Stabilization of Anionic Adducts in Negative Ion Electrospray Mass Spectrometry

Yang Cai and Richard B. Cole*

Department of Chemistry, University of New Orleans, New Orleans, Louisiana 70148

Attachment of small anions to neutral molecules is an important ionization mechanism in negative ion electrospray mass spectrometry. In this report, the tendency for different anions to remain attached to selected analyte compound classes has been systematically investigated. A rationale for the formation and stability of preferred anionic adducts is proposed in light of thermodynamic considerations. A series of collision-induced dissociation experiments reveals that the *gas-phase basicities* of the deprotonated analyte molecule ([M − H]⁻) and the anion moiety play important roles in determining the stability of anionic adducts. Adducts of the form [M − H]⁻···H⁺···[anion]⁻ manifest increased stability when the two anions have similar gas-phase basicities. Within certain limitations, the difference in $\Delta G°$ values for proton combination with [M − H]⁻ and with [anion]⁻ can be used as a first-order predictor of adduct stability. In addition, stability increases with the rising gas-phase basicities of the two moieties. The specific interaction between a small inorganic anion (bisulfate) and a neutral analyte molecule (α-D-glucose) in the form of multiple hydrogen bonding has also been affirmed by computer modeling to contribute to the stability of some anionic adducts. Last, the gas-phase basicity of deprotonated α-D-glucose (i.e., the gas-phase acidity of α-D-glucose) is determined by a "bracketing method" to be in the range of 1373–1407 kJ/mol.

The decade of the 1990s witnessed the rapid development of electrospray mass spectrometry (ES-MS) and its wide range of applications especially in biological and related areas.[1–3] Despite the enormous growth in ES-MS applications, certain aspects of the fundamental mechanisms of ion formation in the electrospray process are not fully understood and remain under contention.[4–6] Attachment of small inorganic anions to neutral molecules is an important ionization mechanism in negative ion electrospray mass spectrometry. Although the coupling of the electrospray source

to mass spectrometry was largely established in a study by Yamashita and Fenn[7] that examined anionic species including the iodide adduct of acetone, anion attachment in negative ion ES-MS has been far less investigated than cation attachment in positive ion ES-MS. A disadvantage of negative ion ES operation is that, compared to the positive ion mode, it is more susceptible to electrical (corona) discharge[8–11] because of the relative ease in which electrons can escape from the sharp edges of the metal capillary held at high (negative) voltage. Moreover, the driving force leading to formation of anionic adducts, as well as their stabilities once formed, may be inferior to those of cationic (e.g., proton or sodium) adducts in the positive ion mode.

Addition of certain salts such as Ph₄PCl[12] to the sample solution may promote analyte adduct formation with the introduced anion, but the conductivity increase resulting from a higher salt concentration can lead to signal suppression.[13–15] Nonetheless, reports of chloride attachment to glycolipids,[16] glycerophosphocholine lipids,[17] glycosphingolipids,[18] and a noncovalent complex,[12] as well as cyanide attachment to fullerenes[19–22] have appeared in the literature.

The formation of anionic adducts in negative ion ES can be influenced by a variety of factors.[11,13–15,23,24] In addition to experimental conditions such as the capillary voltage and the bath gas composition,[11] gas-phase properties of the analyte[13,14,24] and solution-phase considerations[15,23,24] are also considered to be important.

(1) *Biochemical and Biotechnological Applications of Electrospray Mass Spectrometry*; Snyder, A. P., Ed.; ACS Symposium Series 619; American Chemical Society: Washington, DC, 1996.
(2) *Electrospray Ionization Mass Spectrometry; Fundamentals, Instrumentation & Applications*; Cole, R. B., Ed.; Wiley: New York, 1997.
(3) *Mass Spectrometry of Biological Materials*, 2nd ed.; Larsen, B. S., McEwen, C. N., Eds.; Dekker: New York, 1998.
(4) Kebarle, P. *J. Mass Spectrom* **2000**, *35* (7), 804–817.
(5) Cole, R. B. *J. Mass Spectrom* **2000**, *35* (7), 763–772.
(6) Fernandez De la Mora, J.; Van Berkel, G. J.; Enke, C. G.; Cole, R. B.; Martinez-Sanchez, M.; Fenn, J. B. *J. Mass Spectrom.* **2000**, *35* (8), 939–952.

