Structural and Energetic Constraints on Gas Phase Hydrogen/Deuterium Exchange Reactions of Protonated Peptides with D_2O, CD_3OD, CD_3CO_2D, and ND_3

Sherrie Campbell, M. T. Rodgers, Elaine M. Marzluff, and J. L. Beauchamp*

Contribution No. 8960, Beckman Institute California Institute of Technology Pasadena, California 91125

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Deuterium exchange has been used extensively in mass spectrometric studies to determine the number of labile sites in simple molecules[1] and, more recently, to infer structural features of complex biomolecules.[2,3] While examples of solution H/D exchange of biomolecules followed by mass spectrometric analysis are abundant,[4] fewer studies have considered H/D exchange in the gas phase.[2,5-7] We have examined H/D exchange reactions of protonated glycine oligomers (Gly_nH^+, n = 1-5) and several small peptides using an external ion source FT-ICR mass spectrometer.[8] Exchange kinetics are highly dependent on peptide structure and the properties, mainly the basicity, of the exchange reagents [D_2O (166),[9] CD_3OD (182), CD_3CO_2D (190), and ND_3 (204)]. We report implications which these studies have for mechanisms of gas phase H/D exchange reactions of protonated peptides.

The small oligomers Gly_nH^+ with $n \leq 3$ readily exchange labile hydrogens with the reagent gases, with exchange rates generally increasing with reagent basicity.[10] For the larger oligomers Gly_4H^+ and Gly_5H^+ reacting with D_2O, and to a lesser extent CD_3OD, facile exchange abruptly halts and only one deuterium is slowly incorporated into the molecule (e.g., Figure 1).[11] In contrast, ND_3 exchanges all labile hydrogens on every glycine oligomer studied. CD_3CO_2D exhibits behavior intermediate between that of D_2O and ND_3, exchanging several but not all of the labile hydrogens of the larger oligomers.[12]

It is of interest to explore the occurrence of multiple exchanges in a single collision event. Continuous ejection of the first exchange product of Gly_3H^+ with D_2O inhibits further exchange (Figure 2a). It follows that D_2O partakes in predominantly single exchanges. In contrast, continuous ejection of the first exchange product of Gly_3H^+ with ND_3 does not inhibit further exchange (Figure 2b). Multiple exchanges in a single collision event are prevalent with ND_3.

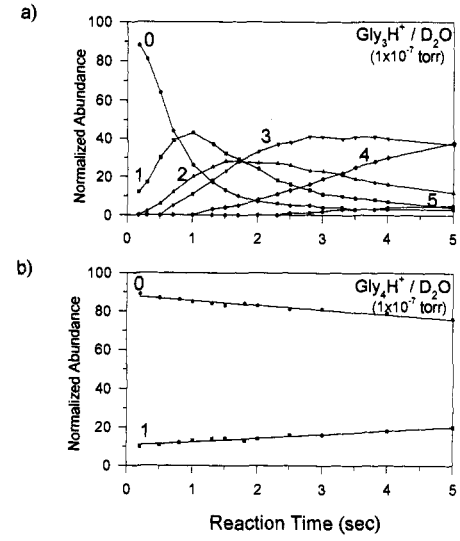

Figure 1. Temporal variation of exchange products of Gly_nH^+. (a) Gly_3H^+ readily exchanges five of six labile hydrogens with D_2O. (b) Gly_4H^+ slowly exchanges a single hydrogen with D_2O. The number of deuterium atoms incorporated into each species is indicated.

