H/D exchange with D_2O, whereas the other two peptides with blocked termini did not, implying that a salt bridge was involved in the H/D exchange process. Ion mobility measurements and complementary molecular modeling unambiguously identified the 300 K structures of all three protonated peptides as charge solvation structures, not salt bridges. High-level density functional theory calculations indicated the global minimum of $(AARAA)H^+$ was a charge solvation structure with the lowest-energy salt bridge structure 4.8 kcal/mol higher in energy. Uptake of the first five water molecules of hydration at 260 K showed near identical propensities for all three peptides consistent with a common structural motif. Quantitative measurements of $\Delta H°$ and $\Delta S°$ for the first two waters of hydration were very similar for all three peptides, again suggestive of a common structure. A detailed search of the potential energy surface for the singly hydrated $(AARAA)H^+$ using molecular mechanics and density functional theory approaches indicated a charge solvation structure was the global minimum, but now the lowest-energy salt bridge structure was only 1.8 kcal/mol higher in energy. Importantly, a low-energy transition state connecting the charge solvation and the salt bridge structures was found where the D_2O molecule facilitated H/D exchange via the relay mechanism. This "relay" transition state was 7 kcal/mol below the $(AARAA)H^+ + D_2O$ asymptotic energy, suggesting that facile H/D exchange could occur in this system. There was no equivalent low-lying relay mechanism transition state for the $(Ac\text{-}AARAA)H^+$ and $(AARAA\text{-}OMe)H^+$ peptides, consistent with the fact that H/D exchange was not observed. Hence, the combined experimental and theoretical methods confirmed that a salt bridge was involved in the H/D exchange by D_2O of $(AARAA)H^+$, but it existed only as a kinetic intermediate, not as a global minimum structure. These findings suggest that caution must be observed in drawing structural conclusions from H/D exchange only. A prescription is given here for understanding both the structural and H/D exchange mechanistic aspects of bare and singly hydrated peptides.

Introduction

In chemistry there is a strong structure−function correlation, and in biochemistry this connection is especially strong. The chemical landscape in biological systems is varied, from the hydrophobic environment in and alongside cell walls to the hydrophilic solutions of varying composition within and outside cellular structures. To fully understand peptide and protein function, their structural responses to these myriad influences must be assessed. Few, if any, unambiguous structural studies at the molecular level are carried out in vivo primarily due to inadequate sensitivity, but also due to poorly controlled environmental conditions. The most detailed structural studies are done either in vitro by NMR or other spectroscopies or in crystalline form by X-ray or other scattering probes.[1] In both instances, the revealed structural features are often invoked to explain peptide and protein function in the much more complex cellular workplace. Regardless of the validity of this approach in specific cases, important structural information is obtained that is valid in the medium of the experiment.

From a fundamental standpoint, it is important to benchmark structural and other molecular properties in a solvent-free environment. A good example of the efficacy of this approach is found in the quantitative determination of gas-phase basicities[2] and acidities[3] pioneered in the 1970s. These new solvent-free measurements induced organic chemists to rethink how acid

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(1) For a good discussion of the various condensed phase methods, see: Creighton T. E. *Proteins*, 2nd ed.; Freeman: New York, 1992.

10.1021/ja037204a CCC: $25.00

and base properties related to both the geometric and electronic properties of molecules. It became clear that earlier models were strongly solvent-dependent[2−6] and thus did not correctly reflect intrinsic molecular properties. A parallel situation is currently emerging in structural studies of biopolymers. Only in the past decade or so has it been possible to extract intact biopolymers from solution into the solvent-free gas phase and thus provide the opportunity to probe the intrinsic intramolecular interactions that lead to formation of stable secondary structural motifs and overall tertiary conformations. This revolution has been lead by soft vaporization and ionization techniques such as fast-atom bombardment[7] and, more importantly, electrospray ionization (ESI)[8] and matrix-assisted laser desorption/ionization (MALDI).[9] When these ionization sources are coupled to the versatile and sensitive techniques of modern mass spectrometry, a broad range of structural properties can be probed.

The initial focus of mass spectrometric methods was in primary structure determination utilizing both accurate mass measurements and collisional dissociation (MS-MS).[10] These methods are currently being streamlined to handle the immense throughput issues associated with genomics, proteomics, and general combinatorial approaches to chemistry and biochemistry.[11] There is also a strong push to develop computer algorithms that can unambiguously identify proteins from a minimum of MS-MS or other structural data.[12]

Alongside this effort is the development of methods that can probe secondary and tertiary structure of peptides and (eventually) proteins in solvent-free environments. In solution, H/D exchange of amide backbone hydrogens can be detected using NMR and used to determine "exposed" regions of protein in favorable cases.[13] Analogous experiments can be performed using mass spectrometry[14] to ascertain the number of backbone and side-chain exchanges, but the actual site of exchange can be difficult to unambiguously determine. In the gas-phase, H/D exchange can also be studied[15] and detected by observing an increase in mass as D is substituted for H. If D_2O is used as the exchange reagent, then energetics requires that a "relay" mechanism be used wherein a charged site (H-atom donor) and a basic site (D-atom acceptor) can be simultaneously accessed by the D_2O molecule.[16] Different sites will have different rates of exchange requiring at least qualitative rate studies be done to assist in interpreting the data. Several early studies suggested H/D exchange results could be used to imply structural information about the underlying peptide.[17] A model that incorporates the minimal required elements for interpreting gas-phase H/D exchange data has been developed.[18]

One major difference between solution-phase H/D exchange and gas-phase H/D exchange is that the D_2O molecule might influence the peptide conformation in the gas phase. This is an interesting issue in its own right as the D_2O molecule represents the first step toward solvation by water.[19−22] Of course, any conformational perturbations caused by the D_2O molecule could also compromise structural inferences drawn from H/D exchange results. These coupled issues will comprise the main focus of this paper. Here we will compare H/D exchange results on a family of pentapeptides with ion mobility results on the same molecules (AARAA, Ac-AARAA, and AARAA-OMe). At issue will be whether $(AARAA)H^+$ exists as a salt bridge with both the N-terminus and arginine protonated and the C-terminus deprotonated or whether it exists as a charge solvation structure with the charge only on the arginine side chain. The molecules with blocked termini exclude salt bridge formation and act as controls. Salt bridge and zwitterion formation is a lively topic in the gas phase where solvent stabilization of the charge is not present,[23−27] and criteria for salt bridge formation are still being developed.[28] The molecules chosen for this study are small enough to be investigated by high-level ab initio calculations to further explore the implications from the experiments reported here. The results of these calculations provide insight into the stability of various conformations and also into the detailed mechanism of the H/D exchange process itself.

