Alkali Metal Ion Binding to Amino Acids Versus Their Methyl Esters: Affinity Trends and Structural Changes in the Gas Phase

Jody M. Talley,[a] Blas A. Cerda,[a, c] Gilles Ohanessian,*[b] and Chrys Wesdemiotis*[a]

Abstract: The relative alkali metal ion (M^+) affinities (binding energies) between seventeen different amino acids (AA) and the corresponding methyl esters (AAOMe) were determined in the gas phase by the kinetic method based on the dissociation of $AA-M^+-AAOMe$ heterodimers (M = Li, Na, K, Cs). With the exception of proline, the Li^+, Na^+, and K^+ affinities of the other aliphatic amino acids increase in the order AA < AAOMe, while their Cs^+ affinities generally decrease in this direction. For aliphatic β-amino acids, which are particularly basic molecules, the order AA > AAOMe is already observed for K^+. Proline binds more strongly than its methyl ester to all M^+ except Li^+. Ab initio calculations on the M^+ complexes of alanine, β-aminoisobutyric acid, proline, glycine methyl ester, alanine methyl ester, and proline methyl ester show that their energetically most favorable complexes result from charge solvation, except for proline which forms salt bridges. The most stable mode of charge solvation depends on the ligand (AA or AAOMe) and, for AA, it gradually changes with metal ion size. Esters chelate all M^+ ions through the amine and carbonyl groups. Amino acids coordinate Li^+ and Na^+ ions through the amine and carbonyl groups as well, but K^+ and Cs^+ ions are coordinated by the O atoms of the carboxyl group. Upon consideration of these differences in favored binding geometries, the theoretically derived relative M^+ affinities between aliphatic AA and AAOMe are in good overall agreement with the above given experimental trends. The majority of side chain functionalized amino acids studied show experimentally the affinity order AA < AAOMe for all M^+ ions, which is consistent with charge solvation. Deviations are only observed with the most basic amino acids lysine and arginine, whose K^+ (for arginine) and Cs^+ complexes (for both) follow the affinity order AA > AAOMe. The latter ranking is attributed to salt bridge formation.

Keywords: alkali metals · amino acids · amino acid esters · charge solvation · kinetic method · mass spectrometry · salt bridges

Introduction

In aqueous media, all common α-amino acids are known to adopt zwitterionic structures with deprotonated carboxyl and protonated amine (or basic side chain) termini.[1] Although these zwitterions are inherently less stable than the corresponding canonical (free acid) tautomers, they interact much more strongly with water than their uncharged tautomers, thereby becoming the lowest energy structures in solution. In contrast, gas-phase α-amino acids exist as free acids because of the absence of stabilizing intermolecular interactions. This has been unequivocally shown for glycine (Gly)[2–7] and phenylalanine (Phe)[8] as well as for the most basic amino acid, arginine (Arg).[9] This indicates that the carboxyl group is not sufficiently acidic for spontaneous intramolecular deprotonation even by the quite basic guanidine group of arginine.[10]

The intrinsic stability of amino acid zwitterions is increased significantly by complexation with alkali metal ions. Thus, the glycine zwitterion is 60–80 kJ mol^{-1} less stable than the free acid according to theory;[4, 11] however, the energy difference between zwitterionic and canonical (i.e. charge-solvated) $[glycine+Na]^+$ tautomers is reduced to only 8–12 kJ mol^{-1}, primarily because of the formation of a salt bridge between the carboxylate terminus and the metal cation.[11–14] For $[arginine+alkali\ metal]^+$ complexes, the relative energy between zwitterionic and charge-solvated isomers depends on the metal ion (M^+). Density functional theory (DFT) and kinetic experiments on the dissociation of $[Arg+M]^+$ carried out by Williams et al.,[15] as well as our assessment of the $Arg-M^+$ binding energies relative to those of pertinent

[a] Dr. C. Wesdemiotis, J. M. Talley, Dr. B. A. Cerda
Department of Chemistry, The University of Akron
Akron, OH 44325-3601 (USA)
Fax: (+1) 330-972-7370
E-mail: wesdemiotis@uakron.edu

[b] Dr. G. Ohanessian
Laboratoire des Mécanismes Réactionnels
URA 1307 du CNRS
Ecole Polytechnique, 91128 Palaiseau Cedex (France)
Fax: (+33) 1-6933-3041
E-mail: gilles@dcmr.polytechnique.fr

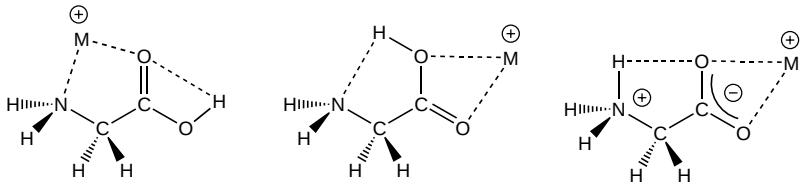
[c] Dr. B. A. Cerda
Present address: PerkinElmer Life Sciences
3985 Eastern Road, Norton, Ohio 44203-6215 (USA)

  0947-6539/02/0806-1377 $ 17.50+.50/0

zwitterionic and charge-solvated analogues,[16] showed that the smaller alkali metal ions (Li^+, Na^+) favor charge solvation by canonical Arg while the larger ions (K^+ and beyond) favor the formation of a salt bridge with zwitterionic Arg. On the other hand, ion mobility studies and parallel DFT calculations by Bowers and co-workers reached the opposite conclusion for the Na^+ and Rb^+ complexes of glycine and *C*- or *N*-methylated glycines, that is, the stability of salt bridges decreases with increasing size of the alkali metal ion.[17]

The possible structures of $[Gly+M]^+$ complexes have been the subject of several computational studies.[11–14, 17–19] The comprehensive ab initio calculations by Hoyau and Ohanessian,[13] which included all alkali metal ions, identified three low-energy isomers: two that involve charge solvation (**I** and **II**) and one (**III**) with a salt bridge. The most stable form is **I** with Li^+ and Na^+; **I** and **II** are degenerate with K^+, while **II** becomes slightly more stable than **I** with Rb^+ and Cs^+. In all cases, zwitterionic **III** is higher in energy by $\approx 6-14$ kJ mol^{-1}.[13] The investigation of *C*- and *N*-methylated glycines by Bowers et al.[17] further revealed that the stability of zwitterionic structure **III**, relative to charge-solvated structures **I** or **II**, increases almost linearly with the proton affinity (*PA*) of the amino acid. A proton affinity above ≈ 910 kJ mol^{-1} was found to result in salt bridge formation (i.e. structure **III**).[17] Based on this correlation, it was predicted which amino acids would produce zwitterionic complexes when chelating a given alkali metal ion. For example, all M^+ complexes of the aliphatic amino acids Ile and Pro, and the side chain substituted amino acids Phe, His, Lys, and Arg should be salt-bridged.[17] This prediction is, however, at odds with experimental data on lithiated and sodiated Arg (vide supra)[15, 16] and with recent ab initio and experimental studies of Phe, whose Na^+ and K^+ complexes were found to contain the free acid.[20, 21] In these latter cases, the ability of the side chain to provide an extra coordination site outweighs its proton affinity increasing effect, resulting in a higher stability for charge-solvated than zwitterionic arrangements. A similar preference for charge-solvated structures has been reported for M^+ complexes of the side chain functionalized amino acids Cys,[16, 22] Ser,[16, 22] Tyr,[16, 20, 21] and Trp,[16, 20, 21] for which no reliable prediction based on proton affinity could be made by Bowers et al.[17]

In the present investigation, the structures of the Li^+, Na^+, K^+, and Cs^+ adducts of a number of α- and β-amino acids with and without side chain substituents are probed by thermochemistry experiments and by ab initio theory. The kinetic method[23] is used to compare the $AA-M^+$ binding energies to those of the corresponding methyl esters, AAOMe, which are unable to form zwitterions. The premise of these comparisons is that the relative M^+ affinity between AA and AAOMe will reflect the binding mode in $[AA+M]^+$. If both AA and AAOMe coordinate M^+ by means of charge solvation at equivalent binding sites, the ester is expected to bind more strongly as a result of the electron-donating properties of the methyl group. In contrast, a zwitterionic AA should form a stronger bond to M^+ than (always canonical) AAOMe because of the attractive ionic interactions resulting from the formation of a salt bridge. Our measurements are complemented by theory to elucidate the lowest energy structures of the metalated acid and ester. As will be demonstrated, such computational data are essential for the interpretation of the experimental trends, which are complicated by the existence of two different isomers for charge-solvated $[AA+M]^+$ (vide supra).



