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Proton Transfer Reactivity of Large Multiply Charged Ions

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Abstract

Charge-charge interactions dramatically influence the dissociation and proton transfer reactivity of large multiply protonated ions. In combination with tandem mass spectrometry, proton transfer reactions have been used to determine the charge state of an ion and to increase the effective mass resolution of electrospray ionization mass spectra. A model for the proton transfer reactivity of multiply protonated ions, in which protons are assigned to specific sites in an ion based on the intrinsic reactivity of the site and the sum of point-charge Coulomb interactions between charges, is discussed. In combination with experimentally measured rates of proton transfer to bases of known gas-phase basicity, information about the intramolecular electrostatic interactions, gas-phase ion conformation and maximum charge state of an ion produced by electrospray ionization can be obtained.

Keywords

proton transfer reactivity; large multiply charged ions

INTRODUCTION

Electrostatic interactions play a key role in the function, conformation and stability of biomolecules and have been implicated in interactions such as enzyme–substrate binding, receptor–ligand interactions, ion channels and allosteric control. In a uniform environment, electrostatic interactions are decreased or shielded by a value given by the dielectric constant of the surrounding medium. Values for typical solvents range from ~2 for hydrocarbons such as cyclohexane to ~80 for water. For non-homogeneous media, such as a protein in solution, electrostatic interactions are shielded both by the surrounding medium and by the molecule itself. Both macroscopic^{1–6} and microscopic^{7–9} models have been proposed to account for electrostatic interactions for proteins in solution. In the macroscopic dielectric continuum model, set forth by Tanford and Kirkwood¹ in 1957, the molecule and surrounding solvent are treated as two uniform media with distinct dielectric constants. This model is used extensively today in modified form^{2,3} for applications such as calculating pK_a values for molecules with multiple charge sites^{4–6} and providing detailed information on electrostatic surface potentials around protein binding sites.³ For a protein in water, a dielectric constant of ~80 is used for the surroundings. Values for the protein interior typically used in this model range from 1 to 5, although much higher values have been reported. These values are based largely on measured dielectric constants of dry crystalline protein powders and analogues which range from ~2 to 5, although absorbed water increases this value dramatically.^{10,11}

Although the dielectric continuum model has been used extensively, its usefulness and that of other models have been hotly debated.^{1–9} A key limitation of this model is the treatment of the electronic properties of the molecule using a bulk dielectric constant, rather than explicitly taking into account the effects of the multiple discrete dipoles, quadrupoles, etc., present in the molecule. The low dielectric used for the protein itself reduces the effectiveness of this method for modeling charges buried in the interior of the protein.⁹ A microscopic model that eliminates bulk dielectric constants has been put forth by Warshel and co-workers.^{7–9} From this model, a dielectric constant that depends on the position of the charge in the protein is calculated, with

values as large as 10 obtained for sites of catalytic importance.¹² These models and their relative merits are discussed in detail elsewhere.^{3,7}

In the gas phase, electrostatic interactions play an even more dramatic role in the reactivity of ions owing to the low dielectric of the surrounding medium (vacuum permittivity = 1). Multiply charged ions, such as those produced by electrospray ionization,^{13,14} are well suited to the investigation of the electrostatic properties of biomolecules in the absence of solvent. Electrostatic interactions have been implicated in both the increased rates of proton transfer^{15–20} as well as ion dissociation.^{21–24} For example, McLuckey *et al.*¹⁵ found that rates of proton transfer from cytochrome *c* to neutral dimethylamine increased with increasing charge state. Similar results have been reported for ubiquitin ions.¹⁶ Proton transfer reactions of all charge states formed by electrospray ionization with neutral molecules prior to their introduction into the mass spectrometer have been measured by several groups.^{17–20} Ikonontou and Kobarle¹⁷ showed that semi-quantitative rates for ion–molecule reactions could be obtained in an external reaction chamber. Smith and co-workers^{18–20} have employed a ‘Y-tube’ inlet reactor to study the proton transfer reactivity of disulfide intact and disulfide reduced proteins and the temperature dependence of these reactions.²⁰ In combination with tandem mass spectrometry, proton transfer reactions have been used to improve the effective mass resolution²⁵ and to determine the charge state of product ions from their known shift in charge and measured shift in m/z .^{26–28}

Smith and co-workers^{29–31} have taken advantage of both the remeasurement capabilities of a Fourier transform mass spectrometer^{32,33} and the change in charge state observed for individual ions in order to obtain their mass. During the non-destructive detection event, an individual polyethylene glycol ion was found to undergo stepwise loss of an individual charge (presumably Na^+) three times over the course of a 60 s transient.²⁹ From the resulting shift in m/z ratio, the mass of the individual ion was found to be 6.527 MDa. By added ammonia to the cell, proton transfer from individual multiply protonated ions of bovine albumin was found to occur.³⁰ This produced the charge state shift necessary to obtain the mass of eight individual ions over the course of 14 remeasurements. A time-resolved ion correlation method has been demonstrated;³¹ the application of this method to the simultaneous study of even larger numbers of individual ions appears promising.

Mass spectrometric methods have been used to generate and study the physical properties of small multiply charge ions for many years.^{34–36} Less is known about the chemistry of larger multiply charged ions. With the advent of electrospray ionization,^{13,14} matrix-assisted laser desorption/ionization^{37,38} and massive cluster ion impact,^{39,40} it is now possible to form multiply charged ions of molecules with molecular masses in the hundreds of megadaltons.⁴¹ From the increased reactivity of multiply protonated ions, it should be possible to extract information about the charge–charge interactions in the gas phase, including effects on thermochemical properties, and ultimately relate these values to the effective intramolecular shielding of biomolecules in solution. This review summarizes progress to date of a model that we have been developing to fit experimental observations of proton transfer reactivity of multiply protonated ions in the gas phase.^{42–47} This review is not comprehensive, nor is the model presented here tremendously sophisticated. However, this model does provide an improved understanding of these types of gas-phase reactions and offers predictive capabilities.

POINT CHARGE MODEL

Point charges

In this model,^{42–47} the reactivity of a multiply protonated ion is calculated from the intrinsic reactivity of the reaction center and the influence of intramolecular Coulomb repulsion from other charges present on the ion as given by the equation

$$GB^{\text{app}} \approx GB_{\text{intrinsic}, t} - \sum_{i=1}^n \frac{q^2}{(4\pi\epsilon_0)\epsilon_{\text{F}}r_{i,t}} \quad (1)$$

where GB^{app} and $GB_{\text{intrinsic}}$ are the apparent and intrinsic gas-phase basicity of a given charge site, respectively, r is the separation distance between charges, ϵ_0 is the vacuum permittivity and ϵ_{F} is the effective shielding of charges as measured by proton transfer reactions in the gas phase. The latter value includes all effects not explicitly treated in this model, including the intrinsic shielding of the ion in vacuum, effects of ion–molecule potential and an assumption about ion conformation (see below).⁴⁴ Implicit in this model are two key assumptions: (i) that charges are localized and can be treated as point charges and (ii) that the influence of a second charge can be modeled entirely by adding a simple Coulomb potential to an ion–molecule potential with a negligible effect from ion–induced dipole or ion–permanent dipole potentials due to the presence of the additional charge. While not necessarily appropriate for multiply charged ions formed by removal or addition of electrons, the first assumption is reasonable for many multiply protonated ions in that the charge will be associated primarily on the atom which is protonated. Although charge delocalization does occur, as clearly evidenced by the increasing basicity for methyl to propylamine,⁴⁸ this delocalization for many types of molecules can be relatively small compared with the charge separation distance. To the extent that the delocalization is spherically symmetric, a charge can be accurately represented as a point charge in the center of the sphere. Hence there should be some effective charge site that should be reasonably approximated by a point charge. This point charge interaction has also been used in models set forth by Fenn and co-workers^{49,50} to explain the maximum charging of polyethylene glycol ions and by Rockwood *et al.*⁵¹ to understand the dissociation of multiply charged ions.