Experimental and Theoretical Methods

A number of different experimental and theoretical methods have been applied to the systems studied here. Gas-phase H/D exchange experiments were carried out at the University of Arizona at Tucson on a quadrupole ion trap mass spectrometer. Cross section and hydration measurements were performed at the University of California at Santa Barbara on a custom built mass spectrometer equipped with a drift cell. The structure and the mechanism of the H/D exchange reaction of protonated AARAA have been investigated using quantum chemical

(2) (a) The first quantitative proton affinities obtained from equilibrium measurements are reported in Bowers, M. T.; Aue, D. H.; Webb, H. M.; McIver, R. T. *J. Am. Chem. Soc.* **1971**, *93*, 4314. (b) For an early review and discussion, see Aue, D. H.; Bowers, M. T. Stabilities of Positive Ions from Equilibrium Gas-Phase Basicity Measurements. In *Gas Phase Ion Chemistry*; Bowers, M. T., Ed.; Academic Press: New York, 1979; Vol. 2, pp 2−52.
(3) For a good discussion and early review of quantitative gas-phase acidities determined by equilibrium methods, see Bartmess, J. E.; McIver, R. T., Jr. The Gas Phase Acidity Scale. In *Gas Phase Ion Chemistry*; Bowers, M. T., Ed.; Academic Press: New York, 1979; Vol. 2, pp 88−123.
(4) Munson, M. J. B. *J. Am. Chem. Soc.* **1965**, *87*, 2332.
(5) (a) Brauman, J. I.; Blair, L. K. *J. Am. Chem. Soc.* **1968**, *90*, 5636. (b) Brauman, J. I.; Riveras, J. M.; Blair, L. K. *J. Am. Chem. Soc.* **1971**, *93*, 3914.
(6) (a) Aue, D. H.; Webb, H. M.; Bowers, M. T. *J. Am. Chem. Soc.* **1976**, *98*, 318. (b) Aue, D. H.; Webb, H. M.; Bowers, M. T.; Liotta, C. L.; Hopkins, H. P. *J. Am. Chem. Soc.* **1976**, *98*, 854.
(7) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *J. Chem. Soc., Chem. Commun.* **1981**, 325.
(8) Fenn, J. B.; Mann, N.; Merg, C. K.; Wong, S. F. *Mass Spectrom. Rev.* **1990**, *9*, 37.
(9) Hillenkamp, F.; Karas, M.; Beavis, R. C.; Chait, B. T. *Anal. Chem.* **1991**, *63*, 1193A.
(10) See, for example, Jennings, K. R. *Int. J. Mass Spectrom.* **2000**, *200*, 479.
(11) See, for example, Smith, R. D. *Int. J. Mass Spectrom.* **2000**, *200*, 509.
(12) (a) Eng, J. K.; McCormack, A. L.; Yates, J. R., III. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 976. (b) Creasy, D. J.; Cottrell, D. M.; Perkins, J. S.; Pappin, D. N. *Electrophoresis* **1999**, *20*, 3551. (c) Keller, A.; Nesvizhskii, A. I.; Kolker, E.; Aebersold, R. *Anal. Chem.* **2002**, *74*, 5383.
(13) Bai, X.; Sosnick, T. K.; Mayre, L.; Englander, S. W. *Science* **1995**, *269*, 192 and references therein.
(14) (a) Chait, B. T. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 214. (b) Katta, V.; Chait, B. T. *J. Am. Chem. Soc.* **1993**, *115*, 6317. (c) Smith, D. L.; Deng, Y.; Zhang, Z. *J. Mass Spectrom.* **1997**, *32*, 135.
(15) (a) Winger, B. E.; Light-Wahl, K. J.; Rockwood, A. L.; Smith, R. D. *J. Am. Chem. Soc.* **1992**, *114*, 5897. (b) Cheng, X.; Fenselau, C. *Int. J. Mass Spectrom.* **1992**, *122*, 109.
(16) Campbell, S.; Rodgers, M. T.; Marzluff, E. M.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 12840.
(17) Freitas, M. A.; Marshall, A. G. *Int. J. Mass Spectrom.* **1999**, *183*, 221.
(18) Wyttenbach, T.; Bowers, M. T. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 9.
(19) Moet-Ner, M.; Field, F. H. *J. Am. Chem. Soc.* **1974**, *96*, 3168.
(20) Klassen, J. S.; Blades, A. T.; Kebarle, P. *J. Phys. Chem.* **1995**, *99*, 15509.
(21) Kohtani, M.; Jarrold, M. F. *J. Am. Chem. Soc.* **2002**, *124*, 11148.
(22) Liu, D.; Wyttenbach, T.; Barran, P. E.; Bowers, M. T. *J. Am. Chem. Soc.* **2003**, *125*, 8458.
(23) Price, W. D.; Jockusch, R. A.; Williams, E. R. *J. Am. Chem. Soc.* **1997**, *119*, 11988.
(24) Wyttenbach, T.; von Helden, G.; Bowers, M. T. *J. Am. Chem. Soc.* **1996**, *118*, 8355.
(25) Wyttenbach, T.; Bushnell, J. E.; Bowers, M. T. *J. Am. Chem. Soc.* **1998**, *120*, 5098.
(26) Wyttenbach, T.; Witt, M.; Bowers, M. T. *Int. J. Mass Spectrom.* **1999**, *183*, 243.
(27) Chapo, C. J.; Paul, J. B.; Provencal, R. A.; Roth, K.; Saykally, R. J. *J. Am. Chem. Soc.* **1998**, *120*, 12956.
(28) Wyttenbach, T.; Witt, M.; Bowers, M. T. *J. Am. Chem. Soc.* **2000**, *122*, 3458.

calculations at the Deutsches Krebsforschungszentrum at Heidelberg. Both the experimental and theoretical techniques are briefly described below.

