Selective Binding of Crown Ethers to Protonated Peptides Can Be Used To Probe Mechanisms of H/D Exchange and Collision-Induced Dissociation Reactions in the Gas Phase

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Received January 5, 1998

Abstract: Selective binding of crown ethers to model protonated peptides is utilized to study the site selectivity and mechanisms of gas-phase hydrogen/deuterium exchange reactions with ND_3 in an external ion source FT-ICR mass spectrometer. Mechanisms for H/D exchange reactions in the gas phase can be classified into two different types: Type I involving direct participation of the labile protons at the charge site and Type II in which the charge site at most plays only an ancillary role in the process (e.g., salt bridge formation). Localization of the labile proton at the charge site by crown ether attachment inhibits Type I processes, as evidenced by a dramatic reduction in the rates of H/D exchange. For example, crown ether attachment to protonated ethylenediamine and 1,4-diaminobutane inhibits H/D exchange reactions, while the free protonated species undergo rapid exchange of all five labile hydrogens. Type II processes are still observed with the crown ether adducts. Both the amide and the carboxyl hydrogens of peptides exchange via a Type II process for which a salt bridge mechanism has been proposed. In the salt bridge mechanism, the charge site may play an important role by stabilizing a charge separated ion pair. Immobilization of the labile proton by crown ether attachment does not eliminate this stabilization. Charge localization by crown ether attachment also affects the dissociation processes of protonated peptides, inhibiting charge directed mechanisms where endothermic proton transfer from the most basic group to a less basic site is a prerequisite for fragmentation. Collisional activation of the crown ether complex with protonated GGDPG and GGI results in no backbone cleavage in the peptide, while the free protonated peptides lead to cleavage at the C-terminus side of aspartic acid and the second glycine, respectively.

Introduction

With the development of "soft" ionization methods such as FAB (fast atom bombardment),[1] MALDI (matrix assisted laser desorption ionization),[2] and electrospray[3] to generate intact biological molecules, numerous mass spectrometric approaches to obtain sequence and structural information have been attempted, including gas-phase H/D exchange and various fragmentation methods.

H/D exchange has long been used in solution chemistry to probe conformations of peptides and proteins.[4] H/D exchange processes have been monitored with NMR, and more recently mass spectrometry.[5] H/D exchange reactions have also been used to infer peptide conformations in the gas phase[6] and determine the number of active hydrogens.[7] While destructive probes such as collision-induced dissociation (CID)[8] yield information on compositions and amino acid sequences, H/D exchange experiments are potentially more useful for elucidating the conformations of folded peptide ions in the gas phase.

Proper interpretation of H/D exchange experiments requires a detailed knowledge of the reaction mechanisms involved, and studies of glycine oligomers as model systems have been reported from our laboratory.[9] On the basis of these studies, we would like to propose a general classification of possible mechanisms for H/D exchange. We will consider H/D exchange processes in which the labile proton at the charge site is intimately involved as Type I exchange mechanisms. For example, exchange of the three hydrogens on the protonated N-terminus amino group of glycine oligomers with ND_3 as the reagent gas has been shown to proceed via an "onium" mechanism in which the endothermicity of proton transfer to ammonia is compensated by intermolecular solvation of the resultant ammonium ion. We will refer to processes in which protons at the charge site are not directly involved as Type II mechanisms. An example is the salt bridge mechanism in which proton transfer to a basic exchange reagent leads to formation of a charge separated ion pair that can be stabilized by favorable

(1) Barber, M.; Boddoli, R. D.; Sedgwick, R. D.; Tyler, A. N. *J. Chem. Soc., Chem. Commun*. **1981**, 325.

(2) (a) Karas, M.; Bachmann, D.; Bahr, U.; Hillenkamp, F. *Int. J. Mass Spectrom. Ion Proc*ess **1987**, *78*, 53. (b) Hillenkamp, F.; Karas, M.; Beavis, R. C.; Chait, B. T. *Anal. Chem*. **1991**, *63*, 1193A.

(3) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64.

(4) Werstiuk, N. H. *Isotopes in the Physical and Biomedical Sciences*; Elsevier: Amsterdam, 1987; Vol. 1, p 122.

(5) Miranker, A.; Robinson, C. V.; Radford, S. E.; Aplin, R. T.; Dobson, C. M. *Science* **1993**, *262*, 896.

(6) (a) Suckau, D.; Shi, Y.; Beu, S. C.; Senko, M. W.; Quinn, J. P.; Wampler, F. M.; McLafferty, F. W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 790. (b) Winger, B. E.; Light-Wahl, K. J.; Rockwood, A. L.; Smith, R. D. *J. Am. Chem. Soc.* **1992**, *114*, 5897. (c) Valentine, S. J.; Clemmer, D. E. *J. Am. Chem. Soc.* **1997**, *119*, 3558.

(7) Ranasinghe, A.; Cooks, R. G.; Sethi, S. K. *Org. Mass Spectrom.* **1992**, *27*, 77.

(8) (a) Johnson, R. S.; Martin, S. A.; Biemann, K. *Int. J. Mass Spectrom. Ion Processes* **1988**, *86*, 137. (b) Hunt, D. F.; Yates, J. R., III; Shabanowitz, J.; Winston, S.; Hauer, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6233. (c) Schwartz, B. L.; Bursey, M. M. *Biol. Mass Spectrom.* **1991**, *21*, 92.