The Coulomb potential is a relatively long-range interaction, falling off as $1/r$. In contrast, the attractive ion–induced dipole and ion–permanent dipole interactions are relatively short-range, falling off as $(1/r)^4$ and $(1/r)^2$, respectively. If a charge is localized $>10\text{\AA}$ away from an ion–molecule reaction center, the influence of this charge on the ion–induced dipole and ion–permanent dipole interaction potential at the reaction site will be small.⁴² Thus, for localized charges with long separation distances, the Coulomb potential should be approximately additive to the normal ion–molecule potential at a given site and the second assumption noted above should apply. At shorter charge separation distances, or for reactions with large molecules, this approximation becomes less valid. Procedures for improving this model are discussed below.

Definitions

Proton transfer reactions of singly protonated ions have been investigated for many years. The relevant thermo-chemical values are the gas-phase basicity (GB) and the proton affinity (PA), defined in the reaction

Models have been successfully developed to account for the reactivity of singly protonated ions,⁵² and numerous experimental methods have been developed to measure GB , including equilibrium,^{53,54} bracketing^{55–58} and kinetic methods.^{59–61} In each of these methods, the relative affinity of an unknown molecule for a proton is compared with that of a neutral molecule with known GB or PA . From calculations or measurements of the relative entropies,

values for the *PA* can be obtained. These methods are reviewed and discussed in depth elsewhere.^{52,62}

In contrast, measurement of the *GB* of charged species is made more complicated by the presence of an activation barrier to thermoneutral reactions which arises from the separation of the two charged products. The *GB* and *PA* of a charged ion, MH^+ , is defined in the reaction

A reaction potential for the reverse of reaction (3) has been calculated by Gill and Radom.⁶³

Reaction potentials

To illustrate the effects of the reverse activation barrier on ion–molecule reactions, consider a molecule, *M*, that is symmetrical and has two identical sites of protonation, separated by at least 10 Å. Reaction (a) (Fig. 1) shows a potential representing proton transfer from MH^+ to a base, *A*, which has a *PA* identical with that of *M* so that proton transfer from MH^+ to *A* is thermoneutral (for now, assume $\Delta S^\circ = 0$ so that $PA = GB$). Consider this same molecule with two protons that do not interact; the hypothetical potential for proton transfer to *A* is shown in reaction (d). The difference in energy between (a) and (d) represents an ‘intrinsic’ *PA* of the protonation site in *M*, or the *PA* of the reaction center in the absence of any intramolecular electrostatic interactions ($= PA(A)$). If the charges in MH_2^{2+} interact, then the *PA* of the MH^+ ion will be reduced. Proton transfer from the MH_2^{2+} ion to some base, *C*, which has a *PA* identical with that of MH^+ (reaction (b)) will be kinetically slow owing to the presence of the large reverse activation barrier that arises from the separation of the two charged products, MH^+ and CH^+ . Proton transfer reactions will become kinetically favorable when the barrier is reduced; this will occur for reactions with bases that have higher *PA*. This is illustrated for some base, *B*, which is sufficiently basic to lower E_a , to a negligible value (reaction (c)). Hence, the value measured in a bracketing or kinetic experiment is the kinetic or ‘apparent’ *PA*.^{64, 65} This apparent *PA* is larger than the true thermodynamic *PA* by a value approximately equal to the reverse activation barrier. Reactions with bases that have *PA*s between those of *B* and *C* will be kinetically slow, and these bases will appear to be less basic than MH^+ . The base *B* is the first base that appears to be more basic than MH^+ in these reactions, hence the term ‘apparent’ affinity or basicity is used to describe the value obtained from these bracketing measurements.⁶⁸

We have proposed that differences ($PA_{\text{intrinsic}}(M) - PA^{\text{app}}(MH^+)$) for a series of related ions which differ only by the distance between charges should parallel the difference in Coulomb energy in these ions within the limits discuss here and in Ref. 44, i.e. additive ion–molecule and Coulomb potentials, charge separation distances > 10 Å, and similar shape potentials for reactions with different bases (see below). That is ($PA_{\text{intrinsic}}(M) - PA^{\text{app}}(MH^+)$) is directly proportional to the Coulomb energy in the MH_2^{2+} ion (Eqn (1)).

Gas-phase basicity of charged species

Bohme and co-workers^{64–67} have investigated the proton-transfer chemistry of fullerene dications. From bracketing measurements, Bohme and co-workers⁶⁴ were the first to report an apparent gas-phase acidity of $C_{60}H^{2+}$ ($= GB^{\text{app}}(C_{60}^{+})$). As a first approximation, the reverse activation barrier for this reaction was estimated to be the energy required to bring two point charges from infinite distance to a distance corresponding to the molecular diameter. An effective dielectric of 1 was assumed for these calculations. The true gas-phase acidity of

$C_{60}H^{2+}$ was determined by subtracting the calculated barrier height from the apparent gas-phase acidity. The same approach was subsequently used to calculate the GB of protonated diaminoalkanes.⁴³ These values, as reported, are lower limits since the reverse activation barrier itself will be less than the Coulomb repulsion in the MH_2^{2+} ion.^{44,64,65,69}

From semi-empirical calculations, Bursey and Pederson⁶⁹ concluded that the barrier height for bringing protonated ammonia and trimethylamine to within reaction distance of monoprotonated 1,3-diaminopropane in an elongated conformation was 77% and 92% of a simple point-charge interaction. Compton and co-workers^{70,71} have modeled the interaction of $C_{60}^- + e^-$ classically, treating C_{60} as a sphere of uniform dielectric material with a charge in the center, to obtain a potential for these reactions. Calculations based on adding a point charge Coulomb potential and Bowers ADO ion–molecule potential indicate that the position of the barrier will be shifted several ångströms away from an equilibrium position in an MH_2^{2+} ion for typical bases used in these experiments.⁴⁴ The height of the reverse activation barrier will be ~ 60–80% of the true Coulomb repulsion in these ions. The height and position of the barrier will be influenced by the physical properties of the bases used in these experiments, primarily the polarizability and dipole moment. A more detailed evaluation of the effects of well depth, polarizability, etc., on these potentials is given in Ref. 44.

A recent *ab initio* study of proton transfer from doubly protonated 1,7-diaminoheptane to ammonia has been reported by Gronert.⁷² From these calculations, a one-dimensional potential was obtained with a reverse activation barrier ~55% of that calculated from a point-charge Coulomb interaction using the interchange distance in the double protonated ion. Interestingly, although the *ab initio* calculations are at a significantly higher level of theory, both the position and height of the barrier obtained are within the range of those reported earlier for similar reactions, based on adding a Coulomb potential to an ADO ion–molecule potential.⁴⁴ The origin of the lower barrier than that calculated based on the distance between charges in the doubly protonated ion is due to the position of the barrier which is shifted from the potential minimum by several ångströms.^{44,72} In earlier work, the shift in barrier position was underestimated.⁴³ We have not explicitly included the effect of the barrier position or height in our model for larger biomolecules, since assumptions about ion conformation, which also change the interchange separation distance, are currently necessary. Both of these factors are incorporated in the value of ϵ_r . However, for smaller ions, such as the doubly protonated 1,*n*-diaminoalkanes, the position of the barrier can be readily taken into account to provide a more accurate value of the thermodynamic basicity,⁷² although the one-dimensional potentials may overly simplify the dynamics of these reactions (see below).