Quadrupole Ion Trap Mass Spectrometer. A Finnigan LCQ electrospray/quadrupole ion trap located in the Mass Spectrometry Facility at the University of Arizona was modified to perform ion−molecule reactions. Using a design similar to that of Gronert,[29] we mixed helium with a neutral reagent (D_2O) prior to delivery to the ion trap. An accurate determination of the partial pressure of added reagent gas to the system is difficult but was estimated by Vachet et al.[30] by comparison of deprotonation and dissociation reactions (in a similarly modified ion trap). In a similar manner, we estimated a partial water pressure of $1-2 \times 10^{-5}$ Torr with helium, bringing the total cell pressure to 1×10^{-3} Torr.

To offset the uncertainty of the absolute partial pressure of added D_2O to the helium background gas, all H/D exchange experiments were conducted sequentially without changing the experimental conditions. This process minimized any variations in the trap conditions. Experiments performed early in the session gave the same results when repeated at the end of the session. The advanced scan features of the Finnigan ion trap were utilized, and ions subjected to H/D exchange were selected with a mass window of 10 amu and subsequently trapped with the neutral reagent for the maximum time allowed by the software (10 000 ms) prior to detection.

Ion Mobility Mass Spectrometer. The instrument used in this study to measure collision cross sections and hydration energies of ions has previously been described.[31] Briefly, ions are generated by nanoelectrospray ionization and injected into an ion funnel. The ion funnel is the interface to the vacuum system and can also be used as an ion storage device to convert a continuous ion beam into a short ion pulse for cross section measurements. Ions are injected into the drift cell, where they are quickly thermalized by collisions with the helium buffer gas present at a pressure of several Torr. In the hydration experiments, a continuous beam of ions is injected into the drift cell and thermalization occurs by collisions with water. Arrival time distributions (ATDs) at the detector are measured as a function of drift time voltage across the cell. These measurements yield mobilities that are converted to cross sections using equations derived by kinetic theory.[32]

In the hydration experiments, equilibria (eq 1) yielded equilibrium constants (eq 2) which can be converted to $\Delta G°$ values (eq 3). Equilibrium was verified by varying the drift time across the cell by over a factor of 2 with no observed change in ion intensity ratios.[22] By plotting $\Delta G°$ vs T, the $\Delta H°$ and $\Delta S°$ values are obtained from the intercept and slope, respectively (eq 4).

$$MH^+\cdot(H_2O)_{n-1} + H_2O \rightleftarrows MH^+\cdot(H_2O)_n \quad (1)$$

$$K_{eq} = \frac{[MH^+\cdot(H_2O)_n]\cdot 760\ \text{Torr}}{[MH^+\cdot(H_2O)_{n-1}]\ p(H_2O)} \quad (2)$$

$$\Delta G° = -RT \ln K_{eq} \quad (3)$$

$$\Delta G° = \Delta H° - T\Delta S° \quad (4)$$

Molecular Mechanics and Molecular Dynamics. One hundred candidate structures of each peptide were generated by molecular mechanics methods using the AMBER force field[33] and an annealing protocol previously described.[24] Orientation-averaged projection cross sections were obtained using atomic collision radii parametrized to account for the ion−helium interaction potential.[34] The cross sections reported in this work are average values of structures in the lowest 3 kcal/mol range. Reported error bars represent the maximum and minimum cross sections obtained over this range of structures.

Potential Energy Scan/DFT Calculations. A recently developed conformational search engine[35] devised specifically to deal with protonated peptides was used to scan the potential energy surface (PES) of protonated AARAA and its water complex. These calculations started with molecular dynamics simulations on charge-solvated (CS) and salt bridge (SB) species of $(AARAA)H^+$ using the Insight II program (Biosym Technologies, San Diego, CA) in conjunction with the AMBER force field[33] modified in-house to enable the study of amide oxygen protonated species. During the dynamics calculations, structures were stored every 50 fs for further refinement, applying full geometry optimization using the same force fields. The optimized structures were then analyzed by a conformer family search program developed at Heidelberg. This program is able to group optimized structures into families for which the most important characteristic torsion angles of the molecule are similar. The most stable species in the families were then fully optimized at the HF/3-21G, B3LYP/6-31G(d), and finally at the B3LYP/6-31+G(d,p) levels. For all ab initio calculations, the Gaussian[36] set of programs was used. To gain insight into the mechanism of the H/D exchange reactions, transition structures were located for some selected water complexes of protonated AARAA, and intrinsic reaction path (IRC) calculations were performed for the transition structures (TS) obtained to ensure the proper minima were connected by the TS investigated. Basis set superposition errors (BSSE) for the water binding energy were estimated by the counterpoise correction method.[37]

Throughout the paper the following structural and energetic denotations are used. The first characters represent either charge solvation (CS) or salt bridge (SB) structures. Because of the large number of different species obtained during the scan of the PES of protonated AARAA, we had to drastically prune the field for detailed discussion. The selection was made primarily according to the energy of the species. Higher-energy structures are described in detail only if they are involved in the H/D exchange process. The energy ordering of the structures considered here is indicated by the letters of alphabet. The water complex of the CS and SB structures are denoted by adding "W" to the notation described above. For example, CS_A represents the lowest-energy species of charge solvated $(AARAA)H^+$, and WSB_B represents a salt bridge $(AARAA)H^+\cdots H_2O$ structure with an energy above that of WSB_A.

