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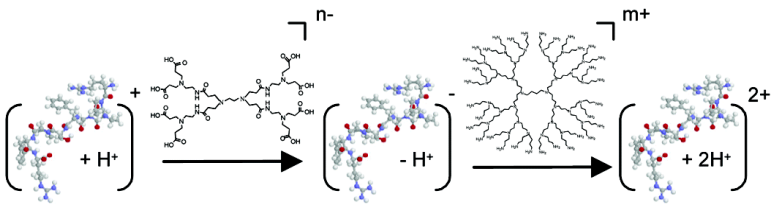

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Two Ion/Ion Charge Inversion Steps To Form a Doubly Protonated Peptide from a Singly Protonated Peptide in the Gas Phase

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Protonated peptides in the gas phase play an important role in life sciences research because they are used, in conjunction with mass spectrometry and tandem mass spectrometry, for protein identification.[1] In the case of tandem mass spectrometry, for example, the dissociation patterns of activated protonated peptides are used to obtain primary sequence information to identify the peptides. Gaseous peptide and protein ions are commonly formed either via matrix-assisted laser desorption ionization[2] or electrospray ionization.[3] The former typically yields singly protonated species whereas the latter often yields a distribution of charge states composed of the molecule of interest with various numbers of excess protons. Neither ionization method allows for the arbitrary determination of peptide/protein ion charge state. The charge states are determined both by the nature of the polypeptide, such as numbers and identities of basic residues, and by the mechanisms for ionization. The preferred dissociation channels of a peptide or protein ion are highly dependent upon the charge state of the ion.[4] It is therefore desirable to develop means for manipulating the charge states of ions independent of the optimal conditions for initial ion formation.

The reduction of the charge states of initially highly charged ions can be effected via ion/molecule reaction chemistry[5] or via ion/ion reaction chemistry.[6] However, increasing the charge state of a positive ion is a much more challenging proposition. Doing so in a single ion/ion reaction encounter of the type:

$$MH_n^{n+} + BH^+ \rightarrow MH_{n+1}^{(n+1)+} + B \qquad (1)$$

is of low probability for both thermodynamic and kinetic reasons. In many cases, reaction 1 is expected to be endothermic. Of greater importance is the fact that the interaction potential associated with the reaction is repulsive at long range such that it is highly unlikely that the reactants could approach one another sufficiently close to give rise to proton transfer. Only low impact parameter collisions at high translational energy could give rise to the desired products. Another option for increasing the charge state of a gaseous positive ion via a single encounter is via some form of impact ionization, such as electron ionization,[7] photoionization, or collisional ionization. However, the higher charge state ions are formed by electron ejection rather than by additional protonation.

We demonstrate here a two-step ion/ion reaction approach that provides means for increasing ion charge states by increasing the number of excess protons. The example demonstrated here involves converting a singly protonated peptide to doubly protonated peptide. The overall approach can potentially be efficient because all steps are highly exothermic and have large cross-sections.[6a] The sequence is illustrated below for positive ions:

$$(M + H)^+ + (N - nH)^{n-} \rightarrow (M - H)^- + (N - (n-2)H)^{(n-2)-} \qquad (2)$$

$$(M - H)^- + (R + mH)^{m+} \rightarrow (M + 2H)^{2+} + (R + (m-2)H)^{(m-2)+} \qquad (3)$$

Reaction of an $(M + H)^+$ ion with a doubly charged anion can give rise to charge inversion of the peptide to give the singly deprotonated ion $(M - H)^-$. Such a reaction is probable when the molecule has both basic (e.g., N-terminus and basic residues) and acidic (e.g., carboxy terminus and acidic residues) functional groups. The next step is to react the singly charged anion with a highly charged reagent cation. The reaction of these species will allow for charge to be shared between the two reactants. When they come apart, the net positive charge of the system will be partitioned between the products with the possibility that M will carry more than one positive charge. The net effect is an increase in the charge of M from +1 to some higher charge state. Just as with the first step, this reaction is highly exothermic and of high cross-section. The extent of charging will depend both upon the nature of M (e.g., how many basic sites are available, their relative strengths, the size and conformational flexibility of the peptide/protein, etc.) and upon the nature and charge of the reagent ion. Ideally, the reagent will be highly charged and the protonated sites will be weak relative to those in the biopolymer.