(7) Yamashita, M.; Fenn, J. *J. Phys. Chem.* **1984**, *88*, 4671–4675.
(8) Cole, R. B.; Harrata, A. K. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 536–539.
(9) Cole, R. B.; Harrata, A. K. *J. Am. Soc. Mass. Spectrom.* **1993**, *4*, 546–556.
(10) Wampler, F. M.; Blades, A. T.; Kebarle, P. *J. Am Soc. Mass Spectrom.* **1993**, *4*, 289–295.
(11) Straub, R. F.; Voyknser, R. D. *J. Am Soc. Mass Spectrom.* **1993**, *4*, 578–587.
(12) Cheng, X.; Gao, Q.; Smith, R. D.; Simanek, E. E.; Mammen, M.; Whitesides, G. M. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 312–316.
(13) Tang, L.; Kebarle, P. *Anal. Chem.* **1991**, *63*, 2709–2715.
(14) Tang, L.; Kebarle, P. *Anal. Chem.* **1993**, *65*, 3654–3668.
(15) Wang, G.; Cole, R. B. *Anal. Chem.* **1994**, *66*, 3702–3708.
(16) Ii, T.; Ohashi, Y.; Ogawa, T.; Nagai, Y. *Org. Mass Spectrom.* **1993**, *28*, 927–928.
(17) Harrison, K. A.; Murphy, R. C. *J. Mass Spectrom* **1995**, *30*, 1772.
(18) Zhu, J.; Li, Y.-T.; Li, S.-C.; Cole, R. B. *Glycobiology* **1999**, *9* (10), 985–993.
(19) Khairallah, G.; Peel, J. B. *J. Phys. Chem. A* **1997**, *101*, 6770–6774.
(20) Khairallah, G.; Peel, J. B. *Chem. Commun. (Cambridge)* **1997**, (3), 253–254.
(21) Khairallah, G.; Peel, J. B. *Int. J. Mass Spectrom.* **2000**, *194* (2/3), 115–120.
(22) Peel, J. B. *Aust. J. Phys* **1996**, *49* (2), 435–43.
(23) Cole, R. B.; Zhu, J. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 607–611.
(24) Zhu, J.; Cole, R. B. *J. Am. Soc. Mass Spectrom* **2000**, *11* (11), 932–941.

Recent reports[24-26] have established that gas-phase processes that occur as the final solvent droplets depart are critical to determining the exact distribution of ions observed in the ES-MS spectrum. These studies indicate that gas-phase thermodynamic considerations are extremely relevant to governing where charge carriers ultimately reside. However, the gas-phase stability of the anionic adduct itself, which plays an essential role in determining the propensity for survival of anionic adduct species, has not been extensively explored. In this report, we have systematically investigated the tendency for different anions to remain attached to selected analyte compound classes. The rationale for the stabilization of preferred anionic adducts is considered in light of thermodynamic considerations and a computer modeling approach.

EXPERIMENTAL SECTION

All experiments were performed on a Quattro II triple quadrupole mass spectrometer (Micromass, Inc., Manchester, U.K.) equipped with an electrospray source. The ES capillary voltage was maintained between −2.0 and −3.0 kV. Sample solutions were introduced into the electrospray source at a flow rate of 2−4 μL/min with nitrogen as both nebulizing gas and drying gas. Low-pressure argon gas (0.1−0.2 mTorr) was used as the collision gas to achieve collision-induced dissociation (CID) under single collision conditions. Collision energies were set to nearly uniform values in the center-of-mass frame of reference (E_{com}) for all anionic adducts during all comparison studies. No correction was made for any small differences in collision cross section. All chemicals were purchased commercially and dissolved in methanol/water (9:1 v/v) without further purification. Mass spectra were acquired and processed using MassLynx software (version 3.0, Micromass).

RESULTS AND DISCUSSION

Previous ES studies[23,24,27] as well as matrix-assisted laser desorption/ionization (MALDI) studies[28,29] have indicated that anion attachment may be particularly useful for analyses involving oligosaccharides. These carbohydrate compounds are not highly acidic; hence, they may not readily form [M − H]⁻ ions in high yield, especially in the absence of a base to aid deprotonation. ES-MS signals for *low-acidity* analytes stand to improve the most via the use of the anion attachment approach, but the factors that contribute to the stability of anionic adducts are not fully understood. It is often the case that certain anionic adducts are stable only under certain conditions, such as when a low "cone voltage" is employed. In the ES source, "cone voltage" refers to the potential difference between the "cone" and the subsequently encountered "skimmer". Elevated cone voltages promote fragmentation[30] because the higher translational energy imparted to ions results in more energetic collisions with neutral gas molecules
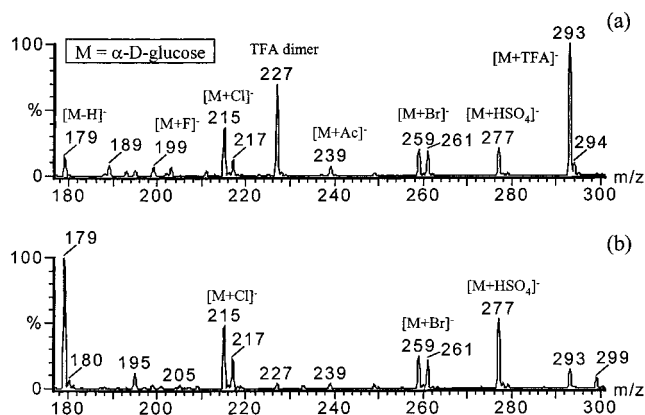

Figure 1. α-D-Glucose (1 mM) with fluoride, chloride, acetate, bromide, trifluoroacetate (TFA) (ammonium salt, 0.01 mM each), and bisulfate [(NH₄)₂SO₄, 0.01 mM] at (a) cone voltage of 8 and (b) 35 V. At high cone voltage chloride, bisulfate, and bromide adduct peaks remain strong, while fluoride, acetate, and TFA adduct peaks decrease considerably.

in a process that is often referred to as "in-source" or "nozzle−skimmer" CID. Low-energy in-source CID serves to break apart residual cluster ions, while high energies promote covalent bond cleavages. Adduct species, such as those resulting from attachment of small inorganic anions to neutral (e.g., carbohydrate) molecules, may not survive at high cone voltages when the electrostatic interaction between the ion and the neutral analyte molecule is not sufficiently strong, leading to observation of the naked anion. In other cases, the attaching anion may be lost as a neutral, taking a proton or other small cation in tow.[23,24] However, when selected α-D-glucose anionic adducts are examined, some extremely stable anionic adducts are observed to survive at high cone voltages.

