A Direct Comparison of "First" and "Second" Gas Phase Basicities of the Octapeptide RPPGFSPF

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Abstract: A modified kinetic method was used in combination with MIKE spectrometry to obtain the value of 191.7 ± 2.3 kcal/mol for the gas phase basicity of the protonated octapeptide RPPGFSPF (des-Arg^9-bradykinin). This was found to be 63.1 ± 3.0 kcal/mol lower than the gas phase basicity of the neutral peptide (254.8 ± 2.0 kcal/mol) determined by the kinetic method on the same instrument. Molecular mechanics studies support the conclusion that this difference reflects both conformational changes and interproton electrostatic repulsion. The experiments and the molecular modeling calculations suggest that protons in multiply charged peptides are not necessarily located on the most basic sites, if the alternative of greater intercharge distance is available.

Introduction

The introduction of the electrospray ionization technique by Fenn and co-workers[1] has given researchers a powerful tool to produce multiply charged ions of biomolecules and study their behavior in the gas phase. This solvent-free environment offers the unique possibility to study intrinsic conformational and thermochemical properties of peptides and proteins unaffected by polar solvent molecules. The relative stabilities of the gas phase conformations of peptides and proteins are suggested by entropy measurements associated with proton binding,[2] by target capture experiments,[3] by collisional cross section/ion mobility experiments with multiply protonated biomolecules,[4,5] and by H/D exchange experiments.[6,7] The relevance of gas phase conformations to the chemistry of proteins in solution is still being debated in the literature.[7,8] Elucidation of the relation between gas phase and aqueous conformations will help to better understand the role of solvent in protein folding and conformation.[9-11] Although the three-dimensional structures of many peptides and proteins in aqueous solutions and crystals have been extensively characterized by means of NMR, X-ray crystallography, and other techniques,[12] the available information on gas phase conformations is limited as yet. The situation may be improved, however, by combining mass spectral methods such as ion mobility methods, H/D exchange, and kinetic energy release (KER) spectrometry[13-15] with molecular modeling methods.[5,15,16]

Determination of gas phase basicities of peptides and proteins provides important information which is analogous to measurements of pK_a in solution. The values of pK_a are greatly affected by intramolecular interactions,[17] and the conversion of the denatured form to the native one maximizes these interactions.[18] Therefore, titration studies may be used to probe conformational changes. Gurd and co-workers[19] have demonstrated that pK_a values of the N- and C-terminals of pentapeptides GGXGG are dependent on the particular aminoacid X (Asp, Glu, Tyr, His, Lys or Arg), which suggests that conformational effects may be important even for rather small peptides.

Most biologically relevant peptides and proteins have more than one ionizable group and carry several charges in solution, and gas phase chemists are also challenged to study thermochemical properties of peptides and proteins with different numbers of charges. Among the most interesting aspects of the thermochemistry of multiply charged molecules are electrostatic repulsion[20] and its potential influence on fragmentation, conformation and ion-molecule reactions in the gas phase.

McLuckey, Glish, and Van Berkel[21] have suggested that proton transfer reactions involving large multiply protonated

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molecules, for example

$$MH_2^{2+} + B \rightarrow MH^+ + BH^+ \quad (1)$$

proceed through the formation of a proton-bound complex [B···H⁺···MH⁺]. The energy hypersurface of this complex has an unusual shape due to the Coulombic field and an unusually high number of degrees of freedom. In this case, the Coulombic interaction imposes a reverse activation energy barrier for reaction 1. This means that even an exothermic reaction 1 may not proceed at thermal energies due to the reverse activation energy barrier. This would result in a higher experimental value of GB(MH^+), measured by the bracketing method. Bohme and co-workers applied the bracketing method to determine the gas phase acidities of a fullerene dication[22] $C_{60}H^{\bullet 2+}$ and derivatized fullerene dications.[23] They measured experimentally the value of "apparent" gas phase acidities of dications $GA_{app}(MH^{2+})$, offset by the height of the reverse activation energy barrier δ. The value of the latter was calculated as an electrostatic repulsion energy between the two like charges at distances corresponding to maximum dimensions of the molecules studied (8.4−10 Å). The gas phase acidities of the dications were then determined as $GA(MH^{2+}) = GA_{app}(MH^{2+}) - \delta$. In contrast, Gross and Williams[24] have proposed that Coulombic repulsion may be estimated as $\delta = GB(M) - GB_{app}(MH^+)$ assuming the molecule's conformation is not altered by multiple protonation. These contradictory hypotheses appear to derive from the use of balanced energy diagrams[22,23] and the diagrams[24] that assume a short-range interaction between MH^+ and BH^+ to be negligible.

