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Structurally related non-covalent complexes examined by quadrupole ion trap (QIT) MS^2 and infrared multiphoton dissociation Fourier transform ion cyclotron resonance mass spectrometry IRMPD-FT-ICR MS: evidence for salt-bridge structures in the gas phase

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Abstract

The gas-phase structures of a series of monomeric, homo- and heterodimeric sodium adduct ions of structurally related synthetic compounds M_n [Gua^+–NH–$(CH_2)_n$–COO^-] with n = 1, 2, 3, 5 and Gua = guanidiniocarbonyl pyrrole were investigated by various MS techniques. The compounds M_n are zwitterions in solution and have a strong tendency to aggregate in polar solvents.

First, quadrupole ion trap (QIT) collision induced dissociation (CID) product ion experiments with $[M_n + Na]^+$ ions (n = 1, 2, 3, 5) and [arginine + Na]$^+$ were conducted. The fragmentation behavior of the sodium adduct ions provides indirect evidence for a change in structure varying from predominantly charge-solvation of non-ionic molecules (M_1, M_2 and arginine), to salt-bridge interactions of zwitterionic structures of M_n for n = 3, 5.

Second, the sodium affinities (ΔH_{Na^+}) of the compounds M_n were related to the known literature value of arginine by examination of the CID fragmentation behavior of heterodimer ions $[M_n$ + arginine + Na$]^+$ and $[M_n + M_m + Na]^+$ ($n \neq m$ and n, m = 2, 3, 5) in a QIT. The relative ordering of sodium affinities (ΔH_{Na^+}): $M_5 \geq$ arginine $> M_3 > M_2$ can be deduced from the relative abundances of $[M_{n,m} + Na]^+$ and [arg + Na]$^+$ product ions. The maximum sodium affinity of M_5 relative to the reference value of arginine strongly supports the assumption of a gas-phase zwitterionic structure.

Third, the dimeric sodium adduct ions $[2M_n + Na]^+$ of M_2, M_3 and M_5 dissociate upon IR activation in FT-ICR MS exclusively into the respective monomeric sodium adduct ion $[M_n + Na]^+$. Hence, the establishment of a relative ordering of the gas-phase dissociation energy barriers E_a^{laser} for the disruption of the non-covalent bond of the complexes by IRMPD-FT-ICR MS was conducted. We find the dimeric complex ion $[2M_3 + Na]^+$ more stable than the respective complexes of M_2 and M_5. Hence, the stability of the examined complex ions $[2M_n + Na]^+$ is obviously strongly determined by the various possible non-covalent interactions between the two respective molecules M_n. The MS study supports the assumption that M_n molecules with $n \geq 3$ are able to conserve zwitterionic structures in the gas phase.

Keywords: FT-ICR; Gas-phase zwitterions; IRMPD

1. Introduction

Electrospray ionization mass spectrometry (ESI-MS) is perfectly suited to the study of weakly bound, non-covalent

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complexes, through the sensitive examination of conformational changes[1–3]. The measurement of absolute and relative dissociation constants[4–7] and the determination of the accurate mass and the stoichiometry of labile complexes are also possible [1,8,9]. There are numerous examples of the successful ESI-MS examination of non-covalently bound species. These range from low mass complexes comprising small organic molecules[10,11], amino acids or peptides [12] to high mass supramolecular assemblies [13–15], protein complexes with inhibitors, cofactors, metal ions and other peptides and enzyme–substrate pairings [16–18]. In addition, the character and strength of the non-covalent interaction can be probed and quantified in the gas phase [19]. However, the question on the comparability of solution- and gas-phase properties of non-covalent complexes is still an issue of constant scientific debate [20–22]. Still unclear is the extent of structural changes as a result of solvent removal and the interactions that govern stability of the desolvated species in the gas phase [21,23]. Correct predictions or a quantification of the magnitude of the changes are difficult or impossible. What is known is that hydrophobic interactions in contrast to van der Waals interactions, that are identical in solution and the gas phase, can be strong in the solution-phase but seem to play a minor role in the gas phase, since such interactions are mainly attributed to the role of solvent [24,25]. Consequently, hydrophobic interactions are rarely considered to be responsible for non-covalent complex stabilization in vacuum [26,1]. Contrarily, electrostatic forces (ion–ion, ion–dipole, dipole–dipole interactions), decreased in solution by the dielectric constant of the solvent, are greatly strengthened in a solvent-free environment. Hence, complexes held together by electrostatic interactions are extremely tightly bound in the gas phase after loss of the solvate shell [26–29]. Consequently, the nature of interaction stabilizing non-covalent complexes can differ substantially in the gas and solution phases.

In this context, the obvious question arises whether compounds (e.g., peptides or α-amino acids) that are predominantly zwitterions in aqueous solution (at least at their isoelectric points) can conserve salt-bridge structures in the gas phase. The solution-phase preference of amino acids for zwitterionic structures is mainly the result of significant solvation effects of polar or protic media that are able to stabilize charges. The lack of this stabilization by solvent in the vacuum supports the common assumption that zwitterions, or salt-bridge structures are rarely found in the gas phase where the corresponding nonionic, neutral structures should be predominantly present [30–32]. However, this issue has been addressed recently by different experimental strategies, such as H/D exchange [33–36], ion mobility [37,38], the kinetic method [39–41], high-pressure mass spectrometry [42], BIRD [43–47] and theoretical approaches with density functional theory (DFT) [48–53] and ab initio calculations [42,54]. The results of these studies have sometimes been contradictory, and remain the subject of debate.

In principle, the stability of a zwitterion in the gas phase is determined by the basicity and acidity of the functional groups involved: the more basic the base, and the more acidic the acid, the more stable the zwitterion [37]. Consequently, arginine with its extremely basic guanidine group should be the most likely amino acid to be able to generate zwitterionic structures in the gas phase. Theoretical calculations and experiments expect the arginine zwitterion in the gas phase to be intrinsically equal to or a little less stable than the neutral form [44,54,32]. However, gas-phase zwitterions should be stabilized to some extent by the presence of an additional charge (e.g., an ion), thereby forming a salt bridge, or by specific non-covalent interactions with nearby molecules [55]. Theory and QIT experiments suggest that arginine dimers and oligomers are held together by salt bridges between the guanidinium and carboxylate groups of arginine zwitterions [52,51]. The protonated arginine dimer $[(\text{arginine})_2 + \text{H}]^+$ is proposed to comprise a zwitterion and a neutral arginine molecule [44,50]. Even the exceptional stability of the protonated serine octamer $[(\text{serine})_8 + \text{H}]^+$ gas-phase cluster, discussed in the context of homochirality, was explained by electrostatic interactions of eight serine zwitterions [53]. Theoretical calculations suggest that the stabilizing contribution of only two water molecules is sufficient to make the zwitterionic structure of glycine more favorable than its nonionic form in the respective gaseous glycine cluster [56]. In another study on cationized hydrated valine adduct ions, experimental and theoretical results led to a similar conclusion that valine in $[\text{valine} + \text{Li} + (\text{H}_2\text{O})_3]^+$ is best represented by a zwitterionic form [57]. Also, intramolecular charge solvation in proteins and peptides has been shown to provide enough stabilization for gas-phase salt-bridge structures [58,50,34,59,60]. Further studies indicated that the size of the additional ion [37,38,61,40] as well as the character of the functionalized amino acid side chain strongly influence the gas-phase structure of the amino acid in the respective cationized adduct ion [39,48,49,42]. It was shown that charge stabilization of nonionic amino acid molecules predominates in protonated and sodiated adduct ions, whereas salt-bridged structures of amino acid zwitterions are postulated for adducts with alkali ions such as potassium, rubidium and cesium. These conclusions were deduced from cation affinity studies by Cooks' kinetic method [62–64], DFT calculations [40], low-energy CID and BIRD experiments [61].