Gronert's calculations provide addition support for the validity of the two assumptions in our model: that a Coulomb interaction in large multiply protonated ions can be approximated using point charges, and that the interaction potential can be approximated by adding an ion–molecule potential to a Coulomb potential. How accurately the difference ($PA_{\text{intrinsic}}(M) - PA^{\text{app}}(MH^+)$) parallels the Coulomb repulsion in a series of related MH_2^{2+} ions that differ by the distance between charges depends on the above approximations and also on how the shape of the potential is influenced by the physical properties of the different bases used to bracket the proton transfer reactivity. Our simple calculations indicate that this effect for typical bases should be relatively small.⁴⁴

Kinetic energy release measurements

Accurate measurements of kinetic energy release distributions of the separating charged products can potentially provide a useful measure of the value of the reverse activation barrier. From this value, the interchange separation distances in the transition-state structure has been determined for a variety of small ions.^{73–77} Applying this method to larger biomolecule ions, Kaltashov *et al.*⁷⁸ measured a value of 13.6 kcal mol⁻¹ (1 kcal = 4.184 kJ) for the kinetic

energy released upon dissociating a complex of doubly protonated bradykinin and leucine-enkephalin in a mass-analyzed ion kinetic energy (MIKE) experiment. In combination with measurements of GB^{app} using the kinetic method, a value for the $GB(MH^+)$ of 217.8 kcal mol⁻¹ for bradykinin was reported. The authors equated the measured kinetic energy release to the Coulomb energy in the doubly protonated ion and used this value to calculate a distance between charges of ~24 Å, using a point-charge Coulomb potential calculated with the vacuum permittivity. This same method was applied to doubly protonated des-Arg⁹-bradykinin.⁷⁹ Adams *et al.*⁸⁰ recently measured kinetic energy distributions for dissociation of doubly protonated angiotensin II and provided a thorough analysis of the optimum method to extract the reverse activation energies from these data.

Key assumptions in these measurements are that the excess energy in the activated complex does not partition into translational energy of the separating products, and that the energy resulting from this barrier is partitioned into translational energy of the separating charged products and not into internal modes. Because of the dominance of Coulomb interactions at large separation distances, the latter assumption may be reasonable for larger ions with significant inter-charge distance, particularly when one of the products is small. Effects of intrinsic shielding and the position and height of the barrier must also be taken into account to obtain information on the Coulomb repulsion in the original ion. Full knowledge of the multi-dimensional potential energy surface is required for a complete understanding of these charge separation reactions. Nevertheless, useful information can be obtained from experimental measurements of both rates of proton transfer and kinetic energy release.

EXPERIMENTAL EVIDENCE FOR A POINT CHARGE MODEL

An ideal system for investigating intramolecular electrostatic interactions are 1,*n*-diaminoalkanes (DA), which have been studied both in solution^{5,6} and in the gas phase.⁴³ The charges in the doubly protonated ion are significantly localized and the distance between charges can be systematically varied by changing *n*. In the gas phase, the singly protonated diaminoalkane is unsuitable as a reference for the $PA_{intrinsic}$ since this ion cyclizes, and both nitrogens interact to solvate the proton, resulting in an increased basicity. However, the proton transfer reactivity of monoaminoalkanes (MA) of the same length can be used to provide an estimate of the intrinsic reactivity of the protonation site in diaminoalkanes, a method first used by Bowers and co-workers⁸¹ and Yamdagni and Kebarle⁸² to measure the free energy of cyclization for the protonated diaminoalkanes. The GB of the monoaminoalkanes is 211.2 kcal mol⁻¹ for *n* = 7–10 and 12. In contrast, the GB^{app} of the protonated 1,*n*-diaminoalkanes increases from 181.8 to 191.5 kcal mol⁻¹ over this same range. Calculations indicate that the difference in entropy of deprotonation of the singly protonated MA vs doubly protonated DA is relatively small (<1 kcal mol⁻¹),⁴³ although these simple calculations may underestimate this value. Thus, the difference $GB(MA) - GB^{app}(DAH^+) \approx PA(MA) - PA^{app}(DAH^+) \approx 27\text{--}20$ kcal mol⁻¹ for *n* = 7–12. From these values and estimates of the charge separation distance in the transition state for dissociation⁸³ obtained from molecular modeling and semi-empirical calculations, $\epsilon_r = 1.01 \pm 0.07$ is obtained from a point-charge Coulomb potential. This indicates that the interaction of a second charge localized > 10 Å from a reaction center follows a $1/r$ potential for charge separation distances of 10–17 Å, and thus the intramolecular electrostatic interactions can be represented using a point-charge Coulomb model. Moreover, these results show that these ions are largely extended or linear and that $-(CH_2)_n-$ provides negligible shielding between the charges in the gas phase. That is, the electrostatic interactions take place predominantly through vacuum. Calculations using classical electrostatic interactions in which the molecule between the charges is treated as a sphere with a uniform dielectric of 2 surrounded by vacuum result in an effective dielectric polarizability of ~1.1, in reasonable agreement with the experimentally measured value.⁸⁴

Kebarle and co-workers⁸⁵ measured the free energy change of hydration in doubly protonated diaminoalkanes for $n = 6, 8, 10$ and 12 with different numbers of water molecules attached. For doubly protonated ions with three water molecules attached, this value decreased from 8.3 to $7.4 \text{ kcal mol}^{-1}$ for $n = 6-12$. This value for propylamine with two water molecules attached was $6.7 \text{ kcal mol}^{-1}$ (this compares with the hydration energy for ions with the same number of water molecules per charge site). These results show that the presence of a second charge does influence the ion-molecule interactions at the charge site even at charge separation distances $> 10 \text{ \AA}$. Thus, additional charges do influence the ion-molecule potentials and thus $GB_{\text{intrinsic}}$, in agreement with calculations,^{42,72} although this effect appears to be small. This is not currently taken into account in the model presented here.

It should be noted that there is significant ambiguity in the choice of the proton transfer reaction rate cut-off used for assigning the GB^{app} in these experiments. Rates of proton transfer from multiply protonated ions typically do not change nearly as abruptly with increasing basicity of the neutral reference base as do those of the corresponding singly protonated ions. The rate we use to assign the GB^{app} is somewhat arbitrary and significantly below the collision rate. It is selected based on the ability to clearly distinguish between a 'reaction' and 'no reaction' (Fig. 2, for example). The upper limit to the rate that can be accurately measured is limited by the time required to accumulate sufficient ions in the cell and the pressure of the base required for accurate pressure measurement. Provided that the cut-off is used consistently, differences in the reactivity of a similar series of ions should directly parallel differences in the Coulomb energy; a different cut-off would change the value of ϵ_r calculated.

The results for the diaminoalkanes raise an intriguing question regarding the true value of $PA(\text{MH}^+)$.⁴⁴ If the difference $PA(\text{MA}) - PA^{\text{app}}(\text{DAH}^+)$ is close to that calculated using a point-charge Coulomb interaction using the distance between charges in the doubly protonated ion (implied by a value of $\epsilon_r = 1.01$), then the difference $PA(\text{MA}) - PA(\text{DAH}^+)$ must be even larger than this value by the height of the reverse activation barrier (Fig. 3). Gronert⁷² reported that Coulomb repulsion in the double protonated 1,7-diaminoheptane reduces the thermodynamic acidity by $\sim 34-38 \text{ kcal mol}^{-1}$; a value of 33 kcal mol^{-1} is calculated based on point charges Coulomb interaction and distance between charges in the doubly protonated ion.⁷² If a faster rate is used to bracket the GB^{app} (resulting in a higher value), then these values are consistent with the calculated barrier height.⁷² However, the slow reactions measured in these experiments should not be observable if the barrier height is the same for these reactions. This could be due to an artifact in the experiment or it could indicate that the barrier height may be lower for these slow reaction.⁴⁴

An additional factor that has not been previously considered is that the distance between charges were calculated based on minimized zero K structures which are fully elongated and have the maximum possible separation between charges.⁴³ At room temperature, significant molecular motion occurs,^{80,86} which results in bending of the ion and thus a decreased charge separation distance. The small fraction of ions with internal energies corresponding to the higher energy tail of the Boltzmann distribution would bend more, resulting in an even higher reactivity for these ions, i.e. they would have lower apparent basicity. It should be noted that this higher energy population is continuously being replenished on the time-frame of these experiments via non-reactive collisions as well as absorption of blackbody infrared radiation generated by the chamber walls.⁸⁷⁻⁸⁹ It is even possible that some cyclization of the diaminoalkane occurs during the transition state for dissociation. This would lower the barrier for these reactions, although one would not necessarily expect that this would result in a $1/r$ GB^{app} relationship with increasing n . The rates of reactions of these doubly protonated ions cannot be fully accounted for by the simple static one-dimensional potential described above. A more systematic treatment of the entire multi-dimensional potential energy surface is

required to account for the kinetics of multiply protonated ions, particularly for larger biomolecules.