Electrostatic interactions must also be considered when the kinetic method[25] is applied to charged molecules. Recently we developed a modified kinetic method to study charged molecules,[15] which takes into account the electrostatic interactions in the complex [B···H⁺···MH⁺]. To determine a "second" gas phase basicity of a peptide M (i.e., GB(MH^+)), we measure relative rates of unimolecular decompositions of metastable doubly charged protonated dimers, formed by M and a molecule of a reference base B

$$^+HM \cdots H^+ \cdots B \xrightarrow{k_1} MH_2^{2+} + B \quad (2)$$

$$^+HM \cdots H^+ \cdots B \xrightarrow{k_2} MH^+ + BH^+ \quad (3)$$

The ratio of the unimolecular rate constants k_1 and k_2 is a function of the gas phase basicity of a reference base GB(B), the second gas phase basicity of the peptide GB(MH^+), the value of the reverse activation energy value δ, and the effective temperature of the dimer T_{eff}[15]

$$\ln(k_1/k_2) = \ln([MH_2^{2+}]/[MH^+]) = (GB(MH^+) - GB(B) + \delta)/RT_{eff} = (GB_{app}(MH^+) - GB(B))/RT_{eff} \quad (4)$$

The value of the "apparent" gas phase basicity of the protonated peptide $GB_{app}(MH^+) = GB(MH^+) + \delta$ can then be determined when several structurally similar reference bases B_i are used. The value of the reverse activation energy barrier δ can be rather accurately measured as a kinetic energy release (KER) in a charge separation reaction[15] 3. Experimentally measured values of $GB_{app}(MH^+)$ and δ then provide the value of the second gas phase basicity of the peptide. In addition, a combination of KER measurements and molecular dynamics calculations may allow us to locate the charges on the multiply protonated molecule.[15] In the present work we measure both the first and the second gas phase basicities of an octapeptide RPPGFSPF and demonstrate that conformational changes as well as Coulombic repulsion influence the second gas phase basicity.

Experimental Section

Methods. All experiments were performed on a JEOL HX110/HX110 (JEOL, Tokyo, Japan) four sector mass spectrometer (EBEB geometry) equipped with a standard fast atom bombardment (FAB) source and an electrospray ion source (Analytica of Branford, Branford, CT). Doubly charged dimers were generated by electrospray ionization. Solutions of peptide mixtures in water/methanol/acetic acid were electrosprayed at 1 μL/min, and the temperature of the interface capillary was kept at ca. 120 °C. Selecting rather mild conditions of spray ionization allowed us to achieve heterodimer intensities as high as 20% of those of the monomer ions. Singly charged dimers were produced by FAB. Normally 1 μL of a peptide solution in water/methanol/TFA and 1 μL of an organic base were mixed in a glycerol/thioglycerol (1:1) matrix to obtain a high intensity signal of the dimer. A heterodimer of interest was selected by the first two sectors of the mass spectrometer and introduced into the collision cell. No collision gas was added to the cell, so that only the decomposition of metastable ions was observed. Mass spectra were acquired using E scans only. Normally 25 to 100 scans were acquired for each spectrum. To maximize the sensitivity and ion transmission, the energy slit of the second electrostatic analyzer was kept wide open to acquire full-scan range MIKE spectra, from which a $[MH^+]/[MH_2^{2+}]$ ratio was then deduced. However, the energy slit was then set to a fine energy resolution to record the shape of MH^+ peak, from which a KER value was deduced. All spectra were recorded using JEOL MP7000 data system.

Molecular modeling studies were performed using CHARMm22/QUANTA4.0 macromolecular modeling program (Molecular Simulations, Inc., Waltham, MA) on a Silicon Graphics Personal Iris workstation (Silicon Graphics, Inc., Mountain View, CA).

