Salt Bridge Chemistry Applied to Gas-Phase Peptide Sequencing: Selective Fragmentation of Sodiated Gas-Phase Peptide Ions Adjacent to Aspartic Acid Residues

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Abstract: Salt bridge chemistry has recently been realized as a determining factor in the structures and reaction dynamics of biological molecules in the gas phase. In this paper, we further investigate salt bridge chemistry in studies of the low-energy collision-induced dissociation (CID) of sodiated peptides. MALDI and electrospray ionization are used to generate singly and multiply charged sodiated peptides which are analyzed by using an external ion source Fourier transform ion cyclotron resonance mass spectrometer. Of particular interest is the observation that sodiated peptides exhibit highly selective cleavage at aspartic acid residues. Sodiated peptides that lack acidic residues, however, undergo sequential cleavages from the C-terminus on low-energy CID. We propose a mechanism for cleavage at aspartic acid residues that involves a salt bridge intermediate in which the sodium ion stabilizes the ion pair formed by proton transfer from aspartic acid to the adjacent amide nitrogen. This proposal is supported by ab initio calculations to quantify the reaction energetics. In several instances the less selective low-energy fragmentation processes of the protonated peptides have also been investigated for comparison.

Introduction

The development of rapid and sensitive methods to obtain sequence information of biopolymers remains an active area of research by biologists and chemists both in solution and gas phase. The automated Edman technique[1] remains the most widely used and reliable method to date for protein sequencing. The relatively recent invention of "soft" ionization methods such as FAB (fast atom bombardment),[2] MALDI (matrix assisted laser desorption ionization),[3] and ESI (electrospray ionization)[4] to generate intact biological molecular ions in the gas phase has led to the development of numerous mass spectrometric approaches to obtain sequence and structural information of these systems in the gas phase. For instance, Chait and coworkers[5] have successfully demonstrated the use of MALDI-MS in conjunction with modified Edman degradation in solution (known as the "ladder" method). C-terminal sequencing utilizing enzymatic digestion of peptides has been demonstrated with use of carboxypeptidase Y followed by analysis in MALDI-MS.[6] Recently, Wilm[7] et al. have combined gel electrophoresis with nanoelectrospray[8] quadrupole mass spectrometry to sequence proteins with femtomoles of sample.

To accomplish true gas-phase sequencing, requiring no preliminary chemical or enzymatic processing in solution, various fragmentation methods of the quasimolecular ions in the gas phase have been developed in the past decade, including collision-induced dissociation (CID),[9] surface-induced dissociation (SID),[10] and infrared multiphoton dissociation (IRMPD), both with a CO_2 laser[11] and by blackbody radiation.[12] CID, the most widely employed technique for investigating the primary structure of biopolymer ions in the gas phase, has been proved simple and effective with FT-ICR (or FTMS)[13] and ion trap[14] mass spectrometers. With the capability for nondestructive ion detection, FT-ICR provides unique MS^n capabilities coupled with quadrupolar excitation to axialize product ions to the center of ICR cell which were brought to off axis from previous cycle of CID.[13] CID in FT-ICR usually employs mass selection of ions of interest using a series of frequency-sweep waveforms or SWIFT (Stored Waveform Inverse Fourier Transform),[15] followed by activation via collisions with target gases (N_2 or Ar). Among several well-defined CID techniques, SORI[16] (sustained off-resonance irradiation) has proven to be easy to implement, efficient, and highly selective in activation.

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SORI slowly increases the internal energy of selected ions, effecting dissociation near threshold.

For the practical application of mass spectrometric techniques to gas-phase sequencing of peptides it is of particular interest to develop methods which provide controllable and predictable fragmentation processes. Mass spectrometric sequencing of large peptides in the gas phase can be problematic due to the broad isotopic envelope that results mainly from ^{13}C and ^{15}N, which can prevent accurate determination of the mass of the molecular ion and make it difficult to identify the mass of a single amino acid if it is cleaved from the parent molecular ion. Recently, an accurate measurement of protein molecular mass using a ^{13}C, ^{15}N double-depletion technique has been successfully demonstrated.[17] As proposed previously,[18] it is desirable to have a method that provides selective cleavage at specific amino acids as well as a method that sequentially removes amino acids one at a time from either the C− or N−terminus. The selective cleavage would yield smaller and more manageable peptides for further gas-phase manipulation, providing an analogy to an enzymatic digest in solution. There have been a few studies published in which preferential cleavage at specific amino acid residues has been observed. For instance, dominant cleavages at the amide bonds adjacent to proline residues were observed with multiply charged proteins in a triple-quadrupole mass spectrometer[19] and FT-ICR.[20] Facile gas-phase cleavages at the C-terminus side of acidic residues[14,21] and histidine residues[22] were also observed in CID of protonated peptides and a complex involving Zn^{2+} and a neutral peptide that has histidine residues, respectively. In previous work[18] from our group, we have thoroughly investigated dissociation dynamics associated with the preferential cleavage at aspartic acid residues using several model compounds. We have proposed a salt bridge mechanism in which a highly localized charge, in most cases involving protonated arginine, stabilizes an ion pair formed by proton transfer from aspartic acid to the adjacent amide nitrogen. CID of protonated peptides that did not contain an arginine typically gave rise to several competing processes, with a loss in selectivity for cleavage at aspartic acid residues. For example, CID on protonated GGDGG yielded mainly dehydrated ions, along with two b-type fragments.[23] In contrast, protonated RGDGG cleaves specifically at the aspartic acid residue. These studies led to the expectation that a metal ion might replace protonated arginine as a local charge and give similar selective cleavage at acidic residues.

Here we are reporting novel observations of selective cleavage at aspartic acid residues from SORI-CAD of sodiated model peptides. For these exploratory and mechanistic studies we have selected several small peptides for investigation. These include leucine enkephalin (YGGFL, no acidic residues) and several peptides with acidic residues up to 16 amino acids in length. Sodiated adducts of these peptides were generated by both MALDI and ESI, including singly and multiply charged species. Several of the peptides are investigated as protonated species for comparison. Potential applications to sequencing of large peptides are discussed.