(9) (a) Campbell, S.; Rodgers, M. T.; Marzluff, E. M.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1994**, *116*, 9765. (b) Campbell, S.; Rodgers, M. T.; Marzluff, E. M.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 12840.

S0002-7863(98)00056-0 CCC: $15.00
Published on Web 05/27/1998

interaction with a proximal charge (e.g., the protonated N-termius). The N-terminus protons, however, are not directly involved in the exchange process. Exchange of carboxylic acid and amide hydrogens in glycine oligomers may proceed via a Type II salt bridge mechanism.[10]

Previous work from our group found that ND_3 was the most efficient reagent studied for promoting H/D exchange of protonated glycine oligomers.[9] Both semiempirical[9,11] and ab initio calculations[12] indicate that glycine oligomers are protonated on the N-terminus and then fold such that the charge site is solvated by intramolecular hydrogen bonding to carbonyl oxygens. The single-crystal X-ray structure of a polyhydrate of the 18-crown-6 ether complex of the salt n-$C_3H_7NH_3^+F^-$ has been reported.[13] The crown ether complexes the cation C_3H_7-NH_3^+ in a layered structure with water polyhedra. There is no hydrogen bonding observed between the protonated amine and water molecules present in the hydrate. This indicates that 18-crown-6 encapsulates a protonated primary amine in a manner that even mitigates against additional hydrogen bond formation to the site of protonation. By forming a complex of the protonated glycine oligomers with an appropriate crown ether, it should be possible to localize and encapsulate the labile protons, inhibiting Type I H/D exchange processes. In this paper, we report such studies of H/D exchanges of singly protonated glycine oligomer−crown ether complexes with ND_3, which confirm this expectation.

Fragmentation methods to obtain primary structural information of biopolymers with mass spectrometric techniques include collision-induced dissociation (CID),[14] surface-induced dissociation (SID),[15] and infrared multiphoton dissociation (IRMPD), either by a CO_2 laser[16] or by blackbody radiation.[17] CID, the most widely employed technique in mass spectrometry, has been used for investigating the primary structures of biopolymers in the gas phase. Of particular interest is the possibility of selective cleavage at certain amino acid residues by CID. Smith and co-workers,[18] for example, have observed dominant cleavages at the amide bonds adjacent to proline residues with CID of multiply charged proteins. Preferential cleavages at the aspartic acid residues were also observed in CID of protonated peptides.[19] In previous works from our laboratory, we have thoroughly investigated dissociation energetics associated with preferential cleavage at aspartic acid residues using several model peptides with arginine residues and sodiated peptides.[20] We have proposed a mechanism for cleavage at aspartic acid residues that involves a salt bridge intermediate where a local charge of either a protonated arginine residue or a sodium ion stabilizes the ion pair formed by proton transfer from aspartic acid to the adjacent amide nitrogen.

It is known that more energy is often required for fragmentation of peptides that have basic amino acid residues such as arginine than peptides with no basic residues.[21] The absence of strongly basic residues which inhibit proton mobility and the presence of extensive intramolecular hydrogen bonding of a labile proton with other basic sites in a peptide correlates with lower energy thresholds and higher yields for the corresponding fragments.[22] These conditions exemplify what has become known as the mobile proton model where facile intramolecular proton transfers, usually through a hydrogen bond network, yield a rapidly interconverting population of structures from which charge directed fragmentation occurs. The inhibition of proton mobility by crown ether attachment provides a unique opportunity to study the mechanisms of dissociation. This is demonstrated in the present study, where CID of the protonated peptides GGDPG and GGI, with and without crown ether, is compared.

Experimental Section

Experiments were performed in an external ion source FT-ICR mass spectrometer that has been described in detail elsewhere,[23] and only a brief description will be given here. For this study the spectrometer is operated with a Cs ion bombardment source. An octopole ion guide transports ions to a $2 \times 2 \times 3$ in. ICR cell, which is located inside a 7-T superconducting magnet. The instrument has three regions of differential cryogenic pumping, resulting in a residual background pressure of $\sim 5 \times 10^{-10}$ Torr in the detection region.

Complexes of protonated peptide and 18-crown-6 ether (18C6)[24] were generated from an acidic glycerol matrix by 5 kV Cs ion bombardment and accumulated in the ICR cell for 100 ms. Ion species of interest were selected by conventional isolation techniques and allowed to undergo H/D exchange. Interestingly, in addition to abundant complex formation, we observed that addition of crown ether to the glycerol matrix enhanced the yield of uncomplexed protonated peptides as well. The static pressures of ND_3 used in the exchange reactions were in the range of 1×10^{-7} and 2×10^{-7} Torr. Accurate pressure measurement is achieved by calibrating a Schulz-Phelps ionization gauge[25] attached directly on the ICR cell with a capacitance manometer connected to the cell through a static port. The isotopic purity of ND_3 in the vacuum chamber ranged from 94 to 97 atom %.[9]

SORI-CID (sustained off-resonance irradiation collision-induced dissociation)[26] was used to activate low-energy fragmentation pathways. In previous work from our group,[27] this activation method has proved to be a reliable and efficient technique to investigate dissociation dynamics of small peptides and nucleotides in the gas phase. Collisions are carried out against a static nitrogen gas pressure of 10^{-8} Torr.

(10) For amide hydrogen exchange a "tautomer" mechanism was suggested in ref 9, which involves proton transfer from the N-terminus to the amide carbonyl in concert with transfer of the amide proton to ammonia. Exchange could occur by a salt bridge mechanism if the proton is not transferred from the N-terminus.