Materials. Bradykinin fragments [1-8] and [2-9], peptides GGG, AGG, LGG, GLG, GGL, GGFL, ALAL, FGGF, and AGSE were purchased from Sigma Chemical Co. (St. Louis, MO). The primary structures of des-Arg^9-bradykinin and des-Arg^1-bradykinin were verified by CID MS/MS. Organic reference bases dipropylamine, dibutylamine, di-*sec*-butylamine, *N,N*-diethylmethylamine, 1,1,3,3-tetramethylguanidine (TMG), 1,5,7-triazabicyclo[4.4.0]dec-5-ene (HMPP), 1,5-diazabicyclo[5.4.0]undec-7-ene (DBU), and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). All chemicals were used without further purification. Gas phase basicity values of the organic reference bases are taken from the compilation by Lias and co-workers[26] and from work of Gal and co-workers.[27]

Results and Discussion

First Gas Phase Basicity of Bradykinin Fragment [1-8]. Bradykinin fragment [1-8] (peptide RPPGFSPF) has only one basic residue (Arg^1). The first gas phase basicity of this peptide (i.e., the basicity of the neutral peptide) was measured using the classical kinetic method.[25] Figure 1 shows a typical decomposition spectrum of a metastable proton-bound dimer, formed by this peptide and the organic reference base (HMPP). The $[BH^+]/[MH^+]$ ratio for each of the reference bases used (DBN, DBU, and HMPP) was calculated using the peaks' areas

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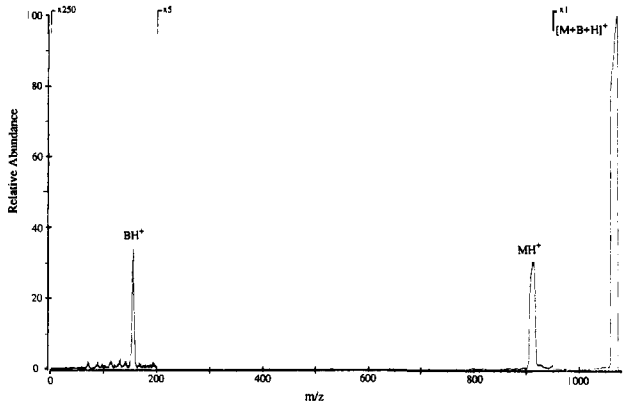

Figure 1. A MIKE spectrum of a unimolecular dissociation of a metastable proton-bound dimer $[M + B + H]^+$ formed by bradykinin fragment [1-8] (M) and HMPP (B).

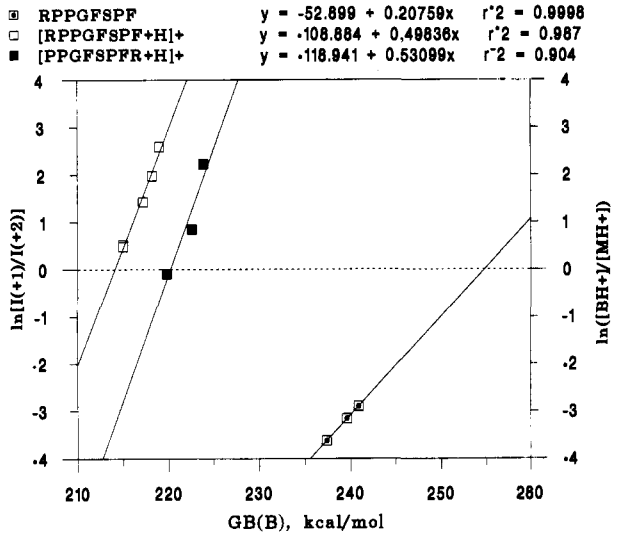

Figure 2. Plots of the logarithms of relative rates of unimolecular dissociation of proton-bound dimers $[RPPGFSPF + B + 2H]^{2+}$ (□), $[PPGFSPFR + B + 2H]^{2+}$ (■), and $[RPPGFSPF + B + H]^+$ (⊡).

and plotted against the gas phase basicities of the guanidines[27] (see Figure 2). Although the gas phase basicity of the peptide is apparently much higher than that of the most basic commercially available reference base (HMPP), the dynamic range of the MIKE spectrum was sufficient to provide a rather good estimation of GB(M). The experimental points fit the straight line with a correlation coefficient $r^2 = 0.9998$. The intercept of this plot with the zero-point abscissae line gives the value of the gas phase basicity of bradykinin fragment [1-8] GB(M) = 254.8 ± 2.0 kcal/mol.

