The Analyst
FULL PAPER

Zwitterionic *vs.* charge-solvated structures in the binding of arginine to alkali metal ions in the gas phase

Blas A. Cerda and Chrys Wesdemiotis

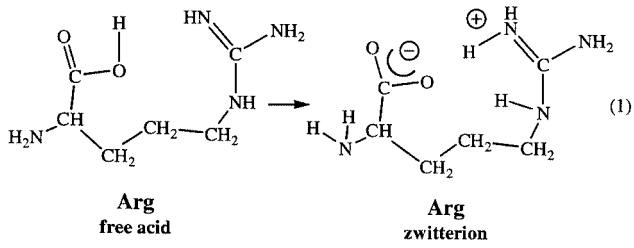
Department of Chemistry, The University of Akron, Akron, OH 44325-3601, USA.
E-mail: wesdemiotis@uakron.edu

Received 22nd November 1999, Accepted 7th February 2000
Published on the Web 8th March 2000

The relative alkali metal ion (M^+) affinities between a side-chain functionalized amino acid in its canonical form (free acid) and the corresponding methyl ester (M = Li, Na, K, Cs), and between a zwitterionic amino acid and isomeric alkyl esters (M = Na), are determined in the gas phase based on the dissociation of A_1–M^+–A_2 heterodimers, in which A_1 and A_2 represent ester and/or amino acid ligands (kinetic method). With all dimers studied, the affinities increase in the order free acid < ester < zwitterion. With this information at hand, the M^+ affinities of arginine, which may bind M^+ as a free acid or as a zwitterion, are compared with those of arginine methyl ester, which cannot form a zwitterion, and with those of the amino acid betaine, a permanent zwitterion. These experiments indicate that the Li^+ and Na^+ complexes contain the free acid form of arginine (charge solvation), whereas the complexes with the larger K^+ and Cs^+ ions contain zwitterionic arginine (salt bridge).

Introduction

It is well established that amino acids and peptides have zwitterionic structures in aqueous solution, where the separated charges can be stabilized by hydrogen bonding with water molecules. In the gas phase, zwitterions and salt bridges are less common, but can nevertheless be formed if the structural features present allow for adequate *intra*molecular solvation.[1–3] The formation of a gas phase zwitterion from an amino acid involves intramolecular deprotonation of the carboxyl group, which should be most facile with the amino acid of highest intrinsic basicity, *viz.* arginine.[4,5] However, recent IR cavity ringdown spectroscopy experiments have unambiguously shown that even arginine exists as the free acid in the gas phase [eqn. (1)].[6] The generation of zwitterionic arginine from the free acid entails replacement of the carboxyl O–H bond with a guanidinium N–H bond [eqn. (1)], a process endothermic by 363 kJ mol^{-1} according to the published gas phase acidity[7] and proton affinity[4] of arginine. The aforementioned IR results provide evidence that zwitterionic arginine is not stabilized sufficiently ($\geq$363 kJ mol^{-1}) by self-solvation in order to become the most stable conformer in the gaseous state.



(1)

The difference in thermodynamic stability between zwitterionic and free acid forms of arginine and other amino acids may be reduced or reversed by introducing extra *inter*molecular interactions. For example, theory predicts that glycine, which is not a zwitterion in the gas phase,[8–14] attains a zwitterionic conformation after the addition of just one[13] or two[12] water molecules. Counterions can also stabilize zwitterionic arrangements through the formation of salt bridges. Thus, zwitterionic [glycine + $Na]^+$ has been found computationally to be only ~10 kJ mol^{-1} less stable than the corresponding charge-solvated complex, while the isolated zwitterion is >70 kJ mol^{-1} less stable than the free acid.[12–14] In a related case, Williams and coworkers[15] showed that the proton-bound dimer of arginine contains a salt bridge in which a zwitterionic arginine interacts with a protonated arginine unit, the latter acting both as counterion and solvent. Ion mobility studies by Bowers and coworkers[16] have suggested that alkali cationized arginine also adopts a salt-bridged structure. Most recently, Williams and coworkers[17] concluded from the dissociation characteristics of [arginine + alkali metal]$^+$ complexes and parallel molecular orbital calculations that the metal ion size determines whether zwitterionic arrangements become more stable than charge-solvated structures. In the present investigation, the gas phase structures of several [arginine + $M]^+$ complexes (M = Li, Na, K, Cs) are probed by thermochemistry experiments. The kinetic method[18] is used to compare the arginine–M^+ bond energies with those between M^+ and (a) the corresponding methyl esters, which are unable to form zwitterions, and (b) the amino acid betaine, which is a permanent zwitterion. These comparisons confirm that the formation of stable salt bridges within [arginine + $M]^+$ depends on the identity of the alkali metal ion which greatly affects the ion's binding site.

