Structure of Gramicidin S $(M + H + X)^{2+}$ Ions (X = Li, Na, K) Probed by Proton Transfer Reactions

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Abstract: Proton transfer reactions of $(M + 2H)^{2+}$ and $(M + H + X)^{2+}$ ions (X = Li, Na, K) of gramicidin S with four reference bases are measured. From the kinetics of proton transfer, the apparent gas-phase basicities of the $(M + H)^+$ and $(M + X)^+$ ions are assigned values of 219.1 ± 2.8 and 223.3 ± 2.4 kcal/mol, respectively. We attribute the higher apparent basicity of the $(M + X)^+$ ions to a larger separation between charges. From this difference in basicities is calculated a charge separation distance of ~11.5 Å in the $(M + H + X)^{2+}$ ions using a simple point charge model. This value is consistent with a structure in which the alkali metal ion is attached to the exterior surface of the peptide and is in excellent agreement with structures obtained by molecular modeling. Rates of proton transfer from $(M + H + X)^{2+}$ to dipropylamine decrease as the size of the alkali metal ion increases. This indicates that the binding site is the same for each of these metal ions.

Introduction

Alkali metal cation binding plays an important role in the function of many biological systems, such as in ion transport across cell membranes.[1] In the gas phase, mass spectrometry has been used to obtain information about alkali metal binding interactions in a variety of molecules including peptides,[2] proteins,[3] carbohydrates,[4] and crown ethers.[5] In both solution and the gas phase, the attached metal ions are usually charged. Because of the relatively long range $1/r$ Coulomb interaction (where r is the distance from the charge), the cation can significantly influence the chemistry of distant sites in the ion. Such electrostatic interactions have been implicated as one of the most influential driving forces in the function of biomolecules.[6] These interactions play an even more dramatic role in the reactivity of charged biomolecule ions in the gas phase due to the absence of the shielding effects of the surrounding solvent.[7−13] Recent calculations[7] indicate that charge−charge repulsion is the primary driving force in the increased rates of proton transfer[8−12] and dissociation[13] observed for multiply protonated ions.

We have investigated the proton transfer reactivity of doubly protonated gramicidin S[9] and 1,*n*-diaminoalkanes (n = 7−10, 12),[10] as well as multiply protonated cytochrome *c*[11] and lysozyme ions.[12] These reactions can be fitted to the simple point charge model of eq 1,

$$\text{Coulomb energy} = \sum_{i=1}^{n} \frac{q^2}{(4\pi\epsilon_0)\epsilon_r r_{i,t}} \approx \text{GB}_{\text{intrinsic}}(\text{M}) - \text{GB}^{\text{app}}(\text{M}+n\text{H})^{n+} \quad (1)$$

where $GB_{intrinsic}(M)$ is the gas-phase basicity of a neutral molecule protonated at site t, $GB^{app}(M+nH)^{n+}$ is the apparent gas-phase basicity of the $(M + nH)^{n+}$ ion, and ϵ_r reflects both the shielding between charges and the potential energy surface of the proton transfer reaction.[11] Here, we report the effect of alkali metal cations on the proton transfer reactivity of $(M + H + X)^{2+}$ ions (X = Li, Na, K) of the peptide gramicidin S. From these measurements, the distance between charge centers is determined and information about the ion structure and alkali metal binding sites is obtained.

Experimental Section

All measurements are performed on an external ion source Fourier-transform mass spectrometer described previously.[9] Ions are produced by electrospray ionization from a 75%/25% methanol/water solution containing gramicidin S (1.1×10^{-4} M) and individual alkali metal salts (lithium chloride, sodium, potassium, rubidium, or cesium acetate,

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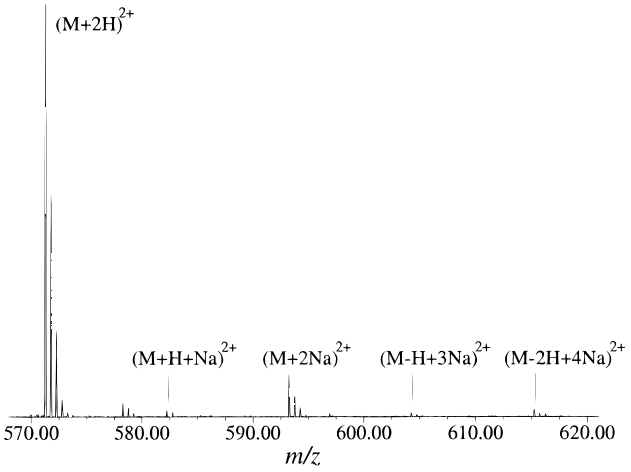

Figure 1. Electrospray ionization mass spectrum of the isolated doubly charged ions produced from a 75%/25% methanol/water solution of gramicidin S and sodium acetate.