The first system we examined (Figure 1) consists of 1 mM α-D-glucose and equimolar amounts (0.01 mM) of six ammonium salts: ammonium fluoride, ammonium chloride, ammonium acetate, ammonium bromide, ammonium sulfate, and ammonium trifluoroacetate (TFA). The presence of excess α-D-glucose permits all anions to form adducts with α-D-glucose, as evidenced by the fact that, at low cone voltage (8 V), all six anions, fluoride, chloride, acetate, bromide, bisulfate, and trifluoroacetate, can form clearly detectable adducts in the negative ion ES spectrum (Figure 1a). However, when the cone voltage is raised to a higher value (35 V), only chloride, bromide, and bisulfate α-D-glucose adducts survive in high yield, while the abundances of other anionic adducts decrease considerably (Figure 1b). The observation of very stable anionic adducts in the conventional ES mass spectra prompted further inquiry into gas-phase factors that promote the stability of anionic adducts.

Even though stable anionic adducts are found in the ES source, "in-source" CID is perhaps not the best tool to examine the stability of anionic adducts. The pressure of residual gas in the region between cone and skimmer in the ES source is typically about 0.01 Torr, but it is hard to monitor and control precisely. The composition of residual gas is also ambiguous and very hard to manipulate. On the other hand, CID in the central hexapole of a "triple quadrupole" mass spectrometer is a better controlled means to study the influence of gas-phase factors on the stability of anionic adducts. To achieve single-collision conditions, low-

(25) Schnier, P. D.; Gross, D. S.; Williams, E. R. *J. Am. Chem. Soc.* **1995**, *117*, 6747−6757.
(26) Amad, Ma'an. H.; Cech, N. B.; Jackson, G. S.; Enke, C. G. *J. Mass Spectrom.* **2000**, *35*, 784−789.
(27) Zhu, J.; Cole, R. B. *J. Am. Soc. Mass. Spectrom.* **2001**, *12*, 1193-1204.
(28) Wong, A. W.; Cancilla, M. T.; Voss, L. R.; Lebrilla, C. B. *Anal. Chem* **1999**, *71*, 205−211.
(29) Wong, A. W.; Wang, H.; Lebrilla, C. B. *Anal. Chem.* **2000**, *72*, 1419−1425.
(30) Wang, G.; Cole, R. B. In *Electrospray Ionization Mass Spectrometry: Fundamentals, Instrumentation & Applications*; Cole, R. B., Ed.; John Wiley & Sons: New York, 1997; pp 137−174.
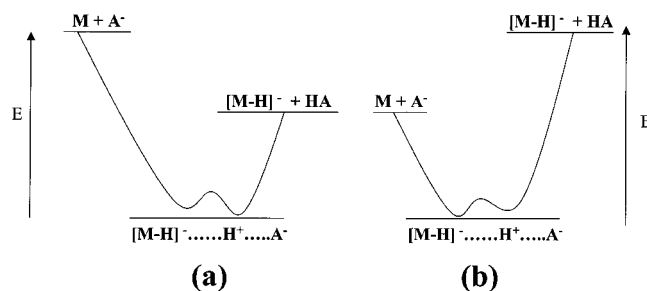
Figure 2. Decomposition of anionic adducts by CID either in the collision cell or after the cone (in-source CID). (a) Anion A^- has a higher gas-phase basicity than deprotonated analyte $[M - H]^-$, and (b) deprotonated analyte $[M - H]^-$ has a higher gas-phase basicity than anion A^-.

pressure argon (0.1–0.2 m Torr) was introduced into the central hexapole that serves as the collision cell. The pressure of argon was adjusted by a needle valve and monitored by a gauge external to the hexapole.

When sites with permanent positive charges are not present, anion attachment is favored to occur at electron-deficient atoms bound to electronegative atoms such as the hydrogens in hydroxyl groups. Adduct species of this type composed of a small inorganic anion $[A]^-$ and a neutral analyte molecule, M, can be considered to exist as "proton-bound mixed dimers of anions" of the form $[M - H]^-\cdots H^+\cdots[A]^-$. Upon low-energy collision in the gas phase, the anionic adduct undergoes simple dissociation to form $[A]^- + M$ or alternatively, $[M - H]^- + HA$. The gas-phase basicities of the anion, defined as the negative of the free energy $(-\Delta G_{A^-})$ released from the combination of an anion and a proton, will determine which low-energy decomposition pathway for the anionic adduct is favored. In the case where the small inorganic anion $[A]^-$ has a higher gas-phase basicity (i.e., larger value of $-\Delta G$) than $[M - H]^-$, then the production of deprotonated analyte molecule $[M - H]^-$ is favored (Figure 2a). In the reverse situation, the production of anion $[A]^-$ is favored (Figure 2b).

