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Increasing the Negative Charge of a Macroanion in the Gas Phase via Sequential Charge Inversion Reactions

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Abstract

Protonated and deprotonated biological molecules in the gas phase play an important role in life sciences research. The structural information accessible from the ions is highly dependent upon their charge states. Therefore, it is desirable to develop means for increasing absolute charge states, particularly for ionization methods, such as MALDI, that yield relatively low charge ions. The work presented here demonstrates the formation of a doubly deprotonated polypeptide or oligonucleotide ion (dianion) from a singly deprotonated analogue via two sequential ion/ion proton-transfer reactions involving charge inversion. The high exoergicity and the large cross section arising from the long-range attractive Coulomb potential of ion/ion reactions make this process plausible. In this example, an overall efficiency of conversion of singly charged ions to doubly charged ions of roughly 8% for polypeptide was noted while lower efficiency (roughly 2%) observed with an oligonucleotide is likely due to a greater degree of neutralization. No other approach to increasing the net negative charge of an anion in the gas phase has as yet been reported.

Gas-phase ions have long played an important role in chemical analysis via their surrogacy for molecules of interest. Measurement of the mass-to-charge ratios of the ions allows for the determination of atomic and molecular weights, for example, and the fragmentation of polyatomic ions reveals structural information. These uses for gaseous ions have been extended to relatively large nonvolatile molecules, such as those derived from biological systems, with the introduction of electrospray ionization[1] and matrix-assisted laser desorption.[2] The former method frequently forms multiply charged ions from large molecules with multiple readily ionized sites whereas the latter method usually forms predominantly singly charged ions. The multiple charging of large molecules can be advantageous or problematic, depending upon the analytical situation. For example, multiple charging can complicate mass measurement when mixtures of ions with various masses and charges are present. This situation typically prevails, for example, when electrospray is applied to a mixture of biological molecules, such as peptides and oligonucleotides. However, multiple charging relaxes the upper mass-to-charge limit requirements of a mass analyzer and can also result in higher detection efficiency than observed for singly charged ions. Furthermore, the structural information that can be derived from a gas-phase macroion is highly dependent upon the charge of the parent ion. It has been demonstrated in many cases that complementary structural information can be obtained from the dissociation of different parent ion charge states.[3,4] Dissociation of singly charged ions often yields dominant losses of small molecules, such as water and ammonia, which provide little useful structural information.

In recognition of the desirability for manipulation of the charge-state distribution of gaseous ions, various measures have been developed to alter the charges of ions formed via electrospray ionization. The manipulation of solvent and interface conditions, for example, can significantly affect the distribution of charges.[5] Methods have also been developed to manipulate the charge

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states of ions in the gas phase after, or in conjunction with, the ionization step by exposing multiply protonated ions to strong neutral bases[6] or to anions.[7,8] In the negative ion mode, the analogous ion/molecule reactions with gaseous neutral acids[9] or ion/ion reactions with positive ions[8,10] have been demonstrated. The latter approaches rely on exoergic proton- or electron-transfer reactions to reduce the charge states of ions formed via electrospray. A number of useful analytical applications have been demonstrated using these strategies for charge-state reduction.[4,10] The development of simple and efficient means for increasing the absolute charge of an ion after the ionization process, however, is a much more challenging proposition than charge-state reduction. Nevertheless, it is desirable to develop means for increasing absolute charge states, particularly for ionization methods, such as MALDI, that yield relatively low charge ions.

Increasing the absolute charge state of a gaseous cation can be accomplished by electron removal via some form of impact ionization, such as electron impact,[11] to yield multiply charged cation radicals. The protonation of a protonated molecule to yield a multiply protonated species has not been demonstrated and suffers from the disadvantage of requiring the long-range repulsive Coulomb potential to be overcome. The cross section for such a process is expected to be low even when sufficient translational energy is present. We have recently shown that it is possible to convert a singly protonated species into a doubly protonated species via two sequential ion/ion reactions involving charge inversion.[12] Each ion/ion reaction is highly exoergic and is expected to have a large cross section arising from the long-range attractive Coulomb potential. In this paper, we demonstrate the formation of a doubly deprotonated polypeptide or oligonucleotide (dianion) from their respective singly deprotonated ions via an analogous procedure. In contrast with the cation case, there is no impact ionization analogue for increasing the net negative charge of an anion.