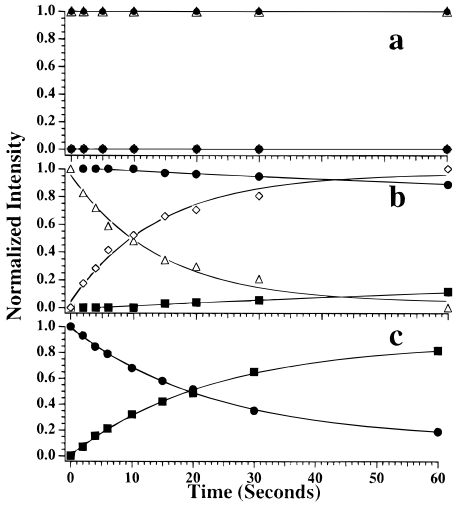

Figure 2. Normalized intensity as a function of time for the reaction of $(M + H + Li)^{2+}$ and $(M + 2H)^{2+}$ with three reference bases, (a) *tert*-butylamine, (b) diethylamine, and (c) dipropylamine; $(M + 2H)^{2+}$ (△), $(M + H)^{+}$ (◇), $(M + H + Li)^{2+}$ (●), $(M + Li)^{+}$ (■). The rate of reaction of the doubly protonated ion with dipropylamine was too rapid to be measured under these conditions.

2.5×10^{-3} M). The molecular ions, $(M + 2H)^{2+}$ and $(M + H + X)^{2+}$, are isolated in the cell and reacted with the neutral reference bases *tert*-butylamine (GB = 216.7 kcal/mol), diethylamine (GB = 221.4 kcal/mol), dipropylamine (GB = 225.2 kcal/mol), and triethylamine (GB = 227.5 kcal/mol)[14] at a static pressure of 1×10^{-7} Torr. Reaction times are varied from 0 to 60 s. These data are fitted to pseudo-first-order kinetics from which rate constants for proton transfer are obtained. Because the alkali metal complexes are investigated individually, the rate constant for each $(M + H + X)^{2+}$ ion is normalized to that of the $(M + 2H)^{2+}$ also present in the cell in these experiments. This corrects for any small changes in instrumental conditions, such as base pressure, that may occur over the course of these experiments. The rate constant for $(M + 2H)^{2+}$ is divided by 2 to normalize for the number of protons. The apparent gas-phase basicity (GB^{app}) of these ions is assigned a value halfway between the GB of the two bases which bracket the absolute rate constant of 1×10^{-11} $cm^3/(mol \cdot s)$.

This cutoff for assigning the GB^{app} is significantly lower than the collision rate of $\sim 10^8$ $cm^3/(mol \cdot s)$ for this peptide. For doubly protonated 1,*n*-diaminoalkanes, the rates of proton transfer did not change as abruptly with increasing basicity of the neutral reference base as did those of the corresponding singly protonated ions.[10] Similar results are observed for multiply protonated protein ions.[11,12] This suggests that the potential energy surface for proton transfer from multiply protonated ions is relatively complex. The cutoff for assigning GB^{app} is somewhat arbitrary and is chosen because the distinction between "reaction" and "no reaction" is relatively clear (see, for example, Figure 2). Information based on this method, such as the distance between charges presented here, depends on differences in the reactivity of related ions or ion series. Provided the cutoff is consistent, our conclusions are not significantly affected by the choice of this value.

Ion structures are obtained by molecular modeling using the AMBER forcefield in BatchMin (version 4.5, Columbia University Chemistry Department) on an IBM RS/6000 computer. A series of geometry minimization and dynamics simulations up to 600 ps at various temperatures are used. In addition, a Monte Carlo conformational search using the lowest energy structure from the dynamics simulations is performed.

Results and Discussion

The electrospray mass spectra obtained from solutions of gramicidin S and alkali metal salts typically contain a variety of molecular ions. The singly charged ions are predominantly alkali metal attached; the abundance of $(M + H)^{+}$ is less than 10% of that of $(M + X)^{+}$. In contrast, $(M + 2H)^{2+}$ is the predominant doubly charged species. The abundance of $(M + H + X)^{2+}$ varies with each alkali metal and with electrospray interface conditions, but is typically small as shown in Figure 1 for the solution containing sodium. The abundance of $(M + 2Na)^{2+}$ is greater than that of $(M + H + Na)^{2+}$, suggesting that the former ion is more stable, although differences in solution conditions, such as pH, may influence these relative abundances. Additional doubly charged ions corresponding to attachment of more than two alkali metals are also observed, *e.g.*, $(M - 2H + 4Na)^{2+}$.[2b,3b] The $(M + H + X)^{2+}$ ions for X = Rb and Cs were formed in insufficient quantities to measure rate constants for proton transfer.