Experimental Procedures and Calculations

All experiments were performed in an external ion source 7-T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer that has been described in detail previously.[24] Briefly, the instrument is equipped with a radio frequency only octopole to guide the ions produced by external ion sources to a rectangular $2 \times 2 \times 3$ in. closed cell through three stages of differential pumping. We used both MALDI and electrospray ionization methods for this work.

For the MALDI source, we implemented a simple arrangement in which a stainless steel probe tip is directly inserted into the octopole ion guide. A nitrogen laser (VSL 337 ND; Laser Science Inc., Cambridge, MA) at a wavelength of 337 nm and a pulse width of 3 ns was focused onto the probe tip on which cocrystallized sample and matrix were deposited and used for ion desorption. This setup simplifies the optimization procedures for signal compared to other types of external MALDI sources which employ ion optics to direct the ion clouds formed by laser desorption. Desorbed peptide ions are constrained by the octopole and transported to the ICR cell. Octopole radio frequency voltages used are dependent on ion mass, but are usually in the range of 300−500 V at 1.2 MHz with zero dc offset voltage on the octopole rods. Since MALDI ions are produced with excess kinetic energy[25] corresponding to an average velocity of approximately 750 m s^{-1}, argon collision gas is introduced into the octopole guide and the cell through a pulsed valve (VAC-1250PSIA, General Valve Co., Fairfield, NJ) to reduce the kinetic energy of the ions. With backing pressures of 50−100 mTorr, the pulsed valve is allowed to open for 1.5 ms and the pressure in the cell increases to the low 10^{-6} Torr region and then decreases to the low 10^{-9} Torr region before the detection event. Seventy milliseconds after the introduction of the Ar cooling gas, the laser was triggered to generate ions. Initially, the trapping plates are kept at 10 V and ramped down to 3 V before detection.

The electrospray source used was purchased from Analytica of Branford Inc. (Branford, CT). Ions are continuously generated by the source. An electromechanical shutter, which is located between the source and the octopole guide and operated by a TTL signal from the OMEGA data system (Ionspec Inc., Irvine, CA), allows ions to pass to the ICR cell for 2 s. This prevents unnecessary contamination of the octopole ion guide from sample deposition and allows the attainment of lower pressures in the differentially pumped cell region. Pressure readouts from an ion gauge calibrated against a MKS (Model 390) pressure transducer were high 10^{-10} Torr with the shutter closed and low 10^{-8} Torr with the shutter opened. Argon gas was again introduced into the system for ion cooling three times during the ion accumulation. During ion accumulation the trapping voltages were kept at 5 V and then ramped down to 3.5 V to perform the reported experiments.

SORI was used to activate low-energy fragmentation pathways. In previous work from our group, 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.[26] Collisions are carried out against pulsing argon gas pressure of high 10^{-5} Torr for 1 s, which corresponds to approximately 10^3 collisions. Radio frequency excitation voltages employed are typically in the range 1.5−2.5 V peak-to-peak. In SORI activation, the center-of-mass collision energy can be adjusted by two variables, excitation voltage and frequency difference ($\Delta\omega$) between cyclotron and excitation frequency. For smaller molecules such as GGDGG, $\Delta\omega$ corresponding to one mass unit difference was used. A larger difference was used for larger molecules. It is our experience that changing the excitation frequency provides better control of the collision energy than changing excitation voltage. With SORI excitation average collision energies in the center of mass were usually around 1 eV. Multiple collisions for an extended time

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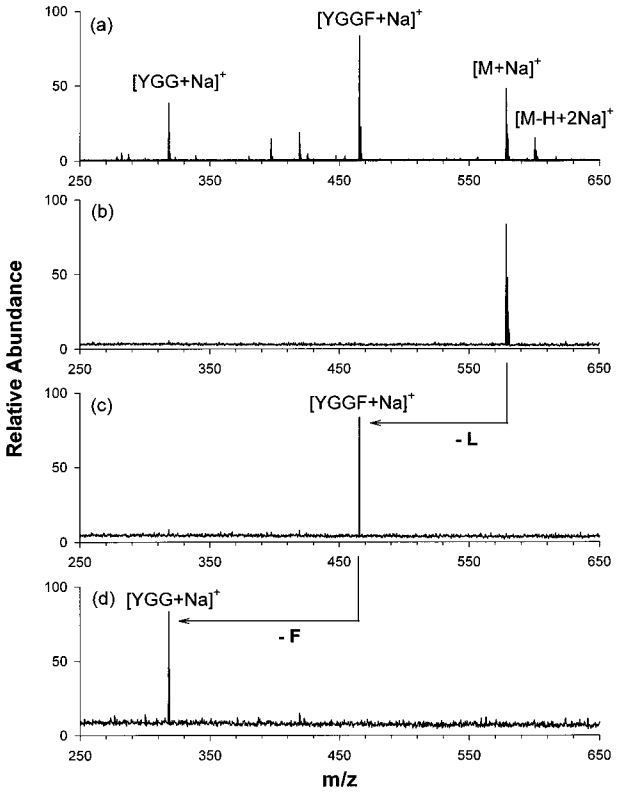

Figure 1. Low-energy dissociation pathways of sodiated YGGFL. (a) Mass spectrum generated by MALDI. (b) Isolation of the sodiated peptide. (c) MS^2 spectrum of the sodiated peptide. Off-resonance excitation leads to loss of leucine (L) residue from the C-terminus. (d) MS^3 spectrum of the sodiated peptide. Off-resonance excitation leads to loss of an additional amino acid (phenylalanine, F) from the C-terminus.