EXPERIMENTAL SECTION

The synthetic peptide GLSDGEWQQVLNVWGK was purchased from Synpep (Dublin, CA) and the oligonucleotide 5′-d(AAAAAA)-3′ (A_6) was custom-synthesized by Integrated DNA Technologies (Coralville, IA). Piperidine, imidazole, and dendrimers were obtained from Aldrich (Milwaukee, WI). Solutions of peptide (1 mg/mL) and poly(propylenimine) (1,4-diamisobutane (DAB) core) dendrimers (generation 4) (5 mg/mL) were prepared for nanoelectrospray in aqueous 1 and 5% acetic acid for positive ions. A solution of carboxylate-terminated polyamidoamine (PAMAM) dendrimers (generation 3.5) was made in 5% NH_4OH with a concentration of 16 mg/mL for negative ions. The DNA sample was prepared in 25 mM piperidine and 25 mM imidazole solution.

The charge inversion experiments were performed on a Finnigan-MAT (San Jose, CA) ion trap mass spectrometer equipped with three independent ESI sources and ion optics that allow sequential injection of ions from any of the three sources. A full description of this instrument can be found in the literature.[13] Briefly, the peptide or DNA anions formed by ESI were introduced into the trap followed by the isolation of the singly charged anion. This ion was subjected to the proton-transfer charge inversion reactions with cations derived from DAB dendrimers from the second ESI source to give rise to the singly charged positive analogue. In the next step, anions of PAMAM dendrimers generated by the third ESI source were accumulated and stored with the singly charged positive peptide or DNA ions to undergo a second charge inversion process.

RESULTS AND DISCUSSION

The general strategy for increasing gaseous ion charge states via ion/ion proton-transfer reactions involves inversion of the singly charged ion to a singly charged ion of opposite

polarity followed by a second charge inversion step to bring the ions back to the original polarity but with at least some of them carrying a higher net charge than the original ion. In the case of a singly charged negative parent ion, the process can be represented by the following two steps:

$$(M - H)^- + (A + xH)^{x+} \rightarrow (M + mH)^{m+} + (A + (x - m - 1)H)^{(x-m-1)+} \quad (1)$$

$$(M + mH)^{m+} + (B - yH)^{y-} \rightarrow (M - nH)^{n-} + (B - (y - m - n)H)^{(y-n-m)-} \quad (2)$$

where M represents the analyte molecule of interest, A represents a reagent molecule used to form cations to transfer protons to the analyte anion(s); and B represents a reagent molecule used to form anions to abstract protons from the analyte cation(s). For the purpose of charge-state manipulation, it is important that A and B ions react exclusively via proton transfer. Other reactions, such as complex formation[14] or fragmentation, could complicate spectral interpretation and reduce the overall reaction efficiency. We have found that dendrimer cations and anions generally react predominantly or exclusively via proton transfer with polypeptide cations and anions.

Figure 1 illustrates the conversion of singly charged polypeptide anions to doubly charged anions via a sequential charge inversion process. Spectra are shown that reflect various aspects of the process. Figure 1a shows the isolated singly charged anion derived from electrospray of the peptide GLSDGEWQQVLN-VWGK. Figure 1b shows the electrospray mass spectrum of positive ions derived from electrospray of DAB dendrimer cations that were used as reagents for the first charge inversion step (i.e., they serve as the $(A + xH)^{x+}$ ions in step 1). Figure 1c shows the positive ion spectrum after the $(M - H)^-$ ions were reacted with the generation 4 DAB ions for 700 ms and after the residual DAB ions were removed from the ion trap to leave the $(M + H)^+$ ion. This ion was then subjected to reaction with anions derived from a sample of generation 3.5 PAMAM dendrimer for another 700 ms. This sample gives a broad poorly resolved signal in the negative electrospray spectrum. Data collected for earlier generation PAMAM dendrimer samples show that a complex mixture of species is observed that includes a distribution of charge states with mixtures of sodium and proton counterions as well as ions arising from synthesis failure products.[15] The resolving power of the ion trap used in these studies is insufficient to separate this mixture of species. Nevertheless, apparently all of the constituents of the PAMAM ion mixture react with the peptide cation via proton transfer because only proton-transfer products are observed. (Modifications of the equipment to allow for improved mass selection of reagent ions are planned that will minimize the background that arises from residual reagent anions.) The spectrum that resulted from the reaction of the $(M + H)^+$ ion formed in the first charge inversion step with the PAMAM dendrimer ions is shown in Figure 1d. This spectrum shows both $(M - 2H)^{2-}$ and $(M - H)^-$ ions as well as broad unresolved peaks arising from residual PAMAM dendrimer ions. Figure 1e represents the results of a control experiment in which no cations were admitted for the first charge inversion step while all other steps of the experiment were unchanged. The data of Figures 1d and 1e show that the dianions observed in (d) are formed only when the first charge inversion step takes place.