Experimental

All experiments were performed with a modified Micromass AutoSpec tandem mass spectrometer of E_1BE_2 geometry.[19] Alkali metal ion-bound heterodimers of amino acids and/or their derivatives, designated as A_1–M^+–A_2 or [A_1 + $A_2]M^+$, were produced by fast atom bombardment (FAB) with 12 keV Cs^+ ions. These precursor ions were accelerated to 8 keV, mass-selected by E_1B (MS-1), and allowed to dissociate spontaneously in the field-free region between B and E_2. The fragment ions formed there were mass-analyzed by E_2 (MS-2) and recorded in the corresponding metastable ion (MI) spectra. Approximately 50–100 scans were accumulated for each [A_1 + $A_2]M^+$ precursor ion, leading to error limits of ≤10% for the relative fragment ion abundances. The amino acids, derivatives, metal salts and FAB matrices were purchased from Aldrich or

Sigma and were used as received. Samples were prepared by mixing saturated solutions (in the matrix) of the proper amino acid(s), derivative and trifluoroacetate or halide salt (source of M^+). The MI spectra were replicated in at least two different matrices, which included glycerol, 3-nitrobenzyl alcohol and monothioglycerol.

Results and discussion

The alkali metal ion affinities of selected amino acids, including arginine, and their methyl esters were evaluated by the kinetic method.[18] The use of this method in the derivation of relative and absolute metal ion binding energies has been described in detail elsewhere.[20,21] Briefly, $[A_1 + A_2]M^+$ heterodimers are generated, where A_1 and A_2 are two M^+-bound amino acids and/or derivatives, and the relative dissociation rates of the dimers into the individual metallated monomers [k_1/k_2 in eqn. (2)] are measured. For a given $[A_1 + A_2]M^+$ ion, k_1/k_2 is identical with the abundance ratio of $[A_1]M^+$ *vs.* $[A_2]M^+$ in the MI spectrum of $[A_1 + A_2]M^+$. The dissociations of eqn. (2) involve simple electrostatic bond cleavages, which generally proceed without reverse activation energy.[18,20,21] In such a case, the natural logarithm of k_1/k_2 is proportional to the difference in free energy of M^+ attachment between A_1 and A_2, $\Delta(\Delta G°_M)$, as shown in eqn. (3).[18b,21] The proportionality factor RT_{eff} contains the gas constant, *R*, and the effective temperature, T_{eff}, a measure of the decomposing dimer ions' internal energy.[22] If A_1 and A_2 have closely related structures, their entropies of M^+ attachment are usually similar, *i.e.* $\Delta(\Delta S°_M) \approx 0$,[21,23] and the experimentally measured k_1/k_2 ratio also reflects the relative M^+ affinity of A_1 and A_2, *i.e.* the difference of bond enthalpies, $\Delta(\Delta H°_M)$, between the A_1–M^+ and A_2–M^+ bonds [eqn. (3)].[18,20] This study employs *relative* affinities (in the form of the k_1/k_2 ratio) to distinguish zwitterionic from charge-solvated structures, as outlined below.