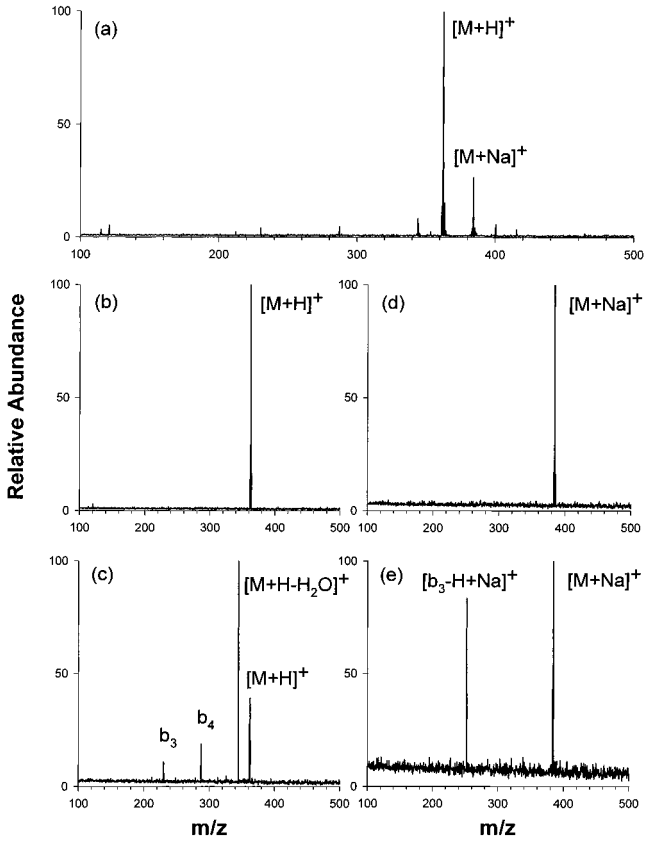

Figure 2. Comparison of low-energy dissociation pathways of protonated and sodiated GGDGG. (a) Mass spectrum generated by electrospray. (b) Isolation of the protonated peptide. (c) MS^2 spectrum of the protonated peptide. Off-resonance excitation of protonated GGDGG results in several competing fragmentation pathways, including dehydration as well as backbone cleavages (b_3 and b_4 fragments). (d) Isolation of sodiated peptide. (e) MS^2 spectrum of sodiated peptide. Off-resonance excitation of the sodiated peptide yields highly selective fragmentation at the aspartic acid. The same solution was used for both experiments.

lead to accumulation of sufficient internal energy to promote fragmentation.[9b]

All peptides, except GGDGG and GGEGG, were purchased from Sigma Chemical Company (St. Louis, MO) and used without further purification. GGDGG and GGEGG were synthesized by the Biopolymer Synthesis Facility in the Beckman Institute at the California Institute of Technology. For MALDI experiments, sample solutions were prepared by mixing 1 mol/L of 2,5-dihydroxybenzoic acid (DHB) in ethanol, 0.03 mol/L of peptides in water/acetonitrile (3:7 V/V), and 1 mol/L of D-fructose in water with a mixing ratio of 6:3:2. The co-matrix D-fructose is known to reduce fragmentation during the ionization process.[27] An aliquot (~1.5 μL) of the sample solution was deposited onto the probe tip and allowed to air-dry at room temperature. Electrospray solutions were prepared by dissolving peptides in methanol/water (1:1) solution at a concentration of 10 pmol/μL and continuously sprayed at 1.5 μL/min flow rate with use of a syringe pump (Harvard Apparatus, Model 22, South Natick, MA); no acids were added. Some samples gave sodiated as well as protonated peptides with no addition of NaCl. To generate sodiated peptides, however, the electrospray solution was usually mixed with an equal volume of 50 pmol/μL NaCl solution in methanol/water (1:1).

To address the reaction energetics of the fragmentation at the C-terminus side of aspartic acid of sodiated peptides, ab initio calculations combined with molecular mechanics calculations have been performed. Molecular mechanics calculations were performed with the Cerius^2 software package[28] running on Origin2000 (Silicon Graphics Inc.). Extensive conformer searches were executed by a random searching scheme to generate 1000 different conformers for Asp-Gly and sodiated Asp-Gly, which were individually subjected to energy minimization with use of the universal force field.[29] Molecular mechanics calculations produced several optimized geometries for Asp-Gly and sodiated Asp-Gly that were selected on the basis of their relative energies. These structures were further optimized at the HF/LAV3P* [30] level with the PS-GVB software package.[31] More refined energies for the optimized structures were obtained at HF/6-31G**.

Results

YGGFL. Figure 1 shows the low-energy dissociation pathways for sodiated YGGFL generated by MALDI. Off-resonance collisional activation leads to sequential removal of amino acids from the C-terminus. The fragment ions likely have the structure of a peptide one residue shorter than the initial peptide ion. Thus the product ions are subject to further fragmentation steps upon activation. The sequential cleavage stops with three amino acids remaining. Further activation leads to loss of sodium ion. Glish and co-workers[32] have observed

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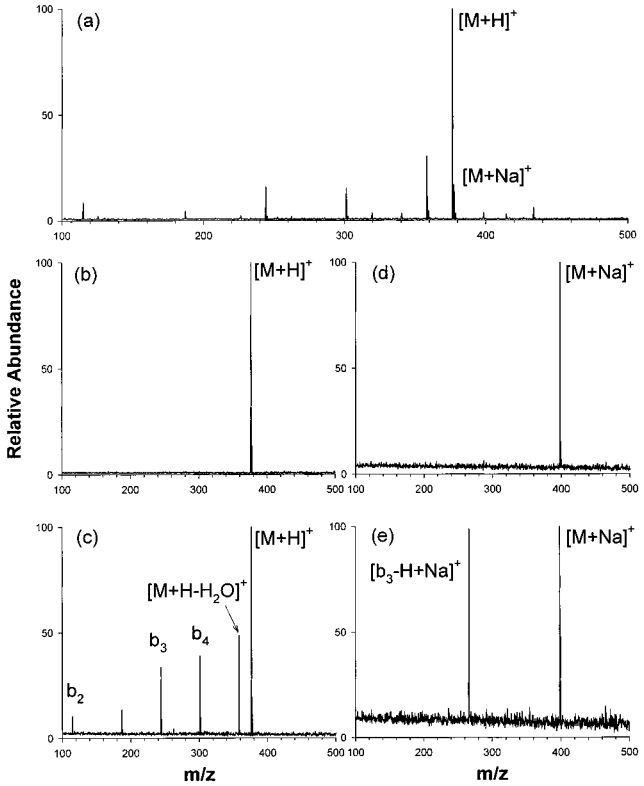