Figure 1 summarizes results for an experiment in which two steps of charge inversion were used to form $(M + 2H)^{2+}$ ions from $(M + H)^+$ ions.[8,9] The first step of the experiment involved the accumulation of bradykinin ions formed via positive electrospray ionization in a quadrupole ion trap followed by isolation of the $(M + H)^+$ ion. The resulting mass spectrum is shown in Figure 1a. A population of anions formed via negative electrospray ionization of a carboxylate-terminated polyamidoamine dendrimer (generation 0.5) (PAMAM) was then admitted into the ion trap and allowed to react with the bradykinin $(M + H)^+$ ions. The electrospray mass spectrum of the PAMAM reagent anions (no cations present) is shown in Figure 1b. After reaction with the bradykinin $(M + H)^+$ ions, both residual PAMAM anions and $(M - H)^-$ bradykinin anions were observed in the spectrum (data not shown). The bradykinin $(M - H)^-$ ions were then isolated and a population of poly(propylenimine) (1,4-diaminobutane (DAB) core, generation 4) dendrimer cations formed via positive ion electrospray was admitted into the ion trap and allowed to react with the anions. The resulting product ion spectrum is shown in Figure 1c. For comparison, the spectrum resulting from the sequence used to acquire the spectrum of Figure 1c but without the admission of anions is shown in Figure 1d. The latter spectrum shows the mixture of bradykinin $(M + H)^+$ and DAB dendrimer cations that results from the sequential injection of positive ions without the intervening anion accumulation and ion/ion reaction period. The position of the $(M + 2H)^{2+}$ bradykinin ion on the mass-to-charge scale, if it were present, is indicated by an arrow. Several major differences can be noted in the comparison of Figures 1c and 1d. First, the charge state distribution of the DAB dendrimer ions has shifted to

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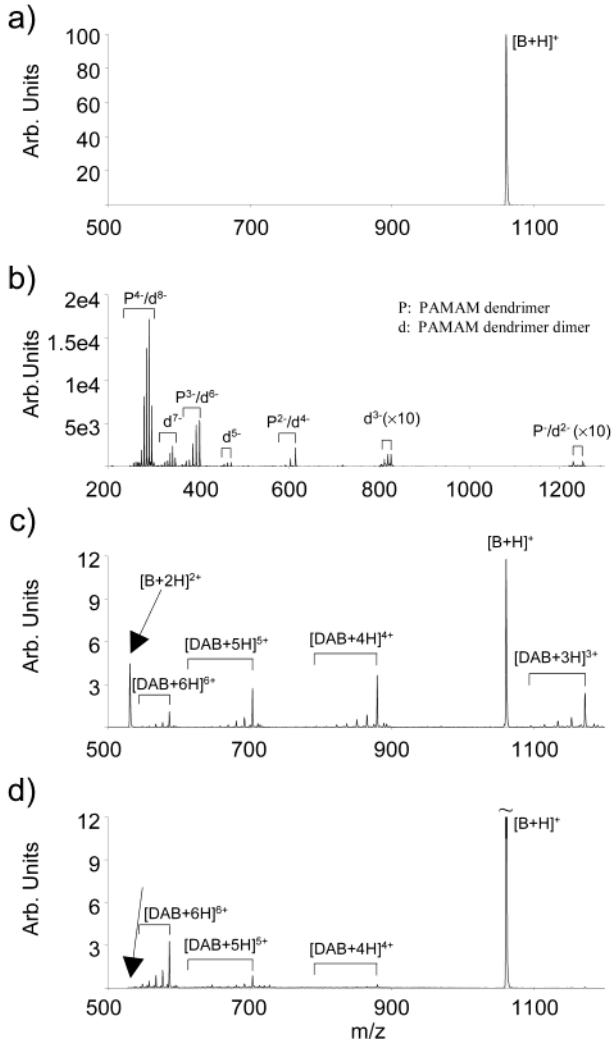