$$A_1{-}\overset{+}{M}{-}A_2 \xrightarrow{k_1} A_1{-}M^+ + A_2 \quad (2a)$$

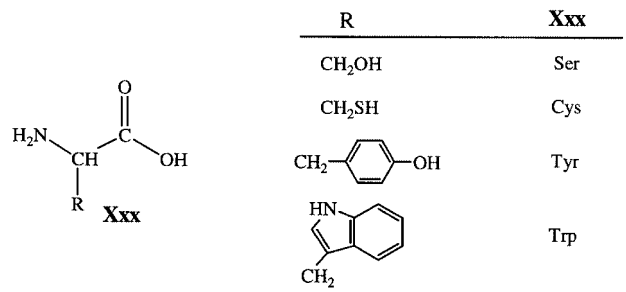
$$A_1{-}\overset{+}{M}{-}A_2 \xrightarrow{k_2} A_1 + {}^+M{-}A_2 \quad (2b)$$

$$\ln\frac{k_1}{k_2} = \frac{\Delta(\Delta G°_M)}{RT_{eff}} = \frac{\Delta(\Delta H°_M)}{RT_{eff}} \quad (3)$$

Metal ion binding to free acids *vs.* zwitterions

The alkyl ester of an amino acid is expected to display a higher M^+ affinity than the corresponding free acid if the alkyl group does not interfere with the coordination of the metal ion; in such a case, the higher affinity results from a higher inductive stabilization of the metallated complex. On the other hand, an amino acid carrying a negatively charged carboxylate group (zwitterion) should have a higher M^+ affinity than the corresponding ester due to its larger dipole moment. Thus, comparison of the alkali metal ion affinities of an amino acid and its alkyl ester should allow one to determine whether the corresponding [amino acid + $M]^+$ complex involves a salt bridge (*i.e.* the zwitterionic amino acid) or just charge solvation (*i.e.* the free acid or "canonical" form of the amino acid). To test this hypothesis, the M^+ affinity order free acid < alkyl ester < carboxylate (zwitterion) must first be established for relevantly structured amino acids.

The side-chain functionalized amino acids serine, cysteine, tyrosine and tryptophan, which have computationally been shown to exist as free acids in their complexes with Na^+ or K^+



ions,[24,25] are suitable models for a canonical amino acid. The relative M^+ affinities (M = Li, Na, K, Cs) of these amino acids (Xxx) *vs.* the corresponding methyl esters ($XxxOCH_3$), determined from the MI spectra of [Xxx + XxxOMe]M^+ dimers, are given in Table 1 and a representative MI spectrum is depicted in Fig. 1. With all four amino acids, [XxxOMe]M^+ is more abundant than [Xxx]M^+ indicating that, irrespective of the metal ion, XxxOMe has a higher M^+ affinity than Xxx. These data confirm that the M^+ affinity order free acid < alkyl ester is valid for amino acids with functional side chains. Chain substituents generally participate in the coordination of metal ions.[24–26] When not present (as in aliphatic amino acids), the carboxyl OH group may play a more important role in the stabilization of the metallated complex;[12–14] under these circumstances, esterification may disrupt important hydrogen bonds or introduce steric hindrance, which could in turn lead to a lower M^+ affinity for the alkyl ester *vis-à-vis* the free amino acid.

The order between alkyl ester and carboxylate is determined from the Na^+ affinities of the isomeric molecules β-alanine ethyl ester (β-AlaOEt), sarcosine ethyl ester (SarOEt) and betaine (Bet), relative to anchor glycylalanine (GlyAla); the MI spectra of the corresponding Na^+-bound heterodimers are displayed in Fig. 2. The abundance ratios of the sodiated monomers in these spectra indicate that both β-AlaOEt and SarOEt have lower Na^+ affinities than GlyAla, while Bet exhibits a higher Na^+ affinity than GlyAla. This ranking clearly establishes the affinity order alkyl ester < carboxylate.

Metal ion binding to arginine

Although Arg is not a zwitterion in the gas phase,[6] theory predicts that the energy levels of zwitterionic and canonical conformers of this amino acid differ by only a few kJ mol^{-1};[15]

Table 1 Relative monomer ion abundances ([Xxx]M^+ *vs.* [XxxOMe]M^+) in the MI spectra of [Xxx + XxxOMe]M^+ heterodimers. A higher abundance represents a higher M^+ affinity

Cation	Ser:SerOMe	Cys:CysOMe	Tyr:TyrOMe	Trp:TrpOMe
Li^+	1:43	1:18	1:8	1:13
Na^+	1:33	1:15	1:8	1:10
K^+	1:23	1:12	1:5	1:7
Cs^+	1:8	1:3	1:8	1:6