Results

Quadrupole ion trap mass spectra of mixtures of the three protonated peptides in D_2O are given in Figure 1. In the spectrum obtained for $(AARAA)H^+$ with free termini (Figure

(29) (a) Gronert, S. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 845. (b) Reid, G. E.; O'Hair, R. A.; Styles, M. L.; McFadyen, W. D.; Simpson, R. J. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 1701.
(30) Vachet, R. W.; Hartman, J. A.; Callahan, J. H. *J. Mass Spectrom.* **1998**, *33*, 1209.
(31) Wyttenbach, T.; Kemper, P. R.; Bowers, M. T. *Int. J. Mass Spectrom.* **2001**, *212*, 13.
(32) Mason, E. A.; McDaniel, E. W. *Transport Properties of Ions in Gases*; Wiley: New York, 1988.
(33) Case, D. A.; Pearlman, D. A.; Caldwell, J. W.; Cheatham, T. E., III; Ross, W. S.; Simmerling, C. L.; Darden, T. A.; Merz, K. M.; Stanton, R. V.; Cheng, A. L.; Vincent, J. J.; Crowley, M.; Tsui, V.; Radmer, R. J.; Duan, Y.; Pitera, J.; Massova, I.; Seibel, G. L.; Singh, U. C.; Weiner, P. K.; Kollman, P. A. *AMBER 6*; Unversity of California: San Francisco, 1999.
(34) Wyttenbach, T.; von Helden, G.; Batka, J. J.; Carlat, D.; Bowers, M. T. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 275.
(35) (a) Paizs, B.; Lendvay, G.; Vekey, K.; Suhai, S. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 525. (b) Csonka, I. P.; Paizs, B.; Lendvay, G.; Suhai, S. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 417. (c) Paizs, B.; Suhai, S. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 2307. (d) Paizs, B.; Suhai, S.; Hargittai, B.; Hruby, V. J.; Somogyi, A. *Int. J. Mass Spectrom.* **2002**, *219*, 203.
(36) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94*, revision A.5; Gaussian, Inc.: Pittsburgh, PA, 1995.
(37) Boys, S. F.; Bernardi, F. *Mol. Phys.* **1970**, *19*, 553.

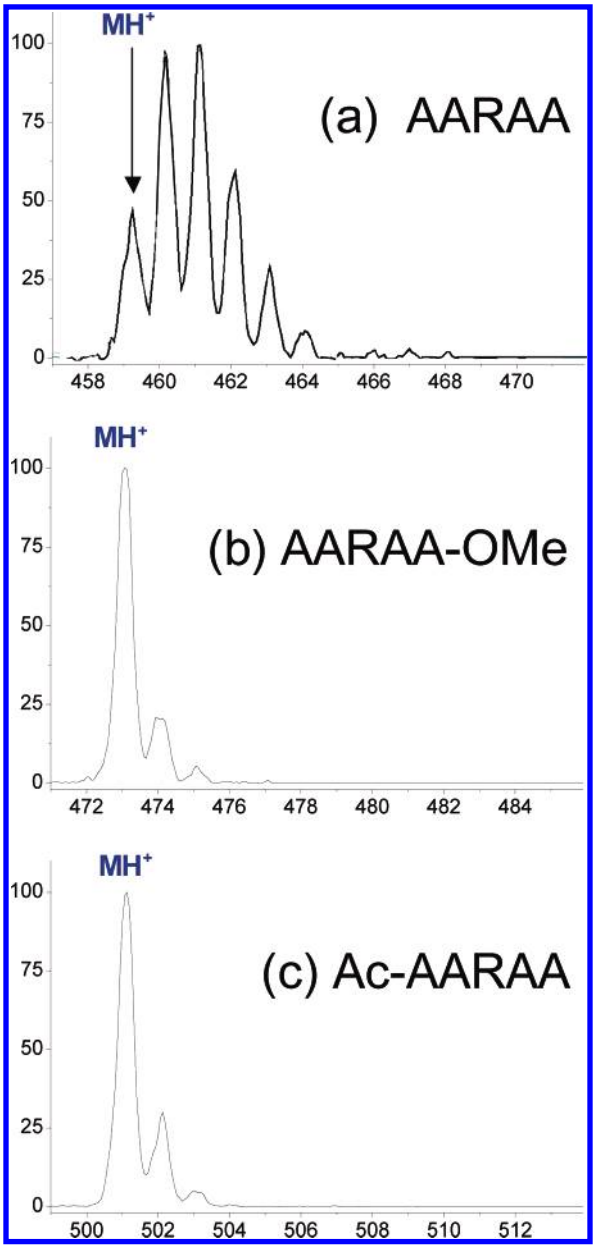


Figure 1. H/D exchange mass spectra of protonated AARAA (a), AARAA-OMe (b), and Ac-AARAA (c) using D_2O as exchange reagent ($\sim 10^{-5}$ Torr, ~10 s trapping time). The all-^{1}H/all-^{12}C monoisotopic species is labeled MH^+. Deuterium uptake is evident as an increase in mass with respect to MH^+.

1a), peaks at 1, 2, 3, 4, and 5 mass units above the nominal mass of the ion are observed, indicating that ^{1}H atoms have been replaced by deuterium (^{2}H). Under identical conditions, the mass spectra of the peptides with one terminus blocked (Figure 1, parts b and c) indicate that essentially no H/D exchange occurs.

The collision cross sections for the $(AARAA)H^+$, $(Ac\text{-}AARAA)H^+$, and $(AARAA\text{-}OMe)H^+$ ions measured in helium by the ion mobility technique are summarized in Figure 2. The somewhat larger ions with blocked termini have proportionately larger cross sections (~153 Å^2) than the smaller $(AARAA)H^+$ ion (~145 Å^2). Also shown in Figure 2 are cross sections calculated for a range of theoretical structures obtained both by molecular modeling and electronic structure calculations. Salt bridge structures for $(AARAA)H^+$ are calculated to be more

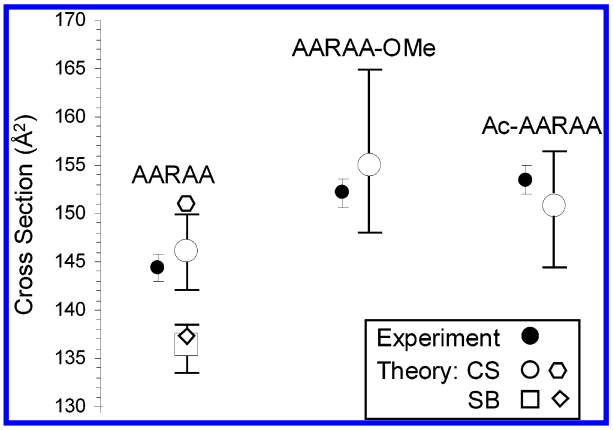


Figure 2. Experimental (solid dots) and theoretical (open symbols) cross sections of $(AARAA)H^+$, $(AARAA\text{-}OMe)H^+$, and $(Ac\text{-}AARAA)H^+$. The average theoretical cross section for AMBER charge solvation (CS) structures is indicated as an open circle and that for AMBER salt bridge (SB) structures as an open square. Cross sections for the DFT structures shown in Figure 3 are indicated as a hexagon (CS_A) and as a diamond (SB_A). The error bars give the cross sectional spread in the lowest 3 kcal/mol of the AMBER calculated structures.