I **II** **III**

Experimental Section

The kinetic method experiments involved the formation of M^+-bound heterodimers between amino acids and the corresponding methyl esters, $AA-M^+-AAOMe$, and measurement of the metastable ion (MI) and/or collisionally activated dissociation (CAD) mass spectra of these ions. If the dissociation of the dimers to the individual metalated monomers, $AA-M^+$ and $M^+-AAOMe$, proceeds with rate constants k_{AA} and k_{AAOMe}, respectively, the relative dimer dissociation rate, that is k_{AA}/k_{AAOMe}, is equal to the abundance ratio of $AA-M^+$ versus $M^+-AAOMe$ in the corresponding MI or CAD spectrum.[23–26] For dimers that dissociate with no reverse activation energies (which applies to ligand detachment from alkali metal ion complexes),[23, 25, 26] the ratio k_{AA}/k_{AAOMe} provides thermochemical information on the binding of M^+ to AA versus AAOMe [Eq. (1)].[16, 23–26]

$$\ln(k_{AA}/k_{AAOMe}) = \Delta(\Delta G_M)/RT_{eff} \approx \Delta(\Delta H_M)/RT_{eff} \quad (1)$$

In Equation (1) $\Delta(\Delta G_M)$ and $\Delta(\Delta H_M)$ are the relative M^+ basicity (free energy of binding) and M^+ affinity (binding enthalpy), respectively, between AA and AAOMe, R is the ideal gas constant, and T_{eff} is the effective temperature of the dissociating dimer ions, a proportionality constant that gauges the average internal energy of these ions but also depends on experimental conditions and the binding energy of the dimers.[23c] The relationship $\Delta(\Delta G_M) \approx \Delta(\Delta H_M)$ presupposes that the $AA-M^+$ and $M^+-AAOMe$ bonds have similar entropies, which is generally true for metal ion bonds to structurally similar ligands.[24–26]

MI and CAD spectra were acquired with a Micromass AutoSpec-Q tandem mass spectrometer of E_1BE_2hQ geometry (E/B = electric/magnetic sector, h = RF-only hexapole, Q = quadrupole mass analyzer).[27] $AA-M^+$-AAOMe heterodimers, $AA-M^+-AA$ homodimers and $AA-M^+$ monomers (also designated as $[AA+M]^+$) were generated by FAB by the use of various matrices (vide infra) and 12 keV Cs^+ ions as bombarding particles. After acceleration to 8 keV (or as stated), the desired precursor ion was mass-selected by E_1B and allowed to dissociate spontaneously or by collision with He or O_2 (80% transmittance) in the field-free region between B and E_2. The dissociation products were mass analyzed by E_2 and recorded in the corresponding MI or high energy CAD spectrum (MS^2). With a few heterodimers, low-energy CAD spectra were also acquired. For this, $AA-M^+-AAOMe$ was mass-selected by E_1BE_2 and decelerated to a kinetic energy of 5–100 eV for CAD with Ar in the hexapole collision cell; the resulting fragments were subsequently dispersed by Q scans.

Kinetic energy releases of metastably formed $AA-M^+$ and $M^+-AAOMe$ were calculated from peak widths at half height ($T_{0.5}$) by means of

  0947-6539/02/0806-1378 $ 17.50+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 6

established procedures.[28] MS^3 spectra[29] of the $AA-M^+$ fragments from $AA-M^+-AA$ homodimers were obtained by collisionally dissociating the dimer ions in the field-free region preceding E_1 (He, 80% transmittance) and transmitting the $AA-M^+$ products by proper adjustment of E_1B into the field-free region between B and E_2, where their CAD (MS^3) spectra were measured using O_2 as the collision gas (80% transmittance). The acquired MI, CAD, and MS^3 spectra combined 100–500 added scans.

Amino acids, their methyl esters, FAB matrices, and alkali metal salts were purchased from Sigma or Aldrich and were used with no further modification. Samples were prepared by mixing saturated solutions in the matrix of the proper amino acid, amino acid ester, and salt, as described in detail elsewhere.[16, 26] The kinetic measurements with AA-M^+-AAOMe heterodimers were replicated in two different FAB matrices (glycerol and nitrobenzyl alcohol) with a trifluoroacetate salt as the source of M^+. Whenever the experiment indicated a higher M^+ affinity for AA than AAOMe, which can be indicative for the presence of salt bridges (vide infra), two additional matrices (monothioglycerol and a 5:1 mixture of dithiothreitol and dithioerythritol) and an additional source of M^+ (NaI, KBr, or CsCl) were used, which resulted in at least 12 replicates for a particular dimer.

Computational methods: Ab initio calculations were performed with Gaussian 98[30] and two different basis sets, namely 6–31G* for H, C, N, O, and Na, and equivalent nonstandard bases for K and Cs (designated as basis 1); and 6–311+G(2d,2p) for H, C, N, O, and Na, and equivalent nonstandard bases for K and Cs (basis 2). [13] Selected M^+ complexes of alanine (Ala), β-aminoisobutyric acid (β-AIB), proline (Pro), glycine methyl ester (GlyOMe), alanine methyl ester (AlaOMe), and proline methyl ester (ProOMe) were geometry-optimized in structures **I**, **II**, and **III**, which were found to be the lowest energy isomers of M^+-cationized glycine and methylated glycines.[13, 17] A few additional structures were considered for glycine methyl ester (vide infra). Optimum geometries were determined at the HF/basis 1 and MP2/basis 1 levels; zero-point vibrational energies, thermal energies at 298 K and entropies at 298 K were also obtained at these levels. The MP2/basis 1 geometries were additionally energy-minimized at the MP2/basis 2 level in order to obtain more precise relative energies of the isomeric $[AA+M]^+$ or $[AAOMe+M]^+$ complexes. Metal ion binding enthalpies (ΔH_{298}, also referred to as M^+ binding energies or M^+ affinities in the text) and free energies (ΔG_{298}, M^+ basicities) of AA and AAOMe were calculated at the MP2/basis 2//MP2/basis 1 level and were subsequently corrected for basis set superposition errors (BSSE) in the full counterpoise approximation at the MP2/basis 2 level. Absolute ΔH_{298} values obtained by this procedure were shown to be in very good agreement with well-established experimental Na^+ affinities.[22] The method has further yielded a large number of relative Na^+ binding energies that agree very well with ligand-exchange equilibrium constants measured in the gas phase with a Fourier-transform ion cyclotron resonance mass spectrometer.[31]

The Li^+-bound dimer of Pro and ProOMe, a substantially more complex system, was subjected to geometry optimization only at the HF/basis 1 level to keep the computations tractable. The isomers considered involved structures **I** or **III** for the Pro ligand and **I** or **II** for the ProOMe ligand. Final energetics of the dimeric complex were obtained at the MP2/basis 2//HF/basis 1 level.

Results and Discussion

Dissociations and structures of $AA-M^+-AAOMe$ heterodimers: The MI spectra of heterodimers $AA-M^+-AAOMe$ contain dominant signals for $AA-M^+$ and $M^+-AAOMe$, as exemplified by the Cs^+-bound complex of isoleucine and its methyl ester in Figure 1a. All $AA-M^+$ and $M^+-AAOMe$ peaks have a Gaussian shape and a relatively narrow width, corresponding to kinetic energy releases ($T_{0.5}$) of 20 ± 8 meV. Such MI characteristics provide evidence that the $AA-M^+-AAOMe$ complexes dissociate without reverse activation energies,[28] as required in kinetic method applications (vide supra).[23] At the higher internal energies available with CAD, the $AA-M^+-AAOMe$ heterodimers continue to yield mainly $AA-M^+$ and $M^+-AAOMe$, which now dissociate, in part, further to M^+ (Figure 1b). This CAD fragmentation pattern agrees well with the connectivity $AA-M^+-AAOMe$, that is, the AA and AAOMe ligands are loosely bridged by the central metal ion.

An important question in our kinetic method experiments is whether steric hindrance (crowding) in the dimer ions prevents the formation of $AA-M^+$ (or $M^+-AAOMe$) monomers in their most stable structure, thereby leading to conflicting relative M^+ affinities. To address this problem, the CAD (MS^3) spectra of $[AA+M]^+$ ions generated from $AA-M^+-AA$ dimers were compared to reference CAD (MS^2) spectra of $[AA+M]^+$ ions formed by the association of AA with M^+ in the FAB ion source. Homodimers were used for improved MS^3 sensitivity because they are generated with higher intensities than heterodimers. Table 1 lists representative results for Pro and Thr, which carry aliphatic and functional side chains, respectively. In both cases, MS^3 and MS^2 spectra are identical within experimental error.[32] Hence, the $AA-M^+$ monomers emerging from the dissociating dimer ions and those formed associatively have similar structures and the second ligand in the dimer complexes does not appear to significantly alter the coordination of M^+ by AA (for example, from bidentate to monodentate). Nevertheless, subtle changes in the structure of $[AA+M]^+$ (for example, from **I** to **II** or **III**) may be induced by attaching a second polarizable ligand. Since independent reference spectra of isomers **I**, **II**, and **III** are not available for any AA, it is not known whether such changes would be detectable by CAD experiments. More information on this subject is provided by MO calculations, as discussed later.

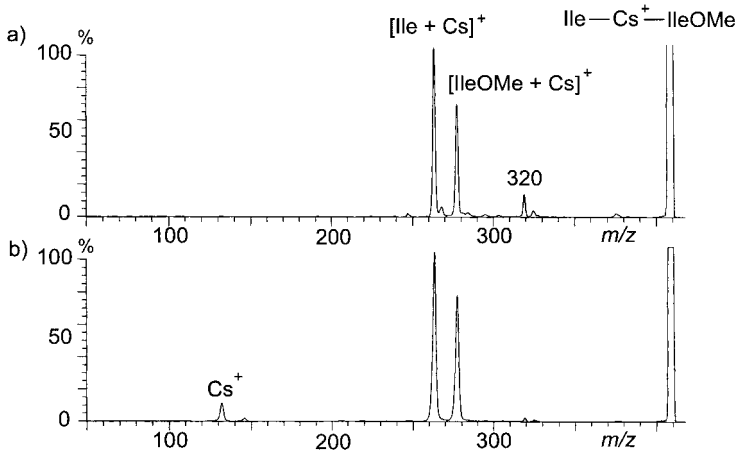


Figure 1. a) MI and b) CAD mass spectra of heterodimer $Ile-Cs^+-IleOMe$ (8.00 keV). The kinetic energies released upon the formation of $[Ile+Cs]^+$ and $[IleOMe+Cs]^+$ under MI conditions are 20 and 23 meV, respectively. The shoulder/tail on the high-mass side of the $[Ile+Cs]^+$ and $[IleOMe+Cs]^+$ signals (Figure 1a) arise from the dissociation of $Ile-Cs^+-IleOMe$ inside the mass-analyzing electric sector (E_2). The narrow peak at m/z 320 is an artifact signal from metastable ion fragmentations in the field-free region between E_1 and B.