Second Gas Phase Basicity of Bradykinin Fragment [1-8]. The "apparent" second gas phase basicity of bradykinin fragment [1-8] was measured using four tripeptides as reference bases (GGG, GGL, GLG, and LGG). Earlier we showed that a carefully chosen set of small peptides can be used as reference bases in the kinetic method measurements.[28] A series of independent experiments has demonstrated that our set of tripeptides can be used as reference bases in kinetic method measurements.[29] Figure 3 shows a typical MIKE spectrum of the decomposition of the metastable doubly charged dimer formed by RPPGFSPF and GGL. The $[MH^+]/[MH^{2+}]$ ratio for

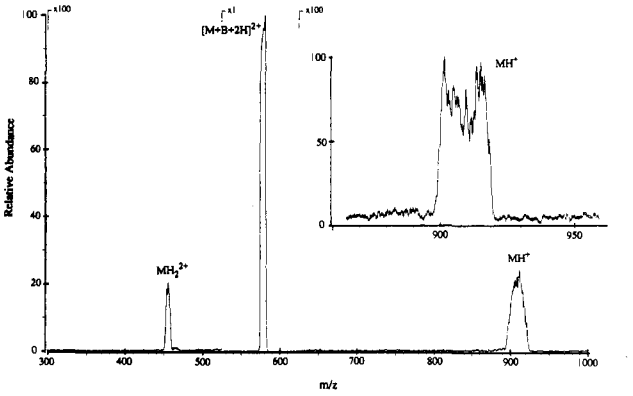

Figure 3. A MIKE spectrum of a unimolecular dissociation of a metastable proton-bound dimer $[M + B + 2H]^{2+}$ formed by bradykinin fragment [1-8] (M) and GLG (B). Energy slit of the second electrostatic analyzer $\beta_2 = 4.0$ mm. On the insert: the MH^+ peak shape, energy slit $\beta_2 = 0.8$ mm.

the dimers formed with each of the reference bases was calculated using the peaks' areas and plotted against the gas phase basicities of the tripeptides[30] (see Figure 2). The five experimental points (measurement with GGG was repeated twice) fit the straight line with a correlation coefficient $r^2 = 0.987$. The intercept of this plot with the zero-point abscissae line gives the value $GB_{app}(MH^+) = 214.1 \pm 2.0$ kcal/mol.

The insert on Figure 3 shows the shape of the MH^+ ion peak obtained with a narrow energy slit in the second electrostatic analyzer of the mass spectrometer. The "dishtopped" shape of this peak suggests that the distribution of released kinetic energy is rather narrow and its maximum is a nonzero value.[31-33] In these circumstances a good estimation of the average value of KER can be obtained using the width of this peak at its half-height[33,34]

$$T = (y^2 m_1{}^2 \mathrm{eV})/(16 x m_2 m_3)\cdot(\Delta E/E)^2 \qquad (5)$$

where V is acceleration voltage, E is the center of the peak of the precursor ion $m_1{}^{x+}$, ΔE is the peak width for the fragment ion $m_2{}^{y+}$, and $m_3 = m_1 - m_2$.

Calculation of the average KER for the charge separation process 3 gives $T = 0.98 \pm 0.04$ eV. Similar measurements have been made using GGG and GLG as reference bases, giving $T = 0.99 \pm 0.04$ and $T = 0.95 \pm 0.04$ eV correspondingly. Based on those measurements, we estimate the reverse activation energy value in (3) as $\delta = 0.97 \pm 0.05$ eV (22.4 ± 2.3 kcal/mol). This gives the value of the second gas phase basicity of bradykinin fragment [1-8], $GB(MH^+) = 191.7 \pm 1.2$ kcal/mol.