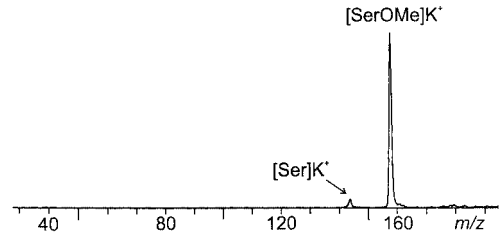
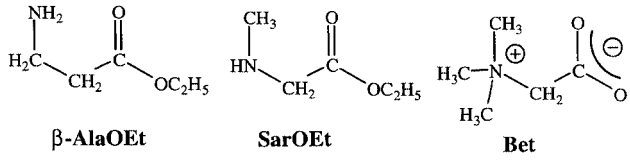


Fig. 1 MI spectrum of the K^+-bound heterodimer of Ser and SerOMe, [Ser + SerOMe]K^+.



for glycine, this difference is >70 kJ mol^{-1}.[12–14] Therefore, compared to Gly, Arg should have a much higher tendency for forming salt-bridged M^+ complexes. The competing dissociations of metastable [Arg + ArgOMe]M^+ (Table 2) indicate that the relative affinities of Arg and ArgOMe depend on the identity of the metal ion. Heterodimers with Li^+ and Na^+ produce more [ArgOMe]M^+ than [Arg]M^+, while those with K^+ and Cs^+ lead to more abundant [Arg]M^+ than [ArgOMe]M^+ (two representative spectra are included in Fig. 3). These findings are consistent with ArgOMe having a higher Li^+ and Na^+ affinity but a lower K^+ and Cs^+ affinity than Arg. Since affinities increase in the order free acid < methyl ester < zwitterion (*vide supra*), the observed trends agree well with the presence of canonical

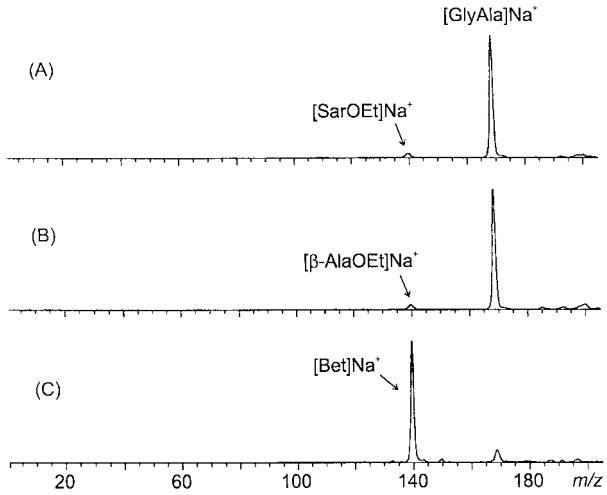


Fig. 2 MI spectra of the Na^+-bound heterodimers of dipeptide GlyAla and the isomeric amino acid derivatives SarOEt, β-AlaOEt and Bet: (A) [SarOEt + GlyAla]Na^+; (B) [β-AlaOEt + GlyAla]Na^+; (C) [Bet + GlyAla]Na^+. The abundance ratios of [isomer]Na^+ *vs.* [GlyAla]Na^+ are (A) 1:36, (B) 1:33 and (C) 10:1.

Table 2 Relative monomer ion abundances ([A_1]M^+ *vs.* [A_2]M^+) resulting from the MI dissociations of [A_1 + A_2]M^+ heterodimers containing arginine, its methyl ester or betaine. A higher abundance represents a higher M^+ affinity

Cation	Arg:ArgOMe	Arg:Bet	ArgOMe:Bet
Li^+	1:9	1:5	1:3
Na^+	1:2	1:7	1:2
K^+	9:1	4:1	1:2
Cs^+	5:1	2:1	1:2

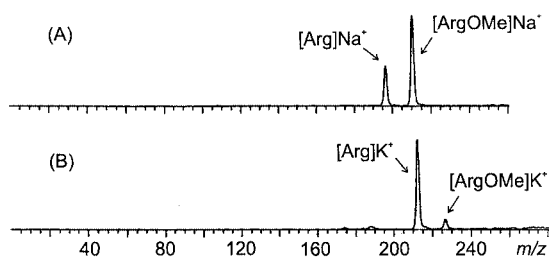


Fig. 3 MI spectra of the Na^+- (A) and K^+-bound (B) heterodimers of Arg and ArgOMe. (A) [Arg + ArgOMe]Na^+ and (B) [Arg + ArgOMe]K^+.

arginine in the Li^+ and Na^+ complexes (charge solvation) but of zwitterionic arginine in the K^+ and Cs^+ complexes (salt bridges).