According to the "kinetic method" for thermochemical determinations,[31–34] the relative abundances I_{A^-} and $I_{[M-H]^-}$ of product ions $[A]^-$ and $[M - H]^-$, respectively, formed by competitive decompositions of $[M - H]^-\cdots H^+\cdots[A]^-$ can be determined by the following equation:[24,32,35,36]

$$\ln(I_{A^-}/I_{[M-H]^-}) = (\Delta G_{A^-} - \Delta G_{[M-H]^-})/(RT_{eff}) =$$

$$(\Delta H_{A^-} - \Delta H_{[M-H]^-})/(RT_{eff}) - (\Delta S_{A^-+M} - \Delta S_{[M-H]^-+HA})/R$$

where the ΔG and ΔH terms refer to the free energy and enthalpy changes, respectively, from the *combination* of the specified anion and a proton; ΔS terms are the entropy changes for the two *combination* pathways leading to adduct formation; and T_{eff} is the effective temperature, an expression of the internal energy of the

(31) Majumdar, T. K.; Clairet, T.; Tabet, J.-C.; Cooks, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 2897–2903.
(32) Cheng, X.; Wu, Z.; Fenselau, C. *J. Am. Chem. Soc.* **1993**, *115*, 4844–4848.
(33) Cooks, R. G.; Patrick, J. S.; Kotiaho, T.; McLuckey, S. A. *Mass Spectrom. Rev* **1994**, *13*,, 287–339.
(34) Cooks, R. G.; Wong, P. S. H. *Acc. Chem. Res.* **1998**, *31*, 379–386.
(35) Cerda, B. A.; Wesdemiotis, C. *J. Am. Chem. Soc.* **1996**, *118*, 11884–11892.
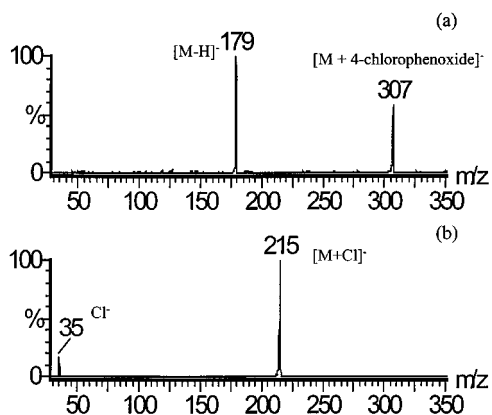(36) Cooks, R. G.; Koskinen, J. T.; Thoma, P. D. *J. Mass Spectrom.* **1999**, *34*, 85–92.

activated anionic adduct. In certain cases, i.e, when $[A]^-$ and $[M - H]^-$ have similar structures, the $\Delta(\Delta S)$ term, i.e., the difference in the entropy changes for the two combination pathways $(\Delta S_{A^-+M} - \Delta S_{[M-H]^-+HA})$, may be negligible, allowing this term to be justifiably ignored; in other cases, the magnitude of the $\Delta(\Delta S)$ term was evaluated,[24,32,35] and it was found to be a very minor contributor to the right side of the equality shown in the above equation.

To accurately treat a mixed dimer of the form $[M - H]^-\cdots H^+\cdots[A]^-$ by the kinetic method, the following assumptions must be met: (1) the reverse activation barriers for both dissociation pathways must be zero; (2) isomeric forms of the mixed dimer must not exist.

In more complicated cases, for example, when the small inorganic anion interacts with more than one hydrogen atom in a neutral analyte molecule, the energy level of such an anionic adduct ("bottom" of the wells in Figure 2) may no longer be simply described. Moreover, the activated complex may lead to two or more forms of $[M - H]^-$ that are isomeric, and their existence precludes straightforward treatment by the kinetic method.

The gas-phase basicities of certain deprotonated molecules, $[M - H]^-$, can be estimated by examining CID spectra revealing decompositions of the anionic adducts, $[M + A]^-$. If $[M - H]^-$ is the main product ion in the low-energy CID spectrum, then its gas-phase basicity is lower than that of the attaching anion. If the $[A]^-$ is the main product ion, then the gas-phase basicity of the deprotonated molecule is higher than that of the anion.[23,24] The gas-phase basicities of the anionic forms of the compounds used in this study are listed in Table 1. We have used a bracketing approach to estimate the gas-phase basicity of deprotonated α-D-glucose, a value that is not available in the literature. The gas-phase basicity of deprotonated α-D-glucose was determined to be between that of 4-chlorophenoxide (1407 kJ/mol) and chloride (1373 kJ/mol) (Figure 3).

The theory underlying the energy diagram depicted in Figure 2 leads us to anticipate that an anionic adduct will exhibit higher stability if the gas-phase basicities of the deprotonated analyte molecule and the anion are made closer to each other. When the gas-phase basicities are very different, one decomposition is necessarily quite favorable, hence, facile. This reasoning is in line with deductions resulting from equilibria measurements[37] of

Figure 3. CID spectra of (a) 4-chlorophenoxide adduct, and (b) chloride adduct of α-D-glucose. The gas-phase basicity of protonated α-D-glucose is determined to be between that of 4-chlorophenoxide (1407 kJ/mol) and chloride (1373 kJ/mol).

hydrogen bond strengths in proton-bound dimers of neutral molecules[38] and of anions.[39–41] Those studies indicated that, within a given adduct, the hydrogen bond became stronger with increasing gas-phase basicity or proton affinity of the moiety engaged in hydrogen bonding. The gas-phase stability of the anionic adduct is relevant to those studies in that hydrogen bond strengths between a chosen anion and electropositive hydrogens on a selected analyte molecule were evaluated. The authors considered either the equilibrium of formation of M···A⁻ adducts or the equilibrium of transfer of anion A⁻ between M and another neutral reference molecule. However, evaluation of the stability of the anionic adduct $[M - H]^-···H^+···[A]^-$ from the perspective of the two competing low-energy decomposition pathways was not the goal of those studies.