Figure 3. Comparison of low-energy dissociation pathways of protonated and sodiated GGEGG. (a) Mass spectrum generated by electrospray. (b) Isolation of the protonated peptide. (c) MS^2 spectrum of the protonated peptide. Off-resonance excitation of protonated GGEGG results in several competing fragmentation pathways, including dehydration as well as backbone cleavages (b_3 and b_4 fragments). (d) Isolation of sodiated peptide. (e) MS^2 spectrum of sodiated peptide. Off-resonance excitation of the sodiated peptide yields highly selective fragmentation at the glutamic acid.

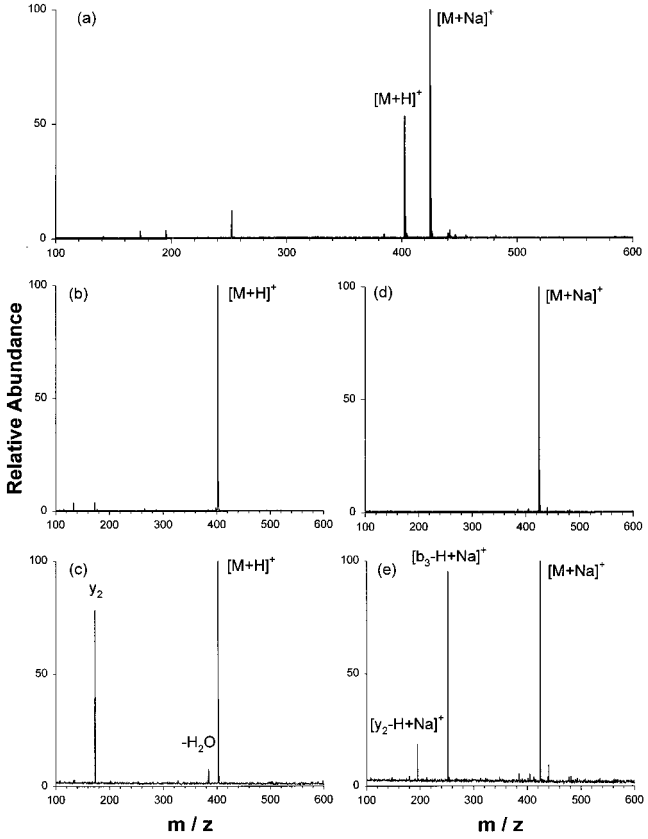

Figure 4. Comparison of low-energy dissociation pathways of protonated and sodiated GGDPG. (a) Mass spectrum generated by electrospray. (b) Isolation of the protonated peptide. (c) MS^2 spectrum of the protonated peptide. Off-resonance excitation of the protonated results in highly selective cleavage at Asp-Pro. (d) Isolation of the sodiated peptide. (e) MS^2 spectrum of the sodiated peptide. Off-resonance excitation of the sodiated peptide yields two products, which result from selective cleavage between the aspartic acid and proline residues.

this sequential fragmentation for alkali cationized leucine enkephalin analogues in a quadrupole ion trap mass spectrometer prior to this study. They recently reported that the C-terminus sequence down to two to three amino acids from the N-terminus could be determined for over 50 peptides.[33] However, one should notice that as with leucine enkephalin, all the peptides used by Glish do not possess acidic residues.

GGDGG. Figure 2 compares the low-energy dissociation pathways of protonated and sodiated GGDGG. These ions are simultaneously generated by electrospray with use of the same sample solution. As shown in previous experiments,[18] off-resonance activation of the protonated peptide results in several competing fragmentation pathways, with dehydration predominating. However, the sodiated peptide exhibits highly selective cleavage of the amide linkage at the C-terminus side of the aspartic acid (D) residue. The mass of the product corresponds to a $[b_3 - H + Na]^+$ ion. Thus with aspartic acid as an internal amino acid, the peptide undergoes selective cleavage at aspartic acid upon activation, rather than sequential cleavage from the C-terminus.

GGEGG. Figure 3 compares low-energy dissociation pathways of protonated and sodiated GGEGG. Again the sodiated peptide undergoes specific cleavage at glutamic acid (Figure 3c) while the protonated peptide yields several fragments from competing dissociation pathways (Figure 3e).

GGDPG. Figure 4 compares low-energy dissociation pathways of protonated and soidated GGDPG. The protonated peptide undergoes highly selective cleavage at the Asp-Pro amide bond, resulting in a y_2 ion. The fragmentation adjacent to proline residues is a cleavage pattern that has been observed to be favorable in protonated peptides.[18−20] This selectivity may result from a higher proton affinity of the tertiary proline amide nitrogen relative to other amide nitrogens. Two products are observed for the sodiated peptide, resulting from specific cleavage of the Asp-Pro amide bond, with sodium ion remaining attached to either of the two fragments. The relative intensity of the two products suggests that the [GGD-OH] fragment has a slightly higher sodium affinity than PG although other factors such as biased trapping efficiencies for fragments with different kinetic energies may be a concern.

GFDLNGGGVG. Highly specific fragmentation at the aspartic acid residue is also observed in CID of sodiated GFDLNGGGVG generated by MALDI (Figure 5). However, the observed product ion is a $[y_7 - H + Na]^+$ ion in this case. These observations are consistent with the expectation that the sodium ion will be bound to the fragment with the higher sodium affinity upon dissociation. It would generally be expected that the larger of the two fragments would more strongly bind sodium ion due to enhanced solvation.