(11) Wu, J. Y.; Lebrilla, C. B. *J. Am. Chem. Soc.* **1993**, *115*, 3270.

(12) (a) Zhang, K.; Zimmerman, D. M.; Chung-Phillips, A.; Cassady, C. J. *J. Am. Chem. Soc.* **1993**, *115*, 10812. (b) Zhang, K.; Cassady, C. J.; Chung-Phillips, A. *J. Am. Chem. Soc.* **1994**, *116*, 11512.

(13) Udachin, K. A.; Lipkowski, J. *Mendeleev Commun.* **1996**, 195.

(14) (a) McLuckey, S. A. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 599. (b) Marzluff, E. M.; Beauchamp, J. L. In *Large Ions: Their Vaporization, Detection and Structural Analysis*; Baer, T., Powis, I., Ng, C. Y., Eds.; Wiley Series on Ion Chemistry and Physics; Wiley: New York, 1996; p 115.

(15) (a) Mabud, MD. A.; Dekrey, M. J.; Cooks, R. G. *Int. J. Mass Spectrom. Ion Process* **1985**, *67*, 285. (b) Ijames, C. F.; Wilkins, C. L. *Anal. Chem.* **1990**, *62*, 1295.

(16) Little, D. P.; Speir, J. P.; Senko, M. W.; O'Connor, P. B.; McLafferty, F. W. *Anal. Chem.* **1994**, *66*, 2809.

(17) Price, W. D.; Schnier, P. D.; Williams, E. R. *Anal. Chem.* **1996**, *68*, 859.

(18) Loo, J. A.; Edmonds, C. G.; Smith, R. D. *Anal. Chem.* **1993**, *65*, 425. For an earlier observation, see: Tomer, K. B.; Crow, F. W.; Gross, M. L. *Anal. Chem.* **1984**, *56*, 880.

(19) Yu, W.; Vath, J. E.; Huberty, M. C.; Martin, S. A. *Anal. Chem.* **1993**, *65*, 3015.

(20) (a) Lee, S.-W.; Kim, H. S.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1998**, *120*, 3188. (b) Marzluff, E. M.; Lee, S.-W.; Beauchamp, J. L. In preparation.

(21) Dongre, A. R.; Jones, J. L.; Somogyi, A.; Wysocki, V. H. *J. Am. Chem. Soc.* **1996**, *118*, 8365.

(22) Klassen, J. S.; Kebarle, P. *J. Am. Chem. Soc.* **1997**, *119*, 6552.

(23) Rodgers, M. T.; Campbell, S.; Marzluff, E. M.; Beauchamp, J. L. *Int. J. Mass Spectrom. Ion Processes* **1994**, *137*, 121.

(24) The 18-Crown-6 ether can make effective bonds with the protonated amino group through its three alternating oxygens, and has large binding constants for NH_4^+ in both water and organic solvents: De Jong, F.; Reinhoudt, D. N. *Stability and Reactivity of Crown-Ether Complexes*; Academic Press: New York, 1981; p 279.

(25) Schulz, G. J.; Phelps, A. V. *Rev. Sci. Instrum.* **1957**, *28*, 1051.

(26) Gauthier, J. W.; Trautman, T. R.; Jacobson, D. B. *Anal. Chim. Acta* **1991**, *246*, 211.

(27) (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.

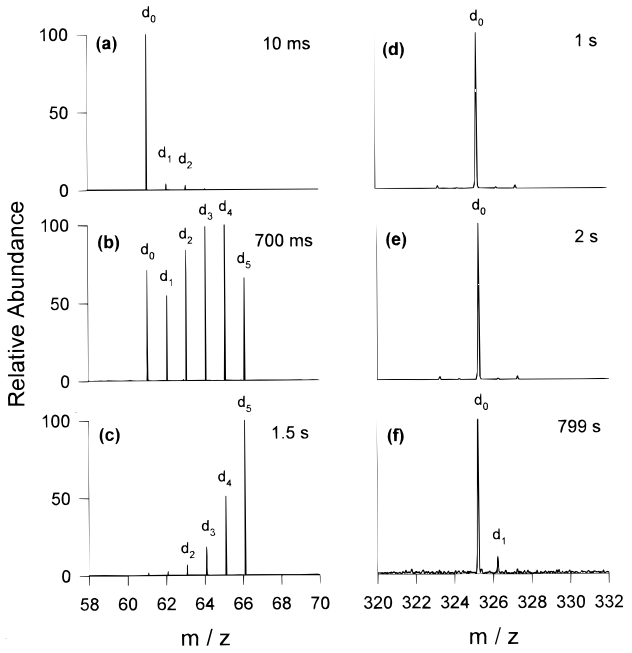


Figure 1. H/D exchange products of protonated ethylenediamine (a−c) and the corresponding 18C6 complex (d−f) with ND_3 at 1.6×10^{-7} Torr at various reaction times. The number of deuterium incorporated, *n*, is indicated as d_n. The small d_1 peak of the crown ether complex might be due in part to the ^{13}C isotopomer resulting from incomplete isolation.