In the current study, we chose para-substituted phenols as the reference neutral compounds to evaluate and compare gas-phase anionic adduct stabilities in light of competing decompositions as revealed in the CID patterns of their fluoride, chloride, bromide, and bisulfate adducts. Para-substituted phenols are excellent candidates to create "proton-bound mixed dimers of anions", where the inorganic anion attaches uniformly to the phenol hydrogen, thus creating ions of the form $[phenoxide]^-···H^+···[A]^-$. The para substituent on the phenyl ring has influence on the gas-phase basicity of the phenoxide oxygen, but in the para position, it does not interact directly with the attaching anion. In this way, for a series of para-substituted phenoxides, any differences in decomposition behavior are attributable to the difference in gas-phase basicity of the phenoxide induced by the para substituent.

The first para-substituted phenol we test is 4-methylphenol (*p*-cresol); the gas-phase basicity of 4-methylphenoxide ($-\Delta G° = 1437$ kJ/mol) is between that of chloride and fluoride. From the CID spectra (Figure 4) it is evident that the 4-methylphenol fluoride adduct yields 4-methylphenoxide as the product, while chloride, bromide, and bisulfate adducts all yield the respective anion as the only readily visible product. Moreover, for this series
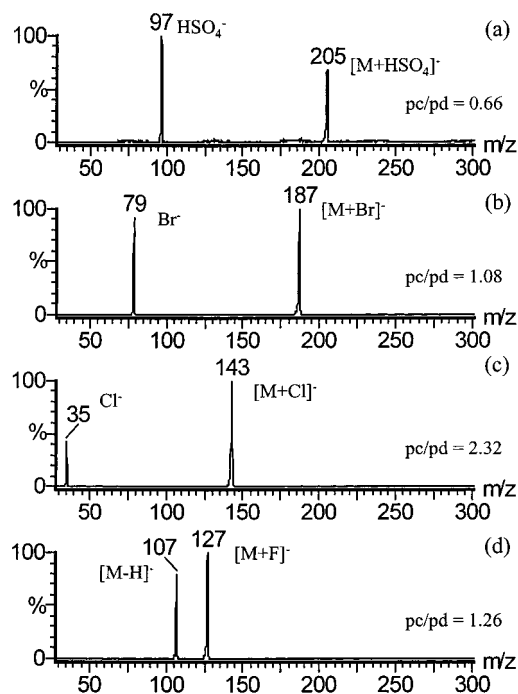
(37) Kebarle, P. *Annu. Rev. Phys. Chem.* **1977**, *28*, 445.
(38) Davidson, W. R.; Sunner, J.; Kebarle, P. *J. Am. Chem. Soc.* **1979**, *101*, 1675–1680.
(39) Larson, J. W.; McMahon, T. B. *J. Am. Chem. Soc.* **1983**, *105*, 2944–2950.
(40) Larson, J. W.; McMahon, T. B. *J. Am. Chem. Soc.* **1984**, *106*, 517–521.
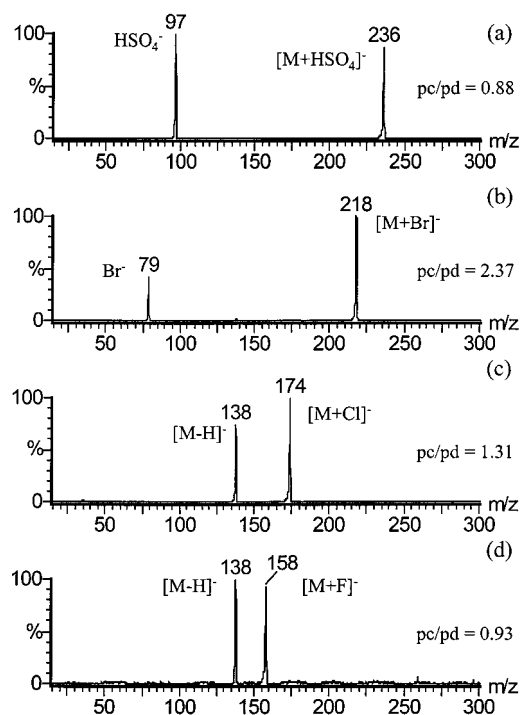(41) Paul, G. J. C.; Kebarle, P. *Can. J. Chem* **1990**, *68*, 2070–2077.

Figure 4. CID spectra of anionic adducts of 4-methylphenol (*p*-cresol) with (a) bisulfate, (b) bromide, (c) chloride, and (d) fluoride. E_{com} was set at 2.0 eV. The gas-phase basicity of 4-methylphenoxide is closest to that of chloride, and the chloride adduct is the most stable. Pc/pd is the precursor/product ion ratio.

of 4-methylphenol anionic adducts, the stability trend (as indicated by the precursor/product ion ratio at constant $E_{com} = 2$ eV) follows the order chloride adduct > bromide adduct > bisulfate adduct. This trend indicates that the probability for precursor ion survival decreases as the gas-phase basicity difference between 4-methylphenoxide and the attaching anion becomes larger. These experimental results point to the conclusion that the anionic adduct is more stable if the gas-phase basicities of the deprotonated analyte molecule and the attaching anion are closer to each other. CID spectra (Figure 5) of 4-nitrophenol anionic adducts with fluoride, chloride, bromide, and bisulfate anions echo this conclusion. In this case, bromide and bisulfate adducts of 4-nitrophenol yield the attaching anion as the dominating product ion, while fluoride and chloride adducts decompose to $[M - H]^-$. This situates the gas-phase basicity of 4-nitrophenoxide ($-\Delta G° = 1343$ kJ/mol) between that of bromide ($-\Delta G° = 1331$ kJ/mol) and chloride ($-\Delta G° = 1373$ kJ/mol). Moreover, among the attaching anions, bromide has the gas-phase basicity closest to that of 4-nitrophenoxide, and the bromide adduct is the most stable one. This reiterates the conclusion that when the gas-phase basicities of the two components in the mixed dimer are more closely matched, the adduct has higher stability.