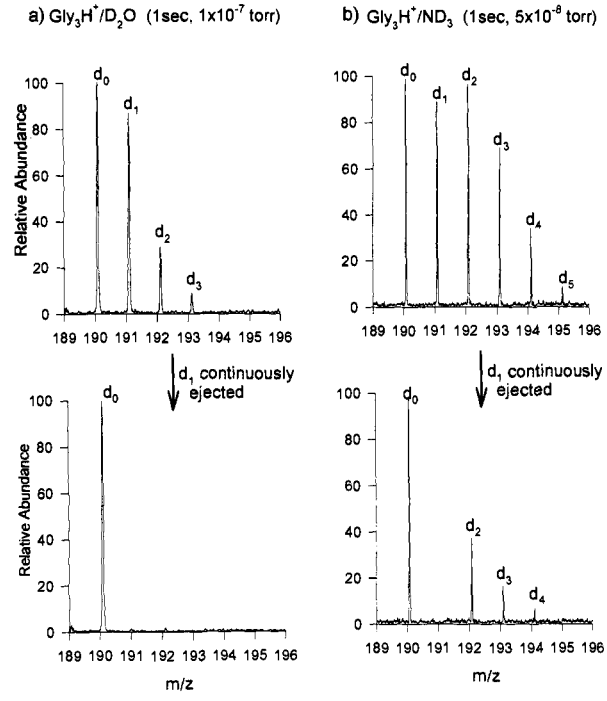

Figure 2. (a) Exchange products of Gly_3H^+ with D_2O. Continuous ejection of the d_1 species (lower spectrum) inhibits higher exchange products. (b) Exchange products of Gly_3H^+ with ND_3. Continuous ejection of the d_1 species (lower separation) still yields higher deuterated products. For all experiments, ion ejection occurs in a time short compared to the time between collisions.

ND_3 is the most effective reagent for facile exchange of labile hydrogens in protonated peptides. Table 1 summarizes H/D exchange reactions of eight selected protonated peptides with ND_3. Peptides which lack basic residues, such as glycine oligomers and leucine enkephalin, exchange all labile hydrogens. Introduction of basic residues such as arginine, with a proton affinity approximately 40 kcal/mol higher than that of ND_3,[13] has a significant impact on exchange. Leucine enkephalin arginine

* Author to whom correspondence should be addressed.

(1) See, for example: Ranasinghe, A.; Cooks, R. G.; Sethi, S. K. *Org. Mass Spectrom.* **1992**, *27*, 77. Hunt, D. F.; Sethi, S. K. *J. Am. Chem. Soc.* **1980**, *102*, 6953.

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(3) Winger, B. E.; Light-Wahl, K. J.; Rockwood, A. L.; Smith, R. D. *J. Am. Chem. Soc.* **1992**, *114*, 5897.

(4) See, for example: Verma, S.; Pomerantz, S. C.; Sethi, S. K.; McCloskey, J. A. *Anal. Chem.* **1986**, *58*, 2898. Katta, V.; Chait, B. T. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 214.

(5) Cheng, X.; Fenselau, C. *Int. J. Mass Spectrom. Ion Processes* **1992**, *122*, 109.

(6) (a) Gard, E.; Willard, D.; Green, M. K.; Bregar, J.; Lebrilla, C. B. *Org. Mass Spectrom.* **1993**, *28*, 1632. (b) Gard, E.; Green, M. K.; Bregar, J.; Lebrilla, C. B. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 614.

(7) Gross, D. S.; Williams, E. R. Submitted for publication.

(8) For a description of the instrument and operating conditions, see: (a) Campbell, S.; Marzluff, E. M.; Rodgers, M. T.; Beauchamp, J. L.; Rempe, M. E.; Schwinck, K. F.; Lichtenberger, D. L. *J. Am. Chem. Soc.* **1994**, *116*, 5257. (b) Marzluff, E. M.; Campbell, S.; Rodgers, M. T.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1994**, *116*, 7787. Singly protonated peptides were generated by bombardment of a matrix containing peptide, glycerol, and trifluoroacetic acid with 5 keV Cs^+.

(9) Proton affinities in kcal/mol from the following: Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17*(1).

(10) Reaction of $GlyH^+$ with D_2O is the only exception, with 1 slow exchange.

(11) The possibility of forming protonated zwitterions was ruled out when similar exchange results were observed for Gly_nH^+ methyl esters.