We present data from the investigation of a series of structurally related synthetic carboxylate receptor molecules M_n $[\text{Gua}^+\text{–NH–}(\text{CH}_2)_n\text{–COO}^-]$ with n = 1, 2, 3, 5 and Gua = guanidinio-carbonyl pyrrole (see Fig. 1). These compounds

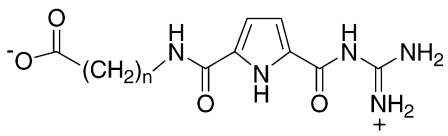

Fig. 1. Solution-phase zwitterionic structures of the compounds M_n (n = 1, 2, 3, 5).

are zwitterions in solution and have a strong tendency to aggregate in polar solvents. The stability, and even more important, the structure of these aggregates depends upon the length and therefore flexibility of the spacer connecting the two complementary binding groups. We have shown by NMR dilution experiments and ESI-MS studies in combination with molecular modeling that M_1 forms highly stable helical aggregates with nanometer sized dimensions. For the more flexible systems of M_3 and M_5, the aggregation is much weaker leading also to linear oligomers. In contrast, the zwitterion M_2 forms discrete 1:1 head to tail dimers and no oligomers [65]. It is now interesting to investigate the extent to which these differences in solution-phase aggregation are reflected by the corresponding gas-phase behavior of the different zwitterions. Indeed, protonated and sodiated dimeric and also oligomeric complex ions can easily be generated and examined in the gas phase by electrospray MS, showing that this class of zwitterions also aggregates in the gas phase. The question was whether these aggregates are equivalent to those found in solution, and therefore also formed by the self-association of the charged zwitterions, or if within these aggregates in the gas-phase the molecules tend to exist in their neutral form? Furthermore, we wanted to find out whether the relative stability of these aggregates also depends upon the flexibility of the molecules. We therefore investigated the monomer sodium adduct ions $[M_n + Na]^+$ by low-energy CID in a QIT to find evidence for either one of the possible isomeric structures (zwitterionic or non-ionic structures) of M_n on the basis of characteristic fragmentation pathways and variations of fragment abundances [61,66–68].

To determine a relative ordering of sodium affinities of the compounds M_n related to the known affinity of arginine (arg), we examined low energy collisionally induced dissociation (CID) behavior of heterodimer ions of the general structure $[arg + M_n + Na]^+$ and $[M_n + M_m + Na]^+$ ($n \neq m$ and n, m = 2, 3, 5) in a QIT [69,39,40]. The relative ordering of sodium affinity (ΔH_{Na^+}) can be deduced from the relative abundances of the respective product ions, $[M_n + Na]^+$ and $[arg + Na]^+$ [62–64].

Finally, the dimeric complexes $[2M_n + Na]^+$ were selected as precursor ions for IRMPD tandem FT-ICR MS/MS experiments to determine the stability of these aggregates. The selected non-covalent complexes dissociate exclusively to yield the respective monomeric sodium adduct ion $[M_n + Na]^+$. The analogous IRMPD behavior of the structurally closely related complexes establishes a relative ordering of the gas-phase dissociation energy barriers E_a^{laser} for the disruption of the respective non-covalent interaction (E_a^{laser} is determined by measurement of first order dissociation rate constants as a function of infrared CO_2 laser power density) [70]. The prerequisites for the application of IRMPD for the determination of relative stability ordering are assumed to be fulfilled when closely related ions of moderate size (≥550 Da) are activated, and the observed analogous fragmentation reactions are governed by energy exchange of comparable oscillators.

2. Methods

2.1. Experimental

10^{-4} M solutions (DMSO/methanol) of the compounds M_n [Gua^+–NH–$(CH_2)_n$–COO^-] with n = 1, 2, 3, 5 and Gua = guanidinio-carbonyl pyrrole were used for all MS experiments[65]. ESI-MS^n measurements were performed in the quadrupole ion trap (QIT) of a Finnigan MAT 900S double-focusing sector field mass spectrometer with an EB-QIT configuration (Thermo Finnigan, Bremen, Germany). A conventional electrospray ion source was used with a flow rate of 3 μL/min and +3.7 kV voltage applied to the stainless steel tip of the ESI capillary.

All FT-ICR experiments were conducted with a homebuilt, passively shielded, 9.4 T ESI Q FT-ICR instrument [71,72] configured for mass selective external ion accumulation. The samples were infused at a flow rate of 300 nL/min through a 50 μm i.d. fused-silica microelectrospray emitter which had been mechanically ground to a uniform thin-walled tip [73]. The electrosprayed ions were transferred into the mass spectrometer through a Chait-style atmosphere-to-vacuum interface [74]. Ions were externally accumulated in a linear rf-only octopole ion trap for 1–30 s [75]. After accumulation, ions were transferred through multipole ion guides and captured by gated trapping in an open cylindrical cell [76]. The isotopic distribution for the dimeric non-covalent complexes, $[2M_n + Na]^+$, was isolated by a combination of mass-selective external ion accumulation and stored waveform inverse Fourier transform (SWIFT) excitation [77,78]. The selected ions were then heated by infrared irradiation with a Synrad (Model 48-2, Mukilteo, WA) 40 W continuous-wave CO_2 laser ($\lambda = 10^{-6}$ μm) for 1–120 s. The factory-determined laser beam diameter is 3.5 mm. A 2.5× beam expander (to yield a beam diameter of ∼9 mm) was installed to ensure that the IR beam intercepted all of the stored ions in the ICR cell.

Ions were frequency-sweep (“chirp”) [79,80] excited (72–720 kHz at 150 Hz/μs) and detected in direct mode (512 kword time-domain data). Five time-domain data sets were co-added, Hanning apodized, zero-filled once and subjected to fast Fourier transform followed by magnitude calculation. The experimental event sequence was controlled by a modular ICR data acquisition system (MIDAS) [81]. Because an indirectly-heated electron emitter for electron capture dissociation (ECD) is aligned along the central magnetic field axis of the FT-ICR spectrometer, the CO_2 laser is angled 2.5° off-axis through a BaF_2 window.