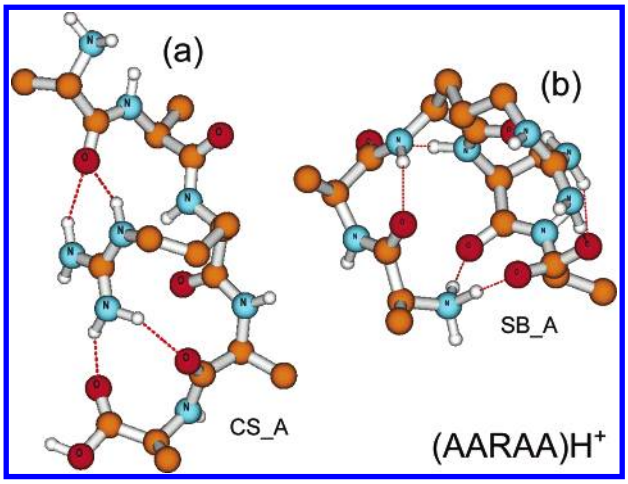


Figure 3. Geometry-optimized $(AARAA)H^+$ structures obtained by a combination of molecular mechanics and electronic structure calculations. (a) Global minimum charge solvation structure. (b) Lowest-energy salt bridge structure (+4.8 kcal/mol). H-atoms, white; C, yellow; N, blue; O, red.

compact with smaller cross sections than charge solvation structures.

The theoretical scan of the PES of protonated AARAA shows that the global minimum of this ion is one of the many stable charge-solvated species found in the calculations (CS_A, Figure 3a, Table 1). Density functional calculations at the B3LYP/6-31+G(d,p) level indicate that the most stable salt bridge structure SB_A (Figure 3b) is one where both the N-terminal amino and the guanidino group are protonated and the C-terminal carboxyl group deprotonated. The relative energy of SB_A with respect to CS_A is +4.8 kcal/mol. Other salt bridge structures considered include species with a protonated backbone amide oxygen instead of a protonated N-terminus, as these species are believed to play a crucial role in the gas-phase H/D exchange of amide hydrogens using D_2O as exchange reagent.[38] However, in the case of $(AARAA)H^+$ these types of salt bridge structures are between 10 and 20 kcal/mol higher in energy depending on which amide oxygen is protonated.

(38) Paizs, B.; Wysocki, V. H.; Bowers, M. T. Unpublished results.

Table 1. Total and Relative Energies for Selected Optimized Structures Calculated at the B3LYP/6-31+G(d,p) Level of Theory

system/structure[a]	B3LYP/6-31+G(d,p) energy (Hartree)	relative energy (kcal/mol)	comments
	(AARAA)H^+		
CS_A	−1596.423905	0.0[b]	Figure 3a
SB_A	−1596.419032	+4.8[b]	Figure 3b
	(AARAA)$H^+\cdots H_2O$		
WCS_A	−1672.878817	−10.8[c,d]	Figure 4a
WSB_A	−1672.879009	−9.0[c]	Figure 4b
WCS_B[e]	−1672.878262	−8.8[c]	Figure 6a
RELAY_TS[e]	−1672.869731	−7.0[c]	Figure 6c
WSB_B[e]	−1672.877503	−7.5[c]	Figure 6b

[a] CS = charge solvation structure, SB = salt bridge structure. An "A" following the dash indicates it is the lowest-energy structure, a "B" a higher energy one, etc. Inclusion of a "W" indicates a water molecule is added. [b] Relative energy is calculated with respect to the total energy of the most stable CS_A species and corrected for ZPE determined at the B3LYP/6-31G(d) level. [c] Relative energy is calculated with respect to the total energy of separated CS_A and water and corrected for ZPE determined at the B3LYP/6-31G(d) level. [d] Water binding energy; BSSE corrected value: −8.9 kcal/mol. [e] Structures related to the H/D exchange relay mechanism (see Figure 6).

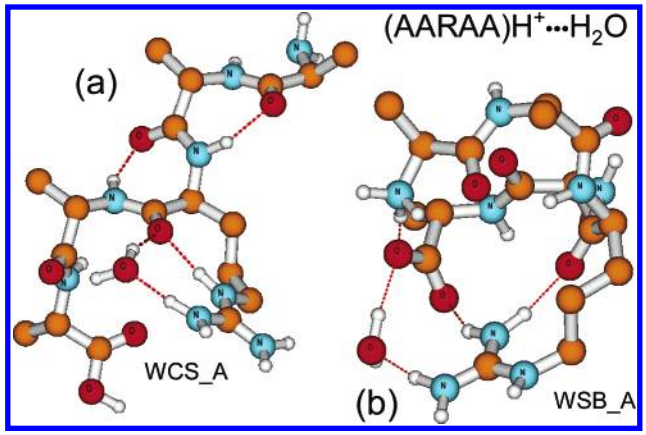


Figure 4. Geometry optimized (AARAA)$H^+\cdots H_2O$ structures obtained by a combination of molecular mechanics and electronic structure calculations. (a) Global minimum charge solvation structure. (b) Lowest-energy salt bridge structure. H-atoms, white; C, yellow; N, blue; O, red.

Scanning the PES of hydrated (AARAA)H^+ indicates that adding a water molecule stabilizes salt bridge structures more than charge solvation structures, but the most stable (AARAA)H+···H2O salt bridge structure (WSB_A, Figure 4b, Table 1) is still 1.8 kcal/mol less stable than its charge solvation counterpart (WCS_A, Figure 4a.). The water binding energy is calculated to be 10.8 kcal/mol at zero-point energy (ZPE)-corrected B3LYP/6-31+G(d,p) level (8.9 kcal/mol after BSSE correction) in reasonably good agreement with the experimental value of 10.2 kcal/mol (Table 2).