All samples except methyl esters of the glycine oligomers, diamines, and GGDPG were purchased from Sigma Chemical Company (St. Louis, MO) and used without further purification. Ethylenediamine and 1,4-diaminobutane were obtained from Aldrich (Milwaukee, WI). Methyl esters of the glycine oligomers were synthesized by dissolving the peptides in a solution of methanol and trifluoroacetic acid (99:1) and stirring overnight. Reaction yields did not exceed 75%, but products could be easily isolated in FT-ICR experiments. GGDPG was synthesized by the Biopolymer Synthesis Facility in the Beckman Institute at the California Institute of Technology. ND_3 was purchased from Matheson (Montgomeryville, PA) with a purity of 99.0 atom % deuterium.

Semiempirical PM3 calculations were performed with the HyperChem software package[28] to assess energetics of crown ether attachment and H/D exchange of amide nitrogens with ND_3. The initial geometries of protonated tryglycine and the corresponding crown ether complex were obtained by using molecular mechanics and the resulting structures were subjected to further geometry optimizations by PM3 calculations.

Results and Discussion

H/D Exchange of Crown Ether Complexes of Diamines. Protonated ethylenediamine and 1,4-diaminobutane, examined as model compounds, undergo rapid H/D exchanges with ND_3, and the reactions proceed to completion in 5 s. This is illustrated for ethylenediamine in Figure 1a−c. The profiles of H/D exchange of the protonated ethylenediamine and their resultant rate constants (Table 1) indicate multiple exchange on single encounters with ND_3. Multiple exchanges have previously been observed in studies of model petides.[9] The probable mechanism of H/D exchange in these systems is a Type I onium ion mechanism involving formation of a cyclic complex in which NHD_3^+ interacts with both terminal amino groups. Figure 2 shows the reaction coordinate diagram for the H/D exchange of protonated ethylenediamine constructed by using PM3 semiempirical calculations. The semiempirical calculations indicate a cyclic intermediate about 20.6 kcal/mol more stable than the reactants. Such a stable intermediate facilitates multiple exchanges. However, when the protonated amino groups of these compounds were complexed with 18C6, no H/D exchanges were observed in 799 s (Figure 1d−f). The small peak at 799 s is less than 5% of total ion abundance and may be due to incomplete ejection of the ^{13}C isotopomer. The results of these control experiments indicate that an isolated amino group will not undergo facile H/D exchange with ND_3.

(28) HyperChem Computational Chemistry Software Package, Version 4.5, Hypercube, Inc., 1995.

Table 1. H/D Exchange Rates for Protonated Glycine Oligomers, Ethylenediamine, 1,4-Diaminobutane, and Their 18-Crown-6 Ether (18C6) Complexes with ND_3

	H/D exchange rates (10^{-12} cm^3 molecule^{-1} s^{-1})[a]						
species	d_1	d_2	d_3	d_4	d_5	d_6	d_7
Gly[b]	270	550	360	110	−[d]	–	–
Gly-18C6	130	*[c]	*	*	–	–	–
Gly_3[b]	890	1410	1190	720	280	47	–
Gly_3-18C6	21	0.30	0.20	*	*	*	–
Gly_3OMe-18C6	0.40	0.25	0.13	*	*	–	–
Gly_4[b]	1230	2110	1300	650	170	76	62
Gly_4-18C6	15	6.7	5.6	2.6	*	*	*
Gly_4OMe-18C6	1.9	1.2	0.54	*	*	*	–
ethylenediamine	410	860	560	470	290	–	–
ethylenediamine-18C6	*	*	*	*	*	–	–
1,4-diaminobutane	160	240	250	180	96	–	–
1,4-diaminobutane-18C6	*	*	*	*	*	–	–

[a] d_n denotes the rate constant for incorporation of the n^{th} deuterium, calculated assuming that only single exchanges occur in each encounter. The uncertainties in the rate constants are estimated to be 20%. This is largely due to the pressure measurement that has been done by a Schulz-Phelps ionization guage mounted just outside of the cell and calibrated with a capacitance manometer connected directly to the cell through a static port.[23] [b] Reference 9. [c] (*) Exchange possible but not observed. [d] (−) Exchange not expected.

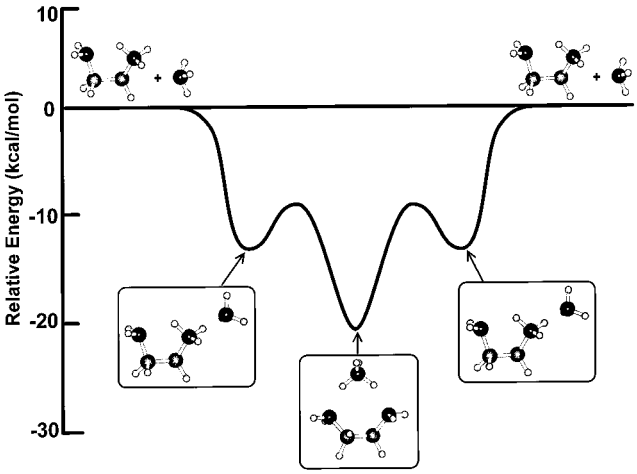


Figure 2. Reaction coordinate diagram for H/D exchange of the protonated ethylenediamine-18C6 complex with ND_3 via an onium ion mechanism. Intermolecular solvation of the ammonium ion by the two amino groups stabilizes the intermediate.