Table 1. CAD mass spectra of AA–Na^+ ions formed by FAB ionization (MS^2) or by CAD of AA–Na^+–AA homodimers (MS^3).[a–c]

[Pro+Na]$^+$ *m/z*	*95/96*[d]	*91*	*70*	*68*	*43*
MS^2	18	13	38	17	14
MS^3	16	14	35	16	19
[Thr+Na]$^+$ *m/z*	124	98/96[d]	79	68	40
MS^2	55	31	5.0	3.3	6.1
MS^3	49	33	7.8	4.3	6.3

[a] Normalized relative intensities ([%], sum of peak heights = 100 %). Uncertainties are ± 1 % in MS^2 and ± 4 % in MS^3 mode. [b] AA–Na^+–AA were accelerated to 8.00 keV. FAB-generated AA–Na^+ were accelerated to 4.15 keV (AA = Pro) or 4.35 keV (AA = Thr), which are the kinetic energies of these ions when formed from 8.00 keV AA–Na^+–AA in the MS^3 mode. [c] The Na^+ fragment (*m/z* 23) is not included because of its low kinetic energy (≤ 0.70 keV); ions of < 1.00 keV suffer from poor transmission and collection efficiencies in our instrument.[32] [d] Unresolved.

Relative M^+ affinities between AA and AAOMe: The larger abundance of Ile–Cs^+ versus Cs^+–IleOMe in the MI spectrum of Ile–Cs^+–IleOMe (Figure 1a) indicates that Cs^+ forms a stronger bond with Ile than IleOMe. As mentioned above, the abundance ratio of AA–M^+ versus M^+–AAOMe equals the ratio of the rate constants with which these fragments are formed from the dimer, namely k_{Ile}/k_{IleOMe}, and the corresponding natural logarithm, $\ln(k_{Ile}/k_{IleOMe})$, reflects the difference in M^+ affinity (binding energy) between AA and AAOMe. Seventeen amino acids were compared this way with the corresponding methyl esters in Li^+-, Na^+-, K^+-, and Cs^+-bound heterodimers. The relative M^+ affinities obtained from these experiments are presented in Table 2 in form of $\ln(k_{AA}/k_{AAOMe})$ ratios;[33] a negative value indicates a higher M^+ binding energy to the ester than the amino acid and vice versa.

Table 2. Relative M^+ affinities between amino acids (AA) and their methyl esters (AAOMe), $\ln(k_{AA}/k_{AAOMe})$,[a] obtained from the dissociation of metastable AA–M^+–AAOMe heterodimers.

Amino acid AA	*PA*(AA)[b] [kJ mol^{-1}]	$\ln(k_{AA}/k_{AAOMe})$[a] Li^+	Na^+	K^+	Cs^+
aliphatic amino acids					
glycine (Gly)	887	−3.95	−3.58	−2.72	−0.34
alanine (Ala)	902	−3.52	−3.08	−2.13	+0.34
valine (Val)	911	−2.99	−2.56	−1.50	+0.34
leucine (Leu)	915	−2.88	−2.42	−1.36	+0.40
isoleucine (Ile)	917	−2.77	−2.27	−1.16	+0.41
β-alanine (β-Ala)	927[c]	−3.08	−2.04	+1.28	+2.82
β-aminoisobutyric acid (β-AIB)	933[d]	−2.79	−1.22	+2.10	+1.71
proline (Pro)	921	−1.77	+0.79	+1.76	+1.98
amino acids with functional side chains					
serine (Ser)[e]	915	−3.77	−3.51	−3.14	−2.12
threonine (Thr)	923	−3.39	−3.33	−3.19	−2.59
cysteine (Cys)[e]	903	−2.87	−2.67	−2.49	−0.92
methionine (Met)	935	−2.88	−2.62	−2.12	−1.36
tyrosine (Tyr)[e]	926	−2.10	−2.03	−1.57	−2.05
tryptophane (Trp)[e]	949	−2.54	−2.30	−1.87	−1.74
histidine (His)	988	−2.71	−2.38	−1.82	−0.79
lysine (Lys)	996	−2.85	−2.23	−0.74	+0.41
arginine (Arg)[e]	1051	−2.20	−0.69	+2.20	+1.61

[a] $\ln(k_{AA}/k_{AAOMe}) \approx [\Delta H_M(AA) - \Delta H_M(AAOMe)]/RT_{eff}$, where ΔH_M is the affinity of the species following in parenthesis (see text); the uncertainties are ± 0.05 at constant experimental conditions and ± 0.20 within months. [b] From Ref. [10], unless noted otherwise. [c] Ref. [33]. [d] Estimated as $PA(\beta\text{-Ala}) + PA(n\text{-propylamine}) - PA(\text{ethylamine})$.[10, 33]

A number of the AA–M^+–AAOMe complexes were subjected to CAD. The abundance ratio [AA–M^+]:[M^+–AAOMe] slightly changes from MI to CAD conditions, as a consequence of the higher effective temperature of collisionally activated dimer ions;[23] however, there are no ratio reversals upon CAD, as illustrated in Figure 1b for Ile–Cs^+–IleOMe. Inversion of the order of relative affinities would have been indicative of a significant difference in the entropies of the bonds compared to the metal-ion bound dimer.[25] Entropy effects upon dissociation of AA–M^+–AAOMe dimers could be appreciable if AA and AAOMe interact differently with M^+, that is the former with a salt bridge and the latter with charge solvation. Theory predicts salt bridge bonding in all Pro–M^+ complexes (vide infra). For this reason, the relative entropy between the Pro–M^+ and M^+–ProOMe bonds was examined more carefully by monitoring the $\ln(k_{Pro}/k_{ProOMe})$ ratio (equal to the logarithm of [Pro–M^+]:[M^+–ProOMe]) as a function of the center-of-mass collision energy (E_{CM}) under low-energy CAD conditions. Increasing E_{CM} increases T_{eff} in Equation (1). Since $\Delta(\Delta H_M)$ and R are constant for a given Pro–M^+–ProOMe dimer, $\ln(k_{Pro}/k_{ProOMe})$ should approach zero as E_{CM} becomes larger, if $\Delta(\Delta G_M) \approx \Delta(\Delta H_M)$, that is, if Pro and ProOMe have similar M^+ binding entropies.[34] Otherwise, $\Delta(\Delta G_M)$ would change with T_{eff}, causing $\ln(k_{Pro}/k_{ProOMe})$ to either cross the zero line or continually increase (if positive) or decrease (if negative), depending on the magnitude of the Pro–M^+ and M^+–ProOMe bond entropies. Figure 2 shows

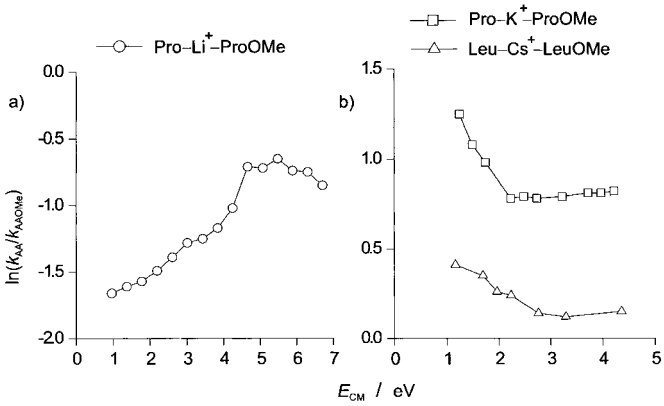


Figure 2. Logarithm of the [AA–M^+]:[M^+–AAOMe] branching ratio, which is equal to $\ln(k_{AA}/k_{AAOMe})$, plotted against the center-of-mass collision energy (E_{CM}) in low-energy CAD spectra of heterodimers AA–M^+–AAOMe (± 0.05). a) Pro–Li^+–ProOMe, b) Pro–K^+–ProOMe and Leu–Cs^+–LeuOMe.

the results obtained for Li^+- and K^+-bound dimers, for which the relative M^+ affinity is negative and positive, respectively (Table 2). In both cases, the $\ln(k_{Pro}/k_{ProOMe})$ term moves in the expected direction. The theoretically expected convergence to zero stops at the higher energies examined, presumably because the E_{CM} fraction transferred to internal energy falls as E_{CM} is raised;[29] nonetheless, the $\ln(k_{Pro}/k_{ProOMe})$ ratios level out with rising E_{CM} *without* crossing the zero line. This is also true for the Cs^+-bound dimer of Leu and LeuOMe (included in Figure 2), where the branching ratio is closer to unity

0947-6539/02/0806-1380 $ 17.50+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 6

(Table 2) and, hence, particularly sensitive to a significant difference in the Leu – M^+ and M^+ – LeuOMe bond entropies. Our combined high-energy (Figure 1b) and low-energy (Figure 2) CAD results verify that the entropies of M^+ attachment to AA and $AAOCH_3$ are similar, as assumed above and corroborated by theory (vide infra).

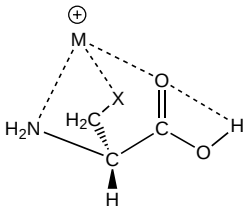
Alkyl esters are stronger Lewis bases than carboxylic acids; this is revealed, inter alia, by their larger proton affinities.[10] Converting the COOH group of a canonical amino acid to $COOCH_3$ would thus increase the M^+ affinity, provided the new methyl group does not interfere with the coordination of M^+ (vide infra). For the majority of systems studied (Table 2), all or most metal ions indeed form stronger bonds with AAOMe than AA, supporting the presence of a COOH group (i.e. charge solvation) in the respective $[AA+M]^+$ complexes. Hence, the experimental data are consistent with charge solvation as the predominant mode of metal ion coordination by gaseous amino acids.