Peptides

Both calculations and experiments indicate that upon protonation, the charge sites in large peptides are solvated by other polar (or polarizable) groups to stabilize the charge further.^{90–93} This results in a significant entropy and enthalpy associated with this reorientation or cyclization upon deprotonation. Unless these terms can be calculated or measured explicitly, an approximation is required to extend this model to such biomolecules. For a symmetrical molecule, if the charge sites do not interact with one another and if the solvation enthalpy and entropy are not significantly influenced by the presence of a second charge localized $> 10 \text{ \AA}$ away, then the entropies of the first and second protonation step should be similar. To the extent that these values are the same, then the difference $GB_{\text{intrinsic}}(\text{M}) - GB^{\text{app}}(\text{MH}^+)$ should reflect the Coulomb energy in the doubly protonated ion within the limits described above.

A system in which this constraint appears to hold is the cyclic and symmetric decapeptide gramicidin S, which has two identical sites of protonation (side-chain of ornithine, $\text{R} = -(\text{CH}_2)_3\text{NH}_2$).⁴² Molecular modeling indicates that the protonated ornithine side-chains are solvated, primarily by the backbone carbonyl oxygens, to a similar extent in both the singly and doubly protonated ions. This is consistent with the measured rates of gas-phase H–D exchange for these ions. Thus, the difference in $GB(\text{M})$ and $GB^{\text{app}}(\text{MH}^+)$ should be proportional to the Coulomb energy in the doubly protonated ion within the limits set out above. This difference is $> 27.9 \text{ kcal mol}^{-1}$ (the singly protonated ion is more basic than the most basic reference molecule commercially available, so that only a lower limit to $GB(\text{M})$ was assigned). From molecular modeling, the distance between charges was estimated to be $\sim 9.5 \text{ \AA}$, consistent with an estimate based on H–D exchange data which indicated that the charges must be separated by approximately the molecular diameter. Combining this with the energy difference leads to an $\epsilon_r < 1.2$ (< 1.4 using a revised basicity scale).^{45,94} This value is again consistent with little shielding of the charge for this small peptide.

With a known value of ϵ_r for a given system, proton transfer reactions can provide a sensitive measure of the distance between charges. For example, the effects of different alkali metals on the proton transfer reaction of gramicidin S $[\text{M} + \text{H} + \text{X}]^{2+}$ where $\text{X} = \text{Li}, \text{Na}$ or K are clearly evident.⁴⁵ Rates of proton transfer to diethylamine and dipropylamine are significantly lower for $\text{X} = \text{Li}, \text{K}$ and Na , than for $[\text{M} + 2\text{H}]^{2+}$, e.g., more than a tenfold difference is observed with dipropylamine. Assuming that the identity of a charge carrier located $\sim 10 \text{ \AA}$ from a protonation site does not effect the $GB_{\text{intrinsic}}$ of this site, i.e. similar charge solvation at this site, then from this difference in reactivity, an increased separation distance of $\sim 2 \text{ \AA}$ for the alkali metal attached ions ($\sim 11.5 \text{ \AA}$) relative to the doubly protonated ion ($9.5 \text{ \AA}$) can be deduced. This indicates that these metal ions bind to the exterior surface of this peptide in the doubly charged ion, consistent with the lowest energy structures obtained by molecular modeling.⁴⁵ The proton transfer rates decrease slightly as the size of the metal ion increases from Li to K. The increased metal ion diameter moves the center of charge further from the protonation site. This is consistent with a similar binding site for these metal ions and also provides further evidence for a point-charge model.

Proteins

To extend this method to larger molecules such as proteins, which have many possible sites of protonation, information about locations of charges and the intrinsic basicity of each of these sites must be obtained. Sites that are protonated in solution are not necessarily protonated in the gas phase. For example, S4 ribosomal protein has 46 basic residues (Arg, Lys, His, N-terminus), but the maximum gas-phase charge state that has been reported in the literature is

only 30+.⁹⁵ Thus, not all basic sites retain protons in the gas phase. In contrast, actin also has 46 basic residues, but the maximum gas-phase charge state is 59+.⁹⁶ Hence sites in addition to these basic residues must be protonated. The individual amino acids Pro, Gln and Trp are basic in the gas phase.⁹⁷ Even backbone sites are relatively basic,⁹⁸ although much less so than the basic residues mentioned. For ions with a multiplicity of similar sites, the number of possible ways to assign the charges increases exponentially with ion size. If one considers each residue as a possible site of protonation, then for cytochrome *c*, which has 104 residues, there are 10^{21} different ways to assign 20 charges. This number increases to $\sim 10^{50}$ for the 45+ charge state of the 260 residue protein carbonic anhydrase.⁴⁶

To attempt to answer the question of where the charges go in a gas-phase protein (charge configuration), the most energetically favorable ways to assign protons are calculated based on estimates of the intrinsic reactivity of each protonation site and a point-charge Coulomb interaction between charges. The Coulomb interaction is a function of ion shape or conformation since this determines the distances between charges. An ion conformation is assumed, and an average GB^{app} is calculated from all the proton configurations that are within 3 kcal mol⁻¹ of the calculated lowest energy configuration. A best fit of these calculated values to those measured experimentally is obtained iteratively by adjusting the value of ϵ_r . Note that the value of ϵ_r depends on the assumed ion conformation. Estimates of the $GB_{intrinsic}$ of each basic residue are obtained from measurements of small peptides containing this residue.⁴⁴ On average, these values are ~ 15 kcal mol⁻¹ more basic than the individual amino acid owing to intramolecular solvation of the charge, a process that is very energetically favorable even in highly charged ions. These calculations are described in detail elsewhere.⁴⁶

For cytochrome *c* electrosprayed from a denaturing solution, a best fit to the experimental values is obtained using $\epsilon_r = 2.0$ with the ion modeled in an extended linear structure (Fig. 4).⁴⁴ Similarly, a value of 4.0 provides a reasonable fit modeling the entire ion as an alpha helix. Modeling the ion using the x-ray crystal structure requires $\epsilon_r \approx 12$, an unreasonably high value.^{99,100} This indicates that these ions must be largely extended under these experimental conditions. The higher value of ϵ_r for this protein than that of the smaller ions is attributable to increased shielding between the charges. This is probably a result of more effective charge delocalization along polarizable groups not directly involved in charge solvation and orientation of polarizable groups such as carbonyl oxygens and aromatic side-chain residues to reduce the electrostatic interactions.

Maximum charge state

The mechanism for ion formation by electrospray ionization has been extensively debated in the literature.^{101–103} Factors such as solution¹⁰³ and gas-phase chemistry,¹⁰⁴ protein conformation^{105,106} and instrumental factors have been found to affect the charge state distributions. Fenn¹⁰¹ has proposed a model based on the charge density on an evaporating droplet to account qualitatively for some of these observations. However, quantitatively determining the maximum charge state and charge state distributions has been a difficult challenge. A common approach to obtain the maximum charge state is simply to count the number of basic sites that are protonated in solution.¹⁰⁷ In many cases, this works fairly well. In others, this can result in either a significant over- or underestimation of charge. For example, a recent study of a series of arginine-containing peptides found no clear relationship between the number of potential charge-bearing sites and the number of charges that an arginine-rich peptide will support.¹⁰⁸

A model to account for the maximum number of sodium ions that polyethylene glycol ions can accommodate was proposed by Fenn and co-workers.^{49,50} In this model, the maximum charge is reached when the Coulomb repulsion by other charges exceeded the binding energy of Na⁺ to polyethylene glycol. This model overestimates the maximum charge state observed

for molecules up to 1 MDa, but underestimates the maximum charge of larger ions. This discrepancy was attributed to a different mechanism for formation of the lower molecular mass vs the larger ions.