The data of Figure 1 demonstrate the net increase in negative charge arising from sequential multiple proton-transfer reactions involving charge inversion. In this case, $(M - 2H)^{2-}$ ions were formed from a singly charged anion. On the basis of signal levels for the singly charged anion prior to the two-step reaction and the signal level of the doubly charged product, the overall efficiency of the process in this case was roughly 8%. This assumes equal detector efficiency for singly charged ions and doubly charged ions. Presumably, a key criterion in

processes of this type is the presence of chemical functionalities that can accommodate the additional charge(s). In this case, the peptide contained three acidic sites (i.e., the C-terminus, one glutamic acid residue, and one aspartic acid residue). Essentially no triply charged anions were observed resulting from the sequential charge inversion process. Current efforts are directed at determining the role that the number and positions of acidic and basic sites play in charge inversion reactions of this type.

The two-step charge inversion process was also carried out with an oligonucleotide. Figure 2 demonstrates the sequential charge inversion for the homopolymer 5′-d(AAAAAA)-3′ by procedures similar to those described above. Briefly, $(A_6 - H)^-$ generated by ESI was isolated and charge inverted to produce $(A_6 + H)^+$, which underwent a second charge inversion step by reacting with PAMAM ions to give rise to $(A_6 - 2H)^{2-}$ and $(A_6 - H)^-$ in Figure 2a. The spectrum in Figure 2b was taken under conditions identical to those for the spectrum in Figure 2a except that no DAB cations were admitted into the trap. The comparison of parts a and b of Figure 2 clearly indicates the formation of doubly charged DNA anions by the two-step charge inversion process. The relatively low efficiency, ~2%, is determined, in part, by the extent to which charges are transferred between analyte and reagent anions. The relative affinities for the charge sites of the analyte and reagent play a major role in the extent to which charge transfer occurs. For this reason, it is of interest to explore further other reagent species that might improve the overall efficiency of charging. Further work along these lines with reagents of different gaseous acidities and basicities is planned.

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References

1. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Science Mass Spectrom Rev 1989;1990;2469:64–71. 37–70. [PubMed: 2675315]
2. Karas M, Hillenkamp F. Hillenkamp F, Karas M, Beavis RC, Chait BT. Anal Chem Anal Chem 1988;1991;6063:2299–2301. 1193A–1202A. [PubMed: 3239801]
3. Dongré AR, Jones JL, Somogyi Á, Wysocki VH. J Am Chem Soc 1996;118:8365–8374.
4. Reid GE, Wu J, Chrisman PA, Wells JM, McLuckey SA. Anal Chem 2001;73:3274–3281. [PubMed: 11476225]
5. Iavarone AT, Williams ER. Lavarone AT, Williams ER. Int J Mass Spectrom J Am Chem Soc 2002;2003;219125:63–72. 2319–2327.
6. McLuckey SA, Van Berkel GJ, Glish GL. Williams ER. J Am Chem Soc J Mass Spectrom 1990;1996;11231:5668–5670. 831–842.
7. Ogorzalek Loo RR, Udseth HR, Smith RD. Stephenson JL Jr, McLuckey SA. Scalf M, Westphall MS, Krause J, Kaufman SL, Smith LM. J Phys Chem J Am Chem Soc Science 1991;1996;1999;95118283:6412–6415. 7390–7397, 194–197.
8. McLuckey SA, Stephenson JL Jr. Mass Spectrom Rev 1998;17:369–407. [PubMed: 10360331]
9. McLuckey SA, Goeringer DE. Ogorzalek Loo RR, Smith RD. Anal Chem J Mass Spectrom 1995;1995;6730:2493–2497. 339–347. [PubMed: 8686879]
10. McLuckey SA, Stephenson JL Jr, O'Hair RAJ. J Am Soc Mass Spectrom 1997;8:148–154.
11. Budnik BA, Zubarev RA. Nielsen ML, Budnik BA, Haselmann KF, Zubarev RA. Chem Phys Lett Int J Mass Spectrom 2000;2003;316226:19–23. 181–187.
12. He M, McLuckey SA. J Am Chem Soc 2003;125:7756–7757. [PubMed: 12822966]
13. Badman ER, Chrisman PA, McLuckey SA. Anal Chem 2002;74:6237–6243. [PubMed: 12510744]
14. Wells JM, Chrisman PA, McLuckey SA. Wells JM, Chrisman PA, McLuckey SA. J Am Chem Soc J Am Chem Soc 2001;2003;123125:12428–12429. 7238–7249. [PubMed: 11734052]