It could be argued that the decrease in relative K^+ and Cs^+ affinity from Arg to $ArgOCH_3$ is not caused by the presence of salt bridges in [Arg]K^+ and [Arg]Cs^+, but to different metal ion coordination environments in the Li^+/Na^+ *vs.* K^+/Cs^+ complexes. K^+ and Cs^+ may still bind to the free acid, but in closer proximity to the carboxyl moiety; the methyl group of the ester could then hinder binding interactions or auxiliary hydrogen bonds, leading to lower binding affinities for the ester *vis-à-vis* the free acid. The likelihood of this scenario can be gauged by the behavior of tryptophan, one of the model amino acids used to determine the free acid *vs.* ester affinity order (*vide supra*). Density functional theory (DFT) calculations by Ryzhov *et al.*[25] indicate that the Na^+ and K^+ complexes have different geometries. Both metal ions are coordinated by the π-electrons of the aromatic substituent and the carbonyl oxygen; Na^+ (but not K^+) also binds to the amino nitrogen, whereas K^+ interacts weakly with the oxygen of the carboxyl OH group which additionally forms a hydrogen bond to the amino group. Despite these differences in binding sites, esterification leads to increases in both Na^+ and K^+ affinity of Trp (Table 1). In fact, all four model amino acids studied, which generally favor charge solvation (*vide supra*), follow the affinity order free acid < methyl ester (Table 1). Based on these results, it is improbable that the lower Li^+/Na^+ but higher K^+/Cs^+ affinities of Arg *vs.* $ArgOCH_3$ originate just from differences in how canonical arginine solvates Li^+/Na^+ *vs.* K^+/Cs^+.

Corroborating evidence for the involvement of zwitterionic arginine in its K^+ and Cs^+ complexes was sought by measuring the M^+ affinities of Arg and ArgOMe *vis-à-vis* betaine (a permanent zwitterion). The MI decompositions of [ArgOMe + Bet]M^+ dimers produce more [Bet]M^+ than [ArgOMe]M^+ for all four metal ions (Table 2). Thus, the carboxylate group of betaine binds all M^+ stronger than the naturally canonical ArgOMe ligand. Since the free acid isomers of amino acids with functional side chains form weaker bonds to metal ions than the corresponding esters (Table 1), betaine must also be a superior M^+ ligand compared to canonical arginine. On the other hand, betaine should have a lower M^+ affinity than zwitterionic arginine which, as a larger, more flexible and more functionalized zwitterion, allows for a better sequestration of M^+ as well as of self-solvation of the resulting complex *via* intramolecular hydrogen bonding (*vide infra*). These expectations are verified when the M^+ affinities of Arg are measured relative to betaine (Table 2); [Arg + Bet]M^+ dimer ions decompose to yield predominantly [Bet]M^+ with Li^+ and Na^+ but [Arg]M^+ with K^+ and Cs^+. The lower Li^+ and Na^+ affinities of Arg *vs.* Bet are consistent with charge solvation (*i.e.* canonical arginine) in [Arg]Li^+ and [Arg]Na^+; analogously, the higher K^+ and Cs^+ affinities of Arg *vs.* Bet agree well with salt-bridged structures in [Arg]K^+ and [Arg]Cs^+, as concluded above from the relative affinities between Arg and ArgOMe.