Owing to the presence of the reverse activation barrier, highly charged ions that are not thermodynamically stable can be kinetically stable with respect to spontaneous unimolecular loss of a charge carrier for long times. For peptide and protein ions, it is possible to account for the maximum charge state observed for multiply protonated ions from the calculated GB^{app} . This is illustrated in Fig. 5, which shows these values for several proteins, all calculated assuming a fully elongated conformation and $\epsilon_r = 2.0$. These calculations indicate that a protein ion should be able to hold significantly more charges than observed without spontaneous loss of a charge. However, if this ion encounters a neutral molecule with greater kinetic affinity for the charge carrier, charge transfer to the neutral molecule will occur. The circled values in Fig. 5 are the highest charge state that has been reported in the literature for these ions when electrosprayed out of methanol-containing solutions. The dashed line at $GB = 174.1 \text{ kcal mol}^{-1}$ indicates the GB of methanol. The correlation between the GB^{app} values of the maximum charge state and the GB of methanol is fairly good. This indicates that the maximum charge state of protein ions formed by electrospray ionization is determined by their gas-phase proton transfer reactivity to solvent molecules.^{44,16} For a series of proteins with more than five published spectra, these calculations indicate the experimentally observed value within 6% average error. For the proteins S4 ribosomal protein and actin, both of which have 46 basic residues, values of 34+ and 56+ are calculated,⁴⁶ respectively, in good agreement with experimentally measured values of 30+ and 59+ reported in the literature.^{95,96}

Similar results are observed for peptides.⁴⁶ For these smaller molecules, $\epsilon_r = 1.3$ provides a better fit to experimentally observed values, indicating that there is less effective shielding for the smaller ions. (There is no obvious reason for a significant difference in the shape of the reaction potential for highly charged peptides vs proteins). It also appears that the maximum charge state of peptides with few basic sites is better correlated with their solution-phase charge. A recent experiment, in which the charge state of a porphyrin ion was determined optically in various stages of the electrospray process, indicated that the doubly protonated ion in solution was converted into a singly protonated ion relatively late in the electrospray process via loss of a charged solvent molecule or cluster.¹⁰⁹ The maximum charge states of a series of starburst polyamidoamine dendrimers with molecular masses up to ~ 1 MDa have been measured and were found to have a linear relationship with $M_r^{2/3}$; from these data, the authors concluded that the maximum charging was controlled by Coulomb effects.¹¹⁰

Charge state distributions can also be significantly influenced by the gas-phase chemistry, but accounting for these distributions provides a greater challenge since other effects, such as solution-phase chemistry and instrumental response, play a role. For example, Winger *et al.*¹⁰⁴ found that by adding water vapor in the inlet/reactor of an electrospray ionization source, the charge distributions for cytochrome *c* shifted from 9+ to 19+ to <9+ to 13+. These charge states have higher GB^{app} values than water, and should not undergo proton transfer under thermal conditions.⁴⁴ However, in the interface region, energetic collisions with solvent molecules can occur. These collisions can provide sufficient energy to drive a reaction over an activation barrier resulting in proton transfer reactions that would not otherwise occur under thermal conditions. In addition, solvent clusters that are more basic than an individual solvent molecule can be present in the interface region. Both of these factors would result in the production of lower charge state ions.

We are currently extending our maximum charge state calculations to both negative ions and cationized species. For example, calculations of the maximum charge state of polyethylene glycol ions using the relative gas-phase sodium affinity of polyethylene glycol vs methanol

and an effective dielectric polarizability of 1.5 (obtained from proton transfer reactivity measurements of a similar polymer¹¹¹) accurately account for the experimental maximum charge states reported by Fenn for ions as large as 0.9 MDa with ~650 charges.¹¹² It should be noted that there are no adjustable parameters used in the calculations for this polymer.

Opposites attract

Reactions of positive and negative ions are different and interesting in that the long-range Coulomb potential between the oppositely charged reactant ions is attractive. This can result in significantly faster reaction rates than ion–molecule reactions for both small and large ions.^{113–117} Ogorzalek-Loo *et al.*^{113,114} used a ‘Y’-shaped capillary inlet reactor in the electrospray interface region to react distributions of multiply charged ions with ions of opposite polarity. Partial neutralization was reported in addition to charge inversion. The ability of trapping instruments to store simultaneously both positive and negative ions makes possible tandem mass spectrometric experiments with oppositely charged ions.^{118,119} McLuckey and co-workers^{115–117} have taken advantage of this capability in a quadrupole ion trap. Proton transfer from protonated pyridine cations to triply and doubly charged anions of DNA was demonstrated to occur with rate constants as high as $10^{-7} \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$.¹¹⁷ Despite the high exothermicity of these reactions, little fragmentation was observed. The high efficiency for these proton transfer reactions makes them well suited for determining charge states of multiply charged ions by inducing a known change in mass and charge.¹¹⁶

Ion conformation

Evidence for the existence of multiple conformations of protein ions has been inferred from a variety of different measurements. Smith and co-workers¹²⁰ observed different H–D exchange rates for native and reduced forms of two proteins when D₂O was introduced into their inlet/reaction capillary. Native proteins exchanged more hydrogens than their reduced counterparts. From both the rates and extent of gas-phase H–D exchange with D₂O measured in a Fourier transform mass spectrometer, McLafferty and co-workers^{121–122} found that distinct isomers of several different proteins exist in the gas phase. For example, eight different isomers of cytochrome *c* were identified; isomers that exhibited lower rates and extents of exchange were interpreted as having a more compact or folded structure analogous to solution H–D exchange results.¹²² Distinct conformers have also been observed via collisional cross-section measurements.^{86,123–127} For example, recent results by Ceilings and Douglas¹²⁴ have indicated the existence of both compact and unfolded forms of myoglobin ions. Ion chromatographic experiments by Bowers and co-workers⁸⁶ indicated that singly sodiated polyethylene glycol and protonated bradykinin ions¹²⁶ are tightly folded to solvate the charge. In similar experiments with cytochrome *c* ions, Jarrold and co-workers¹²⁷ were able to resolve clearly at least three different conformational isomers of the +7 charge state. These isomers fit cross-sections calculated for partially folded structures. Higher charge state ions were found to be consistent with more elongated structures. Sullivan *et al.*¹²⁸ found that large ions formed by ESI, when collided with a surface, produced ‘hillocks’ that could be subsequently imaged by scanning force and tunneling microscopy. Hillocks formed by myoglobin ions electrosprayed from native solutions were circular and more compact than those formed from denaturing solutions, indicating a compact and relatively elongated conformation, respectively.

Because of the long-range Coulomb interaction, proton transfer reactivity provides a sensitive measure of distance between charges. Differences in proton transfer reactivity of disulfide-containing protein ions in which disulfide linkages are intact vs reduced have been observed.^{18,19,47} Smith and co-workers^{18,19} found that the reaction of all charge states of desulfide-intact proteins with neutral basic molecules in the interface region of the mass spectrometer resulted in the production of lower charge states than those obtained with the corresponding disulfide-reduced ions. More significant differences were observed for higher

charge states¹⁸ than for lower charge states.¹⁹ Information about the conformation of these ions can be inferred from a comparison of the measured reactivity of isolated charge states with that calculated with the point charge model.⁴⁷ Figure 6 shows the calculated GB^{app} as a function of charge state for lysozyme ions modeled using both the x-ray crystal structure and as a fully elongated ion. The measured values for each charge state formed from a solution in which the protein is in its native conformation (disulfide-intact, aqueous solution) and denatured (disulfide-reduced, heated methanol-water-acid solution) are indicated by closed squares and open circles, respectively. For most charge states, more than one rate constant was required to fit the kinetic data accurately, indicating the presence of different reactive isomers. A similar observation has been reported for the 12+ charge state of ubiquitin.¹²⁹ Large differences in GB^{app} are observed for ions of the same charge state formed directly from these two solutions. For example, the GB^{app} of the 9+ ion formed from a native solution is 23 kcal mol⁻¹ lower than its disulfide-reduced counterpart. This difference is due to differences in ion conformation; a tightly folded ion has more Coulomb repulsion than one that is largely denatured. Distinct isomers with intermediate reactivity consistent with partially folded ion conformations are observed. Lower charge states not formed directly by the electrospray process can be produced by gas-phase proton transfer reactions. The reactivity of the resulting charge states indicates that the removal of protons in the gas phase can result in either folding or unfolding of protein ions in the complete absence of solvent!^{47,122} Determining the extent of similarities, if any,¹³⁰ between solution and gas-phase conformations, will provide a challenging new area for further investigation.