Amino acids with functionalized side chains: Besides the two most basic amino acids (Lys and Arg) the other side chain functionalized amino acids studied (Ser, Thr, Cys, Met, Tyr, Trp, and His) coordinate all M^+ ions by charge solvation, which is evident from the affinity increases observed upon esterification (Table 2). This finding agrees well with calculations for sodiated Ser,[22] Cys,[22] Phe,[20] Tyr,[20] and Trp,[20] as well as potassiated Phe,[20] Tyr,[20] and Trp.[20] The lowest energy structures of these complexes are predicted to involve tridentate charge solvation by the amino, carbonyl, and side chain groups (**IV**; X represents a functional group),[20, 22] except for $[Trp+K]^+$, where a bidentate coordination by the aromatic and carbonyl groups and weak interactions between the metal ion and the OH group (**V**) is slightly more stable (by $\approx 1\ kJ\,mol^{-1}$).[20] Structures **IV** and **V** also account for the affinity trends of the Thr, Met, and His complexes, for which computational data are not available.

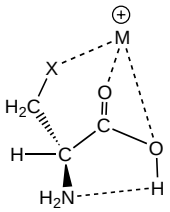
Concerning arginine, the DFT calculations of $[Arg+M]^+$ carried out by Williams et al. have indicated that zwitterionic geometries become more energetically favorable with increasing size of the metal ion.[15] The most stable structure was found to change from a charge-solvated complex for Li^+ (similar to **IV**) to salt-bridged complexes for K^+ and Cs^+ ions (similar to **VI**) with the cross-over occurring with Na^+ for which both types of coordination are very similar in energy.[15] The larger dipole moments of zwitterions, compared to those of canonical analogues, enable them to form stronger bonds with alkali metal ions, as recently shown for betaine (a naturally zwitterionic amino acid) and isomeric nonzwitterionic esters.[16] The higher K^+ and Cs^+ affinities of Arg versus ArgOMe (Table 2) thus indicate that $[Arg+K]^+$ and $[Arg+Cs]^+$ contain salt bridges, in agreement with the theoretical prediction. Conversely, the negative relative affinity between Arg and ArgOMe for the corresponding Li^+ and Na^+ complexes (Table 2) is characteristic for charge solvation in $[Arg+Li]^+$ and $[Arg+Na]^+$; this is also in agreement with the theoretical prediction. For lysine, another basic amino acid with a long, flexible side chain, the relative M^+ affinity versus LysOMe changes from negative to positive first with Cs^+ (Table 2); the later cross-over suggests a lower stability for salt bridges with Lys than Arg, probably because of the lower proton affinity of Lys.[10]

Aliphatic α- and β-amino acids: All Gly – M^+ affinities as well as the Li^+ affinities of all, and the Na^+ and K^+ affinities of most aliphatic AA molecules are smaller than those of the corresponding esters, in agreement with charge solvation in the corresponding $[AA+M]^+$ complexes. In several cases, however, AA is found to bind M^+ more strongly than AAOMe. This is true for all cesiated amino acids except Gly, for potassiated β-alanine and β-aminoisobutyric acid, and for sodiated and potassiated proline (Table 2). The presence of the zwitterionic AA tautomer in these complexes could account for the higher relative affinities against AAOMe, as explained above for arginine. Without supporting theoretical data, similar to those for Arg complexes, alternative scenarios are possible.

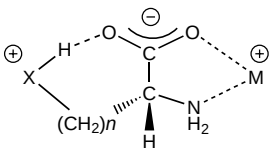
We indicated above that a lower M^+ affinity for canonical AA than for AAOMe is expected if the ester methyl group does not interfere with the coordination of the metal ion; in addition, the methyl group should not obstruct any important stabilizing interactions in the metalated complex. Inspection of structures **I** and **II** as well as **IV** and **V** reveals that esterification would disable hydrogen bonding. With functionalized amino acids, where the side chain substituent is a significant contributor to the binding of M^+ (cf. **IV** and **V**), the removal of these H bonds appears to be compensated by the higher intrinsic basicity of esters versus free acids (vide supra), leading to the affinity order ester > free acid. With aliphatic amino acids (cf. **I** and **II**), the H bonds are a more important stabilizing element of the M^+ complexes and their removal upon esterification could affect the stability of $[AAOMe+M]^+$ to the point that the affinity order reverses to free acid > ester. Isomer **II**, which contains a more stable H bond than **I**, would be most severely destabilized upon esterification.[17] Consequently, the positive relative affinities observed between aliphatic AA and AAOMe (Table 2) cannot be interpreted unequivocally. This impasse is largely alleviated by the computational data discussed below.



IV



V



VI

Ab initio calculations: Several M^+ complexes of three representative amino acids and the methyl esters of glycine and proline were investigated computationally. The AA molecules chosen include alanine (Ala), the simplest amino acid whose relative affinity com-

pared to the corresponding ester changes sign as the size of M^+ increases, β-aminoisobutyric acid (β-AIB), the most basic of the aliphatic amino acids studied, and proline, a unique amino acid bearing a cyclic secondary amine and showing a higher M^+ affinity than ProOMe for all M^+ ions except Li^+ (Table 2). The study of a β-amino acid further allows us to elucidate the effect of a longer chain that can chelate M^+ or form an H bond over a larger ring than the chain of an α-amino acid. Each complex was optimized in structures **I**, **II**, and **III**, which have been identified as the lowest energy isomers of alkali metal ion cationized glycine in earlier work.[13, 17] Additionally, calculations were performed on the dimer Pro – Li^+ – ProOMe in order to assess how a second M^+ ligand (AAOMe) affects the relative stabilities of the various $[AA+M]^+$ isomers. The results of the calculations are summarized in Tables 3 – 9.

Alanine complexes: For $[Ala+Na]^+$, charge-solvated isomer **I** and zwitterionic isomer **III** are essentially degenerate, while isomer **II** is significantly higher in energy (cf. ΔE_e entries in Table 3). With thermal corrections added (ΔE_{298}), **III** becomes less stable than **I** (by 3 kJ mol^{-1}) and **II** remains as the highest energy isomer (+7 kJ mol^{-1}). After consideration of the corresponding Na^+ complexation entropies (S_{298}), the 298 K difference in free energy (ΔG_{298}) between **I** and **III** is still 1 kJ mol^{-1} in favor of charge solvation (i.e. **I**). The trends reverse for $[Ala+K]^+$ and $[Ala+Cs]^+$, where charge-solvated **II** now becomes the most stable isomer, lying considerably lower in energy than either **I** or **III** (Table 3). At 298 K, the energy difference between most stable charge-solvated and zwitterionic isomers increases continually from Na^+ (+3 kJ mol^{-1}) to Cs^+ (+14 kJ mol^{-1}), as previously observed for glycine[13] and methylated glycines,[17] and in contrast to arginine, for which DFT calculations revealed exactly the opposite.[15] Furthermore, ΔE_{298} and ΔG_{298} follow the same order and are fairly similar because of the comparable entropies of isomers **I**, **II**, and **III**. Overall, the results predict that salt bridges become less favorable with increasing size of the metal ion;[17] such a result makes it unlikely that the inversion of the relative affinity between Ala and AlaOMe from K^+ to Cs^+ (Table 2) originates from the formation of a salt bridge in $[Ala+Cs]^+$. To validate this conclusion, calculations were also performed on the K^+ and Cs^+ complexes of β-AIB, for which the affinity order AA > AAOMe is already observed with K^+ (Table 2).

β-Aminoisobutyric acid complexes: The optimized geometries of isomers **I**–**III** of $[\beta\text{-AIB}+K]^+$ and $[\beta\text{-AIB}+Cs]^+$ are displayed in Figure 3. The type of binding interactions in these isomers is identical to that in the respective complexes of Ala and Gly. The ΔE_e values (Table 4) clearly show that the

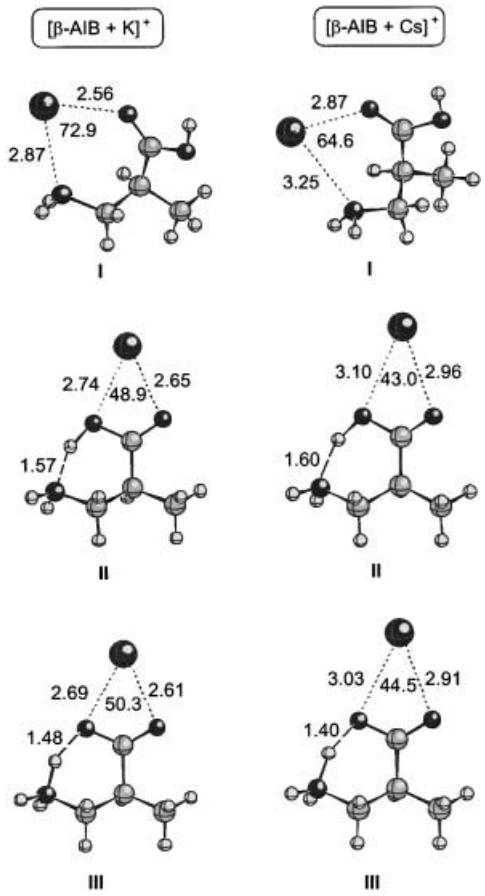


Figure 3. Optimized structures of isomers **I**–**III** of $[\beta\text{-AIB}+K]^+$ (left column) and $[\beta\text{-AIB}+Cs]^+$ (right column) at the MP2/basis 1 level. Important bond lengths [Å] and bond angles [°] are marked on the structures. The H-O-M angles of structures **III** are 163.0° and 161.4° in the K^+ and Cs^+ complexes, respectively.