2.2. Kinetic method

The kinetic method is an approximate procedure for the determination of thermochemical properties such as sodium affinities (ΔH_{Na^+}) based on the rates of competitive dissociations of mass-selected cluster ions [62,82,63]. It should be noted that kinetic methods provide information about the transition state of the activated precursor species. However,

transition states often (but not necessarily) mirror equilibrium structures of the ground state [38]. To determine sodium affinities of the molecules M_n relative to arginine, we selected the sodium-bound clusters $[M_n + M_m + Na]^+$ and $[M_n + arg + Na]^+$ with $n \neq m$ and n, m = 2, 3, 5 for kinetic analysis. In general, affinities are much less dependent on temperature than free energies (i.e., gas-phase basicities) and are therefore more suitable for establishing correlations between intrinsic thermochemistry and specific structural features. This point is especially important in kinetic method studies in which the actual temperature (T_{eff}) of the activated ions is often poorly defined [39].

$$[M_n + Na]^+ \xleftarrow{k_1} [M_n + M_m + Na]^+ \xrightarrow{k_2} [M_m + Na]^+ \quad (1a)$$

$$[M_n + Na]^+ \xleftarrow{k'_1} [M_n + arg + Na]^+ \xrightarrow{k_3} [arg + Na]^+ \quad (1b)$$

Here, k_1, k'_1, k_2 and k_3 are the rate constants for the competitive dissociations of a chosen precursor cluster ion to yield $[M_n + Na]^+$, $[M_m + Na]^+$ and $[arg + Na]^+$, respectively. The experimental protocol is in accordance with the prerequisites for application of the simplest form of the kinetic method, because we compare competitive dissociation reactions of weakly bound complexes having no other decomposition channels. Hence, it is reasonable to assume that there is no reverse activation barrier to the dissociation involved. Second, we observe and examine dissociations of chemically very similar species so that the differences in the entropy requirements for the competing channels are negligible [83,82]. Consequently, the experimentally determined ratio of fragment ion abundances is directly related to the Na^+ affinity (ΔH_{Na^+}) difference of the two compared molecules (see Eq. (1a) and (1b)).

$$\ln\left(\frac{k_1}{k_2}\right) = \ln\left(\frac{[M_n + Na^+]}{[M_m + Na^+]}\right) \approx \frac{\Delta H_{Na^+}(M_n) - \Delta H_{Na^+}(M_m)}{RT_{eff}} \quad (2)$$

The heterodimer cluster ions $[M_n + M_m + Na]^+$ and $[M_n + arg + Na]^+$ were mass selected and isolated with an isolation window width sufficiently narrow to exclude all but the monoisotopic (i.e., all-^{12}C) ion. Following isolation, the precursor ions were subjected to very low energy collisional activation in the QIT (q_z = 0.250, activation time = 30 ms) by choosing a very weak resonant excitation amplitude (0.2–0.5 V). The MS^2 experiments for the $[M_n + arg + Na]^+$ precursor ions suffered from medium to low signal to noise ratios of the product ions. All experiments were repeated three to five times.

By comparing the respective abundances of the product ions ($[M_n + Na]^+$, $[M_m + Na]^+$ and $[arg + Na]^+$), we deduced the relative ordering of sodium affinities (ΔH_{Na^+}) (see Fig. 6). The validity of the sodium affinity comparisons was verified by performing analogous CID measurements under variable excitation conditions. According to the kinetic method, an increase of the collision energy leads only to a change in the effective temperature (T_{eff}) of the activated ions, and should not influence the sodium affinity (ΔH_{Na^+}) order (data not shown) [69].

2.3. IRMPD

The current models used to describe ion activation by IRMPD [84,85] are inadequate for the calculation of *absolute* activation energy values because of inaccurate estimation of the number and the character of the oscillators involved in energy exchange [70,86,85]. Nevertheless, IRMPD has proven its applicability to arrange closely related ions in relative stability order provided that structurally related ions of moderate molecular size (≥550 Da) are activated and analogous fragmentation channels are examined [87–90]. To identify *relative* activation energies E_a^{laser} of first order gas-phase dissociation reactions by IRMPD, the corresponding rate constants of the fragmentation reactions have to be determined. The rate constants (k_{diss}) and laser power densities (P_{laser}) are applied to Eq. (3) as proposed by Dunbar [84].

$$E_a^{laser} = qh\nu \frac{d \ln k_{diss}}{d \ln P_{laser}} \quad (3)$$

In Eq. (3), E_a^{laser}, is the activation energy (in eV) of the gas-phase dissociation reaction, q is the partition function for the vibrational mode that absorbs the incoming radiation, h is Planck's constant, ν is the laser frequency, k_{diss} is the first-order dissociation rate constant, and P_{laser} is the laser power density (W cm^{-2}). Because q varies slightly with temperature between 1.01 and 1.1, an average value of 1.05 was chosen for the maximum expected range of internal temperature (280–580 K) [84,86,87].

The rate constants of the unimolecular dissociation of $[2M_n + Na]^+$ precursor ions were determined for five laser power densities in the range between 4.5 and 10.3 W cm^{-2}. The measurement of the relative precursor-to-product ion

Table 1
Low energy QIT CID MS^2 experiments for $[M_n + Na]^+$ (n = 1, 2, 3, 5) and $[arg + Na]^+$ precursor ions

Precursor ion	MS^2 product ion experiments: characteristic neutral loss reactions (% relative abundance)
$[arg + Na]^+$	$-H_2O$ (100) $-NH_3$ (10)
$[M_1 + Na]^+$	$-HO_2CCH_2NH_2 \equiv 75$ u (100) $-HN{=}C(NH_2)_2 \equiv 59$ u (20)
$[M_2 + Na]^+$	$-HO_2C(CH_2)_2NH_2 \equiv 89$ u (60) $-HN{=}C(NH_2)_2 \equiv 59$ u (100) $-NH_3$ (10)
$[M_3 + Na]^+$	$-HO_2C(CH_2)_3NH_2 \equiv 103$ u (10) $-HN{=}C(NH_2)_2 \equiv 59$ u (100) $-NH_3$ (10)
$[M_5 + Na]^+$	$-HO_2C(CH_2)_5NH_2 \equiv 131$ u (5) $-HN{=}C(NH_2)_2 \equiv 59$ u (100) $-NH_3$ (5)

abundance ($[2M_n + Na]^+/[M_n + Na]^+$) was repeated five to seven times after each of four to six irradiation periods ranging from 150 ms to 60 s.