The measured value of δ represents an electrostatic repulsion between the two elementary charges in the transition state of

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(29) The tripeptides chosen (GGG, GGL, GLG, and LGG) are structurally similar and it is intuitively apparent that the topology of the intrapeptide hydrogen bonds is preserved in the series, since small aliphatic chains cannot form hydrogen bonds themselves or significantly affect the conformation of peptides in any other way. To verify this assumption, we remeasured the gas phase basicity of yet another tripeptide, AGG (217.1 kcal/mol[30]) using GGG, GGL, GLG, and LGG as reference bases. The kinetic method measurements yielded the value of the gas phase basicity of AGG 216.9 ± 2.0 kcal/mol, which is only 0.2 kcal/mol lower than that measured by the kinetic method using alkylamines as reference bases (i.e., the two values are the same within the experimental error).
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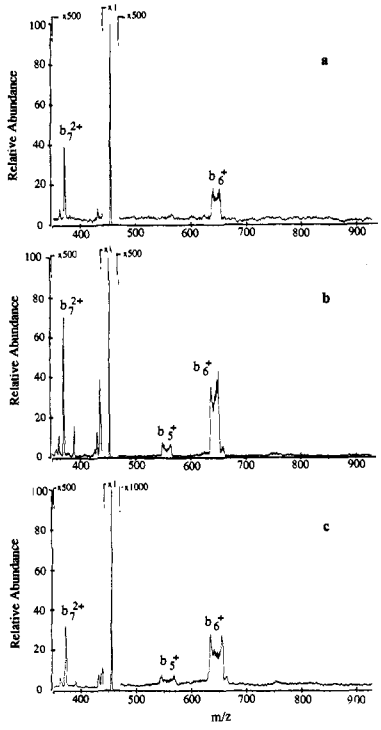

Figure 4. MIKE spectra of metastable doubly charged bradykinin fragment [1-8] ions generated by electrospray, acceleration voltage of MS1 is 5 kV (a), FAB, 5 kV (b), FAB, 2 kV (c).

the complex[15] $[B{-}H^+{-}MH^+]^*$. It is intuitively clear that although the three-dimensional structures of both B and M in this transition complex should be close to those of the separate (isolated) peptides, they still could be influenced by one another. To evaluate the degree of this influence, we measured the values of the electrostatic repulsion within doubly protonated RPPGFSPF by measuring the average KER in the decomposition of metastable MH_2^{2+} ions. The spectrum of the metastable MH_2^{2+} ions generated by electrospray features only one singly charged fragment, b_6^+, in the range of *m/z* above that of the precursor (see Figure 4a). An average value of the KER in this fragmentation, calculated using (5), is $T = 1.01 \pm 0.04$ eV. FAB-generated ions possess more internal energy,[35] so that the spectrum of FAB-generated metastable MH_2^{2+} ions has two singly charged fragments b_6^+ and b_5^+ (see Figure 4b). The average KER values calculated using the shapes of these two peaks, are 0.95 ± 0.04 and 1.01 ± 0.08 eV, respectively. Lowering the acceleration voltage of MS1 from 5 to 2 kV allowed us to increase the flight time of ions by a factor of 1.6 and thus to sample metastable ions with lower internal energies (see Figure 4c). Still, the average KER for the formation of the b_6^+ fragments was not altered ($T = 0.97 \pm 0.05$ eV). The fact that the value of the Coulombic repulsion in MH_2^{2+} is very close to that in the transition state of the complex $[B{-}H^+{-}MH^+]^*$ suggests that the three-dimensional structure of RPPGFSPF is not affected much by the reference peptide (i.e., the solvation of M by B is minimal) and that the only strong interaction between B and M in the transition state of the complex is through the "shared" proton.