Reaction of $(M + 2H)^{2+}$ and $(M + H + X)^{2+}$ with *tert*-butylamine, the weakest base used in this study, resulted in no observable proton transfer for reaction times up to 60 s. With the bases diethylamine and dipropylamine, the proton transfer reactivity of each of the $(M + H + X)^{2+}$ ions is clearly less than that of $(M + 2H)^{2+}$. For example, Figure 2 shows normalized ion abundances as a function of time for the reactions of $(M + H + Li)^{2+}$ and $(M + 2H)^{2+}$ with these three bases. From the measured rate constants of each of these ions (Table 1), we assign the GB^{app} of $(M + H)^{+}$ to be 219.1 ± 2.8 kcal/mol[15] and the GB^{app} of $(M + X)^{+}$ for X = Li, Na, and K to be 223.3 ± 2.4 kcal/mol. Thus, the $(M + X)^{+}$ ions have a GB^{app} approximately 4.2 kcal/mol greater than $(M + H)^{+}$.

The origin of the difference in GB^{app} of these ions was investigated by molecular modeling. Molecular dynamics simulations of $(M + H + Na)^{2+}$ at 500 K resulted in detachment of Na^{+} after 100 ps. At 300 K, a structure in which the sodium is attached to an exterior surface backbone carbonyl oxygen of the peptide and is solvated by the side chains of both the unprotonated ornithine and a phenylalanine was found to be stable for greater than 600 ps (Figure 3a). The distance between charges is between 10.7 and 11.2 Å at this temperature. Previous molecular modeling (molecular dynamics simulations

(14) All GB values are from the basicity scale of Meot-Ner (Mautner) and Sieck (Meot-Ner (Mautner), M.; Sieck, W. *J. Am. Chem. Soc.* **1991**, *113*, 4448−4460), normalized to the scale of Lias et al. (Lias, S. G.; Liebman, J. F.; Levin, R. D. *J. Phys. Chem. Ref. Data* **1984**, *13*, 695−808).

(15) The basicity of the $(M + H)^{+}$ ion is between the GB of *tert*-butylamine and that of diethylamine, as reported previously (ref 9). The higher value of GB^{app} reported here reflects the revised basicity scale (refs 14). The difference in GB^{app} between $(M + H)^{+}$ and $(M + X)^{+}$ and, hence, our conclusions, are not affected by this change.

Table 1. Rates of Proton Transfer ($\times 10^{-11}$ cm^3/(mol·s)) for the Reaction of Gramicidin S $(M + 2H)^{2+}$ and $(M + H + X)^{2+}$ Ions (X = Li, Na, K) with Four Reference Bases[a]

ref base (GB, kcal/mol)	$(M + H + Li)^{2+}$	$(M + H + Na)^{2+}$	$(M + H + K)^{2+}$	$(M + 2H)^{2+}$
tert-butylamine (216.7 ± 1.5)	<0.0026	<0.0080	<0.0078	<0.0046
diethylamine (221.4 ± 1.5)	0.32 ± 0.04	0.068 ± 0.02	<0.072	4.4 ± 0.4
dipropylamine (225.2 ± 1.5)	2.6 ± 0.4	2.4 ± 0.4	1.8 ± 0.2	>26
triethylamine (227.5 ± 1.5)	>52	>52	>52	>26
ionic radius[b]	Li^+ = 0.9 Å	Na^+ = 1.1 Å	K^+ = 1.4 Å	

[a] The rates for the doubly protonated ion have been divided by 2 to normalize for the number of protons. [b] From ref 16.

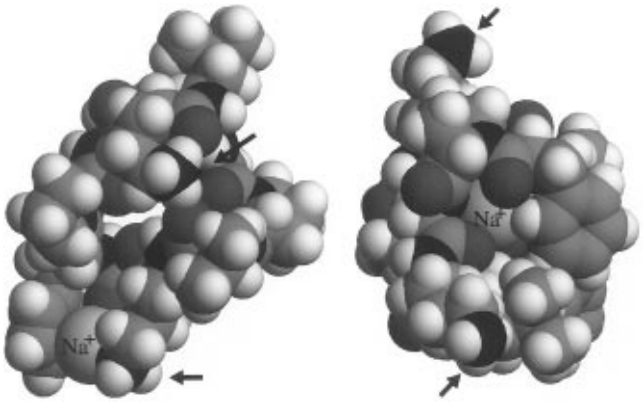

Figure 3. Molecular modeling structures of (a, left) $(M + H + Na)^{2+}$ and (b, right) $(M + Na)^+$ of gramicidin S (cyclo[-Pro-Val-Orn-Leu-D-Phe-]$_2$). N, O, C, and H are shown in gray scale dark to light, respectively, and the Na^+ is labeled. Side-chain ornithine nitrogens are indicated by arrows.