Hydration mass spectra obtained at ∼260 K and 1.3 Torr of water vapor pressure are shown in Figure 5 for all three peptide ions. In all cases, up to ∼5 water molecules are added to the peptide. Values for $\Delta H°$ and $\Delta S°$ of hydration for the first two water molecules are ∼10 and ∼8 kcal/mol and ∼22 and ∼18 cal/mol/K, respectively (Table 2), for all three peptides. Temperature could not be varied over a wide enough range to yield reliable values of $\Delta H°$ and $\Delta S°$ for the higher hydrates.

Discussion

The correlation between the extent of gas-phase H/D exchange observed in the ion trap experiment (Figure 1) and the ability to form a salt bridge (free vs blocked termini) for the three molecules (AARAA)H^+, (Ac-AARAA)H^+, and (AARAA-OMe)H^+ is remarkable, leading to a temptation to conclude that (AARAA)H^+ actually is a salt bridge. Freitas and Marshall[17] also used H/D exchange observations between peptides with and without blocked termini to conclude that a number of bradykinin-derived systems were salt bridges. In those instances, however, the presumed salt bridges did not undergo H/D

Table 2. Experimental Enthalpies and Entropies for the Sequential Addition of *n* Water Molecules to the Protonated Species Indicated[a]

	n	$-\Delta H°$ (kcal/mol)	$-\Delta S°$ (cal/mol/K)
AARAA	1	10.2	23
	2	8.4	18
AARAA-OMe	1	9.4	21
	2	8.4	18
Ac-AARAA	1	9.5	21
	2	8.1	18

[a] Uncertainties are ±0.3 kcal/mol for $\Delta H°$ and ±1 cal/mol/K for $\Delta S°$.

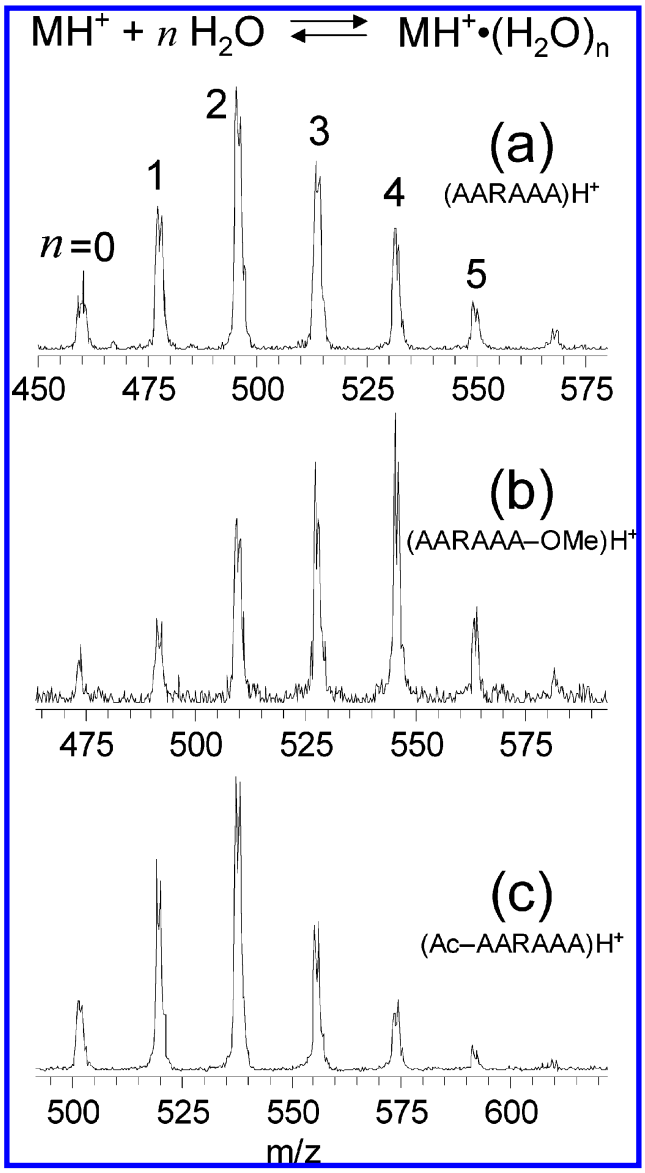
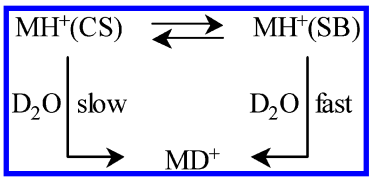


Figure 5. Hydration mass spectra obtained for (AARAA)H^+ (a), (AARAA-OMe)H^+ (b), and (Ac-AARAA)H^+ (c) exposed to 1.3 Torr of water vapor at 260 K under equilibrium conditions.

Scheme 1



exchange, whereas the charge solvation structures underwent extensive exchange. No attempt at theoretically understanding the results was made.

The AARAA system of this study is an ideal candidate for providing a scientific basis for the H/D exchange−salt bridge correlation. In the AARAA molecule there is little ambiguity about the site(s) of protonation and deprotonation in assuming both a charge solvation and a salt bridge structure. The +1 charge state observed in the experiment is also the preferred state in solution (at pH 7). Furthermore, the molecule is small enough to be subjected to sophisticated potential energy scan methods that include extensive electronic structure calculations to obtain theoretical insight about energetics and possible H/D exchange mechanisms. In addition, calculations on $(AARAA)H^+$ indicate that salt bridge structures are on average substantially more compact than charge solvation structures, making an unambiguous assignment based on cross section measurement possible (Figure 2). The experiment clearly agrees with $(AARAA)H^+$ charge solvation structures and disagrees with salt bridges. Hence, under 300 K thermal conditions unsolvated $(AARAA)H^+$ is not a salt bridge, indicating that the gas-phase H/D exchange results are not quite that straightforward to interpret.