Another intriguing question that can be addressed as well concerns the location of the protons in the doubly charged peptide MH_2^{2+}. The two most basic sites of RPPGFSPF are the guanidinium group of Arg[1] and the N-terminus of the peptide. Cassady[16] has pointed out that the C-terminus of a peptide is the third basic side, which has a lower "local" proton affinity than the N-terminus, but is more distant from a highly basic guanidinium group of Arg[1] as a site of proton attachment. To test which group is the most probable site for the second protonation, we performed a molecular modeling study for the two systems: R^{2+}PPGFSPF (the two "extra" protons are on the guanidinium group of Arg[1] and on the N-terminus of the peptide) and R^+PPGFSPF$^+$ (the two "extra" protons are on the guanidinium group of Arg[1] and on the C-terminus). The starting linear conformations were energy minimized to remove possible steric hinderance using CHARMm22 semiempirical force field.[36] The molecular dynamics analysis[36] included heating of each peptide model from 0 to 500 K in 1 ps, equilibrating at 500 K for 1 ps, followed by the dynamics simulation (10 ps, 10^{-3} ps time steps). Every tenth conformation was saved in a dynamics simulation trajectory file. Analysis of the simulation trajectories was accomplished by correlating the intercharge distances and potential energies of the peptide models (see supporting information). The correlation map for R^{2+}PPGFSPF reveals that the most probable intercharge distance in this model is 7.00 Å. This corresponds to an electrostatic repulsion of 2.06 eV, which is twice as high as the Coulombic energy measured experimentally. In contrast, the correlation map for R^+-PPGFSPF$^+$ suggests that its most probable intercharge distance is 14.55 Å. This corresponds to Coulombic energy of 0.99 eV and is strikingly close to the experimentally measured value![37] This suggests that in doubly protonated RPPGFSPF the "extra" protons are located so as to maximize the distance between them, not to occupy the sites with highest "local" proton affinities.

The lowest potential energy conformations of R^{2+}PPGFSPF and R^+PPGFSPF$^+$ from corresponding trajectory files were energy minimized to rms gradients below 0.1. The minimized conformations are shown on Figure 5. Both conformations exhibit significant charge stabilization by hydrogen bonding between the protonation sites and the peptides' backbones (all hydrogen bonds are listed in the legend for Figure 5). It is noteworthy, however, that the total free energy of R^+PPGFSPF$^+$ is 16.9 kcal/mol below that of R^{2+}PPGFSPF.[38] This contributes to stabilization of the second proton on the C-terminus.

Influence of the Position of the Arg Residue on the Second Gas Phase Basicity of the Peptide. Wu and Fenselau[30] showed that variation in the position of the most basic residue along the peptide chain only slightly affects the first gas phase basicity of the peptide. In this work the influence of the position of the most basic residue on the second gas phase basicity was examined. Bradykinin fragment [2-9] (peptide PPGFSPFR) has the same amino acid composition as bradykinin fragment [1-8] but differs by the position of the Arg residue. The "apparent" second gas phase basicity of bradykinin fragment [2-9] has been measured using three tetrapeptides as reference bases (GGFL,

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(37) In our calculations we assume that charge repulsion is negligibly screened within the molecule (i.e., the hypothetical "effective" dielectric constant of the peptide's interior equals to one). We base this assumption on the fact that the two like charges will not polarize the molecule in a "dipolar" fashion but will rather produce a quadrupolar field, which is not likely to generate a strong net dipole. It is also supported by a good correlation of the results of direct measurement of Coulombic repulsion (refs 14 and 15; present work; Adams, J.; Strobel, F.; Reiter, A. Presented at the 43rd ASMS Conference on Mass Spectrometry and Allied Topics, Atlanta, GA, May 21–26, 1995) and the results of molecular modeling studies that assume $\epsilon_{eff} = 1$.

(38) Another possible site for the second protonation is the tertiary nitrogen atom of the Pro[7] residue. A molecular modeling study performed on R^+PPGFSP$^+$F indicated that the most probable intercharge distance in this case is 13.9 Å (Coulombic repulsion 1.04 eV), which is also close to the experimentally found value. The lowest energy structure of R^+-PPGFSP$^+$F is only 6.2 kcal/mol less favorable than that of R^+PPGFSPF$^+$. It is possible that the doubly protonated peptide exists as a mixture of the these isomers in the gas phase.