Comparison of energies required for fragmentation of precursor ions such as $[2M_n + Na]^+$ complexes, requires that the size of the complexes be considered [11,91,92]. The complex of M_5 has more atoms n_a and thus more degrees of freedom N with which to distribute energy relative to the complexes of M_3 and of M_2, meaning it requires more energy to break the non-covalent bond in the former complex than it requires

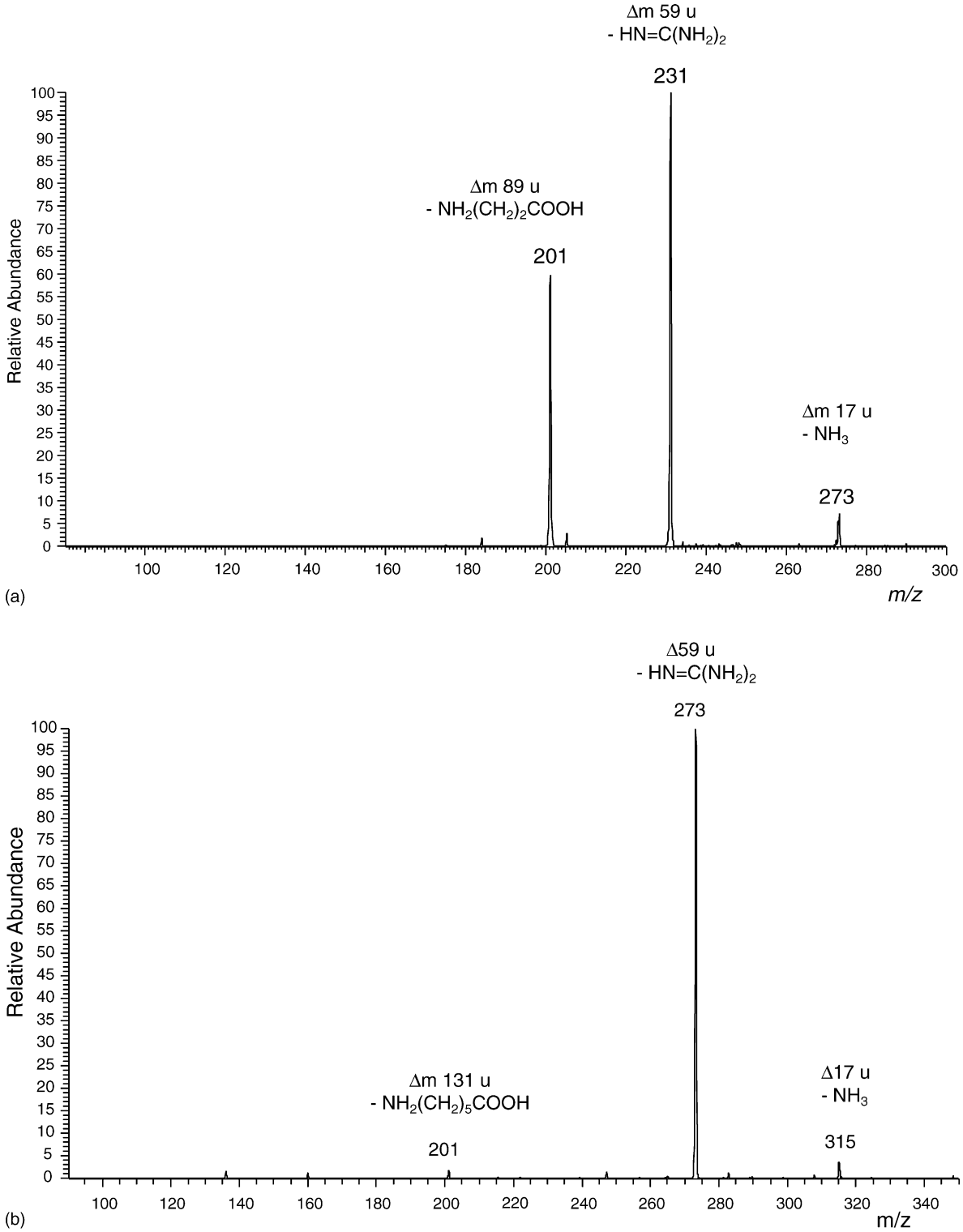

Fig. 2. (a) QIT MS^2 spectra of the precursor ion $[M_2 + Na]^+$ (m/z 290). (b) QIT MS^2 spectra of the precursor ion $[M_5 + Na]^+$ (m/z 332).

for the equivalent non-covalent bond in the latter complexes, within the same time frame.

$$(E_a^{laser})_{corrected} = E_a^{laser}\left(\frac{N_{reference}}{N_{complex}}\right) \quad (4)$$

This kinetic shift problem is compensated by correcting the relative activation energy barriers for the number of degrees of freedom ($n = 3n_a - 6$) in the complexes (see Eq. (4)) [11]. The number of degrees of freedom of the complex $[2M_3 + Na]^+$ was chosen as $N_{reference}$ for the scaling of the other two complexes (see Table 1. Supplementary material).

3. Results and discussion

3.1. Quadrupole ion trap low energy CID of $[M_n + Na]^+$ and $[arg + Na]^+$ precursor ions

In Table 1 and Fig. 2, three characteristic fragmentation reactions of $[M_n + Na]^+$ precursor ions in the QIT MS^2 experiments are documented. All sodium adduct ions of M_n derivatives lose guanidine (59 u), aminoalkylcarboxylic acid $NH_2(CH_2)_nCOOH$, or ammonia (17 u). The product ion signals for these three fragmentation reactions vary in abundance and are sensitive indicators for the structures of M_n in the precursor ions.

The neutral loss of the aminoalkylcarboxylic acid side chain (see Fig. 3) can be reasonably explained by a mechanism based on non-ionic structures of M_n in $[M_n + Na]^+$ ions. A feature of this mechanism is that the sodium ion is associated with the amide carbonyl having a very high cation affinity [67,93]. The attachment of the sodium cation (Lewis acid) to the amide carbonyl leads to a significant withdrawal of electron density at the respective carbon supporting the shift of the free electron pair of the amide nitrogen and establishing an increase in double bond character of the respective amide bond. The subsequent proton transfer sets the stage for the separation of the neutral aminocarbonic acid side chain ($NH_2(CH_2)_nCOOH$: Δm for $M_1 \equiv 75$ u, $M_2 \equiv 89$ u, $M_3 \equiv 103$ u and $M_5 \equiv 131$ u) by cleaving the adjacent amide bond. The high abundance of the product ions resulting from the side chain loss (75 u for M_1 and 89 u for M_2) points strongly toward non-ionic structures of these M_n derivatives with short side chains (see Table 1).

The precursor ions M_1 and M_2 show an abundant neutral loss of 59 u, i.e., guanidine, $HN{=}C(NH_2)_2$. Because M_1 and M_2 have side chains of limited length we assume that intramolecular salt-bridge interactions of zwitterionic structures should be substantially hindered and are therefore unlikely. Thus, we propose a fragmentation mechanism for M_1 and M_2 featuring non-ionic structures shown in Fig. 4a.