Electronic structure calculations support the cross section (ion mobility/molecular mechanics) result (Table 1). The global minimum on the $(AARAA)H^+$ potential surface is the charge solvation structure CS_A shown in Figure 3a, which is 4.8 kcal/mol below the lowest-energy salt bridge structure SB_A (Figure 3b). In a Boltzmann distribution a species with an energy of +4.8 kcal/mol above the ground state has a probability for population of $\sim 10^{-4}$ and would not contribute to the average collision cross section measured for this population. However, under the ion trap experimental conditions ($\sim 10^{-5}$ Torr D_2O, 10 s reaction time) about 5000 collisions occur, indicating that a minor structural component could contribute to the reactivity. Hence, it is possible that ion mobility and gas-phase H/D exchange experiments sample different molecular structures in the overall population (Scheme 1).

If salt bridges are indeed responsible for H/D exchange, the important question that remains is why this is so. To understand the energetic and kinetic details of the H/D exchange process, possible mechanisms have to be evaluated. Unlike ammonia, the water molecule is not basic enough to make proton transfer within the collision complex (to form a peptide$\cdots H_3O^+$ complex) a favorable process. Hence, the relay mechanism[16] must be invoked (Scheme 2).

For $(AARAA)H^+$, the relay mechanism might involve a CS → SB transition with the COOH group being AH and the N-terminal amino group being B. Such a transition leads to the $A^-\cdots H_2O\cdots BH^+$ ion pair present in the SB species.

A scan of the potential energy surface, combined with electronic structure calculations on the $(AARAA)H^+\cdots H_2O$ system, indicates that the global minimum is still a charge

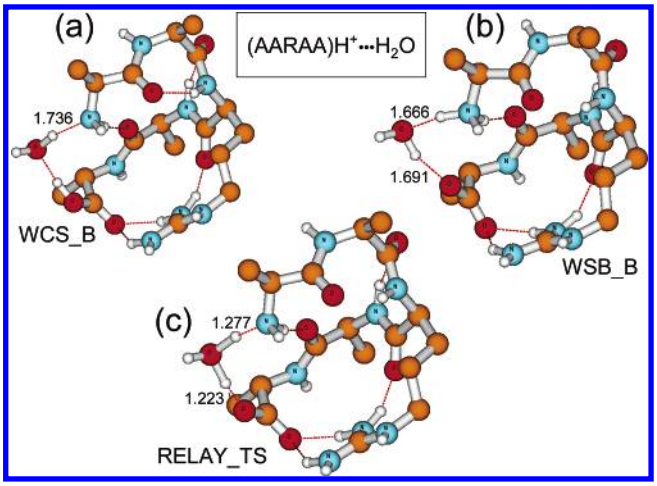
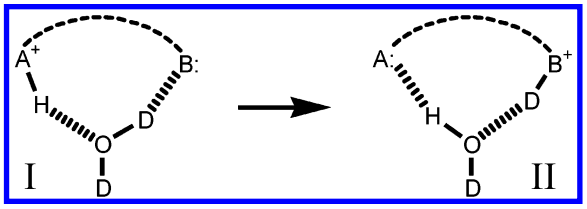


Figure 6. Selected structures of the $(AARAA)H^+\cdots D_2O$ system, where the water molecule has been inserted between the N- and C-termini. Numbers indicate bond lengths in Å. (a) Charge solvated form (WCS_B), (b) salt bridge form (WSB_B), and (c) transition structure (RELAY_TS) of the relay-type H/D exchange reaction between WCS_B and WSB_B. H-atoms, white; C, yellow; N, blue; O, red.

Scheme 2



solvation structure, but the lowest-energy $(AARAA)H^+\cdots H_2O$ salt bridge structure is only 1.8 kcal/mol higher in energy. Hence, addition of the water molecule stabilizes salt bridge structures more than charge solvation structures, a trend that will continue as more water molecules are added (the preferred solution phase structure of $(AARAA)H^+$ in water is a fully hydrated salt bridge). Interestingly, however, the calculations indicate that charge-solvated structures with the water molecule inserted between the N- and C-terminus do exist. Such structures have been found to be favored energetically in an extensive study of hydration of small peptides.[22] Figure 6a shows such a CS structure (WCS_B) located ~9 kcal/mol below the energy level of separated $(AARAA)H^+$ and H_2O.

The relevant question now is: What are the energetics of the relay mechanism starting with the structure shown in Figure 6a? Figure 7 shows schematically a cut through the potential energy surface featuring some of the relevant points on the surface. On the left-hand side are the reactants $(AARAA)H^+$ and D_2O; on the right-hand side are the products $(AARAA)D^+$ and HOD. Between reactants and products lie various structures of the collision complex $(AARAA\cdots D_2O)H^+$. There is a family of charge solvation structures including the global minimum WCS_A and a family of salt bridge structures with the lowest-energy salt bridge structure WSB_A 1.8 kcal/mol above the global minimum. Also shown in Figure 7 are three points relevant to the relay mechanism; the local minimum WCS_B corresponding to the charge-solvated structure set up for the relay mechanism shown in Figure 6a ("I" in Scheme 2), the transition state RELAY_TS corresponding to the saddle point for H/D exchange, and the local minimum WSB_B corresponding to the resulting salt bridge structure ("II" in Scheme 2).

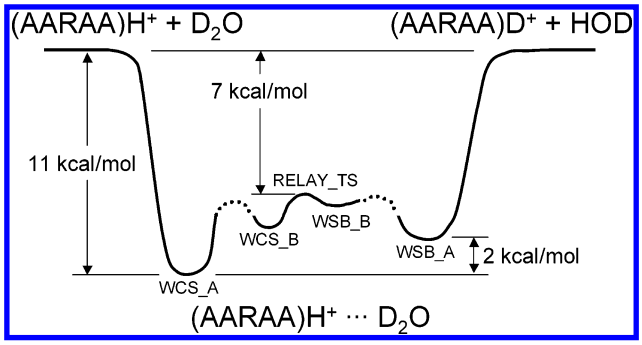


Figure 7. Schematics of the potential energy surface of the [(AARAA)H^+ + H_2O] system showing the relevant structures related to the H/D exchange relay mechanism, charge solvation structure WCS_B, transition state RELAY_TS, and resulting salt bridge structure WSB_B (Figure 6). Dotted lines indicate barriers of unknown height. Energy values indicated are based on B3LYP/6-31+G(d,p) calculations including ZPE correction.