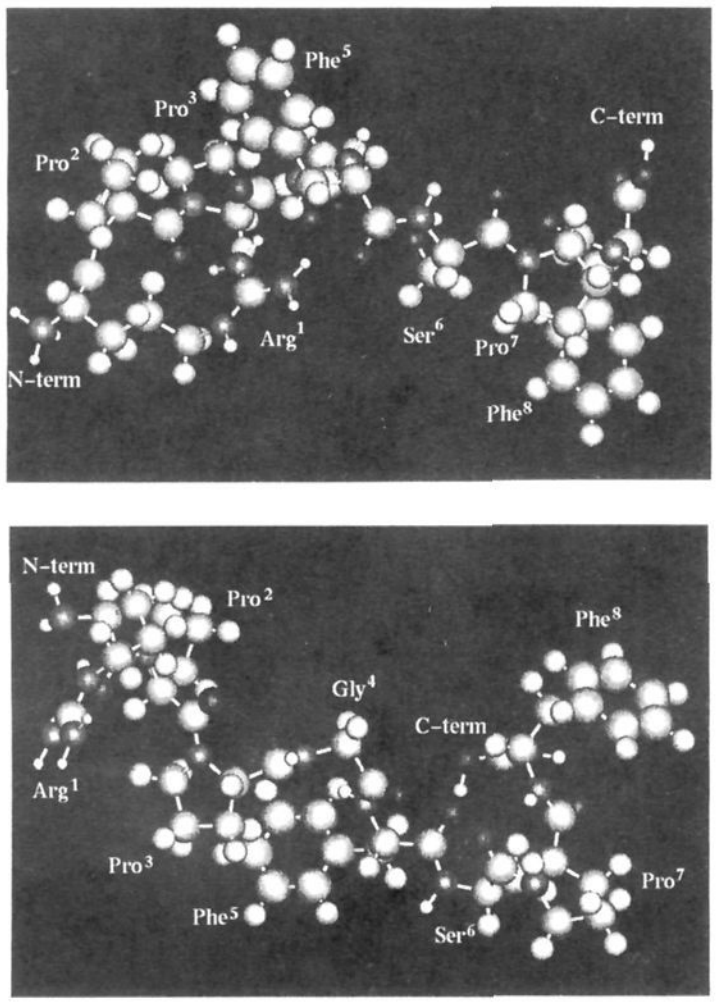

Figure 5. The lowest energy conformations of R^{2+}PPGFSPF (a) and R^{+}PPGFSPF^{+} (b), generated by CHARMm22/QUANTA4.0 macromolecular modeling program. In structure (a) charge-stabilizing hydrogen bonds are formed between the hydrogens of guanidinium group of Arg[1] and the carbonylic oxygens of Pro[2], Pro[3], Gly[4], and Phe[5]. N-terminus is not solvated by the bulk of the peptide. Intercharge distance is 7.06 Å. In structure (b) charge-stabilizing hydrogen bonds are formed between the hydrogen atoms of guanidinium group of Arg[1] and the carbonylic oxygen of Arg[1] and between the hydrogens on the C-terminus and the carbonylic oxygens of Gly[4], Phe[5], Ser[6], and Pro[7]. Intercharge distance is 14.30 Å.

ALAL, and FGGF). The gas phase basicities of these tetrapeptides were determined using the classical kinetic method:[25,39] GB(GGFL) = 219.8 ± 2.0 kcal/mol, GB(ALAL) = 222.6 ± 2.0 kcal/mol, and GB(FGGF) = 223.9 ± 2.0 kcal/mol. Figure 6 shows a typical MIKE spectrum of the decomposition of the metastable doubly charged dimer formed by PPGFSPFR and ALAL. The $[MH^+]/[MH^{2+}]$ ratio for the dimers formed with each of the reference bases was calculated and plotted against the gas phase basicities of the tetrapeptides[39] (see Figure 2). The experimental points fit the straight line with a correlation coefficient $r^2 = 0.904$. The plot gives the value $GB_{app}(MH^+) = 220.2 \pm 2.2$ kcal/mol.

The insert on Figure 6 shows the shape of the MH^+ ion peak obtained with a narrow energy slit in the second electrostatic analyzer of the mass spectrometer. Calculation of an average KER for the charge separation process (3) gives the value of $\delta = 0.97 \pm 0.03$ eV. Similar measurements have been made using GGFL ($\delta = 0.96 \pm 0.03$ eV) and with FGGF ($\delta = 1.00 \pm 0.03$ eV). Based on those measurements, we estimate the reverse activation energy value in (3) as $\delta = 0.98 \pm 0.04$ eV

(39) The gas phase basicities of these tetrapeptides were measured using alkylamines as reference bases: GB(GGFL) = 219.8 ± 2.0 kcal/mol, GB(ALAL) = 222.6 ± 2.0 kcal/mol, and GB(FGGF) = 223.9 ± 2.0 kcal/mol. All three tetrapeptides have only hydrophobic side chains, and it is reasonable to assume that the topology of hydrogen bonding is unaltered in this series. To support this assumption, the gas phase basicity of another tetrapeptide, AGSE, was measured using this set of tetrapeptides as reference bases (giving GB = 221.7 ± 2.0 kcal/mol) and using alkylamines as reference bases (giving GB = 221.5 ± 2.0 kcal/mol). The two values are the same within experimental error, suggesting that GGFL, ALAL, and FGGF can be used as a set of reference bases.