Computationally predicted structures of [Arg]M^+

In a recent study, Williams and coworkers[17] examined the structures of [Arg]M^+ complexes (M = Li, Na, K, Rb, Cs) by density functional theory at the B3LYP/LACVP** level. After considering hundreds of conformers, these investigators showed that the lowest energy [Arg]M^+ complexes contain canonical Arg (charge solvation) if M = Li and zwitterionic Arg (salt bridge) if M = K, Rb or Cs; for M = Na, charge-solvated and salt-bridged structures were of essentially the same energy (Fig. 4). Parallel low-energy collisionally activated dissociation and thermal radiative dissociation experiments by the same research group[17] showed that [Arg]M^+ decomposes *via* loss of water if M = Li and Na, while ammonia loss takes

place if M = K, Rb and Cs. The theoretical and experimental data were interpreted as indicative of charge solvation in $[Arg]Li^+$ and $[Arg]Na^+$ and zwitterionic structures for all other complexes, in excellent agreement with our results from thermochemistry experiments.

Conclusions

The canonical forms of side-chain functionalized amino acids, their methyl esters and zwitterionic amino acids of comparable size have detectably different alkali metal ion affinities, which increase in the order free acid < ester < zwitterion. This thermochemical characteristic has been exploited here to determine the structures of the M^+ adducts of arginine based on the relative M^+ affinities of Arg (which can adopt either a canonical or zwitterionic structure) *vs*. ArgOMe (no zwitterion possible) and Arg *vs*. Bet (a natural zwitterion). These comparisons reveal that $[Arg]Li^+$ and $[Arg]Na^+$ contain the free acid form of arginine, *i.e*. they have charge-solvated structures, and that $[Arg]K^+$ and $[Arg]Cs^+$ contain zwitterionic arginine, *i.e*. a salt-bridged structure.

The computationally predicted structures of $[Arg]M^+$ reveal that charge solvation provides a larger number of ligands to the metal ion than alternative zwitterionic conformations, *cf*. **I** *vs*. **II** and **III** in Fig. 4.[17] The preference of the larger alkali metal ions (K^+/Cs^+) to form salt bridges presumably arises from their lower tendency for intramolecular solvation, a result of their lower charge densities and higher polarizabilities due to their larger sizes. Further, large alkali metal ions can interact simultaneously with both O-atoms of a carboxylate (*cf*. **III**), thereby optimizing stabilizing anion–cation attractive forces and, hence, a salt bridge. Conversely, the smaller alkali metal ions (Li^+/Na^+), which have high charge densities, seek to maximize the solvent shell (and, hence, electron density) around them; this is best achieved by charge solvation (*i.e*. electrostatic interactions), as anionic charges closely packed with other basic sites would lead to destabilizing repulsion forces.

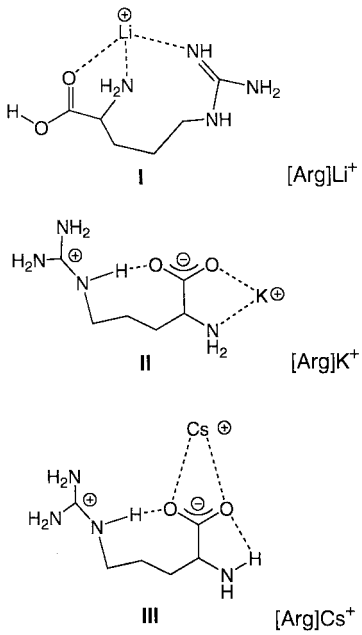


Fig. 4 Computationally predicted most stable structures of $[Arg]Li^+$ (**I**), $[Arg]K^+$ (**II**) and $[Arg]Cs^+$ (**III**), adapted from ref. 17. Structures **II** for $[Arg]Cs^+$ and **III** for $[Arg]K^+$ are only ≤ 1 kJ mol^{-1} higher in energy than the structures shown. For $[Arg]Na^+$, the energy difference between conformers **II** (most stable) and **I** is also very small (< 3 kJ mol^{-1}).

Acknowledgements

Support for this work was obtained from the Ohio Board of Regents and the University of Akron. We thank Dr Michael J. Polce for stimulating discussions and helpful suggestions.