Figure 1. (a) Positive electrospray mass spectrum of bradykinin after isolation of $(M + H)^+$. (b) Negative ion electrospray mass spectrum of PAMAM anions used for the first step of charge inversion. (c) Positive ion spectrum resulting from charge inversion of the bradykinin $(M + H)^+$ ion from reactions with PAMAM anions followed by charge inversion of the bradykinin $(M - H)^-$ ion from reactions with DAB cations. (d) Positive ion mass spectrum resulting from the same process as that used for the spectrum of (c) except that no anions were admitted into the ion trap. Note that the abundances for anions (b) and cations (a, c, d) are not directly comparable.

lower charge states as a result of partial neutralization with the bradykinin anions. Second, the abundance of the $(M + H)^+$ ion is significantly reduced (by roughly a factor of 10) by the admission of the anions. This is presumably due to losses associated with neutralization of part of the $(M + H)^+$ ion population by the PAMAM anions used to form $(M-H)^-$ ions, losses associated with neutralization of the $(M - H)^-$ anions in reactions with DAB cations and losses associated with the fraction of ions formed as $(M + 2H)^{2+}$ ions. Third, there is a clear signal that arises from the formation of the bradykinin $(M + 2H)^{2+}$ ion as a result of a two-step process represented by (2) and (3). (Note that significant background in the region of the bradykinin $(M + 3H)^{3+}$ ions arising from high charge state DAB cations and chemical noise precluded the clear identification of the formation of the triply protonated peptide.) A roughly 200-fold increase over the background signal at the *m*/*z* ratio of the $(M + 2H)^{2+}$ ion in Figure 1d is observed in Figure 1c. The net yield for $(M + H)^+ \rightarrow\rightarrow (M + 2H)^{2+}$ is roughly 5%.

A wide variety of charge inversion processes, including sequential reactions as indicated here, can occur via ion/ion reactions. In this case, all reactions proceeded via proton transfer with no evidence for fragmentation or adduct ion formation. This is desirable from the point of view of charge state manipulation. The extent to which proton transfer competes with complex formation, for example, depends both upon the nature of the reagent species and upon the reaction conditions used in the ion trap. In this case, the dendrimer ions appear to be particularly well-suited for proton transfer reactions. The PAMAM anions abstract protons without appreciable adduct formation and the DAB cations donate protons without appreciable adduct formation. Reaction conditions can also affect the extent to which reactions occur via long-range single proton transfer and via an ion/ion complex. In the case of the process described here, single proton transfer reactions are deleterious in that they can lead to neutralization of the peptide/protein ion of interest. This process can be minimized by choice of reagent ion and by minimizing the relative velocities of the reactants. It is therefore possible that the net efficiencies for increasing peptide/protein charge states via ion/ion reactions can be improved over that shown here upon further exploration of charge transfer reagents and upon optimization of reaction conditions for each charge inversion step. (For example, all of the DAB dendrimer charge states were used for reaction but the $(M + 2H)^{2+}/(M + H)^+$ ratio is expected to increase with DAB ion charge state. Therefore, the selection of high DAB charge states could improve the overall efficiency of formation of the $(M + 2H)^{2+}$ ion.) It is also of interest to develop charge transfer reagents capable of transferring multiple charges to larger polypeptides/proteins. Such a capability can potentially enhance the structural information that can be obtained using ionization techniques that tend to form predominantly singly charged ions from large polypeptides/proteins.

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