H/D Exchange of Crown Ether Complexes of Glycine Oligomers. A comparison of H/D exchange results for protonated triglycine with and without attachment of 18C6 adduct is shown in Figure 3. All six labile hydrogens of the protonated triglycine exchange rapidly with ND_3 (Figure 3a−c), and at 10 s (Figure 3c) the reaction is approaching completion. Under identical conditions, the crown ether adduct has exchanged only a single hydrogen after 10 s (Figure 3e), and after 500 s (Figure 3f) there is evidence that two additional sites slowly exchange. From spectra taken at various delay times, rate constants for H/D exchange were determined by fitting ion abundances to a

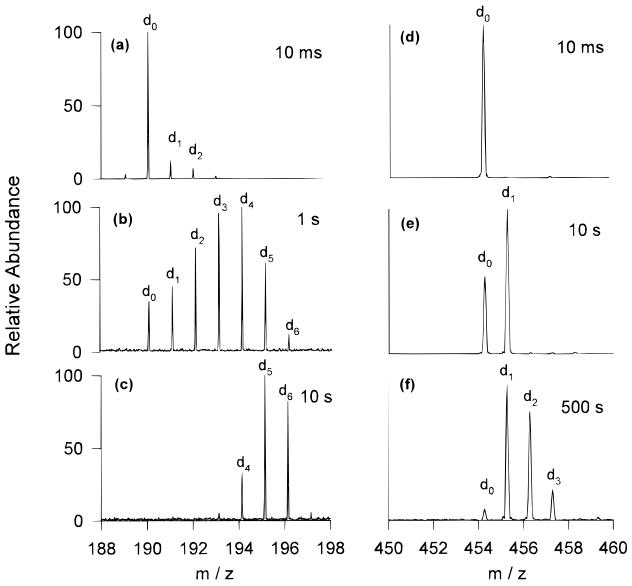


Figure 3. H/D exchange products of protonated triglycine (a−c) and the corresponding 18C6 complex (d−f) with ND_3 at 1.4×10^{-7} Torr at various reaction times. The number of deuterium incorporated, *n*, is indicated as d_n.

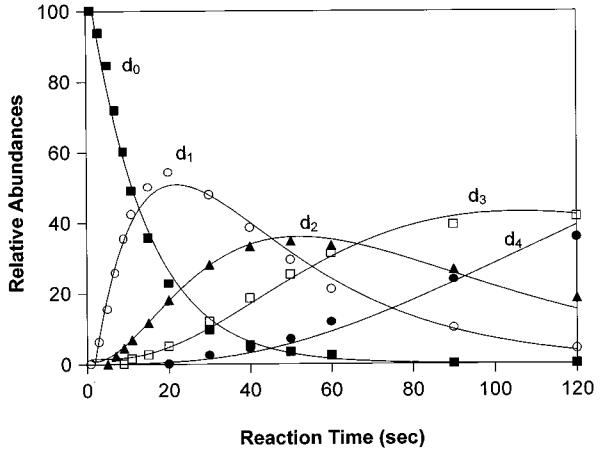


Figure 4. Time plot of H/D exchange products of the Gly_4H^+-18C6 complex with ND_3. Least-squares fits (lines) of the kinetic equation to the experimental data (points) are shown. The number of deuterium incorporated, *n*, is indicated as d_n.

series of $n + 1$ first-order differential equations for *n* exchangeable hydrogens (Figure 4). Table 1 lists the rate constants for the H/D exchange of the 18C6 complexes of several glycine oligomers, ethylenediamine and 1,4-diaminobutane with ND_3.[29] When the crown ether binds to protonated glycine oligomers and esters of the glycine oligomers, the total number of exchanged hydrogens is reduced from the total number of labile hydrogens in the free protonated peptides by 3 and 4, respectively, which supports the conjecture that the crown ether coordinates to the protonated N-terminus amino group and inhibits H/D exchange at that site. The crown ether complex of the methyl ester of Gly_3H^+ was the only exception to this generalization, in which three H/D exchanges were observed, but the intensity of the d_3 peak was less than 8% of the total ion abundance even after 800 s. With the N-terminus protons immobilized by 18C6, Type I reaction pathways which involve proton transfer from the protonated N-terminus to ND_3 or

(29) We were not able to examine diglycine, since the peak due to the 18C6−Cs^+ complex was exceedingly large and overlapped the signal due to Gly_2H^+-18C6.

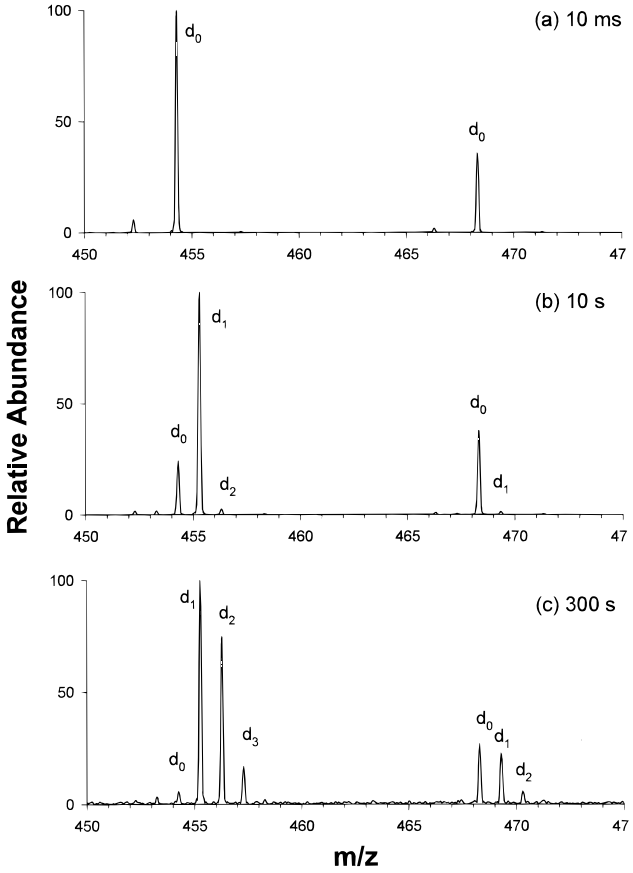


Figure 5. A comparison of H/D exchange results for crown ether complexes of protonated tryglycine and its methyl ester. The number of deuterium incorporated, *n*, is indicated as d_n. The d_1 peak of protonated tryglycine-18C6 corresponds to the C-terminus carboxylic hydrogen, and the other two are attributed to amide hydrogens.