Table 3. Relative electronic energies (ΔE_e), relative energies at 298 K (ΔE_{298}), entropies at 298 K (S_{298}), and relative free energies at 298 K (ΔG_{298}) of isomeric complexes of alanine (Ala) with Na^+, K^+, and Cs^+.[a]

Complex	Isomer	ΔE_e [kJ mol^{-1}]	ΔE_{298} [kJ mol^{-1}]	S_{298} [J mol^{-1} K^{-1}]	ΔG_{298} [kJ mol^{-1}][b]
		MP2/basis 2		MP2/basis 1	
$[Ala+Na]^+$	**I**	0	0	370	0
	II	+10	+7	379	+5
	III	0	+3	374	+1
$[Ala+K]^+$	**I**	+4	+6	384	+8
	II	0	0	392	0
	III	+4	+8	386	+9
$[Ala+Cs]^+$	**I**	+9	+10	404	+13
	II	0	0	411	0
	III	+12	+14	405	+16

[a] See the Experimental Section for a description of the basis 1 and basis 2 sets. The MP2/basis 2 energies refer to geometries optimized with MP2/basis 1. ΔE_{298} were calculated by adding zero-point vibrational energies and thermal energies from the MP2/basis 1 data sets. [b] With MP2/basis 2 energies and MP2/basis 1 entropies.

0947-6539/02/0806-1382 $ 17.50+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 6

Table 4. Relative electronic energies (ΔE_e) of isomeric complexes of β-amino-isobutyric acid (β-AIB) with K^+ and Cs^+. Selected relative energies at 298 K (ΔE_{298}) and entropies at 298 K(S_{298}) are also given.[a]

Complex	Isomer	ΔE_e [kJ mol^{-1}]	ΔE_{298} [kJ mol^{-1}] MP2/basis 2	S_{298} [J mol^{-1} K^{-1}] HF/basis 1
[β-AIB+K]$^+$	**I**	+22		410
	II	0	0	405
	III	+9	+10	400
[β-AIB+Cs]$^+$	**I**	+26		
	II	0		
	III	+14		

[a] See the Experimental Section for a description of the basis 1 and basis 2 sets. The MP2/basis 2 energies refer to geometries optimized with MP2/basis 1. ΔE_{298} were calculated by adding zero-point vibrational energies and thermal energies from MP2/basis 1 data sets.

salt-bridged structures **III** are less stable than structures **II**, in which M^+ is charge-solvated by the carboxyl group of β-AIB; the energy difference is augmented at 298 K, as revealed by the ΔE_{298} value for K^+. Relative to the corresponding Ala complexes, isomer **I** of β-AIB is substantially destabilized compared to **II** (cf. ΔE_e in Tables 3 and 4). Hence, the extra flexibility of a β-amino acid improves the H bond between amino and carboxyl termini (present in **II** and **III**, Figure 3) much more than metal ion sequestration (**I**). Despite the increased proton affinity of β-AIB compared to Ala (Table 2), zwitterionic structures **III** remain less stable than **II** and become less favorable compared to **II** as the size of the metal ion increases (Table 4). These trends corroborate the fact that zwitterionic arrangements cannot be invoked to explain why the relative affinity between β-AIB and β-AIBOMe changes from a negative value (for Li^+/Na^+) to a positive value (for K^+/Cs^+).

Proline (Pro) complexes: In contrast to the amino acids discussed so far, proline preferentially forms zwitterionic complexes with all alkali metal ions (Table 5). Isomer **III** is consistently the lowest energy structure, followed by **II** and **I**.[22] Pro carries a secondary amine and is thus more basic than most aliphatic amino acids,[10] which could promote salt bridge formation within [Pro+M]$^+$. This certainly is not the only reason for proline's high proclivity to form zwitterions because β-AIB is more basic (Table 2), yet it favors charge solvation (Table 4).

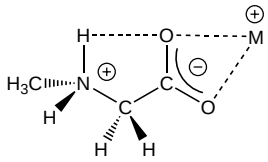
Table 5. Relative electronic energies (ΔE_e) of isomeric complexes of proline (Pro) with Li^+, Na^+, K^+, and Cs^+. Selected relative energies at 298 K (ΔE_{298}) and entropies at 298 K (S_{298}) are also given.[a]

Complex	Isomer	ΔE_e [kJ mol^{-1}]	ΔE_{298} [kJ mol^{-1}] MP2/basis 2	S_{298} [J mol^{-1} K^{-1}][e] HF/basis 1
[Pro+Li]$^+$	**I**	+16	+13[b]	369
	II	[d]		
	III	0	0	371
[Pro+Na]$^+$	**I**	+28	+33[c]	393
	II	[d]		
	III	0	0	388
[Pro+K]$^+$	**I**	+28		409
	II	+19	+14[b]	408
	III	0	0	400
[Pro+Cs]$^+$	**I**	+25	+21[c]	432
	II	0	+6[c]	431
	III	0	0	415

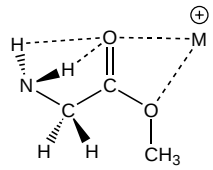
[a] See the Experimental Section for a description of the basis 1 and basis 2 sets. The MP2/basis 2 energies refer to geometries optimized with MP2/basis 1. ΔE_{298} were calculated by adding zero-point vibrational energies and thermal energies from [b] MP2/basis 1 or [c] HF/basis 1 data sets. [d] Collapses to isomer **III**. [e] The entropy of Pro at the HF/basis 1 level is 351 J mol^{-1} K^{-1}.

A zwitterionic [AA+M]$^+$ complex carries positive charges at a proton and metal ion, separated by a negative charge at carboxylate O atoms (+ − +). The most favorable interaction of the charges is achieved in a linear arrangement. A nearly linear + − + geometry is possible if the proton resides on an α-amine group, as in Pro or Gly (cf. H-O-M angle in **III**). With increasing deviation from linearity of the +− and −+ dipoles, the electrostatic stabilization resulting from a salt bridge is reduced. The nearly linear H-O-M orientation in [Pro+M]$^+$ zwitterions[22] constitutes an extra driving force for the formation of salt bridges within these complexes. On the other hand, the somewhat twisted H-O-M angle in β-AIB (Figure 3) could be a reason for the decreased stability of salt bridges with this more basic AA.

Glycine and proline methyl ester complexes: The [GlyOMe+M]$^+$ complexes involving a small (Na^+) or large (Cs^+) alkali metal ion were optimized in structures **I**, **II**, **IIIa**, and **VII** (Figure 4 and Table 6). Esterification obstructs the H bond in **II**, but rotation about the $COOCH_3$ moiety gives rise



IIIa



VII

to a new isomer, **VII**, in which hydrogen bonding between the carbonyl oxygen and the amine hydrogens can take place. Isomer **IIIa** arises from **II** by CH_3^+ migration from the carboxyl to the amine group and resembles **III**, which is formed by an analogous H^+ transfer. Note that **IIIa** corresponds to a zwitterionic sarcosine complex. Although **IIIa** is a particularly stable isomer, the intramolecular rearrangement **II**/**IIIa** requires a very high barrier (Table 6); therefore, **IIIa** can be excluded as a probable structure of the [GlyOMe+M]$^+$ complexes probed in our experiments. From the remaining isomers, **I**, which involves charge solvation by the carbonyl and amine groups (Figure 4), is the most stable structure for both sodiated and cesiated GlyOMe; a similar trend was reported by Bowers et al. for the Na^+ and Rb^+ adducts of GlyOMe.[14] The energy difference between **I** and **II** is +68 and +40 kJ mol^{-1} for M = Na and Cs, respectively (Table 6). For comparison, the corresponding differences between isomers **I** and **II** of [Gly+M]$^+$ are +16 and −7 kJ mol^{-1}.[13] These dramatic changes emphasize the importance of stabilizing hydrogen bonding in certain tautomers of metalated amino acids and the implications of their removal or alteration.[14]

Computational data were also obtained for the [ProOMe+Li]$^+$ complex (Table 7), mainly for use in the

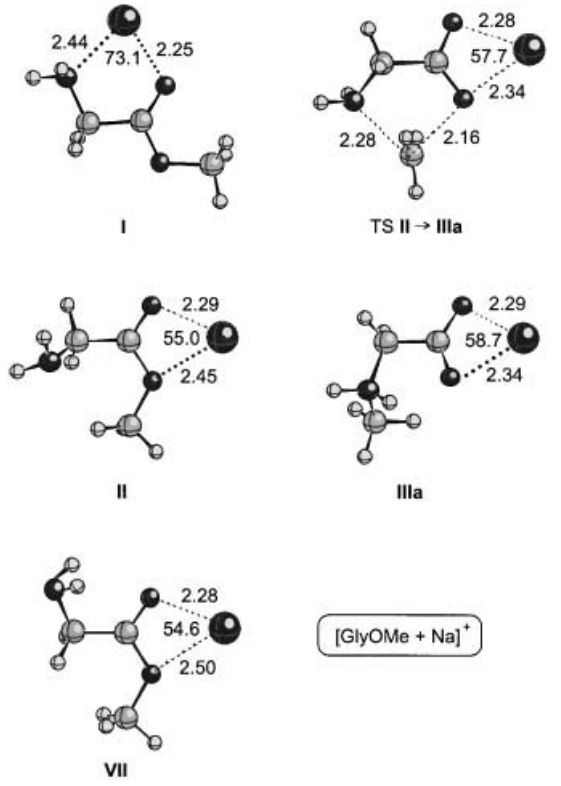


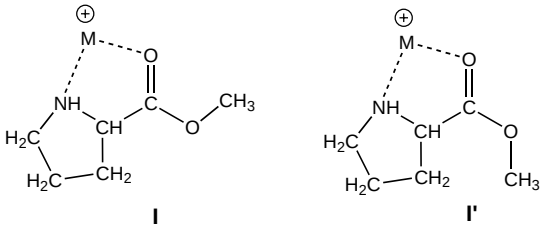
Figure 4. Optimized structures of isomers **I**, **II**, **III a**, and **VII** of $[GlyOMe+Na]^+$ and of the transition state for the isomerization **II** ↔ **III a** at the MP2/basis 1 level. Important bond lengths [Å] and bond angles [°] are marked on the structures.