Fig. 4b shows a mechanism for the characteristic neutral loss of guanidine, $HN{=}C(NH_2)_2$ (59 u), based on zwitterionic structures of the respective M_n molecules with longer side chains ($n = 3, 5$). The alkali metal cation is coordinated

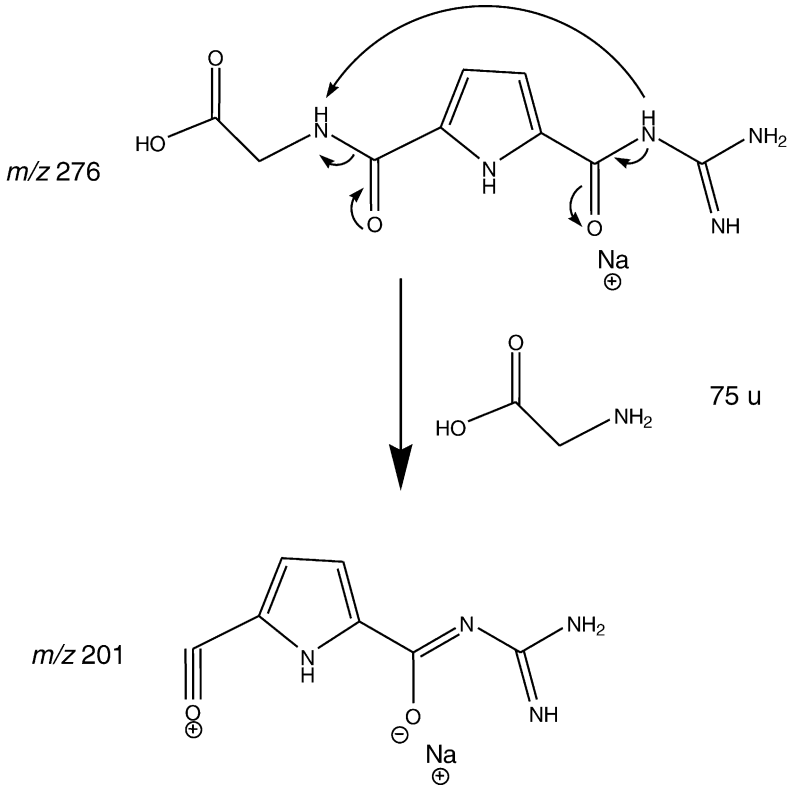

Fig. 3. . Fragmentation mechanism for the neutral loss of $NH_2(CH_2)_nCOOH$ in QIT MS^2 experiments for $[M_n + Na]^+$ ($n = 1, 2, 3, 5$): $[M_1 + Na]^+$ precursor ion at m/z 276 loses NH_2CH_2COOH ($\Delta m = 75$ u).

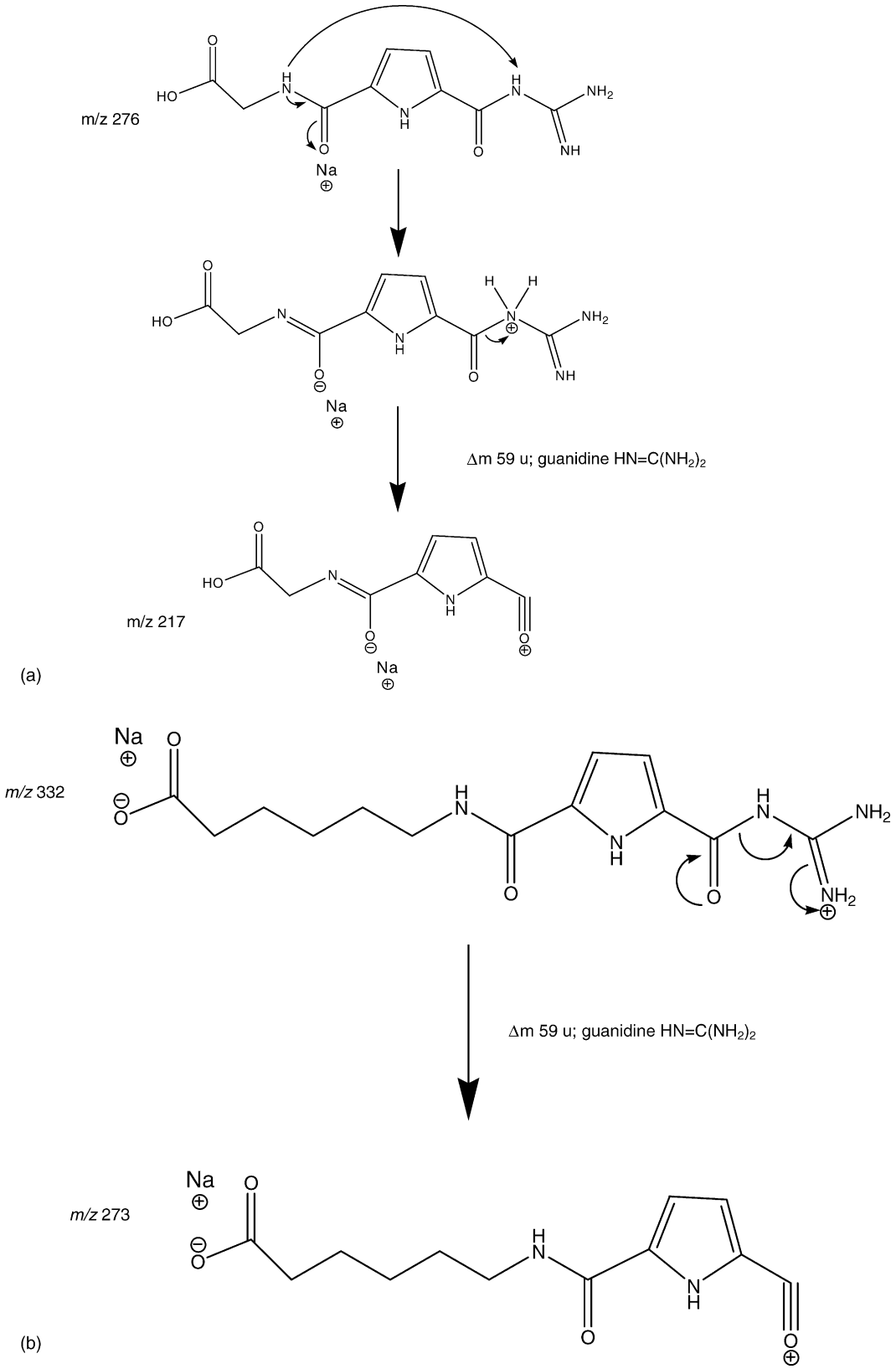

Fig. 4. (a) Fragmentation mechanism for the neutral loss of guanidine in QIT MS^2 experiments for $[M_n + Na]^+$ (n = 1, 2): $[M_1 + Na]^+$ precursor ion at m/z 276 loses guanidine ($HN=C(NH_2)_2$; Δm = 59 u). (b) Fragmentation mechanism for the neutral loss of guanidine in QIT MS^2 experiments for $[M_n + Na]^+$ (n = 3, 5). The formation of an intramolecular salt bridge (i.e., Coulombic interaction of the sodium ion with the carboxylate and the guanidinium functionality) stabilizing the zwitterionic structure is not depicted for reasons of clarity: $[M_5 + Na]^+$ precursor ion at m/z 332 loses guanidine ($HN=C(NH_2)_2$; Δm = 59 u).