FUTURE PROSPECTS

Intramolecular electrostatic interactions clearly play a significant role in the reactivity of multiply charged ions. A number of exciting studies on the effects of charge on the dissociation, collisional cross-sections and ion-ion and ion-molecule reactivity of multiply charged biomolecule ions are yielding new information about the chemistry of these ions. The model of the proton transfer reactivity of multiply protonated ions described here is somewhat simplistic and contains crude approximations. However, it does appear to provide useful information about the effects of molecular structure on intramolecular electrostatic interactions, ion conformation and maximum charge states. The accuracy of this model could be improved by including factors such as nearest neighbor and local folding interactions, and effects of other charges on the values of $GB_{intrinsic}$. Salt-bridge interactions, frequently observed in solution, have recently been observed in gas-phase proton transfer complexes of betaine and ammonia⁹³ and also in arginine-containing peptide ions.^{89,131} This type of interaction, which affects the charge distributions in these ions, is not currently included in this model.

Additional improvements include accounting for the effects of ion conformation on ϵ_r and, ultimately, replacing this bulk value through the use of a microscopic model. For these latter modifications, detailed information about the exact ion conformation is required, which has not yet been obtained for large gas-phase ions. To the extent that vacuum and solution structures are similar, the intrinsic intramolecular shielding at various charge sites in a gas-phase ion could be related to those values in solution and the effect of the surrounding solvent on these values determined. The effects of hydration on the physical properties of multiply charged gas-phase ions is one of the exciting new areas for exploration. Kebarle and co-workers⁸⁵ have measured the free energy of hydration in doubly protonated diaminoalkanes as a function of increasing charge separation and number of water molecules attached. From a similar study done with hydrated peptide ions, information about charge-site solvation was obtained.¹³² A wide range of mass spectrometric techniques have been developed to probe the structure of ions. By extending these measurements to hydrated biomolecule ions, it appears highly

promising that information about the differences between gas-phase and solution-phase structures, and ultimately the influence of solvent on these structures, can be obtained.

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References

1. Tanford C, Kirkwood JG. *J Am Chem Soc* 1957;79:5333.
2. Gilson MK, Honig B. *Biopolymers* 1986;25:2097. [PubMed: 3790703]
3. Honig B, Nicholls A. *Science* 1995;268:1144. [PubMed: 7761829]
4. Bashford D, Case DA, Dalvit C, Tennant L, Wright PE. *Biochemistry* 1993;32:8045. [PubMed: 8347606]
5. Rajasekaran E, Jayaram B, Honig B. *J Am Chem Soc* 1994;116:8238.
6. Potter MJ, Gilson MK, McCammon JA. *J Am Chem Soc* 1981;103:10298.
7. Warshel A, Aqvist J. *Annu Rev Biophys Chem* 1991;20:267.
8. Warshel A, Russell ST. *O Rev Biophys* 1984;17:283.
9. Warshel A, Russell ST, Churg AK. *Proc Natl Acad Sci USA* 1984;81:4785. [PubMed: 6589625]
10. Takashima S, Schwan HP. *J Phys Chem* 1965;69:4176. [PubMed: 5846598]
11. Baker WO, Yager WA. *J Am Chem Soc* 1942;64:2171.
12. King G, Lee FS, Warshel A. *J Chem Phys* 1991;95:4366.
13. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. *Science* 1989;246:64. [PubMed: 2675315]
14. Smith RD, Loo JA, Ogorzalek Loo RR, Busman M, Udseth HR. *Mass Spectrom Rev* 1991;10:359.
15. McLuckey SA, Van Berkel GJ, Glish GL. *J Am Chem Soc* 1990;112:5668.
16. Cassady CJ, Wronka J, Kruppa GH, Laukien FH. *Rapid Commun Mass Spectrom* 1994;8:394. [PubMed: 8025335]
17. Ikonomou MG, Kebarle P. *Int J Mass Spectrom Ion Processes* 1992;117:283.
18. Ogorzalek Loo RR, Smith RD. *J Am Soc Mass Spectrom* 1994;5:207.
19. Ogorzalek Loo RR, Winger BE, Smith RD. *J Am Soc Mass Spectrom* 1994;5:1064.
20. Ogorzalek Loo RR, Smith RD. *J Mass Spectrom* 1995;30:339.
21. Loo JA, Edmonds CG, Udseth HR, Smith RD. *Anal Chim Acta* 1990;241:167.
22. Little DP, Speir JP, Senko MW, O'Connor PB, McLafferty FW. *Anal Chem* 1994;66:2809. [PubMed: 7526742]
23. Ishikawa K, Nishimura T, Koga Y, Niwa Y. *Rapid Commun Mass Spectrom* 1994;8:933. [PubMed: 7696702]
24. Busman M, Rockwood AL, Smith RD. *J Phys Chem* 1992;96:2397.
25. McLuckey SA, Goeringer DE. *Anal Chem* 1995;67:2493. [PubMed: 8686879]
26. McLuckey SA, Glish GL, Van Berkel GJ. *Anal Chem* 1991;63:1971. [PubMed: 1661106]
27. Hunter A, Severs JC, Harris FM, Games DE. *Rapid Commun Mass Spectrom* 1994;8:417. [PubMed: 8025337]
28. Bruce JE, Cheng X, Bakhtiar R, Wu Q, Hofstadler SA, Anderson GA, Smith RD. *J Am Chem Soc* 1994;116:7839.
29. Smith RD, Cheng X, Bruce JE, Hofstadler SA, Anderson GA. *Nature (London)* 1994;369:137.
30. Cheng X, Bakhtiar R, Van Orden S, Smith RD. *Anal Chem* 1994;66:2084. [PubMed: 8067525]