incorporate one of the protons to form intermediate complexes are not possible, resulting in no exchange or significantly slower exchanges.

The rate constants for the first exchanges are 1.3×10^{-10}, 2.1×10^{-11}, and 1.5×10^{-11} cm^3 $molecule^{-1}$ s^{-1} for crown ether complexes of Gly_1H^+, Gly_3H^+, and Gly_4H^+, respectively. The comparison of these data with those of the protonated methyl ester complexes that are an order of magnitude slower makes it clear that the first exchange occurs predominately with the carboxyl hydrogen. Figure 5 shows a comparison of H/D exchange results for crown ether complexes of protonated Gly_3 and its methyl ester with identical experimental conditions. No exchange is observed for the crown ether complex of the methyl ester, while the protonated Gly_3^+-18C6 complex undergoes one exchange. Furthermore, the d_1 and d_2 peaks of methyl ester-18C6 adduct develop almost identically to d_2 and d_3 of protonated Gly_3^+-18C6. Therefore, the first fast exchange of protonated Gly_3^+-18C6 adduct is unambiguously assigned to carboxylic hydrogen at the C-terminus and the remaining two correspond to amide hydrogens. We postulated earlier[9] that the carboxyl hydrogen exchanges via a salt bridge mechanism (Type II), in which the N-terminus charge stabilizes the ion pair formed by proton transfer from the carboxyl group to ND_3. The trend in the first exchange rate constants in crown ether complexes of Gly_1H^+, Gly_3H^+, and Gly_4H^+ may reflect the decreasing stabilization with increasing distance between the charge and the ion pair in these complexes. Furthermore, the exchange rate for Gly_1H^+-18C6 complex is comparable to that of both free Gly_1H^+ and betaine, which were reported in a previous

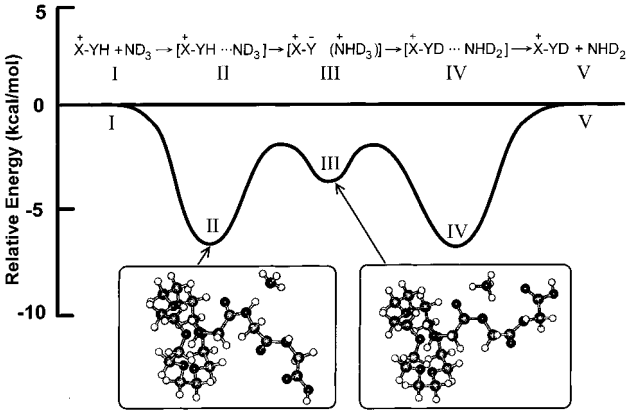


Figure 6. Reaction coordinate diagram for the H/D exchange of amide hydrogen of the Gly_3H^+-18C6 complex with ND_3 via a salt bridge mechanism. Proton transfer from the amide to ND_3 and formation of a salt bridge structure is facilitated by the stabilizing effect of the nearby positively charged N-terminus.

paper[9] to have rate constants of approximately 2×10^{-10} cm^3 molecule^{-1} s^{-1} with ND_3 and proposed to proceed via the salt bridge mechanism.

Semiempirical PM3 calculations were performed to assess ion structures and energetics for several crown ether adducts. For example, the complexation energies of protonated methylamine and protonated triglycine to 18C6 ether are calculated to be 43.0 and 34.1 kcal/mol, respectively. Smaller complexation energy for the 18C6-protonated tryglycine adduct is attributed to loss of the folding energy of protonated tryglycine, which is calculated to be 9.8 kcal/mol.[9b] In addition, calculations indicate that the salt bridge complex of Gly_3H^+-18C6 with ND_3 is 3.3 kcal/mol more stable than separated Gly_3H^+-18C6 and ND_3 (Figure 6). The electrostatic stabilization of the ion pair from the proximal charge can be estimated from the ion−dipole interaction, which is 18.9 kcal/mol when the dielectric constant is set to 1.[30] Clearly this is an important contribution to the stability of the proposed salt bridge intermediate shown in Figure 6.

CID of Crown Ether Complexes. Figure 7 shows the comparison of CID results of protonated GGDPG and its 18C6 adduct. As observed previously,[20] the protonated peptide yields the y_2 fragment predominantly (Scheme 1).[31] No direct cleavage of the peptide occurs in the crown ether complex. Instead, activation of the adduct leads to crown ether loss as the lowest energy dissociation pathway. Once the crown ether is eliminated, further fragmentation of the free protonated GGDPG occurs at the aspartic acid residue (Figure 7e).