to the carboxylate at the end of the aliphatic side chain and a positive charge (i.e., the proton) is located at the basic guanidinium group of the molecule. The zwitterionic structures of M_n rely on the ability of the acylguanidinium functionality to deprotonate the carbonic acid side chain, the stabilization of the gas-phase structures by intramolecular salt-bridge interactions and the stabilizing contribution arising from the presence of the sodium cation. Peptide structures of this type have been implicated recently in other fragmentation processes [67,94,95]. Numerous tandem MS studies of arginine-containing peptides have shown that arginine is preferentially protonated due to its high basicity, and that these protons are nearly immobile [96,66,94]. However, once the positively charged acylguanidinium group is formed, a cleavage of the adjacent amide bond is facilitated, and neutral guanidine (59 u) is expelled (see Fig. 4b). The loss of guanidine gains importance with increasing length of the aliphatic side-chain of the M_n molecules (Table 1). We assume that the increased flexibility of the side-chain of M_3 and M_5 enables intramolecular salt-bridge interactions with substantial stabilization of zwitterionic structures. Consequently, for M_3 and M_5 we favor a mechanism for the neutral loss of guanidine involving zwitterionic structures for the prominent fragmentation reaction.

The sodium adduct ion of arginine $[arg + Na]^+$ loses predominantly H_2O and with minor abundance ammonia (see Table 1). Jockusch et al. proposed fragmentation mechanisms for both characteristic neutral losses (H_2O and NH_3) and explained the high abundance of the $[arg + Na - H_2O]^+$ signal as strong support for a non-ionic structure of arginine in the respective sodium adduct ion. With increasing size of alkali ions, salt-bridged structures of zwitterionic arginine were postulated for the alkali ion adducts $[arg + AM]^+$ (alkali metal cation AM: K^+, Rb^+, Cs^+) indicated by increasing importance of the loss of ammonia [61]. Except for $[M_1 + Na]^+$ all examined precursor ions (i.e., sodium adduct ions of M_2, M_3 and M_5) loose ammonia in QIT MS^2 product ion experiments but the respective product ions are of minor abundance (see Table 1). Although for arginine the low abundance of the product ion referring to the loss of ammonia is taken as a hint for a non-ionic gas-phase structure of the amino acid we favor a fragmentation mechanism involving zwitterionic gas-phase structures at least for the molecules with longer side chains (i.e., M_3 and M_5) to be more appropriate as a stabilization by intramolecular salt bridges seems to be possible (see Fig. 5).

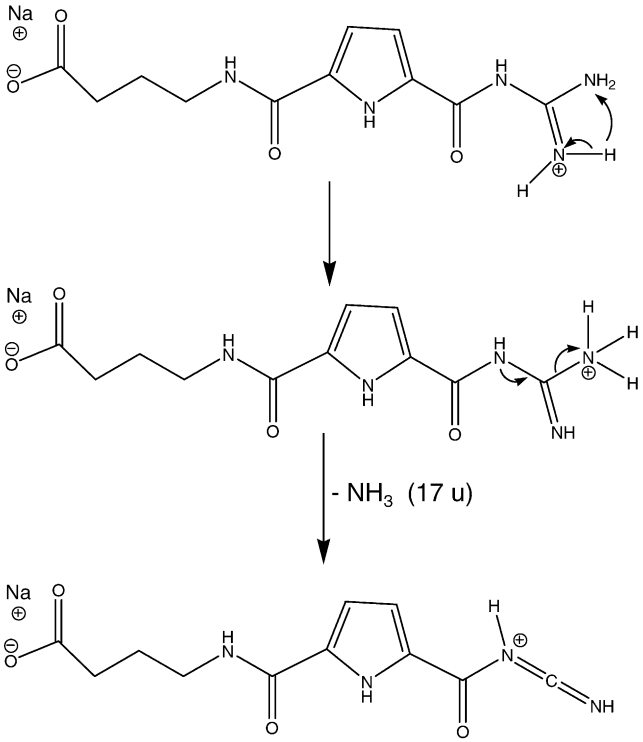

Fig. 5. Fragmentation mechanism for the neutral loss of ammonia NH_3 in QIT MS^2 experiments for $[M_n + Na]^+$ (n = 2, 3, 5): $[M_3 + Na]^+$ precursor ion at m/z 304 loses NH_3 (Δm = 17 u) [61].

3.2. Determination of relative sodium affinities (ΔH_{Na^+}) by the kinetic method

Kish et al. [39] applied the kinetic method to determine ion affinities of α-amino acids and determined a maximum sodium affinity (>225 kJ mol^{-1}) for arginine. In that extensive study, increased sodium affinities are found for α-amino acids with functionalized side chains, especially when amide and electron-rich aromatic groups are available. Obviously, the side chain substituent participates as additional binding ligand in the electrostatic coordination of the metal ion. For proline, the high Na^+ affinity is attributed to the zwitterionic character of the pro-Na^+ bond. Besides the high proton affinity of the secondary amine in proline, the nearly linear geometry of the + − + charges of the salt bridge is assumed to be crucial [40,42]. Hence, the sodium affinities of the molecules M_n related to the respective value of arginine serve as a sensitive measure of the electrostatic interactions in the $[M_n + Na]^+$ ions.

Heterodimers of the compounds M_n (n = 2, 3, 5) could be prepared by ESI-MS from mixed solutions of the respective molecules M_n and arginine. The precursor ions $[M_n + M_m + Na]^+$ and $[M_n + arg + Na]^+$ with $n \neq m$ and n, m = 2, 3, 5 were subjected to MS^2 low energy CID experiments

Table 2
Low energy CID MS^2 QIT experiments for sodium-bound cluster $[M_n + M_m + Na]^+$ and $[M_n + arg + Na]^+$ precursor ions with $n \neq m$ and n, m = 2, 3, 5

Precursor ion	Abundances of MS^2 product ions
$[M_2 + arg + Na]^+$	$[arg + Na]^+$
$[M_3 + arg + Na]^+$	$[arg + Na]^+ \gg [M_3 + Na]^+$
$[M_5 + arg + Na]^+$	$[arg + Na]^+ \leq [M_5 + Na]^+$
$[M_2 + M_3 + Na]^+$	$[M_2 + Na]+ < [M_3 + Na]^+$
$[M_2 + M_5 + Na]^+$	$[M_2 + Na]^+ \ll [M_5 + Na]^+$
$[M_3 + M_5 + Na]^+$	$[M_3 + Na]^+ < [M_5 + Na]^+$

The MS^2 product ion abundances are compared to establish a relative order of sodium affinities (ΔH_{Na^+}).