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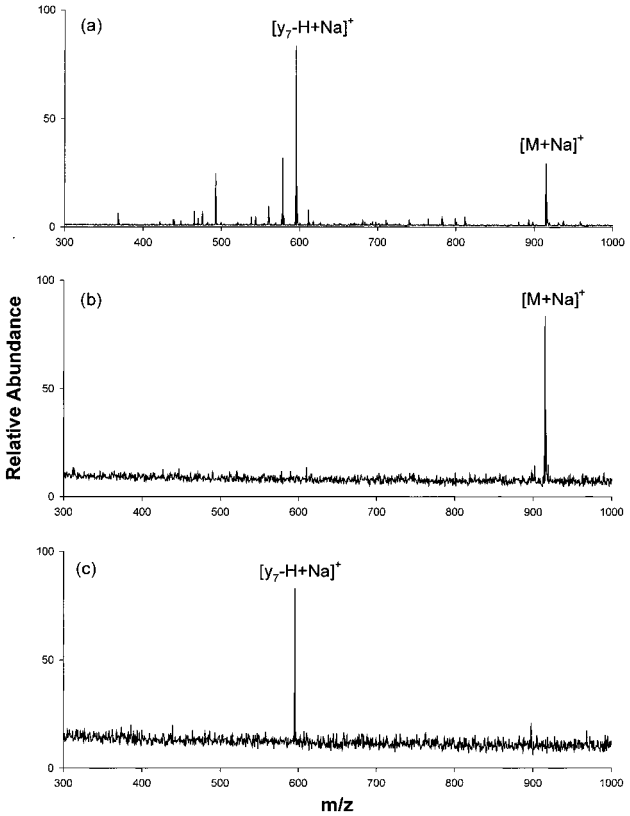

Figure 5. Low-energy dissociation pathway of a sodiated 10-mer peptide containing an aspartic acid residue (GFDLNGGGVG). (a) Mass spectrum generated by MALDI. (b) Isolation of the sodiated peptide. (c) MS^2 spectrum of the sodiated peptide. Off-resonance excitation produces a single product ion, resulting from cleavage of the aspartic acid amide linkage.

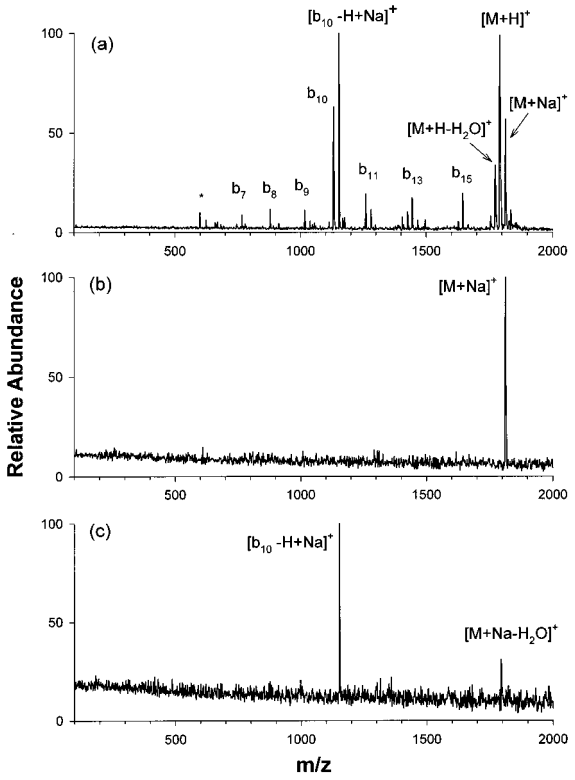

Figure 6. Low-energy dissociation pathways of a sodiated 16-mer peptide (AVSEHNLLHDKGKSIN) that has glutamic acid as well as aspartic acid. (a) Mass spectrum generated by MALDI. (b) Isolation of the sodiated peptide. (c) MS^2 spectrum of the sodiated peptide. Off-resonance excitation results in cleavage only at aspartic acid. No fragment corresponding to cleavage at glutamic acid was observed. Additional experiments indicate that the series of b_n ions observed in (a) results from dissociation of the protonated peptide.

AVSEHNLLHDKGKSIN. In Figure 6 the low-energy dissociation pathway for sodiated hypercalcemia malignancy factor fragment 1−16 (AVSEHNLLHDKGKSIN) generated by MALDI is shown. This peptide has both aspartic and glutamic acid as internal residues. Off-resonance activation yields only a single product, resulting from cleavage of the amide linkage on the C-terminus side of aspartic acid. Interestingly, no competitive cleavage is observed at the glutamic acid (E) residue.

Since ESI usually generates multiply charged ions for larger peptides, it is also of interest to investigate their low-energy fragmentation pathways. It is known, for example, that Coulombic repulsion of charged groups within multiply charged ions can significantly affect fragmentation processes resulting from activation.[34] In Figure 7 low-energy dissociation pathways for doubly protonated AVSEHNLLHDKGKSIN generated by ESI are shown. Off-resonance collisional activation results in several competing dissociation pathways with the fragments (b_{10} and y_6) from cleavage at aspartic acid residue as major products. The charge state of each fragment was determined from the ^{13}C isotope multiplet. Exciting at a lower frequency than the cyclotron frequency (higher mass, +1.5 *m*/*z*) and a higher frequency (lower mass, −1.5 *m*/*z*) generated identical spectra, indicating that no product ions were accidentally ejected. In addition to loss of water, collisional activation of the doubly sodiated peptide yields two singly charged complementary fragments, resulting from selective cleavage at aspartic acid (Figure 8). Again, no cleavage at glutamic acid is observed. The relative intensity of the complementary fragments should be the same. However, the spectrum shows that the $[b_{10} - H + Na]^+$ fragment appears more abundant than $[y_6 - H + Na]^+$, probably due to a biased trapping efficiency.[35]

Discussion

Summary of Observed Cleavage Processes. It is observed that low-energy activation such as SORI on sodiated YGGFL gives sodiated YGGF and YGG upon MS^2 and MS^3, respectively. Glish and co-workers have also demonstrated that CID of sodiated peptides which lack acidic residues results in sequential loss of amino acids from the C-terminus,[32,33] promising the application of mass spectrometric fragmentation to gas-phase sequencing. Renner and Spiteller[36] originally proposed a mechanism for this process, in which the sodium ion interacts with carboxylate at C-terminus and polarizes the carbonyl group of the adjacent amino acid residue, facilitating nucleophilic attack by the carboxylate. Subsequent rearrangements result in losses of a CO and an imine and form a peptide of one less amino acid (Scheme 1).