These observations can be rationalized with reference to Figure 8. Activation initially leads to crown ether elimination as the energetically favorable pathway. RRKM calculations indicate that despite the large number of degrees of freedom in biopolymers, which obligates deposition of high excess internal energy over the threshold energy, competing dissociation pathways with slightly different activation energy can be activated with high selectivity.[14b] In Figure 8, a barrier for crown ether elimination in excess of the reaction endothermicity is shown to account for the expectation that the free protonated peptide can fold to a stable compact form not accessible in the presence of the crown ether. As a result, the activation energy for crown ether loss should be in the range 40−50 kcal/mol, based on the energetics discussed above. Removal of the crown ether allows access to low-energy dissociation pathways of the protonated peptide in charge directed processes involving a

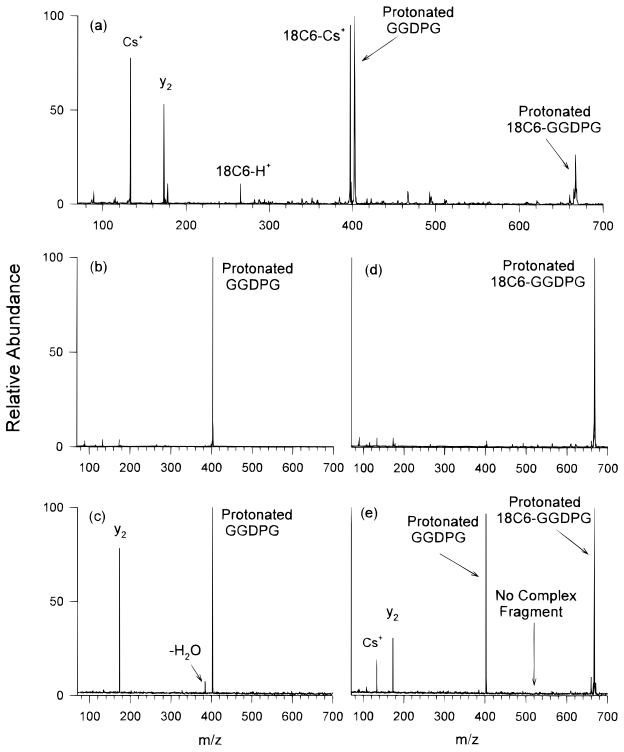


Figure 7. The effect of crown ether attachment on collision-induced dissociation of protonated GGDPG. (a) Protonated GGDPD complexed to 18C6. (b) Isolation of protonated GGDPG. (c) CID of protonated GGDPG at 0.30 eV for 1000 ms against a static gas pressure of nitrogen at 2.9×10^{-8} Torr. Off resonance collisional activation leads to preferential cleavage at the aspartic acid residue. (d) Isolation of crown ether adducts. (e) CID of the crown ether adducts at 0.46 eV for 1000 ms against a static gas pressure of nitrogen at 1.2×10^{-8} Torr. Specific cleavage of the asp-pro amide linkage follows the loss of neutral crown ether.

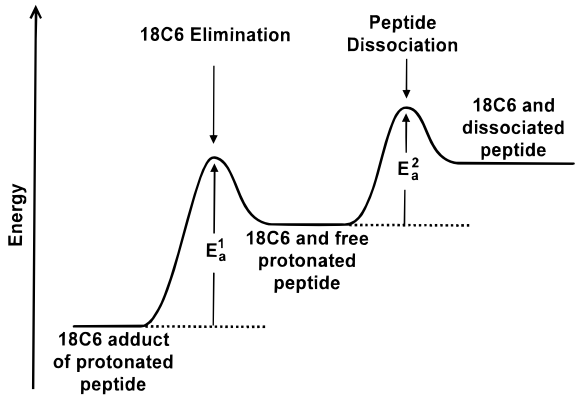
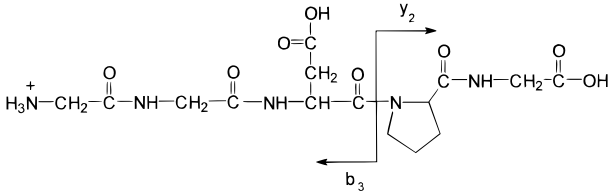


Figure 8. A simplified reaction coordinate diagram to illustrate the effect of crown ether attachment on dissociation of protonated peptides.

Scheme 1



(30) Gross, D. S.; Rodriguesz-Cruz, S. E.; Bock, S.; Williams, E. R. *J. Phys. Chem.* **1995**, *99*, 4034.

(31) In this paper, we are following the nomenclature introduced by Roepstorff and Fohlman: Roepstorff, P.; Fohlman, J. *J. Biomed. Mass Spectrom.* **1984**, *11*, 601.