Table 6. Relative electronic energies (ΔE_e) of isomeric complexes of glycine methyl ester (GlyOMe) with Na^+ and Cs^+.[a]

Complex	Isomer	ΔE_e MP2/basis 1
$[GlyOMe+Na]^+$	**I**	+19
	II	+87
	III a	0
	VII	+99
	TS **II/III a**	+401
$[GlyOMe+Cs]^+$	**I**	+22
	II	+62
	III a	0

[a] See the Experimental Section for a description of the basis 1 set. The MP2/basis 1 energies are for geometries optimized at the same level.

comparison of M^+ binding modes in metalated monomers compared to metalated dimers (see next section). Here, the ester methyl group was optimized in *cis* (**I**) and *trans* (**I′**) orientation with respect to the carbonyl group. Isomer **I** is 33 kJ mol^{-1} more stable than **I′** and both these structures are significantly lower in energy than **II**, in agreement with the results for $[GlyOMe+M]^+$ (Tables 6 and 7).



Li^+-bound dimer of proline and proline methyl ester: One question about the reliability of the kinetic method experiments relates to the nature of the lowest energy isomer of the heterodimer. In a metalated complex with one AA and one AAOMe ligand, will the AA molecule be attached to the metal in the same way as when the ester is absent? If this is not true (and the dimer dissociates without rearrangement), the kinetic method probes an excited state of the $[AA+M]^+$ monomer and, thus, would be inadequate to derive thermodynamic and structural information about the most stable $[AA+M]^+$ geometry. There are two factors which could lead to a structural difference between the monomer and the dimer. First, direct steric repulsions between AA and AAOMe could favor less tightly bound structures, leading, for example, to a bidentate metal–AA chelation in the dimer, in a case where it is tridentate in the monomeric metal–AA complex. Neither the MS^3 experiments described above (Table 1) nor our calculations for the Pro–Li^+–ProOMe dimer (discussed below) support such a scenario. The second factor is electronic: for metal ions with significant charge transfer interactions with AA and AAOMe, a salt-bridge bond with AA could be disfavored, compared to charge solvation, if substantial charge transfer also occurs with AAOMe. For Na^+ and heavier alkali metal ions, charge transfer is small or negligible to cause any measurable effect. Therefore, the only case investigated is that of Li^+ complexes. A first test was reported in a previous paper which compared the relative energies of several isomers of Gly–M^+–Gly to those of Gly–M^+.[13] It was found that the salt bridge structure **III** is disfavored relative to **I** in the dimer, but the energy ordering was not reversed. To provide a more stringent test of this possibility, we carried out calculations on Pro–Li^+–ProOMe isomers. The secondary amine in proline is expected

Table 7. Relative electronic energies (ΔE_e) of isomeric $[ProOMe+Li]^+$ complexes. Selected relative energies at 298 K (ΔE_{298}) and entropies at 298 K (S_{298}) are also given.[a]

Complex	Isomer	ΔE_e [kJ mol^{-1}] HF/basis 1	ΔE_{298} [kJ mol^{-1}] HF/basis 1	ΔE_e [kJ mol^{-1}] MP2/basis 1	ΔE_e [kJ mol^{-1}] MP2/basis 2	S_{298} [J mol^{-1} K^{-1}][b] HF/basis 1
$[ProOMe+Li]^+$	**I**	0	0	0	0	403
	I′	+42	+42	+38	+33	399
	II	+113	+110			421

[a] See the Experimental Section for a description of the basis 1 and basis 2 sets. The energies at each level are for the optimized geometries at the same level, except for the MP2/basis 2 values which refer to geometries optimized with MP2/basis 1. ΔE_{298} were calculated by adding zero-point vibrational energies and thermal energies from the same computational level. [b] The entropies of ProOMe and $[ProOMe+K]^+$ at the HF/basis 1 level are 393 and 444 J mol^{-1} K^{-1}, respectively.

  0947-6539/02/0806-1384 $ 17.50+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 6

to induce larger charge-transfer interactions than the primary amine in glycine. Thus, Pro–Li^+–ProOMe is the most probable dimer to have a different Pro–Li^+ coordination from that present in the monomer. Three isomers of Pro–Li^+–ProOMe were considered, one with binding mode **I** for Pro and ProOMe (denoted **I/I**) and two containing Pro in structure **III** and ProOMe in either structure **I** (isomer **III/I**) or **II** (isomer **III/II**). Their relative energies at the HF/basis 1 level are 0, +13, and +95 kJ mol^{-1}, respectively (Table 8). Given the high energy of **III/II**, only **I/I** and **III/I** were calculated at higher levels. Their optimized geometries are shown in Figure 5. Single-point energy calculations at the

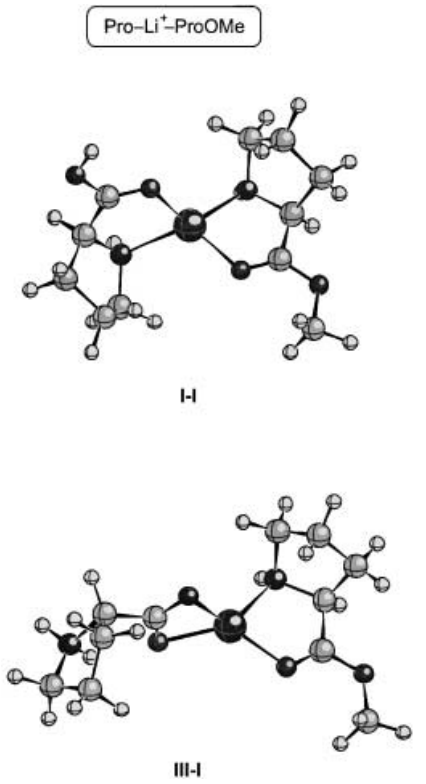


Figure 5. Optimized structures of isomers **I/I** and **III/I** of Pro–Li^+–ProOMe at the HF/basis 1 level.

MP2/basis 2 level yield **I/I** as the lowest energy isomer, but with an insignificant energy difference from **III/I** (+2 kJ mol^{-1}). After addition of zero-point vibrational energies and 298 K thermal corrections computed at the HF/basis 1 level, our best estimate of the relative energy between **I/I** and **III/I** is +6 kJ mol^{-1}; the corresponding error bar is close to the value itself, so that both isomers could be populated in the experiments.

Based on the energy data of [Pro+Li]$^+$ (Table 5) and Pro-M^+–ProOMe (Table 8), the zwitterionic geometry is destabilized in the dimer. This behavior results from the differences in the Li^+–Pro bond lengths in the monomer compared to the dimer. Geometries **I** and **III** involve bidentate coordination of Li^+ by Pro in both the monomer[22] and the dimer (Figure 5). The calculated distances between Li^+ and the two binding sites at the Pro ligand are listed in Table 9. Because of its

Table 9. Distances [Å] between Li^+ and the binding sites in Pro in the [Pro+Li]$^+$ monomer compared to the Pro–Li^+–ProOMe heterodimer.[a]

Isomer	Bond	Monomer		Heterodimer
		HF	MP2	HF
I	Li–N	2.062	2.055	2.150
	Li–O	1.852	1.887	1.946
III	Li–O (N side)	1.952	1.987	2.043
	Li–O (other)	1.912	1.953	2.009

[a] See Figure 5.

complexity, the dimer was optimized at the HF level only. Improving the wave function quality from HF to MP2 for the monomer causes fairly small structural changes, which should barely impact relative energetics. Hence, the HF values are assumed to adequately describe the effect of adding a second ligand to Li^+. The length of each Li^+ bond to Pro (Table 9) increases from the monomer to the dimer by a remarkably constant increment (0.09 Å). This increase is the result of small steric interactions between Pro and ProOMe and a less efficient electron donation to Li^+ by each ligand in the dimer, where the ligands compete for this process. Longer bonds reduce the strength of cation–anion interactions (in salt bridges) more severely than that of electrostatic bonding (in charge solvation), which provides a plausible rationale for the preference of structure **I** in the Pro–Li^+–ProOMe dimer.

The results for Gly–Li^+–Gly[13] and Pro–Li^+–ProOMe show that even in lithiated AA complexes, where the number of ligands around Li^+ exerts the largest effect on the energetics of isomers, the change in relative isomer stabilities from the monomer to the dimer is small. Consequently, in nearly all complexes considered in this paper, the preferred AA binding modes in monomers and heterodimers are most probably the same; it cannot be excluded, however, that a reversal of energy ordering might occur in a limited number of complexes, especially if they involve Li^+ ions and/or AA and AAOMe ligands with very similar M^+ binding energies.

Table 8. Relative electronic energies (ΔE_e) of Pro–Li^+–ProOMe isomers. Selected relative energies at 298 K (ΔE_{298}) and entropies at 298 K (S_{298}) are also given.[a]

Complex	Isomer [b]	ΔE_e [kJ mol^{-1}]	ΔE_{298} [kJ mol^{-1}]	ΔE_e [kJ mol^{-1}]	ΔE_{298} [kJ mol^{-1}]	S_{298} [J mol^{-1} K^{-1}]
		HF/basis 1		MP2/basis 2		HF/basis 1
Pro-Li^+-ProOMe	**I/I**	0	0	0	0	605
	III/I	+13	+17	+2	+6[c]	611
	III/II	+95	+97			649

[a] See the Experimental Section for a description of the basis 1 and basis 2 sets. The energies at both levels are for geometries optimized with HF/basis 1. [b] The first number indicates the binding mode of Pro and the second one the binding mode of ProOMe. [c] Using vibrational frequencies computed at the HF/basis 1 level.