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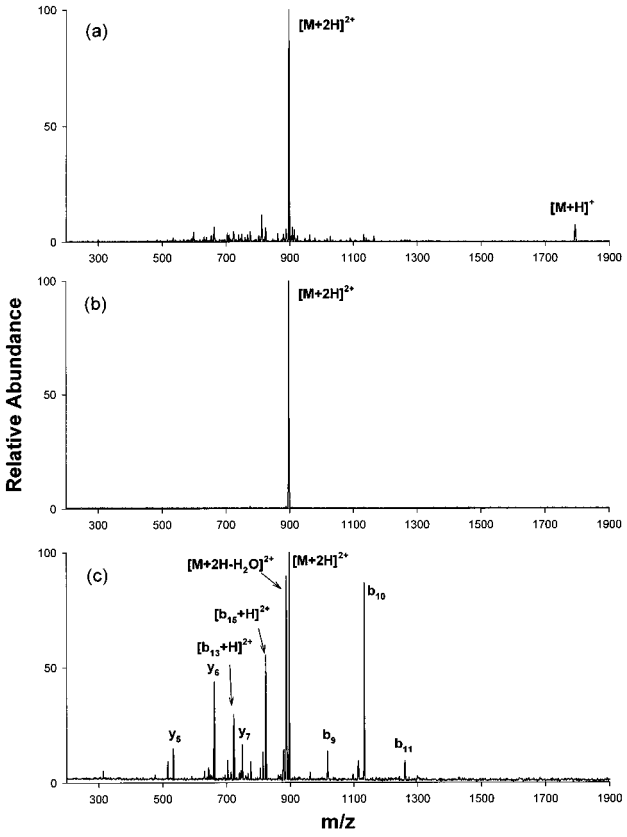


Figure 7. Low-energy dissociation pathways of a doubly protonated 16-mer peptide (AVSEHNLLHDKGKSIN). (a) Mass spectrum generated by electrospray. (b) Isolation of the doubly protonated peptide. (c) MS^2 spectrum of the doubly protonated peptide. Off-resonance excitation results in several competing dissociation pathways with the complementary singly charged fragments (b_{10} and y_6) from cleavage at the aspartic acid residue as major fragments.

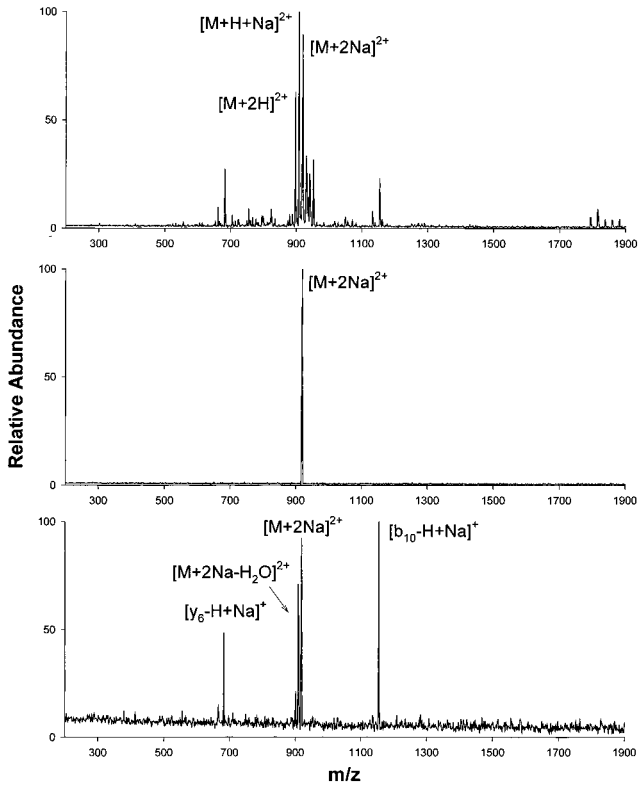


Figure 8. Low-energy dissociation pathways of a doubly sodiated 16-mer peptide (AVSEHNLLHDKGKSIN). (a) Mass spectrum generated by electrospray. With the addition of NaCl to the electrospray solution sodiated peptides can be produced as abundant ions. (b) Isolation of the doubly sodiated peptide. (c) MS^2 spectrum of the doubly sodiated peptide. Off-resonance excitation gives the doubly charged dehydration product ion as well as two complementary singly charged fragments, resulting from selective cleavage at the aspartic acid residue. No cleavage is observed at the glutamic acid residue.

The process in Scheme 1 is inhibited by the presence of an acidic residue in the peptide. The present study demonstrates that sodiated peptides with internal aspartic acid residues undergo highly selective cleavage of the C-terminus amide linkage at aspartic acid. A similar cleavage occurs at glutamic acid, but predominantly at aspartic acid when both residues are present in the peptide. To propose a mechanism for this process we first consider possible structures for sodiated peptides.