31. Bruce JE, Cheng X, Bakhtiar R, Wu Q, Hofstadler SA, Anderson GA, Smith RD. *J Am Chem Soc* 1994;116:7839.
32. Williams ER, Henry KD, McLafferty FW. *J Am Chem Soc* 1990;112:6157.
33. Speir JP, Gorman GS, Pitsenberger CC, Turner CA, Wang PPIJ. *Amster Anal Chem* 1993;65:1746.
34. R. G. Cooks, J. H. Beynon, R. M. Caprioli and G. R. Lester, *Metastable Ions*. Elsevier, Amsterdam (1973).
35. Roth LM, Freiser BS. *Mass Spectrom Rev* 1991;10:303.
36. Harris FM. *Int J Mass Spectrom Ion Processes* 1992;120:1.
37. Hillenkamp F, Karas M, Beavis RC, Chait BT. *Anal Chem* 1991;63:1193A. [PubMed: 1897719]
38. Overberg A, Karas M, Kaufmann R, Hillenkamp F. *Rapid Commun Mass Spectrom* 1990;4:293.
39. Mahoney JF, Perel J, Ruatta SA, Martino PA, Husain S, Lee TD. *Rapid Commun Mass Spectrom* 1991;5:441. [PubMed: 1841643]
40. Mahoney JF, Perel J, Lee TD, Martino PA, Williams P. *J Am Soc Mass Spectrom* 1992;3:311.
41. Chen R, Cheng X, Mitchell DW, Hofstadler SA, Wu Q, Rockwood AL, Sherman MG, Smith RD. *Anal Chem* 1995;67:1159.
42. Gross DS, Williams ER. *J Am Chem Soc* 1995;117:883.
43. Gross DS, Rodriguez-Cruz SE, Bock S, Williams ER. *J Phys Chem* 1995;99:4034.
44. Schnier PD, Gross DS, Williams ER. *J Am Chem Soc* 1995;117:6747.
45. Gross DS, Williams ER. *J Am Chem Soc* 1996;118:202.
46. Schnier PD, Gross DS, Williams ER. *J Am Soc Mass Spectrom* 1995;6:1086.
47. Gross DS, Schnier PD, Rodriguez-Cruz SE, Fagerquist CK, Williams ER. *Proc Natl Acad Sci USA* 1996;93:3143. [PubMed: 8610183]
48. Lias SG, Liebman JF, Levin RD. *J Phys Chem Ref Data* 1984;13:695.
49. Nohmi T, Fenn JB. *J Am Chem Soc* 1992;114:3241.
50. Wong SF, Meng CK, Fenn JB. *J Phys Chem* 1988;92:546.
51. Rockwood AL, Busman M, Smith RD. *Int J Mass Spectrom Ion Processes* 1991;111:103.
52. D. H. Aue and M. T. Bowers, in *Gas-Phase Ion Chemistry*, edited by M. T. Bowers, Vol. 2, p. 1. Academic Press, New York (1979).
53. R. W. Taft, in *Proton Transfer Reactions*, edited by E. F. Caldin and G. Gold, p. 31. Wiley, New York (1975).
54. Kebarle P. *Annu Rev Phys Chem* 1977;28:445.
55. DeFrees DJ, McIver RT, Hehre WJ. *J Am Chem Soc* 1980;102:3334.
56. Beauchamp JL, Holtz D, Woodgate SD, Patt SL. *J Am Chem Soc* 1972;94:2798.
57. Ridge DP. *J Am Chem Soc* 1975;97:5670.
58. Bliznyuk AA, Schaefer HF III, Amster IJ. *J Am Chem Soc* 1993;115:5149.
59. McLuckey SA, Cameron D, Cooks RG. *J Am Chem Soc* 1981;103:1313.
60. Wu Z, Fenselau C. *J Am Soc Mass Spectrom* 1992;3:863.
61. Li XP, Harrison AG. *Org Mass Spectrom* 1993;28:366.
62. Cooks RG, Patrick JS, Kotiaho T, McLuckey SA. *Mass Spectrom Rev* 1994;13:287.
63. Gill PMW, Radom L. *J Am Chem Soc* 1988;110:5311.
64. Petrie S, Javahery G, Wincel H, Wang J, Bohme DK. *J Am Chem Soc* 1993;115:6290.
65. Petrie S, Javahery G, Bohme DK. *Int J Mass Spectrom Ion Processes* 1993;124:145.
66. Javahery G, Petrie S, Wincel H, Wang J, Bohme DK. *J Am Chem Soc* 1993;115:6295.
67. Javahery G, Petrie S, Ketvirtis A, Wang J, Bohme DK. *Int J Mass Spectrom Ion Processes* 1992;116:R7.
68. By definition, $GB(MH^+) = GA(MH_2^{2+})$. Because these proton transfer measurements of multiply charged ions reflect kinetic, not thermodynamic values, the choice of terminology in referring to these kinetic values, i.e. $GB^{app}(MH^+)$ vs $GA^{app}(MH_2^{2+})$, is largely irrelevant as long as the definition used is clearly stated.
69. Bursey MM, Pederson LG. *Org Mass Spectrom* 1992;27:974.

70. Scheller MK, Compton RN, Cederbeum LS. *Science* 1995;270:1160.
71. Jin C, Hettich RL, Compton RN, Tuinman A, Derecskei-Kovacs A, Marynick DS, Dunlap BI. *Phys Rev Lett* 1994;73:2821. [PubMed: 10057204]
72. Gronert S. *J Am Chem Soc* 1996;118:3525.
73. Ast T, Beynon JH, Cooks RG. *Org Mass Spectrom* 1972;6:741.
74. Beynon JH, Caprioli RM, Ast T. *Org Mass Spectrom* 1972;16:273.
75. Caprioli RM, Beynon JH, Ast T. *Org Mass Spectrom* 1971;5:417.
76. Gill PMW, Radom L. *Chem Phys Lett* 1988;147:213.
77. Weiske T, Koch W, Schwarz H. *J Am Chem Soc* 1993;115:6312.
78. Kaltashov IA, Fabris D, Fenselau CC. *J Phys Chem* 1995;99:10046.
79. Kaltashov IA, Fenselau CC. *J Am Chem Soc* 1995;117:9906.
80. Adams J, Strobel FH, Reiter A, Sullards MC. *J Am Soc Mass Spectrom* 1996;7:30.
81. Aue DH, Webb HM, Bowers MT. *J Am Chem Soc* 1973;95:2699.
82. Yamdagni R, Kebarle P. *J Am Chem Soc* 1973;95:3504.
83. As discussed earlier, this separation distance is too small, as indicated by subsequent calculations of the reaction potentials in Refs 44 and 72.
84. W. D. Price and E. R. Williams, unpublished results.
85. Klassen JS, Blades AT, Kebarle P. *J Am Chem Soc* 1994;116:12075.
86. von Helden G, Wyttenbach T, Bowers MT. *Science* 1995;267:1483. [PubMed: 17743549]
87. Thölmann D, Tonner DS, McMahon TB. *J Phys Chem* 1994;98:2002.
88. Dunbar RC. *J Phys Chem* 1994;98:8705.
89. Price WD, Schnier PD, Williams ER. *Anal Chem* 1996;68:859.
90. Wu JY, Lebrilla CB. *J Am Chem Soc* 1993;115:3270.
91. Cheng X, Wu Z, Fenselau C. *J Am Chem Soc* 1993;115:4844.
92. Zhang K, Zimmerman DM, Chung-Phillips A, Cassady CJ. *J Am Chem Soc* 1993;115:10812.
93. Cambell S, Rodgers MT, Marzluff EM, Beauchamp JL. *J Am Chem Soc* 1995;117:12840.
94. The GB values are from the basicity scale of M. Moët-Ner and W. Sieck, *J. Am. Chem. Soc.* 113, 4448 (1991), adjusted to the scale of Lias et al. (Ref. 48).
95. E. E. Gulcicek, S. Shen, J. D. Boyle, C. M. Whitehouse, D. H. Harrison and P. B. Moore in *Proceedings of the 39th ASMS Conference on Mass Spectrometry and Allied Topics*; Nashville, TN, 1991, p. 1245.
96. Smith RD, Loo JA, Ogorzalek Loo RR, Busman M, Udseth HR. *Mass Spectrom Rev* 1991;10:359.
97. Gorman GS, Speir JP, Turner CA, Amster IJ. *J Am Chem Soc* 1992;114:3986.
98. Wu Z, Fenselau C. *Tetrahedron* 1993;49:9197.
99. Simonson T, Perahia D. *J Am Chem Soc* 1995;117:7987.
100. Simonson T, Perahia D. *Proc Natl Acad Sci USA* 1995;92:1082. [PubMed: 7862638]
101. Fenn JB. *J Am Soc Mass Spectrom* 1993;4:524.
102. Ashton DS, Beddell CR, Cooper DJ, Green BN, Oliver RWA. *Org Mass Spectrom* 1993;28:721.
103. LeBlanc JCY, Sui KWM, Guevremont R. *Anal Chem* 1994;66:3289.
104. Winger BE, Light-Wahl KJ, Smith RD. *J Am Soc Mass Spectrom* 1992;3:624.
105. Chowdhury SK, Katta V, Chait BT. *J Am Chem Soc* 1990;112:9012.
106. Loo JA, Edmonds CG, Udseth HR, Smith RD. *Anal Chem* 1990;62:693. [PubMed: 2327585]
107. Covey TR, Bonner RF, Shushan BI, Henion J. *Rapid Commun Mass Spectrom* 1988;2:249. [PubMed: 2577836]
108. Downard KM, Biemann K. *Int J Mass Spectrom Ion Processes* 1995;148:191.
109. Chillier XFD, Monnier A, Bill H, Gulacar FO, Buchs A, McLuckey SA, Van Berkel GJ. *Rapid Commun Mass Spectrom* 1996;10:299.
110. Schwartz BL, Rockwood AL, Smith RD, Tomalia DA, Spindler R. *Rapid Commun Mass Spectrom* 1995;9:1552.