Absolute affinities for Gly, GlyOMe, Ala, and AlaOMe: Based on the foregoing discussions, charge solvation is the preferred mode of alkali metal ion coordination by both aliphatic amino acids and their esters, except proline. The most stable charge-solvated structure is **I** for all $[AAOMe+M]^+$, but varies between **I** and **II** for $[AA+M]^+$, depending on the metal ion. Structure **I** is favored with the smaller Na^+ (and Li^+), while **II** is favored with the larger K^+ and Cs^+. To assess how a structural change in $[AA+M]^+$ affects the relative M^+ affinity between the amino acid and its methyl ester, absolute $AA-M^+$ and $AAOMe-M^+$ binding energies were calculated for the most stable Na^+, K^+, and Cs^+ complexes of Gly and Ala as well as their methyl esters; the results are summarized in Table 10.

M^+ affinities are given with and without basis set superposition error (BSSE) corrections, so that the size of such errors can be appraised. Recent literature has suggested that noncorrected values may be closer to true binding energies.[35, 36] As expected, the larger the metal ion, the larger the orbital overlap with the attached molecule, the larger the BSSE (Table 10). For a given M^+, however, the BSSE values do not depend greatly on the AA or AAOMe molecule. Therefore, relative energetics do not change markedly even though the absolute correctional increments are quite large. The BSSE-corrected data are used in the following discussions.

The Na^+ affinity (ΔH_{298}) increases by 12 kJ mol^{-1} from Gly to GlyOMe and a similar increase of 10 kJ mol^{-1} is observed from Ala to AlaOMe. Na^+ basicities, ΔG_{298}, show comparable changes as a result of the similar entropies of the $AA-Na^+$ and $AAOMe-Na^+$ bonds. The Na^+ complexes of Gly and GlyOMe as well as Ala and AlaOMe involve isomer **I**; hence, when similarly coordinated systems are compared, AAOMe binds more strongly, as expected from the favorable inductive effect of the methyl group. With K^+ and Cs^+, on the other hand, the most stable complexes of $[AAOMe+K/Cs]^+$ involve structure **I**, but those of $[AA+K/Cs]^+$ involve structure **II**. Furthermore, isomer **II** becomes increasingly more stable (relative to other isomers) with increasing size of the metal ion (see ΔE_{298} data in Table 3). These changes affect the corresponding binding energies, especially for Cs^+ (Table 10). In contrast to Na^+ affinities (or basicities), Cs^+ affinities (or basicities) decrease from Gly to GlyOMe and from Ala to AlaOMe by 1 (or 4) and 4 (or 6) kJ mol^{-1}, respectively (Table 10). K^+ binding shows an intermediate behavior: the K^+ affinity and basicity increase from Gly to GlyOMe but decrease from Ala to AlaOMe.

The ΔS_{298} data of Table 10 also show that the entropies of the $AA-M^+$ and $AAOMe-M^+$ bonds are generally similar, but they are not identical. The assumption that the entropies of the $AA-M^+-AAOMe$ dissociations to $AA-M^+$ and $AAOMe-M^+$ mutually cancel is consequently not rigorous and entropy effects may lead to a reversal of the order of very small relative affinities (see the next section).

Theoretical predictions compared to experimental results for aliphatic systems: The computational data permit a direct comparison of the M^+ binding properties of the isomeric GlyOMe and Ala molecules. Based on Table 10, Na^+ is more strongly bound by GlyOMe than Ala, while the opposite is true of Cs^+; with K^+, either ligand forms bonds of approximately equal bond strength. To cross-check this prediction, the Na^+, K^+, and Cs^+ affinities of GlyOMe and Ala were measured relative to anchor *N*-acetylglycine (NAcG) by the use of the heterodimers $GlyOMe-M^+-NAcG$ and $Ala-M^+-NAcG$. The relative M^+ affinities for Ala compared to GlyOMe derived from these experiments are in qualitative agreement with the computationally predicted affinity differences (Table 11).

It is noteworthy that $\Delta(\Delta H_{298})$ and $\Delta(\Delta G_{298})$ have different signs for the $Ala-K^+-GlyOMe$ dimer. The experiment probes $\Delta(\Delta G_{298})$ correctly (both positive), but does not reveal the proper relative K^+ affinity because of entropy effects; the small difference in the $Ala-K^+$ and $GlyOMe-K^+$ bond entropies (Table 10) reverses the $\Delta(\Delta G_{298})$ order because the relative K^+ affinity between Ala and GlyOMe is particularly small, as is also reflected by the corresponding $\ln(k_{Ala}/k_{GlyOMe})$ ratio (+0.04). The ratios for the Na^+- and Cs^+-bound dimers of Ala and GlyOMe (Table 11) as well as all $\ln(k_{AA}/k_{AAOMe})$ ratios listed in Table 2 are considerably larger. In the latter cases, modest entropy effects (Table 10) are unlikely to yield reversed $\Delta(\Delta H_{298})$ and $\Delta(\Delta G_{298})$ orders; this expectation is

Table 10. Calculated M^+ affinities (ΔH_{298}) with and without BSSE corrections, M^+ binding entropies (ΔS_{298}), and M^+ basicities (ΔG_{298}) for the Na^+, K^+, and Cs^+ complexes of glycine (Gly), alanine (Ala), glycine methyl ester (GlyOMe), and alanine methyl ester (AlaOMe).[a]

Complex	Geometry	ΔH_{298} [kJ mol^{-1}]		ΔS_{298} [J mol^{-1} K^{-1}]	ΔG_{298} [kJ mol^{-1}]
		without BSSE	with BSSE		
$[Gly+Na]^+$	**I**	164	154	116	120
$[GlyOMe+Na]^+$	**I**	175	166	117	131
$[Gly+K]^+$	**II**	120	113	104	82
$[GlyOMe+K]^+$	**I**	125	120	110	87
$[Gly+Cs]^+$	**II**	98	92	94	64
$[GlyOMe+Cs]^+$	**I**	97	91	104	60
$[Ala+Na]^+$	**I**	167	158	117	123
$[AlaOMe+Na]^+$	**I**	177	168	117	133
$[Ala+K]^+$	**II**	126	119	102	89
$[AlaOMe+K]^+$	**I**	123	118	110	84
$[Ala+Cs]^+$	**II**	102	96	98	67
$[AlaOMe+Cs]^+$	**I**	99	92	105	61

[a] MP2/basis 2//MP2/basis 1, calculated with entropies from the MP2/basis 1 level. See the Experimental Section for a description of the basis 1 and basis 2 sets. BSSE-corrected ΔH_{298} values were used in the calculation of ΔG_{298}.

0947-6539/02/0806-1386 $ 17.50+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 6

Table 11. Experimental and computational relative M^+ affinities between the isomeric molecules GlyOMe and Ala.

Dimer	Experimental rel. affinity		Ab initio rel. affinity	
	$\ln(k_{GlyOMe}/k_{NAcG})$ or $\ln(k_{Ala}/k_{NAcG})$[a]	$\ln(k_{Ala}/k_{GlyOMe})$[b]	$\Delta(\Delta H_{298})$ [kJ mol^{-1}][c]	$\Delta(\Delta G_{298})$ [kJ mol^{-1}][d]
GlyOMe-Na^+-NAcG	−1.36			
Ala-Na^+-NAcG	−1.57			
Ala-Na^+-GlyOMe		−0.21	−8	−8
GlyOMe-K^+-NAcG	−1.64			
Ala-K^+-NAcG	−1.60			
Ala-K^+-GlyOMe		+0.04	−1	+2
GlyOMe-Cs^+-NAcG	−1.70			
Ala-Cs^+-NAcG	−1.22			
Ala-Cs^+-GlyOMe		+0.48	+5	+7

[a] Rel. M^+ affinity of GlyOMe or Ala versus NAcG (i.e. of bases compared in the heterodimers); k_{GlyOMe}/k_{NAcG} and k_{Ala}/k_{NAcG} were obtained from CAD spectra of the listed dimer ions ($\pm \leq 0.07$). [b] Rel. M^+ affinity of Ala versus GlyOMe, calculated by subtracting $\ln(k_{GlyOMe}/k_{NAcG})$ from $\ln(k_{Ala}/k_{NAcG})$. [c] ΔH_{298}(Ala) − ΔH_{298}(GlyOMe) calculated with values listed in Table 10. [d] ΔG_{298}(Ala) − ΔG_{298}(GlyOMe) calculated with values listed in Table 10.

corroborated by the data for Ala–Na^+–GlyOMe and Ala–Cs^+–GlyOMe in Table 11.

Theory predicts that Na^+ ions should bind more strongly to (aliphatic) AAOMe than AA (Table 10). This is confirmed by the experimental Na^+ affinity differences between AA and AAOMe (Table 2); all are negative, except that of proline, which constitutes a special case (vide infra). Negative relative affinities are obtained for Li^+, too, which closely resembles Na^+ in its chelation preferences.[13] In contrast, theory predicts that Cs^+ ions should bind more weakly to AAOMe than AA (Table 10). Indeed, our experimental relative Cs^+ affinities between AA and AAOMe are generally positive, except that of the Gly/GlyOMe pair. A possible rationale for the latter disagreement is given below.

The largest discrepancy between theory and experiment is observed with K^+. The calculations predict a negative relative affinity for Gly/GlyOMe, but a positive relative affinity for Ala/AlaOMe (which should also hold true for the larger aliphatic amino acids). Experimentally, we observe positive relative affinities only for the β-amino acids (Table 2; Pro is a special case and will be discussed separately). All other AA/AAOMe pairs show negative relative K^+ affinities. The negative (i.e. decreased) values cannot be caused by entropy effects because the entropies of AA–K^+ (**II**) bonds are slightly but consistently lower than those of the corresponding AAOMe–K^+ bonds (Table 10); this gives rise to an increase (not decrease) of the relative K^+ affinity between AA and AAOMe.