Sodiated Peptide Structures. Sodiated GGDPG undergoes four fast deuterium exchanges with ND_3 in the gas phase.[37] To account for this observation we suggest a zwitterionic structure in which the sodium ion forms an ion pair with one of the two carboxyl groups, producing a carboxylate salt with the labile proton on the basic N-terminus amino group. The three equivalent N-terminus hydrogens and the acidic hydrogen of the remaining carboxyl group undergo fast exchanges with ND_3. For a "neutral structure" of sodiated GGDPG where the sodium ion interacts with several of the carbonyls, two fast exchanges of the carboxyl hydrogens and possibly two very slow exchanges of the N-terminus amino hydrogens might be expected. Combining the results of the H/D exchange experiments with the CID results discussed above, one can draw a picture of sodiated GGDPG structures in the gas phase in which the sodium ion mainly interacts with carboxylate of aspartic acid and forms an ion pair. The resultant "mobile" proton may rapidly transfer within the molecule, find basic sites, and induce charge-directed fragmentation upon activation.

(37) Lee, S.-W.; Beauchamp, J. L. In preparation.

Wysocki and co-workers recently investigated the relative energetics of fragmentation of protonated peptides using surface-induced dissociation tandem mass spectrometry and found that more energy is required for fragmentation of peptides that have basic residues such as arginine than peptides that have no basic residues.[38] Combined with other experimental results, they have supported the mobile proton model where rapid intramolecular proton transfers yield a rapidly interconverting population of structures from which charge-directed fragmentation occurs. Charge-directed fragmentation has also been observed in CID studies of an 18-crown-6 ether complex of protonated GGDPG in which cleavage at aspartic acid does not occur for the complex due to encapsulation of the mobile proton by the crown ether.[39] However, activation of the adduct initially leads to crown ether loss as the lowest energy dissociation pathway. Once the crown ether is eliminated, free protonated GGDPG undergoes selective fragmentation as shown in Figure 4c.

Salt bridge chemistry was originally invoked to describe proposed intermediates in H/D exchange studies of protonated glycine oligomers with ND_3.[39] Exchange of carboxylic hydrogen was proposed to occur via a salt bridge mechanism where the ion pair formed by proton transfer from the carboxylic group

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

(39) (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. (c) Lee, S.-W.; Lee, H.-N.; Kim, H. S.; Beauchamp, *J. Am. Chem. Soc.* Submitted for publication.

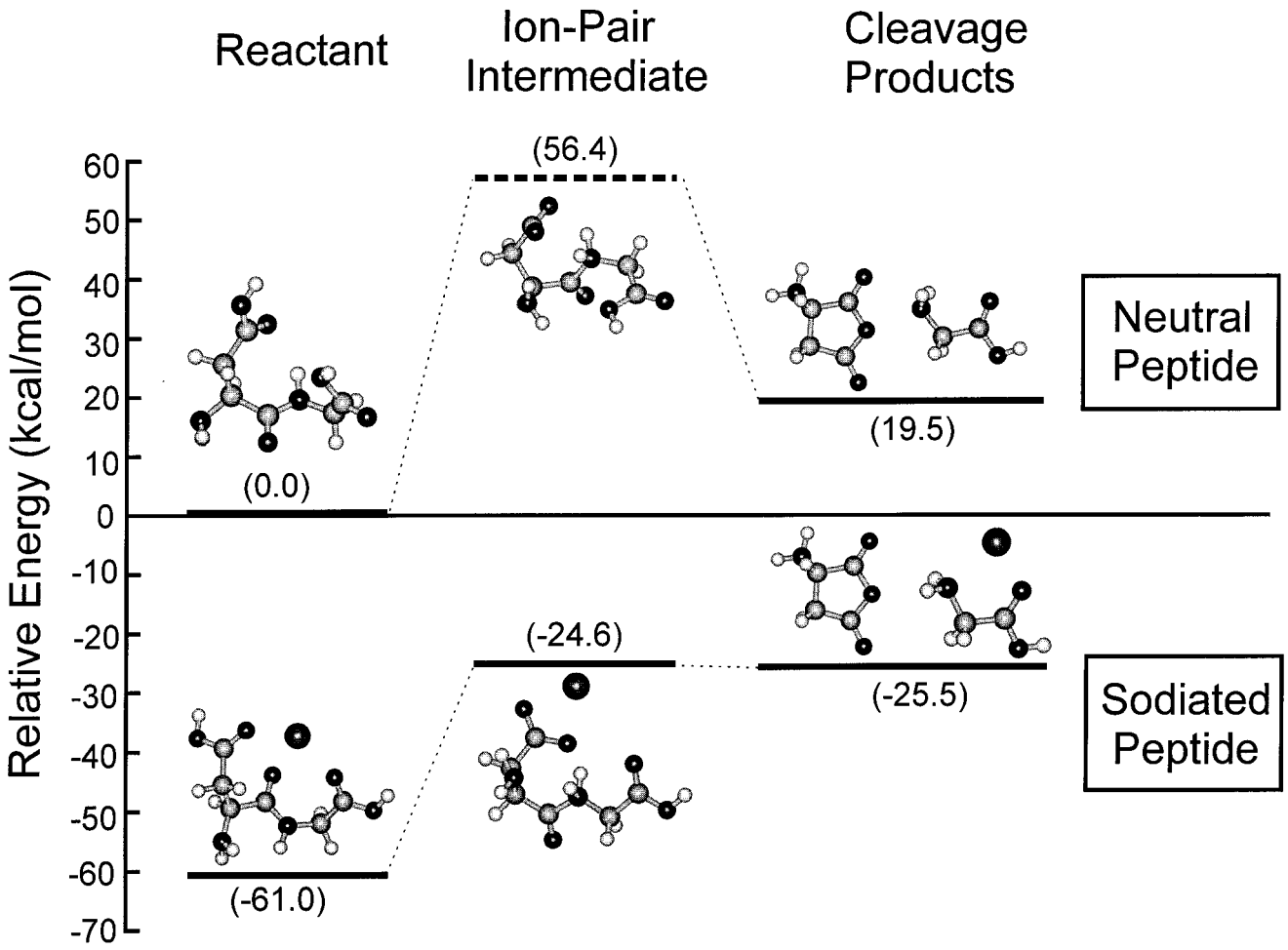
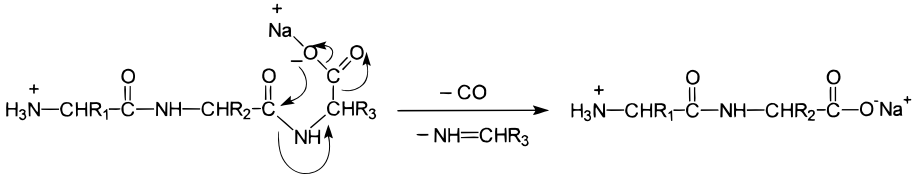