at 800 K) indicates that both protonated ornithine side chains in $(M + 2H)^{2+}$ are solvated by the carbonyl oxygens of the peptide backbone and are separated by ~9.5 Å in the minimized structure.[9] In dynamics simulations for 100 ps at 300 K, the charge separation ranges from 9.4 to 10.1 Å. However, the majority of structures sampled in this simulation have a charge separation comparable to that in the minimized structure. These calculations are consistent with H/D exchange experiments which show that solvation of both charge sites occurs, and that the charge separation is comparable to the peptide diameter (~8−10 Å).[9] The lower stability of $(M + H + Na)^{2+}$ indicated by the molecular modeling is consistent with the significantly lower abundance of this ion in the electrospray mass spectrum.

A Monte Carlo conformation search of $(M + H + Na)^{2+}$ starting with the structure shown in Figure 3a resulted in 17 structures within 3 kcal/mol of the global minimum (1.7 kcal/mol lower in energy than the starting geometry). This suggests that several conformations exist at 300 K, consistent with the molecular dynamics simulations done at this temperature. All the lowest energy structures found in this search have similar solvation of the protonation site. The proton transfer data fit pseudo-first-order kinetics with a single rate constant. Thus, our data are consistent with either a single conformation or multiple conformations which have similar charge separation.

The local charge solvation of the protonation sites in both $(M + H + Na)^{2+}$ and $(M + 2H)^{2+}$ obtained by molecular modeling is similar and shows that these interactions are not significantly influenced by the type of charge carrier (Na^+ or H^+) located ~10 Å away. This strongly indicates that the intrinsic basicity of the protonation site is comparable in these ions. Thus, we conclude that the difference in proton transfer reactivity between $(M + 2H)^{2+}$ and $(M + H + X)^{2+}$ directly corresponds to the difference in Coulomb energy in these ions. From the difference in apparent basicity and the value of the effective dielectric polarizability (ϵ_r) measured previously for gramicidin S[9] ($\epsilon_r < 1.44$ using the revised basicity scale), we calculate the distance between charge centers in $(M + H + X)^{2+}$ to be 2.0 Å greater than that in $(M + 2H)^{2+}$, or ~11.5 Å. This distance is consistent with a structure in which the alkali metal ion is attached to the exterior surface of the peptide.

In contrast, molecular modeling indicates that the sodium in $(M + Na)^+$ is in the center of the backbone ring and significantly solvated (Figure 3b) in a site similar to that of the proton in $(M + H)^+$.[9] The stability of this ion is indicated by the high abundance of $(M + Na)^+$ formed by electrospray and shows that the solvation of sodium in $(M + Na)^+$ and $(M + H + Na)^{2+}$ differs dramatically.

Although the $(M + X)^+$ ions are all assigned the same value of GB^{app}, their rates of proton transfer are not identical. The rates of proton transfer from $(M + H + X)^{2+}$ to dipropylamine are 2.6, 2.4, and 1.8 $\times 10^{-11}$ cm^3/(mol·s) for X = Li, Na, and K, respectively. The ionic radii of these alkali metal cations are 0.9, 1.1, and 1.4 Å, respectively.[16] These results indicate that the alkali metals are coordinated to the same site on the exterior of the peptide. With increasing radius of the alkali metal ion, the center of charge is effectively moved further from the surface of the peptide and, hence, further from the protonation site. This decreases the Coulomb energy in the ion slightly and results in slower rates of proton transfer. The higher abundance of $(M + 2Na)^{2+}$ than $(M + H + Na)^{2+}$ indicates that the Coulomb energy in the former ion is lower. This is consistent with an ion structure in which both sodium ions are bound to the exterior surface of the peptide, resulting in a greater intercharge separation distance and hence higher ion stability.

In conclusion, we have shown that the distance between charge centers in $(M + H + X)^{2+}$ of gramicidin S, obtained by proton transfer measurements, is ~11.5 Å. An ion structure for $(M + H + X)^{2+}$ in which the alkali metal ion is attached to the exterior surface of the peptide is proposed. This structure is consistent with the relative ion stabilities deduced from abundances in the electrospray ionization mass spectrum and is in excellent agreement with the structure obtained by molecular modeling.

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