The RELAY_TS is well below the entrance and exit levels of the reaction (by ~7 kcal/mol). Hence, from an energetic point of view H/D exchange proceeds unhindered via this mechanism.

The data (Figure 1) indicate that the reaction efficiency must be low, however, since there are approximately 5000 collisions of (AARAA)H^+ with D_2O under the experimental conditions and only a fraction of the H-atoms have exchanged. One of the possible bottlenecks could be related to the conformation change from a typical low-energy charge solvation structure (shown in Figure 4a), where the N-terminus is very remote from the C-terminus, to a structure set up for the relay mechanism such as that shown in Figure 6a. The bottleneck does not necessarily have to be caused by a high-energy barrier; it could be entropic in nature. The system might not sample the right geometry (WCS_B in Figure 6a) given the limited time scale given by the lifetime of the [(AARAA)H^+···D_2O]* collision complex. On the other hand, it is probably much easier to find the RELAY_TS starting from a typical salt bridge structure (Figure 3b). Hence, both entropy and an equilibrium largely disfavoring salt bridge structures (Scheme 1) could contribute to the low reaction efficiency.

A mechanism involving the three structures depicted in Figure 6, parts a−c, works for the three hydrogen atoms of the $-NH_2$ and −COOH groups, accounting for the bulk of the H/D exchange observed experimentally (Figure 1).[38,39] If salt bridge structures are inaccessible, which is the case for (Ac-AARAA)H^+ and (AARAA-OMe)H^+,[38,40] the relay mechanism transition state would shift to much higher energies, making the reaction very much less efficient in agreement with the experiment (Figure 1).

The (AARAA)H^+···H_2O binding energy is calculated to be 8.9 kcal/mol after corrections for ZPE and BSSE. This value compares favorably with the experimental value of 10.2 kcal/mol (Table 2), giving confidence in the calculations. Hydration mass spectra obtained for (AARAA)H^+ in the temperature range from 250 to 350 K look very similar to those of (Ac-AARAA)H^+ and (AARAA-OMe)H^+ (Figure 5) although there are minor differences.[41] Values for $\Delta H°$ and $\Delta S°$ of hydration obtained from the temperature dependence also turn out to be similar for the three systems (Table 2), providing no support for a salt bridge structure for (AARAA)H^+·$(H_2O)_{1,2}$. The hydration results are thus consistent with the ion mobility and electronic structure calculation results, which indicate that in the gas-phase (AARAA)H^+ assumes a charge solvation structure.

Conclusions

Our combined experimental and theoretical investigation yielded the following results on the AARAA, Ac-AARAA, and AARAA-OMe family of protonated peptides:

(1) (AARAA)H^+ undergoes H/D exchange with D_2O much more rapidly than (Ac-AARAA)H^+ and (AARAA-OMe)H^+. Since the latter two peptides have blocked termini, these results suggest a salt bridge may be involved in the H/D exchange of (AARAA)H^+.

(2) Experimental cross sections of all three peptides are obtained using ion mobility methods. In all three cases, the cross sections are in excellent agreement with model cross sections for charge solvation structures (<2% deviation). Theoretical cross sections for the salt bridge form are 6% too small, conclusively indicating that (AARAA)H^+ has a charge solvation structure as a global minimum.

(3) All three peptides add the first five water molecules with nearly the same propensity at 260 K and 1.2 Torr water pressure, consistent with common structural forms.

(4) Equilibrium measurements yield essentially the same values of $\Delta H°$ and $\Delta S°$ for all three peptides for addition of the first two waters of hydration, again consistent with a common structural motif.

(5) High-level DFT calculations indicate the global minimum of (AARAA)H^+ is a charge solvation structure. The lowest-energy salt bridge form is 4.8 kcal/mol higher in energy.

(6) DFT calculations give good agreement with the experiment for the binding energy of the first water to (AARAA)H^+, indicating a sufficient level of theory is utilized.

(7) Potential energy scan methods were applied to the (AARAA)H^+·H_2O system. The global minimum was still a charge solvation form of (AARAA)H^+ but the lowest-energy salt bridge form was now only 1.8 kcal/mol higher in energy. The results are consistent with the fact that the fully solvated system will be a salt bridge.

(8) A relay mechanism transition state for H/D exchange was found to be only 4 kcal/mol above the global minimum and, hence, 7 kcal/mol below the asymptotic energy of (AARAA)H^+ + D_2O. A relay mechanism H/D exchange process converts a low-lying charge solvation form of (AARAA)H^+ to a low-lying salt bridge structure (or vice versa). Hence, a salt bridge does appear to be involved in the H/D exchange of (AARAA)H^+ even though it is not the global minimum. The inability of (Ac-AARAA)H^+ and (AARAA-OMe)H^+ to form a low-energy salt bridge upon hydration shuts down H/D exchange in these systems.

(9) H/D exchange is a kinetic method that requires both the addition of a water molecule and a suitable low-energy transition

(39) Additional (very slow) H/D exchange is expected for amide hydrogens. Hydrogen atoms least likely to exchange are those of the guanidino group. A mechanism via structure WSB_A, for example, yields a neutral guanidino group which is energetically extremely unfavorable.

(40) For (Ac-AARAA)H^+, salt bridge formation is potentially possible by protonation of backbone amide oxygens. However, the energetics for H/D exchange involving such structures are equally unfavorable as for the exchange of (AARAA)H^+ amide hydrogens. See Results section.

(41) The three mass spectra in Figure 5 are considered very similar: all spectra show peaks in a mass range of ~100 amu and none exhibit a bimodal distribution. Note that a small change in $\Delta G°$ of 1 kcal/mol yields a change in the relative intensity of two adjacent peaks of more than a factor of 5.

state for the exchange to occur. Caution must be utilized in drawing structural conclusions based on H/D exchange measurements alone. A combination of H/D exchange, cross section measurements, hydration studies, and theoretical modeling is needed to characterize the system and is a powerful combination for extracting both structural and mechanistic information.

Acknowledgment. We gratefully acknowledge funding by The National Science Foundation (U.S.A.) under Grant CHE-0140215 (M.T.B.) and funding by the National Institutes of Health (U.S.A.) under Grant GM RO151387 (V.H.W.).

JA037204A