Figure 9. Energetics of cleavage of the amide linkage in the model system of DG with and without sodium ion (from HG/6-31G**//HF/LAV3P* calculations). The intermediate geometry for the neutral DG was obtained by fixing the positions of the two hydrogens of the protonated amide. Other geometries were obtained by full geometry optimizations. The sodium ion promotes the cleavage reaction by interacting more strongly with the ion pair intermediate than with the reactant.

Scheme 1



to ammonia is stabilized by a proximal protonated basic site (the N-terminus amino group in the case of glycine oligomers). Williams and co-workers have also inferred salt bridge structures for gas-phase peptides in their studies of blackbody infrared radiative dissociation using FTMS.[40] Salt bridge intermediates play a key role in the selective cleavage of protonated peptides at aspartic acid residues.[18]

Mechanism and Energetics of Cleavage at Aspartic Acid. We propose a salt bridge mechanism for the selective cleavage of sodiated peptides at aspartic acid residues. The suggested mechanism invokes intermediacy of an ion pair stabilized by means of salt bridge formation with the sodium ion, as depicted in Scheme 2.

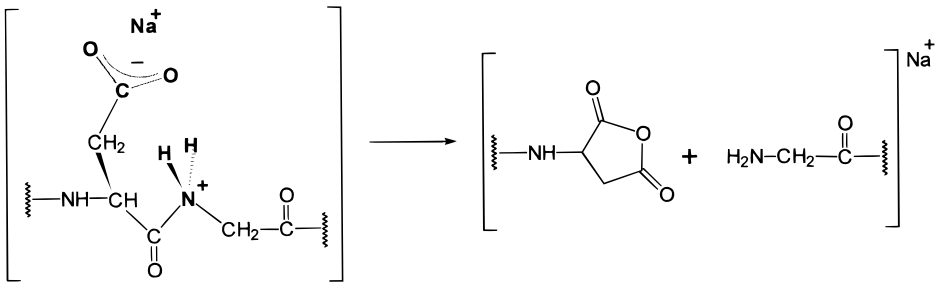
In this process the mobile proton is bonded to the amide nitrogen on the C-terminus side of the aspartic acid residues, which in turn leads to cleavage at this site. With an extra methylene in the side chain, the stabilization afforded a salt bridge intermediate for glutamic acid appears to be less than in the case of aspartic acid, since cleavage at the glutamic acid residue of doubly sodiated hypercalcemia malignancy factor fragment 1−16 does not compete with cleavage at the aspartic acid residue (Figure 8).[41]

Ab initio calculations have been performed to address the energetics of the salt bridge reaction mechanism. Figure 9 shows the energy diagram constructed from the HF/6-31G**/ /HF/LAV3P* calculations using Asp-Gly (DG) with and without

(40) Schnier, P. D.; Price, W. D.; Jockusch, R. A.; Williams, E. R. *J. Am. Chem. Soc.* **1996**, *118*, 7178.

(41) We do observe a $[y_{12} - H + Na]^+$ fragment resulting from cleavage at the C-terminus side of glutamic acid from singly sodiated AVSEHNLLHDKGKSIN using a quadrupole ion trap mass spectrometer (LCQ, Finnigan Co., San Jose, CA). The ratio of $[b_{10} - H + Na]^+$ and $[y_{12} - H + Na]^+$ from the sodiated peptide is about 6:1 favoring cleavage at aspartic acid. The time scale for excitation and dissociation of ions is 3 orders of magnitude less in the ion trap (1 ms) than in the FT-ICR experiment. As a result, significantly higher initial energies are required to produce dissociation in the ion trap, resulting in lower discrimination between processes having only a small difference in activation energy.[9b]

Scheme 2



sodium ion as a model system. The molecular structures shown are optimized geometries at the HF/LAV3P* level and the energies are obtained from single point energy calculations on the optimized geometries using a 6-31G** basis set. The two hydrogens on the amide nitrogen of the ion pair intermediate have been constrained in the case of the neutral DG (no sodium ion present) to prevent proton transfer back to the carboxylate of the aspartic acid residue, with optimization of all other geometrical parameters. The geometry of the salt bridge intermediate for the sodiated DG has been optimized without any constraints. The proximity of a sodium ion to the ion pair formed between the carboxylate and the protonated amide stabilizes the intermediate by a highly favorable ion−dipole interaction. This has been estimated to contribute about 42 kcal/mol of stabilization when the dielectric constant is set to 1.[42] The calculations shown in Figure 9 clearly demonstrate the viability of a salt bridged intermediate, with a reduction of approximately 20 kcal/mol in the energy of the ion pair intermediate compared to cleavage in the absence of the sodium ion. The salt bridge intermediate is a local minimum on the potential energy surface. No attempt has been made to estimate the low barriers that separate this intermediate from reactants and products.

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

Conclusion

FT-ICR experiments have demonstrated highly selective cleavage at aspartic acid residues of sodiated peptides generated by MALDI and electrospray ionization. We propose a salt bridge mechanism in which the sodium ion plays an important role as a localized charge stabilizing an ion pair intermediate formed by proton transfer from the carboxylic group of aspartic acid to the adjacent amide nitrogen. This observation provides the intriguing possibility of mapping aspartic acid residues in proteins. Currently we are investigating this process with more complex species with multiple aspartic acid residues to determine possible factors that might influence preference for cleavage at a particular site. We are also investigating other processes that effect selective cleavage at specific amino acids as well as sites of posttranslational modification.

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