111. S. E. Rodriguez-Cruz and E. R. Williams, in Proceedings of the 44th ASMS Conference on Mass Spectrometry and Allied Topics, Portland, OR, 1996.
112. D. S. Gross, Y. Zhao, P. D. Schnier and E. R. Williams, in Proceedings of the 44th ASMS Conference on Mass Spectrometry and Allied Topics, Portland, OR, 1996.
113. Ogorzalek-Loo RR, Udseth HR, Smith RD. J Phys Chem 1991;95:6412.
114. Ogorzalek-Loo RR, Udseth HR, Smith RD. J Am Soc Mass Spectrom 1992;3:695.
115. Herron WJ, Goeringer DE, McLuckey SA. Rapid Commun Mass Spectrom 1996;10:277.
116. Herron WJ, Goeringer DE, McLuckey SA. Anal Chem 1996;68:257. [PubMed: 9027235]
117. Herron WJ, Goeringer DE, McLuckey SA. J Am Soc Mass Spectrom 1995;6:529.
118. Mather RE, Todd JFJ. Int J Mass Spectrom Ion Processes 1980;33:159.
119. Gorshkov MV, Guan S, Marshall AG. Rapid Commun Mass Spectrom 1993;6:166.
120. Winger BE, Light-Wahl KJ, Rockwood AL, Smith RD. J Am Chem Soc 1992;114:5897.
121. Suckau D, Shi Y, Beu SC, Senko MW, Quinn JP, Wampler FW, McLafferty FW. Proc Natl Acad Sci USA 1993;90:790. [PubMed: 8381533]
122. Wood TD, Chorush RA, Wampler FM, Little DP, O'Connor PB, McLafferty FW. Proc Natl Acad Sci USA 1995;92:2451. [PubMed: 7708663]
123. Covey T, Douglas DJ. J Am Soc Mass Spectrom 1993;4:616.
124. Collings BA, Douglas DJ. J Am Chem Soc 1996;118:4483.
125. Cox KA, Julian RK, Cooks RG, Kaiser RE. J Am Soc Mass Spectrom 1994;5:127–136.
126. von Helden G, Wyttenbach T, Bowers MT. Int J Mass Spectrom Ion Processes 1995;146:349.
127. Clemmer DE, Hudgins RR, Jarrold MF. J Am Chem Soc 1995;117:10141.
128. Sullivan PA, Axelsson J, Altmann S, Quist AP, Sunqvist BUR, Reimann CT. J Am Soc Mass Spectrom 1995;7:329.
129. Cassidy CJ, Carr SR. J Mass Spectrom 1996;31:247. [PubMed: 8799276]
130. Wolynes P. Proc Natl Acad Sci USA 1995;92:2426. [PubMed: 7708658]
131. P. D. Schnier, W. D. Price, R. A. Jockusch and E. R. Williams, J. Am. Chem. Soc. in press.
132. Klassen JS, Blades AT, Kebarle P. J Phys Chem 1995;99:15509.

Figure 1.

Qualitative interaction potentials for proton transfer reactions of singly and doubly protonated M and neutral reference bases (A, B and C), where M is a symmetrical molecule with two identical sites of protonation separated by $>10 \text{ \AA}$. (a) Reaction of MH^+ with A, $PA(\text{M}) - PA(\text{A})$; (b) reaction of MH_2^{2+} with C, $PA(\text{MH}^+) - PA(\text{C})$; (c) reaction of MH_2^{2+} with B, $PA^{\text{app}}(\text{MH}^+) - PA(\text{B})$; (d) the hypothetical reaction of MH_2^{2+} with A in the absence of Coulomb repulsion. From Ref. 44.

Figure 2.

Proton transfer kinetics for gramicidin S $[M + 2H]^{2+}$ reacted with two bases that have different gas-phase basicities; ion abundance vs. time for reactions with *tert*-butylamine (TBA) (top, $GB = 216.7 \text{ kcal mol}^{-1}$, rate $< 0.0046 \times 10^{-11} \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) and diethylamine (DEA) (bottom, $GB = 221.4 \text{ kcal mol}^{-1}$, rate $= 4.4 \times 10^{-11} \text{ cm}^3 \text{ mol}^{-1} \text{ s}^{-1}$); ○, $[M + H]^+$; ●, $[M + 2H]^{2+}$; ▲, $[TBA + H]^+$; ◆, $[DEA + H]^+$. From these kinetic data, the GB^{app} of the $[M + H]^+$ ion (measured from proton transfer reactivity of the $[M + 2H]^{2+}$ ion) is assigned a value between these two bases, or $219 \text{ kcal mol}^{-1}$.^{42,45} From Ref. 42 with minor modification.

Figure 3.

Reaction diagram for the proton transfer reaction of doubly protonated 1,*n*-diaminoalkane (DAH_2^{2+}) with the corresponding monoamine (MA). $\Delta H_{\text{RAB}} = \Delta H_{\text{b,c}}$, $\text{PA(MA)} - \text{PA}^{\text{app}}(\text{DAH}^+) = \Delta H_{\text{c,d}}$ and $\Delta H_{\text{RXN}} = \Delta H_{\text{b,d}}$, where the subscripts b, c and d refer to the energy level of the products of the reactions shown in Fig. 1 and RAB = reverse activation barrier.

Figure 4.

Apparent gas-phase basicity as a function of charge state of cytochrome *c* ions, measured (●), and calculated assuming three different ion conformations: linear extended ($\epsilon_r = 2.0$, ○; $GB_{\text{intrinsic}}$, ▲), linear α -helix ($\epsilon_r = 4.1$, □) and from the x-ray crystal structure ($\epsilon_r = 2.0$, ◆). The dashed line indicates the GB of methanol ($174.1 \text{ kcal mol}^{-1}$) which correlates with the value of GB^{app} calculated for maximum charge state observed experimentally for this ion. The origin of the dip in $GB_{\text{intrinsic}}$ at $n = 15$ is deprotonation of backbone sites in the lowest energy charge configurations for this charge state.⁴⁴ From Ref. 44 with minor modification.

Figure 5.

Calculated apparent gas-phase basicity as a function of charge state for nine molecules modeled as elongated strings and using $\epsilon_r = 2.0$. In order from left to right: melittin ($\bullet$); ubiquitin ($\square$); cytochrome *c* (tuna) ($\blacktriangle$); cytochrome *c* (equine) ($\circ$); ribonuclease A ($\blacklozenge$); hemoglobin (α -chain) ($\circ$); myoglobin ($\bullet$); carbonic anhydrase ($\square$); bovine serum albumin ($\blacklozenge$). The dashed line indicates the *GB* of methanol ($174.1 \text{ kcal mol}^{-1}$); circled values correspond to maximum experimental charge states that have been reported in the literature for each protein. From Ref. 46 with minor modification.

Figure 6.

GB^{app} of $[M + (n - 1)H]^{(n - 1)+}$ (measured by proton transfer from $[M + nH]^{n+}$ ions to neutral reference bases) of disulfide-intact (■) and disulfide-reduced (○) hen egg-white lysozyme. Where more than one reactive conformer is present, its percentage abundance is indicated next to the data point (percentages of the disulfide-intact and disulfide reduced conformers are indicated on the left and right sides of the data points, respectively). Error bars include the uncertainty in the value of GB of the reference bases. Calculated values of GB^{app} of these ions modeled using the x-ray crystal structure coordinates (●) and modeled as a fully elongated one-dimensional string (▲) using $\epsilon_r = 2.0$ are shown. The dashed line indicate the GB of methanol ($174.1 \text{ kcal mol}^{-1}$). From Ref. 47