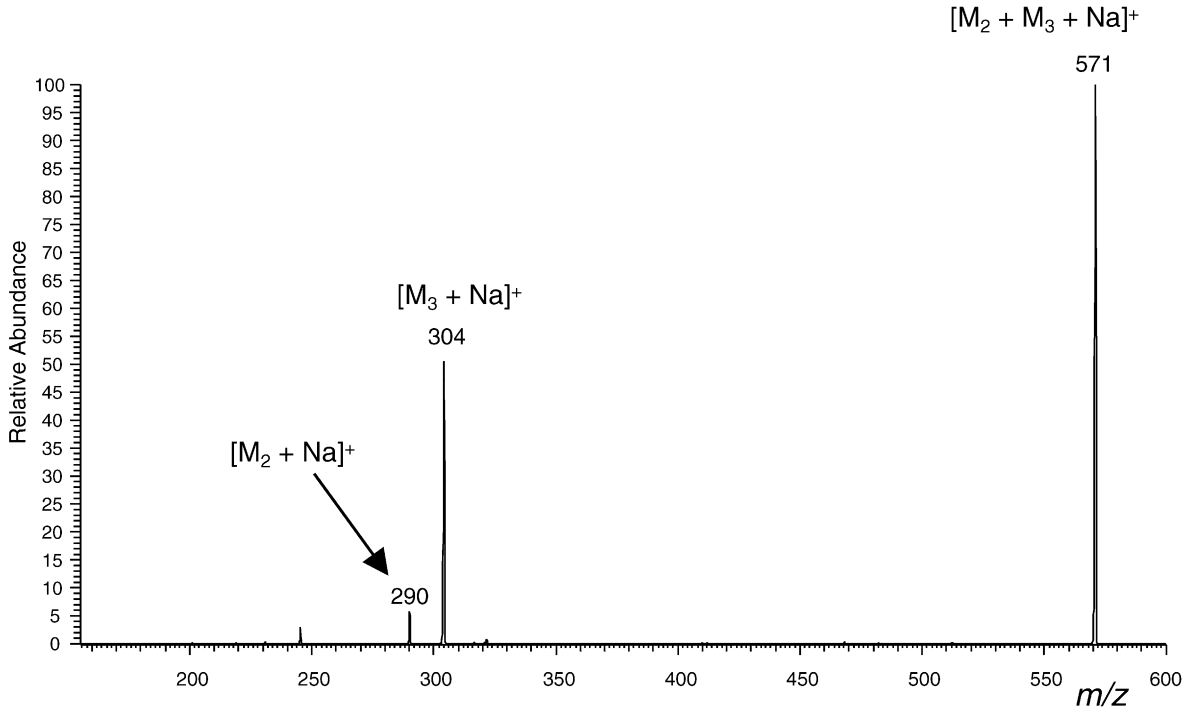

Fig. 6. QIT MS^2 spectrum of the hetero-dimeric ion $[M_2 + M_3 + Na]^+$.

in a QIT and were systematically studied by measuring and relating the abundances of the product ions $[M_n + Na]^+$, $[M_m + Na]^+$ and $[arg + Na]^+$, respectively. The results of these experiments are shown in Table 2 (see also Fig. 6).

A qualitative sodium affinity (ΔH_{Na^+}) order for the molecules M_n may be inferred from the relative abundances ($M_2 < M_3 <$ arginine $\leq M_5$) of the product ions shown in Table 2. Because the sodium affinity of M_n increases with

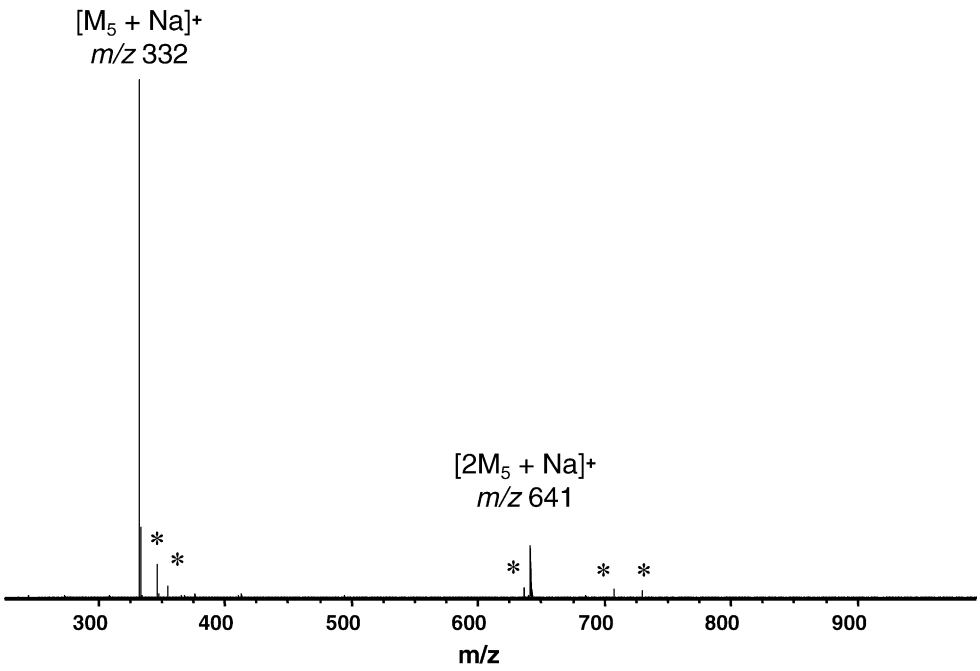

Fig. 7. IRMPD of the precursor ion $[2M_5 + Na]^+$ (*m/z* 641) at 8.9 W cm^{-2} CO_2 laser power, 1500ms irradiation period, detected by FT-ICR MS. Ions marked with an asterisk (*) probably result from IRMPD fragmentation reactions of high mass clusters that are not completely ejected by quadupole and SWIFT mass selection; the signals are generally weak and do not affect the evaluation of precursor/product ion abundance ratios.

increasing length of the side chain, it is reasonable to assume that at least M_5 with a sodium affinity in the range of arginine is very likely able to establish zwitterionic structures with electrostatic salt-bridge interactions to the sodium ion in the complex ion $[M_5 + Na]^+$. Obviously, the length and flexibility of the aliphatic side chain determines the conformational orientations possible, thereby limiting the magnitude of stabilization of zwitterionic structures by intramolecular salt bridges.

3.3. IRMPD of $[2M_n + Na]^+$ precursor ions in an FT-ICR mass spectrometer: determination of the relative energy of activation

Dimeric sodium adduct ions $[2M_n + Na]^+$ were selected and their dissociation behavior upon infrared laser activation was examined. All of the precursor ion complexes dissociate exclusively into the respective monomeric sodium adduct ion $[M_n + Na]^+$ (see, e.g., Fig. 7) and are therefore chosen for comparative study. The homologous IRMPD behavior of the complexes allows the establishment of a relative ordering of the gas-phase dissociation energy barriers E_a^{laser} for the specific disruption of the respective non-covalent interaction [86,87,90,88].