References

1 S. Campbell, M. T. Rodgers, E. M. Marzluff and J. L. Beauchamp, *J. Am. Chem. Soc.*, 1995, **117**, 12840.
2 P. D. Schnier, W. D. Price, R. A. Jockusch and E. R. Williams, *J. Am. Chem. Soc.*, 1996, **118**, 7118.
3 S. G. Summerfield, A. Whiting and S. J. Gaskell, *Int. J. Mass Spectrom. Ion Processes*, 1997, **162**, 149.
4 Z. Wu and C. Fenselau, *Rapid Commun. Mass Spectrom.*, 1992, **6**, 403.
5 E. P. Hunter and S. G. Lias, in *NIST Chemistry WebBook, NIST Standard Reference Database Number 69*, ed. W. G. Mallard and P. J. Linstrom, National Institute of Standards and Technology, Gaithersburg MD, 20899, November 1998 (*http://webbook.nist.gov*).
6 C. J. Chapo, J. B. Paul, R. A. Provencal, K. Roth and R. J. Saykally, *J. Am. Chem. Soc.*, 1998, **120**, 12956.
7 R. A. J. O'Hair, J. H. Bowie and S. Gronert, *Int. J. Mass Spectrom. Ion Processes*, 1992, **117**, 23.
8 M. J. Locke and R. T. McIver Jr., *J. Am. Chem. Soc.*, 1983, **105**, 4226.
9 J. H. Jensen and M. S. Gordon, *J. Am. Chem. Soc.*, 1991, **113**, 7917.
10 A. G. Csaszar, *J. Am. Chem. Soc.*, 1992, **114**, 9568.
11 C. H. Hu, M. Shen and H. F. Schaefer III, *J. Am. Chem. Soc.*, 1993, **115**, 2923.
12 J. H. Jensen and M. S. Gordon, *J. Am. Chem. Soc.*, 1995, **117**, 8159.
13 Y. Ding and K. Krogh-Jespersen, *J. Comp. Chem.*, 1996, **17**, 338.
14 S. Hoyau and G. Ohanessian, *Chem. Eur. J.*, 1998, **4**, 1561.
15 W. D. Price, R. A. Jockusch and E. R. Williams, *J. Am. Chem. Soc.*, 1997, **119**, 11988.
16 T. Wyttenbach, M. Witt and M. T. Bowers, *Int. J. Mass Spectrom.*, 1999, **182/183**, 243.
17 R. A. Jockusch, W. D. Price and E. R. Williams, *J. Phys. Chem. A*, 1999, **103**, 9266.
18 (*a*) R. G. Cooks, J. S. Patrick, T. Kotiaho and S. A. McLuckey, *Mass Spectrom. Rev.*, 1994, **13**, 287. (*b*) R. G. Cooks and P. S. H. Wong, *Acc. Chem. Res.*, 1998, **31**, 379.
19 M. J. Polce, M. M. Cordero, C. Wesdemiotis and P. A. Bott, *Int. J. Mass Spectrom. Ion Processes*, 1992, **113**, 35.
20 B. A. Cerda and C. Wesdemiotis, *J. Am. Chem. Soc.*, 1995, **117**, 9734.
21 (*a*) B. A. Cerda and C. Wesdemiotis, *J. Am. Chem. Soc.*, 1996, **118**, 11884. (*b*) B. A. Cerda, S. Hoyau, G. Ohanessian and C. Wesdemiotis, *J. Am. Chem. Soc.*, 1998, **120**, 2437. (*c*) B. A. Cerda and C. Wesdemiotis, *Int. J. Mass Spectrom.*, 1999, **189**, 189.
22 (*a*) K. Vékey, *J. Mass Spectrom.*, 1996, **31**, 445. (*b*) S. L. Craig, M. Zhong, B. Choo and J. I. Brauman, *J. Phys. Chem.*, 1997, **101**, 19.
23 X. Cheng, Z. Wu and C. Fenselau, *J. Am. Chem. Soc.*, 1993, **115**, 4844.
24 S. Hoyau, K. Norrman, T. B. McMahon and G. Ohanessian, *J. Am. Chem. Soc.*, 1999, **121**, 8864.
25 V. Ryzhov, R. C. Dunbar, B. A. Cerda and C. Wesdemiotis, *J. Am. Soc. Mass Spectrom.*, submitted.
26 S. Hoyau and G. Ohanessian, *J. Am. Chem. Soc.*, 1997, **119**, 2016.

Paper a909220j