It should be noted that when only the difference in *enthalpies* of dissociation according to the two exit channels is considered, any possible contribution to the stability of anionic adducts from the difference in the entropy changes for the two pathways is ignored, and this may not always be justifiable. However, if one can assume that the $\Delta\Delta G$ terms in the previously given equation can be approximated by $\Delta\Delta G°$ obtained from the literature, the abundances of the respective product ions I_{A^-} and $I_{[M–H]^-}$ can be approximated by the difference in gas-phase basicities of $[A]^-$

Figure 5. CID spectra of anionic adducts of 4-nitrophenol with (a) bisulfate, (b) bromide, (c) chloride, and (d) fluoride. E_{com} was set at 2.0 eV. The gas-phase basicity of 4-nitrophenoxide is closest to that of bromide, and the bromide adduct is the most stable. Pc/pd is the precursor/product ion ratio.

Figure 6. Gas-phase stability trends for CID of three different para-substituted phenol homodimers obtained at two distinct collision energies. Diamonds, 4-nitrophenol; squares, 4-chlorophenol; triangles, 4-methylphenol. Hollow symbols, $E_{com} = 1.5$ eV; solid symbols, E_{com}= 2.0 eV. The error bars indicate standard deviations calculated from at least three measurements.

$(-\Delta G^\circ_A)$ and $[M - H]^-$ $(-\Delta G^\circ_{[M-H]^-})$. The results compiled in Figures 4 and 5 indicate that a smaller difference in ΔG° values implies a higher stability for the anionic adduct. Within these limitations, the ΔG° values of $[A]^-$ and $[M - H]^-$ may be used as a first-order predictor of adduct stability.

If one considers that "evening up" the energies required to reach the two exit channels (i.e., adjusting the enthalpies) of dissociation (Figure 2) by narrowing the difference in gas-phase basicities of the two component anions can augment stability of the adduct, then one may ask, what else can be done to render the adduct even more stable? For the case where both decomposition products have roughly equivalent energies, we propose that the anionic adduct should be more stable when both attaching anions have higher gas-phase basicities. That is to say, the deeper the "well" in the energy diagram, the more stable the anionic adduct. Again para-substituted phenols are chosen to test this idea using a mixture of 4-methylphenol, 4-chlorophenol, and 4-nitrophenol, each 0.1 mM dissolved in methanol:water = 9:1 (v/v). This time, anionic adducts in the form of proton-bound *homodimers* of phenoxides (i.e., [phenoxide]$^-$⋯H$^+$⋯[phenoxide]$^-$) were selected for MS/MS experiments. The use of homodimers ensures that the reaction profiles of the two competing low-energy decomposition pathways for a given adduct are identical; i.e., the two exit channels of the energy diagram require equivalent energies. Moreover, the structural similarity of the selected homodimers in the series ensures that the influence of molecular properties on adduct stability, such as the numbers of degree of freedom, any initial internal energy difference due perhaps to variable rates of desolvation, etc., should be minor.

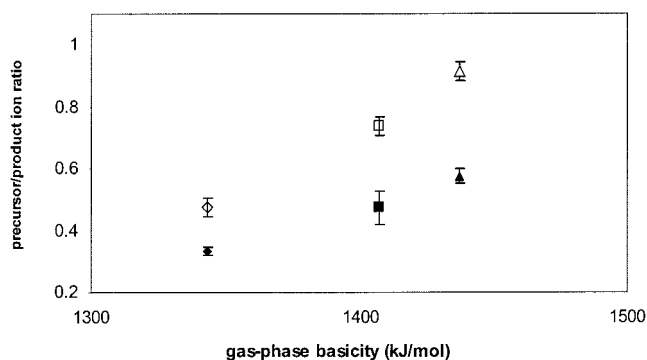The dimer/monomer ratios for low-energy collision-induced decompositions of three sets of homodimers are compared in Figure 6. Because the collision energies were set to be nearly uniform in the center-of-mass frame of reference, this dimer/monomer ratio (i.e., the precursor/product ion ratio) is a reliable indicator of adduct stability (the monomer abundance here actually is the sum of products resulting from two decomposition pathways). In each case, the two competing low-energy decomposition pathways are identical, but the differing gas-phase basicities of phenoxides employed in the three cases clearly influenced the stability of the adduct. Upon CID at either $E_{com} = 1.5$ eV or $E_{com} = 2$ eV, the stability trend for phenoxide dimers is found to be in the order, 4-methylphenoxide $(-\Delta G^\circ = 1437$ kJ/mol) > 4-chlorophenoxide $(-\Delta G^\circ = 1407$ kJ/mol) > 4-nitrophenoxide $(-\Delta G^\circ = 1343$ kJ/mol) (Figure 6). The data obtained for the homologous series of homodimers indicate that a higher gas-phase basicity (i.e., larger value of $-\Delta G^\circ$) of the anion constituting the homodimer improves the stability of the adduct; i.e., the deeper the "well", the more stable the adduct. This implies that, for the case of mixed dimers having closely matched basicities, the adduct is further stabilized when the two anions are each more strongly basic.