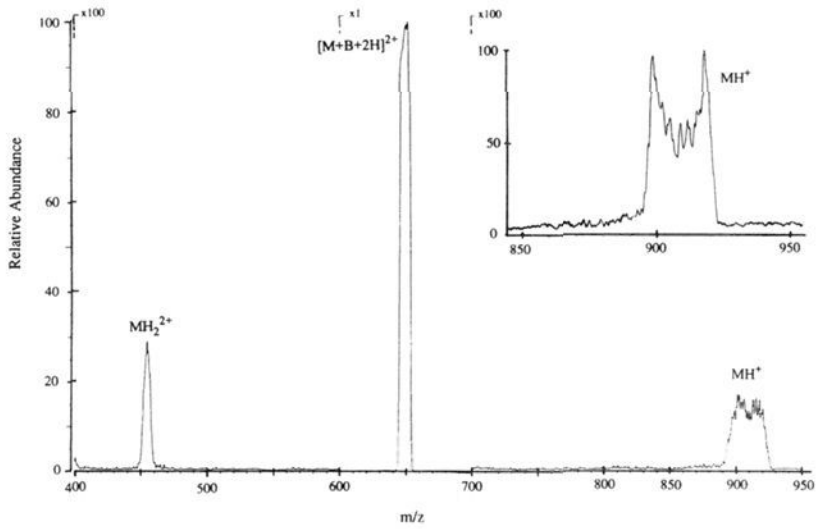

Figure 6. A MIKE spectrum of a unimolecular dissociation of a metastable proton-bound dimer $[M + B + 2H]^{2+}$ formed by bradykinin fragment [2-9] (M) and ALAL (B). Energy slit of the second electrostatic analyzer $\beta_2 = 4.0$ mm. On the insert: the MH^+ peak shape, energy slit $\beta_2 = 0.8$ mm.

(22.6 ± 1.0 kcal/mol). This gives the value of the second gas phase basicity of bradykinin fragment [2-9] $GB(MH^+) = 197.6 \pm 2.4$ kcal/mol, which is significantly higher (by 5.9 kcal/mol) than the second gas phase basicity of bradykinin fragment [1-8]. This is because the two most basic sites of PPGFSPFR (Arg^8 side chain and N-terminus) are separated much further, and the second proton is not forced to a more distant site with a lower local proton affinity.

Conclusions

The difference between the first and the second gas phase basicities of bradykinin fragment [1-8] is 63.1 ± 3.0 kcal/mol (2.74 ± 0.13 eV), which is much higher than the measured Coulombic repulsion between the two charges in the doubly charged peptide.[40] Comparison of the experimentally measured Coulombic repulsion in the doubly charged dimers as well as in monomers with the results of molecular modeling studies suggests that the "extra" protons are not necessarily located on the most basic sites, if Coulombic repulsion is a strong influence. Minimization of Coulombic repulsion may result in protonation of sites with lower "local" proton affinity, leading to a decrease of "second" basicity of the peptide.

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Supporting Information Available: Potential energy−intercharge distance correlation maps for R^{2+}PPGFSPF (a) and R^{+}PPGFSPF^{+} (b), generated by CHARMm22/QUANTA4.0 macromolecular modeling program (1 page). This material is contained in many libraries on microfiche, immediately follows this article in the microfiche version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(40) One of the referees pointed out that found values of GB(M) and $GB(MH^+)$ may correspond to different temperatures, since electrospray-generated ions possess less internal energy than FAB-generated ones. The corresponding difference for Δ_TGB will be, nevertheless, rather small. Indeed, at constant pressure $d(\Delta G) = -\Delta S dT$ and so $\Delta_T(\Delta G) = -\Delta S(T_1 - T_2)$. The upper limit of ΔS is well below 10 cal/mol·K,[2] so even for temperature differences as large as 1000 K Δ_TGB will be much lower than 10 kcal/mol.