15. He M, McLuckey SA. Rapid Commun Mass Spectrom 2004;18:960–972. [PubMed: 15116423]

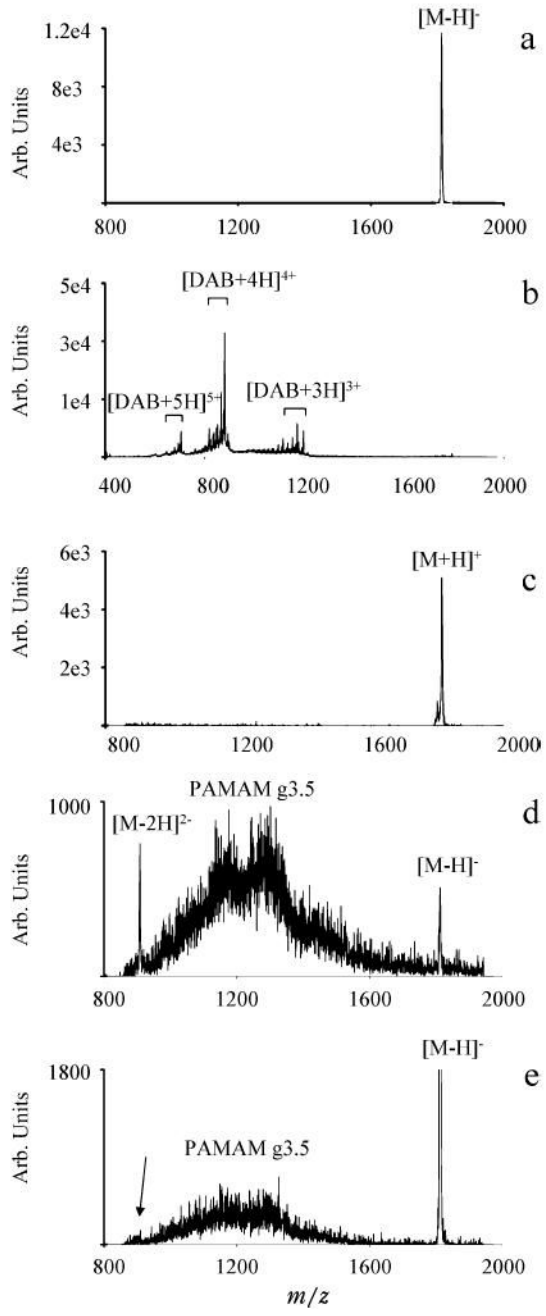

Figure 1.

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

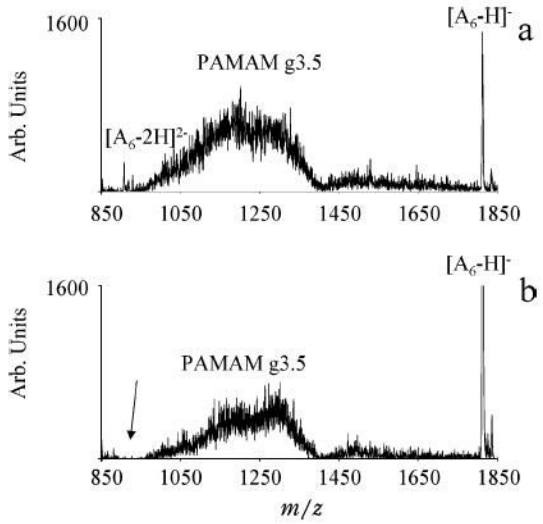

Figure 2.

(a) Negative ion mass spectrum resulting from charge inversion of the oligonucloetide $(A_6 - H)^-$ ion from reactions with DAB cations followed by charge inversion of the oligonucloetide $(A_6 + H)^+$ ion from reactions with PAMAM anions. (b) Negative ion mass spectrum resulting from the same process as that used for the spectrum of (a) except that no cations were admitted into the ion trap.