To situate our observations and conclusions derived from substituted phenols in a wider context, information concerning the hydrogen bond strength of proton-bound halide homodimers of the form X$^-$⋯H$^+$⋯X$^-$ was retrieved from the literature.[39,40,42−44] The hydrogen bond strength of XH⋯X$^-$ was defined as the free energy (ΔG°) required for the reaction XH⋯X$^-$ → HX + X$^-$.[42] Using gas-phase thermodynamic data found in the literature, it can be concluded that the hydrogen bond strength of XH⋯X$^-$

(42) Bartmess, J. E. Negative Ion Energies Data. In *NIST Chemistry WebBook, NIST Standard Reference Database Number 69*; Mallard, W. G., Linstrom, P. J., Eds.; National Institute of Standards and Technology: Gaithersburg MD, 20899 (http://webbook.nist.gov), February 2000.
(43) Yamdagni, R.; Kebarle, P. *Can. J. Chem.* **1974**, *52*, 2449.
(44) Caldwell, G.; Kebarle, P. *Can. J. Chem.* **1985**, *63*, 1399.
(45) Deppmeier, B. J.; Driessen, A. J.; Hehre, W. J.; Klunzinger, P. E.; Le, T.; Yu, J. PC SPARTAN Plus, Version 2.0; Wavefunction Inc.: Irvin, CA, 2000.
(46) Blondel, C.; Cacciani, P.; Delsart, C.; Trainham, R. *Phys. Rev. A* **1989**, *40*, 3698.
(47) Martin, J. D. D.; Hepburn, J. W. *J. Chem. Phys.* **1998**, *109*, 8139−8142.
(48) Fujio, M.; McIver, R. T., Jr.; Taft, R. W. *J. Am. Chem. Soc.* **1981**, *103*, 4017.
(49) Viggiano, A. A.; Henchman, M. J.; Dale, F.; Deakyne, C. A.; Paulson, J. F. *J. Am. Chem. Soc.* **1992**, *114*, 4299.
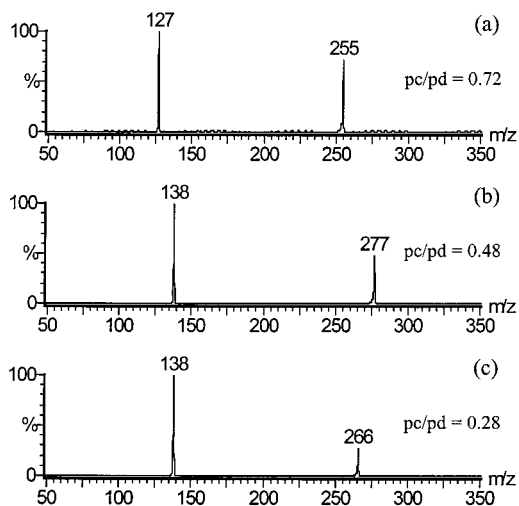(50) Cumming, J. B.; Kebarle, P. *Can. J. Chem.* **1978**, *56*, 1.

Figure 7. CID spectra of homodimers, and the mixed dimer, of 4-chlorophenoxide and 4-nitrophenoxide. (a) Precursor *m/z* 255, 4-chlorophenoxide homodimer; (b) precursor *m/z* 277, 4-nitrophenoxide homodimer; (c) precursor *m/z* 266, 4-chlorophenoxide and 4-nitrophenoxide mixed dimer. E_{com} was set at 1.5 eV. Pc/pd is the precursor/product ion ratio.

follows the trend, FH···F⁻ (134 kJ/mol[39]) > ClH···Cl⁻ (69.9 [43] or 66.9 kJ/mol[40]) > BrH···Br⁻ (58 kJ/mol[44]). This gas-phase hydrogen bond strength trend for small halide ion homodimers is a reflection of the increasing gas-phase basicity of the employed anion. This correlation is analogous to the trends observed for the much larger substituted phenoxide homodimers employed in our experiments, and it supports the conclusion that anionic adducts will be more stable if both proton-bound anions are each more basic.

For a final direct comparison of the stability of mixed dimers versus homodimers, we examined a solution containing equimolar amounts of 4-chlorophenoxide and 4-nitrophenoxide. From this solution, the two respective homodimers and the mixed dimer were observed in the conventional ES mass spectrum. The CID spectra of these three precursors obtained under identical collision gas pressure and energy (center-of-mass frame of reference, E_{com} = 1.5 eV) are shown in Figure 7. Under virtually identical conditions, the stability trend of the mixed dimer and homodimers is 4-chlorophenoxide homodimer > 4-nitrophenoxide homodimer > the mixed dimer of the two compounds. In comparing the two homodimers, the higher stability of the 4-chlorophenoxide homodimer relative to the 4-nitrophenoxide homodimer is attributed to the higher gas-phase basicity of the former. But the fact that the mixed dimer is less stable than either homodimer suggests that having closely matched energies of dissociation is even more important for stability than raising the absolute value of the gas-phase basicity of only one of the component anions. Notably, because the collision cross section decreases in moving from the 4-nitrophenoxide homodimer (Figure 7b) to the mixed dimer (Figure 7c), the slightly variable cross section can be eliminated as a contributor to the decreased stability of the mixed dimer. The above stability conclusions were affirmed in an analogous experiment employing 4-methylphenol and 4-nitrophenol, where the stability trend was found to be 4-methylphenoxide homodimer > 4-nitrophenoxide homodimer > the mixed dimer.