(12) Fast ion bombardment can generate ions with excess vibrational energy. A range of experiments were conducted to explore the effect of both translational and vibrational energy on the rate and extent of H/D exchange with only small effects being observed. Consistent with this result is the observation that semi-log plots for the rate of disappearance of the protonated glycine oligomers were linear over 2 orders of magnitude.

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Table 1. Summary of H/D Exchange Results of Selected Protonated Peptides with ND_3

peptide	sequence	exchangeable sites	exchanges obsd[a]
glycine oligomers	Gly_n (n = 1–5)	$n+3$	$(n+3)$ f
leucine enkephalin	Tyr-Gly-Gly-Phe-Leu	9	5 f, 4 m
leucine enkephalin arginine	Tyr-Gly-Gly-Phe-Leu-Arg	14	5 m, 1 s
bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	18	none

[a] f = fast, $k > 1 \times 10^{-10}$; m = medium, $1 \times 10^{-10} > k > 1 \times 10^{-11}$; s = slow, $k < 1 \times 10^{-11}$ cm^3 molecule^{-1} s^{-1}.

readily exchanges five of 14 labile hydrogens and one more slowly,[14] while bradykinin with two arginine residues does not undergo exchange with our conditions.

The proton affinities of Gly_n vary smoothly from 211.6 to 231.8 kcal/mol for n = 1–5.[15] We have considered the contribution which folding makes to this increase. Separate AM1 calculations[16] were carried out for the protonated species in an extended β-sheet and in a compact hydrogen-bonded configuration. The extended form experiences a significant increase in proton affinity (approximately 5 kcal/mol) only in proceeding from Gly to Gly_2. The regular increase in proton affinity predicted and observed for Gly_n (n = 3–5) comes mainly from folding the peptide to solvate the charge site through intramolecular hydrogen bonding.[17-19] For Gly_5H^+, AM1 calculations predict that the folding stabilization accounts for 83% of the proton affinity increase relative to glycine.[19]

When the mechanism of H/D exchange is discussed, an initial reaction intermediate comprising a chemically activated hydrogen-bonded complex[20] is usually invoked (Figure 3). We propose that the mechanism of H/D exchange for Gly_nH^+ with ND_3 involves molecular choreography in which an endothermic proton transfer is rendered energetically feasible by solvation of the resultant ammonium ion to compensate for loss of folding stabilization. AM1 calculations show that this onium ion mechanism is especially favorable for ammonia, as the solvated ammonium ion complex with Gly_3 is 27 kcal/mol more stable than the reactants (Figure 3a).[21] Multiple exchanges are favorable with this potential energy surface.

In contrast, calculations indicate that a solvated D_3O^+ complex with Gly_3 is energetically unfavorable (Figure 3b). The proton affinity of D_2O is too low and the energy recovered by solvation of the hydronium ion is insufficient to overcome the endothermicity of proton transfer from any of the glycine oligomers. We propose a relay mechanism for facile H/D exchange of protonated peptides with D_2O in which a proton is shuttled from the site of protonation onto D_2O in concert with the transfer of a deuteron from D_2O to a slightly less basic site on the molecule (an amide carbonyl in Figure 3b). The relay mechanism makes proton transfer viable within a molecule using the chemical activation (E^*, Figure 3) provided by hydrogen bonding. It seems reasonable that the relay surface in Figure 3b is less conducive to multiple exchanges. AM1 calculations indicate that with ND_3 relay intermediates are energetically feasible but readily collapse to the more stable solvated ammonium ion (species **IIIa**).