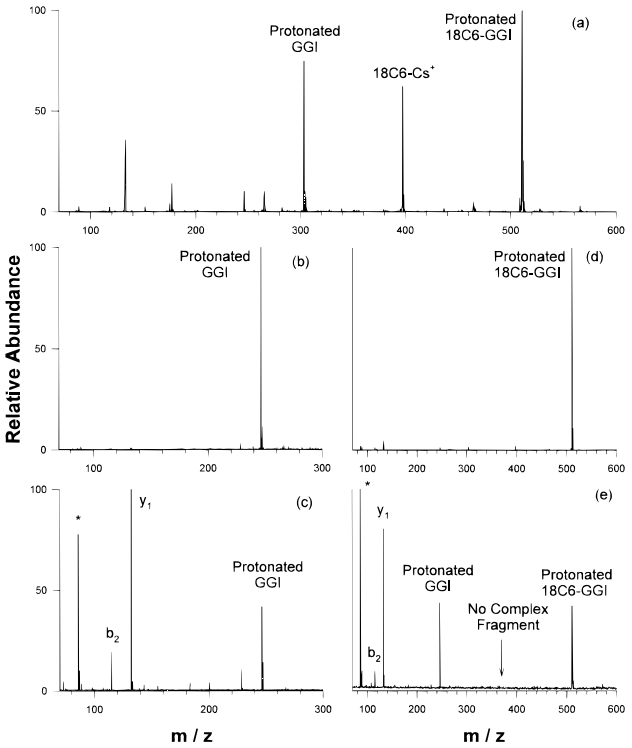


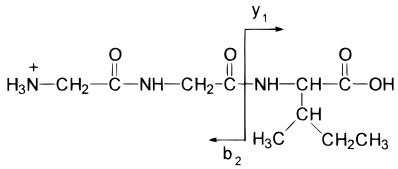
Figure 9. The effect of crown ether attachment on collision-induced dissociation of protonated GGI. (a) Protonated GGI complexted to 18C6. (b) Isolation of protonated GGI. (c) CID of protonated GGI at 1.35 eV for 1000 ms against a static gas pressure of nitrogen at 1.2×10^{-8} Torr. (d) Isolation of crown ether adducts. (e) CID of the crown ether adducts at 0.74 eV for 1000 ms against a static gas pressure of nitrogen at 2.1×10^{-8} Torr. No fragment corresponding to 18C6-b_2 is noted, while the free peptide produces b_2 and y_1. The absence of crown ether adduct of the b_2 fragment suggests that the crown ether is lost before fragmentation occurs. The peak denoted by * corresponds to an a-type fragment from the y_1 ion.

mobile proton. Such processes are expected to dominate the product spectrum if $E_a^2 < E_a^1$, even though the total excitation energy available to the dissociation of free peptide will be reduced by the energy of complexation of the crown ether to the protonated peptide. This is a general consequence of the dissociation dynamics of large molecules,[14b] where internal excitation many times the activation energy is required to produce observed dissociation rates ($1-10\ s^{-1}$ for typical FT-ICR studies). For large molecules, dissociation rates for processes with similar activation parameters are comparable for species of different size when the *average energy per vibrational degree of freedom* is comparable. Following crown ether loss the average energy per vibrational degree of freedom in the free protonated peptide will only be slightly less than in the reactant. With these considerations, it is not surprising that the resultant internal energy of protonated GGDPG is sufficient to promote fragmentation at the asp-pro amide linkage in a substantial fraction of the dissociation events.[32]

Figure 9 shows another example of the effect of crown ether attachment on CID. Without crown ether, protonated GGI yields b_2 and y_1 complementary fragments (Scheme 2). Crown ether attachment, however, inhibits this fragmentation pathway, confirmed by the absence of crown ether complex of a b_2 fragment ion. Off resonance activation leads to the detachment of crown ether from the peptide, followed by the characteristic fragmentation of the free peptide. These examples clearly indicate that immobilization of the labile proton prevents low-energy dissociation pathways via inhibition of a charge-directed mechanism.

(32) The crown ether adduct of GGDPG might be able to undergo cleavage of the asp-pro linkage by a salt bridge mechanism. Apparently the activation energy for this process is higher than that for crown ether elimination.

Scheme 2



Conclusion

These experiments have shown that crown ether adducts of protonated peptides can be useful in elucidating the mechanisms of H/D exchange reactions and collision-induced dissociation reaction in the gas phase by encapsulating and immobilizing labile protons. From the comparisons of H/D exchange kinetics of free peptides and complexed peptides (Table 1), we observed that Type I processes, including onium ion mechanism and tautomer mechanism where the labile protons at the charge site are directly involved, are dominantly operative in H/D exchange of free protonated peptides and diamines in parallel with the Type II process, but are effectively inhibited by crown ether attachment on the charge site, resulting in dramatic reduction of exchange rates or no exchange. Both the amide and the carboxyl hydrogens of peptides exchange via a Type II process when the labile proton at the charge site is immobilized by crown ether attachment. The Type II process, which does not directly involve the labile proton, occurs by a salt bridge mechanism, in which the proximal charge plays an important role by stabilizing the charge-separated ion pair. By using the encapsulating properties of crown ethers to advantage, we have shown that the localization of the labile proton has a significant effect on fragmentation processes which require the participation of a mobile proton. Furthermore, crown ether attachment can protect a fragile molecule against fragmentation via low-energy charge-directed pathways. As noted in the Experimental Section, the presence of the crown ether enhanced the yield of ions sputtered from the glycerol matrix by Cs^+ bombardment. Adduct formation that immobilizes the labile protons may also serve to inhibit processes such as ion−ion recombination and proton transfer, which might reduce ion yields in the sputtering process. Although we have not explored the phenomenon in the present study, we also note the intriguing possibility of in situ generation of selectively labeled peptides using crown ether adducts, by carrying out H/D exchange followed by CID to remove the crown ether.

Acknowledgment. This work was supported in part by the National Science Foundation under Grant No. CHE-9727566. We gratefully acknowledge the financial support of H.L. from an NIH-NRSA Human Genome traineeship. Funds for instrument development have also been provided by ARPA and the DOD-URI program (ONR-N0014-92-J-1901). We are also indebted to the Beckman Foundation and Institute for the initial funding and continuing support of the research facilities.

JA980056H