The gas-phase stability considerations deduced in the above CID experiments can be used to rationalize the exceptional
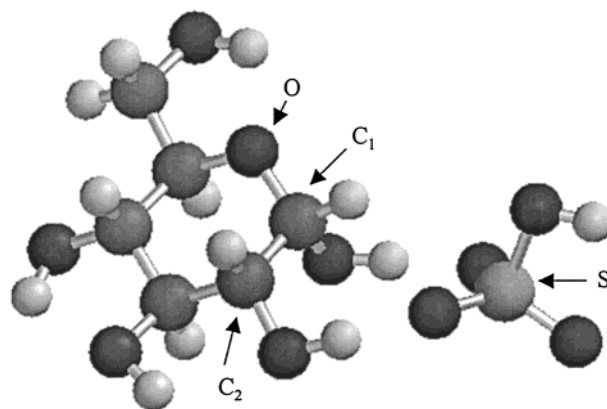

Figure 8. Gas-phase ab initio RHF 6-31+G* structure of the bisulfate adduct of α-D-glucose. Bisulfate forms hydrogen bonds with hydroxyl hydrogens on C1 (hydrogen bond length 1.705 Å) and C2 (hydrogen bond length 1.641 Å)

stability of chloride and bromide adducts of α-D-glucose shown in Figure 1b. The gas-phase basicity of α-D-glucose has been determined to be in the range of 1373−1407 kJ/mol, and it is thus closer to the gas-phase basicity of chloride (1373 kJ/mol) than to that of bromide (1332 kJ/mol). This fact contributes to the extra stability of the α-D-glucose chloride adduct over that of the α-D-glucose bromide adduct. However, the extraordinary stability of the α-D-glucose bisulfate adduct (see Figure 1) cannot be explained solely by gas-phase basicity factors, because the difference in gas-phase basicities of bisulfate and deprotonated α-D-glucose is quite large. We propose that bisulfate attachment to α-D-glucose cannot be considered as a simple proton-bound mixed dimer of anions. Instead, bisulfate may form more than one hydrogen bond with hydroxyl hydrogens on α-D-glucose, and the multiple hydrogen bonding may contribute to the extra stability of the adduct.

The gas-phase structure of the α-D-glucose bisulfate adduct has been optimized by ab initio RHF 6-31+G* calculation via PC SPARTAN Plus version 2.0.[45] The ab initio calculation started from a conformation first selected via a molecular mechanics MMFF94 conformer search that was then further optimized by semiempirical AM1 calculation. The obtained stable ab initio gas-phase structure of the α-D-glucose bisulfate adduct (Figure 8) indicates that bisulfate forms hydrogen bonds with C1 and C2 hydroxyl hydrogens. The multiple hydrogen bonding between bisulfate and α-D-glucose augments the stability of the anionic adduct. Moreover, it appears that, in this case, the contribution of multiple hydrogen bonding to the stability of the α-D-glucose bisulfate adduct even surpasses the contributions due to the gas-phase basicity factors described above. Bisulfate adducts of small saccharides have also been observed and studied in MALDI experiments.[28] Because MALDI is considered to impart a higher amount of energy to desorbed species than ES, anionic adducts are less likely to survive in MALDI. Our explanations concerning the factors that influence the gas-phase stability of anionic adducts, including both gas-phase basicity and multiple hydrogen-bonding factors, certainly also apply to gas-phase ions formed via MALDI. We propose that multiple hydrogen bonding is perhaps the key factor contributing to the success in observing bisulfate adducts of saccharides via MALDI. Investigation of the role of multiple

hydrogen bonding in forming and stabilizing anionic adducts is in progress in our laboratory.

CONCLUSION

The stabilization of anionic adducts in negative mode ES is governed by a combination of several phenomena. While the solution-phase affinity of an analyte molecule for an anion will set up an initial equilibrium prior to electrospray, gas-phase considerations will have the last word in determining adduct stability as the final solvent droplets depart. Seven sets of experiments have been conducted to elucidate the gas-phase basicity factors that influence the stability of anionic adducts of the form $[M - H]^-\cdots H^+\cdots[A]^-$. First, the stability trend of mixed dimers composed of a para-substituted phenoxide and a small inorganic anion establishes that the adduct is more stable if the gas-phase basicities of the two components in the mixed dimer are closely matched. Second, the stability trend of para-substituted phenoxide homodimers establishes that the adduct is further stabilized when the two anions are each more strongly basic. Third, by comparing these same homodimers with mixed dimers, one deduces that disrupting a balance in basicity by increasing the basicity of only one component can lower overall stability of the adduct. Moreover, computer modeling results illustrate the favorable stability of certain anionic adducts engaged in multiple hydrogen bonding. The existence of higher levels of hydrogen bonding between a neutral molecule and the attaching small inorganic anion can raise the stability of an anionic adduct even when gas-phase basicity considerations are not optimal.