Inconsistencies between theory and experiment are limited to K^+ complexes and the Cs^+ complexes of Gly/GlyOMe, that is to cases where the structures of $[AA+M]^+$ and $[AAOMe+M]^+$ are distinct and the theoretical $\Delta(\Delta H_{298})$ and $\Delta(\Delta G_{298})$ values are rather small. For example, the Cs^+ affinities and basicities of Gly and GlyOMe differ by only +1 and +4 kJ mol^{-1}, or +1 and +6%, respectively; similarly, the relative K^+ affinities and basicities of Ala and AlaOMe differ by +1 and +5 kJ mol^{-1}, or +1 and +6%, respectively. Perhaps, such small differences are not calculated accurately with the basis sets and levels of theory used. Furthermore, small relative affinities are most sensitive to subtle changes in the relative stability of structures **I** and **II** in the heterodimer. It is conceivable that isomer **I** becomes more stable than **II** within the dimer (as shown for **I** compared to **III** in Pro complexes), thereby leading to a switch in the affinity order for AA compared to AAOMe. Unfortunately, in order to resolve this dilemma, the necessary calculations on K^+- or Cs^+-bound dimers are not feasible at the higher levels of theory employed in this study.

Our calculations finally predict all $[Pro+M]^+$ complexes to be salt bridges (i.e. zwitterionic). This prediction is consistent with the kinetic method experiments for the Na^+, K^+, and Cs^+ complexes, which show the affinity order Pro > ProOMe (Table 2); as stated above, a zwitterionic amino acid is bound more strongly by M^+ than its (canonical) methyl ester. For Li^+, on the other hand, the experimentally determined affinity order is Pro < ProOMe (Table 2). The reason for this change is that the dimer Pro–Li^+–ProOMe contains proline in geometry **I** (vide supra), which leads to the observed Li^+ affinity order, Pro < ProOMe. The experimental result also points out that the isomerization **I/III** within the dissociating dimer requires more energy than cleavage of ProOMe to form Pro–Li^+ in structure **I**.

Conclusion

Our kinetic method experiments reveal that the binding affinities of amino acids to alkali metal ions generally increase upon their conversion to methyl esters. This is the normal trend expected from the higher intrinsic basicity of AAOMe compared to AA ligands.[10] Nevertheless, AA may also bind more strongly to M^+ than AAOMe, especially with larger metal ions. According to theory, the affinity order AA > AAOMe can be caused by AA becoming zwitterionic within the $[AA+M]^+$ complex (structure **III**); the attractive ionic interactions in the resulting salt bridge lead to the higher affinity of AA compared to AAOMe. This case applies to proline complexes and selected M^+ complexes of the most basic amino acids lysine and arginine. Theory further indicates that a stronger AA–M^+ than AAOMe–M^+ bond can alternatively be the consequence of M^+ receiving better charge solvation by AA than by AAOMe. This is possible when the charge-solvated structure **II** becomes the lowest energy $[AA+M]^+$ isomer, which in turn applies to AA complexes with the larger alkali metal ions (K^+ and, especially, Cs^+). Overall, the combined computational and experimental data show that charge solvation is the most common intrinsic chelation mode of M^+ by amino acids. With the exception of proline, aliphatic amino acids as basic as β-aminoisobutyric acid ($PA = 933$ kJ mol^{-1}) and side chain functionalized amino acids as basic as histidine ($PA = 988$ kJ mol^{-1}) coordinate M^+ by bidentate or tridentate charge

solvation rather than through a salt bridge. Important determinants for the formation of zwitterionic complexes are a high proton affinity for AA as well as linearity for the $+-+$ charges. Proline[22] and *N*-methylated glycines[17] provide both these features, facilitating the formation of salt-bridged complexes. When the most basic AA site moves to a flexible side chain, however, a linear $+-+$ geometry is not achievable; in this case, significantly higher *PA* values than those of aliphatic amino acids (Table 2) are necessary to make zwitterionic $[AA+M]^+$ more stable than alternative charge-solvated complexes.

Acknowledgements

We thank Dr. Michael J. Polce and In-Su Hahn for stimulating discussions and experimental assistance. We are grateful to the National Science Foundation (CHE-9725003 and 0111128), the Ohio Board of Regents, and the University of Akron for generous financial support. We thank Ecole Polytechnique for financial support to C.W. of a stay in Palaiseau, and the Institut de Développement et de Ressources en Informatique Scientifique du CNRS for a generous grant of computer time (grant no. 000543).

[1] G. L. Zubay, *Biochemistry*, 2nd ed., Macmillan, New York, **1988**.
[2] M. J. Locke, R. T. McIver, *J. Am. Chem. Soc.* **1983**, *105*, 4226.
[3] J. H. Jensen, M. S. Gordon, *J. Am. Chem. Soc.* **1991**, *113*, 7917.
[4] J. H. Jensen, M. S. Gordon, *J. Am. Chem. Soc.* **1995**, *117*, 8159.
[5] F. J. Lovas, Y. Kawashima, J. -U. Grabow, R. D. Suenram, G. T. Fraser, E. Hirota, *Astrophys. J.* **1995**, *455*, L201.
[6] Y. Ding, K. Krogh-Jespersen, *J. Comp. Chem.* **1996**, *17*, 338.
[7] F. Huisken, O. Wehrhan, A. Yu. Ivanov, S. A. Kransnokutski, *J. Chem. Phys.* **1999**, *111*, 2978.
[8] L. C. Snoek, E. G. Robertson, R. T. Kroemer, J. P. Simons, *Chem. Phys. Lett.* **2000**, *321*, 49.
[9] a) C. J. Chapo, J. B. Paul, R. A. Provencal, K. Roth, R. J. Saykally, *J. Am. Chem. Soc.* **1998**, *120*, 12956; b) A. Melo, M. J. Ramos, W. B. Floriano, J. A. N. F. Gomes, J. F. R. Leao, A. L. Magalhaes, B. Maigret, M. C. Mascimento, N. Reuter, *THEOCHEM* **1999**, *463*, 81.
[10] E. P. L. Hunter, S. G. Lias, *J. Phys. Chem. Ref. Data* **1998**, *27*, 413.
[11] T. Wyttenbach, J. E. Bushnell, M. T. Bowers, *J. Am. Chem. Soc.* **1998**, *120*, 5098.
[12] F. Jensen, *J. Am. Chem. Soc.* **1992**, *114*, 9533.
[13] S. Hoyau, G. Ohanessian, *Chem. Eur. J.* **1998**, *4*, 1561.
[14] T. Wyttenbach, M. Witt, M. T. Bowers, *Int. J. Mass Spectrom.* **1999**, *182/183*, 243.
[15] R. A. Jockusch, W. D. Price, E. R. Williams, *J. Phys. Chem. A* **1999**, *103*, 9266.
[16] B. A. Cerda, C. Wesdemiotis, *Analyst* **2000**, *125*, 657.
[17] T. Wyttenbach, M. Witt, M. T. Bowers, *J. Am. Chem. Soc.* **2000**, *122*, 3458.
[18] S. Bouchonnet, Y. Hoppilliard, *Org. Mass Spectrom.* **1992**, *27*, 71.
[19] D. Yu, A. Rauk, D. A. Armstrong, *J. Am. Chem. Soc.* **1995**, *117*, 1789.
[20] R. C. Dunbar, *J. Phys. Chem. A* **2000**, *104*, 8067.
[21] V. Ryzhov, R. C. Dunbar, B. A. Cerda, C. Wesdemiotis, *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 1037.
[22] S. Hoyau. K. Norrman, T. B. McMahon, G. Ohanessian, *J. Am. Chem. Soc.* **1999**, *121*, 8864.
[23] a) R. G. Cooks, J. S. Patrick, T. Kotiaho, S. A. McLuckey, *Mass Spectrom. Rev.* **1994**, *13*, 287; b) R. G. Cooks, P. S. H. Wong, *Acc. Chem. Res.* **1998**, *31*, 379; c) R. G. Cooks, J. T. Koskinen, P. D. Thomas, *J. Mass Spectrom.* **1999**, *34*, 85.
[24] B. A. Cerda, C. Wesdemiotis, *J. Am. Chem. Soc.* **1995**, *117*, 9734.
[25] B. A. Cerda, C. Wesdemiotis, *J. Am. Chem. Soc.* **1996**, *118*, 11884.
[26] B. A. Cerda, S. Hoyau, G. Ohanessian, C. Wesdemiotis, *J. Am. Chem. Soc.* **1998**, *120*, 2437.
[27] M. J. Polce, M. M. Cordero, C. Wesdemiotis, P. A. Bott, *Int. J. Mass Spectrom. Ion Processes* **1992**, *113*, 35.
[28] a) R. G. Cooks, J. H. Beynon, R. M. Caprioli, G. R. Lester, *Metastable Ions*, Elsevier, Amsterdam, 1973; b) J. L. Holmes, *Org. Mass Spectrom.* **1985**, *20*, 169.
[29] K. L. Busch, G. L. Glish, S. A. McLuckey, *Mass Spectrometry*, VCH, New York, **1988**.
[30] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheesemann, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challocombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian98, Gaussian, Inc., Pittsburgh, PA, **1998**.
[31] T. B. McMahon, G. Ohanessian, *Chem. Eur. J.* **2000**, *6*, 2931.
[32] S. Beranova, C. Wesdemiotis, *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 1093.
[33] I. Hahn, C. Wesdemiotis, *J. Phys. Chem.* submitted.
[34] X. Cheng, Z. Wu, C. Fenselau, *J. Am. Chem. Soc.* **1993**, *115*, 4844.
[35] a) D. Feller, *Chem. Phys. Lett.* **2000**, *322*, 543; b) D. Feller, D. A. Dixon, J. B. Nicholas, *J. Phys. Chem. A* **2000**, *104*, 11414.
[36] F. M. Siu, N. L. Ma, C. W. Tsang, *J. Chem. Phys.* **2001**, *114*, 7045.

Received: August 31, 2000
Revised: November 19, 2001 [F2702]

  0947-6539/02/0806-1388 $ 17.50+.50/0