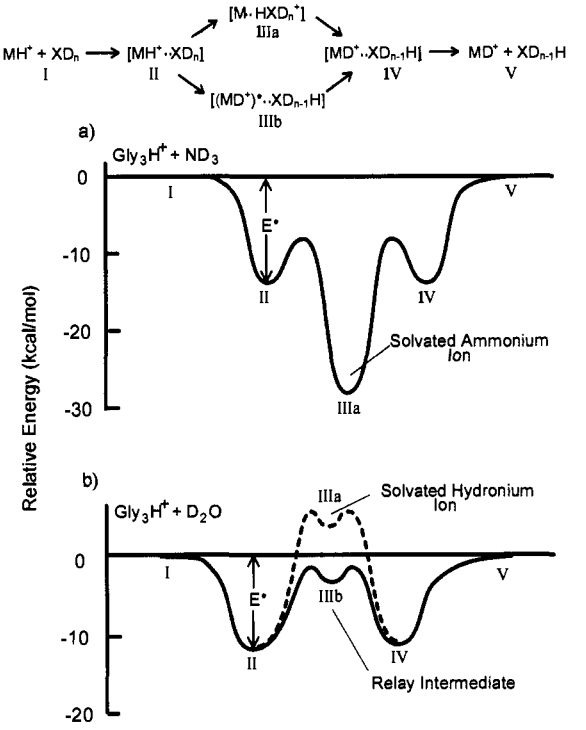

Figure 3. (a) Potential energy surface for H/D exchange of Gly_3H^+ and ND_3 via an onium ion mechanism. (b) Potential energy surfaces for H/D exchange of Gly_3H^+ and D_2O via an onium ion and relay mechanism. E^* is the chemical activation provided by hydrogen bond formation in the nascent complex **II**.

Molecular dynamics simulations show that D_2O hydrogen bonds with Gly_3H^+ and disrupts the secondary structure, causing the internal hydrogen bonds to rupture.[19] This exposes labile hydrogen sites and facilitates exchange by the relay mechanism. Similar calculations show that the more extensive intramolecular hydrogen bonding of Gly_4H^+ cannot be disrupted, and D_2O is unable to undergo facile exchange with this (or any larger) oligomer.

Strongly basic residues complicate the simple mechanistic picture of H/D exchange due to their high proton affinities, typically resulting from the ability to delocalize charge on a functional group. The resultant disparity in functional group basicities mitigates against the relay mechanism for H/D exchange. Five sites on leucine enkephalin arginine, presumably the five labile hydrogens of the side chain, readily exchange with ND_3. AM1 calculations show that the protonated arginine side chain is not strongly solvated by the peptide due to the diffuse nature of the charge site, and reaction with ND_3 to form a solvated ammonium ion is slightly exothermic. Protonated bradykinin with two arginine groups does not exchange with ND_3. Calculations predict that a protonated arginine side chain is completely encapsulated and not readily accessible to an approaching ND_3.[19,22]

It is clear from the simple examples discussed here that the temptation to assign gas phase structures of complex species from H/D exchange results must be approached with caution. We are currently undertaking investigations to further elucidate the relay and onium ion exchange mechanisms with more complex singly and multiply protonated peptides and oligonucleotides.

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(14) Our results for leucine enkephalin and leucine enkephalin arginine are consistent with those reported in ref 5.
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(16) (a) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J.-J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902. (b) Dewar, M. J. S.; Dieter, K. M. *J. Am. Chem. Soc.* **1986**, *108*, 8075. Calculations were performed using Hyperchem Computational Software Package, ver. 4.0 (Hypercube Inc., 1994).
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(18) Zhang, K.; Zimmerman, D. M.; Chung-Phillips, A.; Cassady, C. J. *J. Am. Chem. Soc.* **1993**, *115*, 10812.
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(20) Meot-Ner, M. *Molecular Structures and Energetics*; Liebman, J., Greenberg, A., Eds.; VCH Publishers: New York, 1986; Vol. 4, pp 71–103.
(21) Energies of the reactants and intermediates shown in Figure 3a,b were obtained using AM1 calculations.[16] The heights of barriers between intermediates are not known.
(22) A second structure of similar energy is predicted, with two protonated arginines interacting with the deprotonated C-terminus, forming a salt bridge (Barlow, D. J.; Thornton, J. M. *J. Mol. Biol.* **1983**, *168*, 867). The onium ion mechanism would require that the energetically favorable salt bridge be disrupted. See also: Salvino, J. M.; Seoane, P. R.; Dolle, R. E. *J. Comput. Chem.* **1993**, *4*, 438.