Fig. 8 shows the plots of experimental data to determine the rate constants for the dissociation of the precursor ion $[2M_2 + Na]^+$. Analogous figures for the precursor ions $[2M_n + Na]^+$ ($n = 3, 5$) are provided in the supplementary material. The rate constants and laser power densities are substituted into Eq. (3) to determine the energy of activation. It should be noted that the E_a^{laser} in Eq. (3) is not an absolute and must be used only as a relative value [90,88]. Fig. 9 shows a plot of the natural log of the dissociation rate constant k_{diss} for each respective laser power density versus the natural log of the laser power density in watts per area. The relative activation energy E_a^{laser} for the dissociation of the three precursor ions was calculated from the slope of each line in Fig. 9. The inherent kinetic shift, due to differing number of degrees of freedom in the three activated complexes $[2M_n + Na]^+$ of M_2, M_3 and M_5 was corrected according to Eq. (4) [11]. The $(E_a^{laser})_{corrected}$ for the dissociation of the $[2M_n + Na]^+$ complexes, calculated from Eq. (4), was 0.69 eV (67 kJ mol^{-1}) for M_2, 0.84 eV (81 kJ mol^{-1}) for M_3 and 0.49 eV (47 kJ mol^{-1}) for M_5.

The data for $n = 3$ and the corresponding linear fit ($R^2 = 0.9628$) in Fig. 9 require close and careful evaluation. Here, the dissociation rate constant of $n = 3$ at the laser power density of 4.5 W cm^{-2} is crucial because the fit is very much determined by that data point. The profiles of natural logarithm of the relative precursor ion abundances vs. time for the three complexes at the same laser power density of 4.5 W cm^{-2} are compared in Fig. 10. Obviously, $[2M_3 + Na]^+$ is substantially more stable than the respective complexes of M_2 and M_5, which is generally true for all laser power densities applied. Thus, the questioned linear fit for $n = 3$ in Fig. 9, having an acceptable coefficient of determination of 0.9628, correctly reflects the experimentally determined superior stability of the complex $[2M_3 + Na]^+$ because the steepest slope of the fit consequently leads to the highest value of E_a^{laser} for M_3 relative to M_2 and M_5.

Dunbar originally derived Eq. (3) to describe the photodissociation of styrene ions of relatively low molecular weight and demonstrated its successful application [84]. For ions containing relatively few atoms, it is reasonable to assume that only one vibrational mode is mainly responsible for the energy exchange with the laser photons. Since large molecules provide many more oscillators it is very likely that many available vibrational modes are involved in energy absorption at or near the applied laser frequency. Consequently, Eq. (3) tends to underestimate energy barriers for

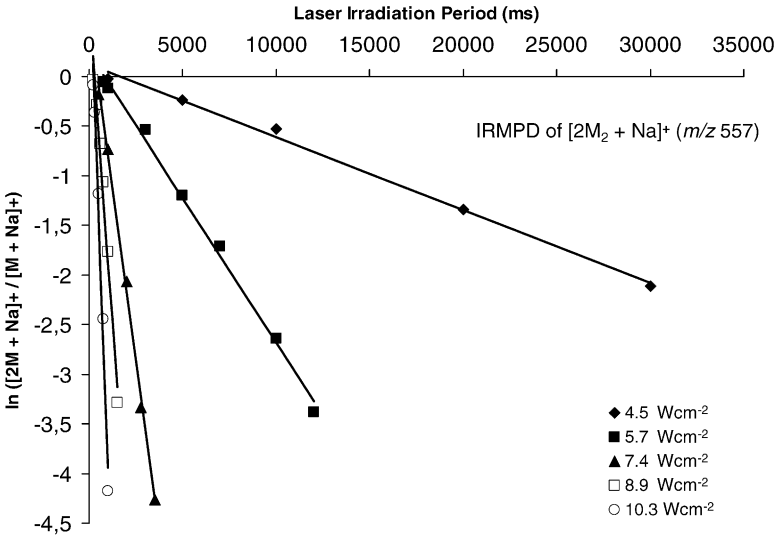

Fig. 8. Natural logarithm of the abundance of the precursor ion $[2M_2 + Na]^+$ (m/z 557) relative to the abundance of the primary fragment $[M_2 + Na]^+$ (m/z 290), vs. laser irradiation period, for each of five indicated laser power densities.

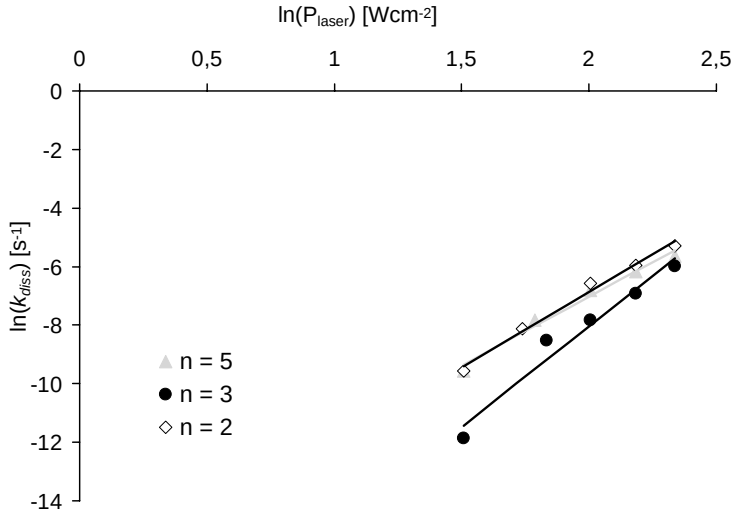

Fig. 9. Natural logarithm of the first-order dissociation rate constant, k_{diss} [s^{-1}], for each respective laser power density vs. the natural logarithm of the laser power density, (P_{laser}) [$W\,cm^{-2}$] for the precursor ions $[2M_n + Na]^+$ with n = 2, 3, 5. The slope of each line yields the relative activation energy for the unimolecular dissociation of the respective dimeric sodium adduct ion (see Eq. (3)).

the photodissociation of larger molecules [87,70]. However, for a series of structurally related precursor ion derivatives with similar vibrational frequencies, it appears reasonable to assume that the IRMPD-determined E_a^{laser} values provide a reliable ladder of *relative* activation energies for a chosen fragmentation reaction [87]. The experimentally determined activation energy for the dissociation of the non-covalent interaction of the $[2M_3 + Na]^+$ precursor ion is substantially higher than that of the respective complex ions of M_2 and M_5. The molecules M_2, M_3 and M_5 have very closely related structures. Hence, the basicity of the guanidio-carbonyl pyrrole functionality is expected to be very similar. Obviously, the difference in relative activation energy observed for the dissociation of the cationized dimeric complexes is related to different conformations of the complexes limited or determined by the flexibility and the length of the side chain. The short M_2 side chain probably limits the variety of conformations able to stabilize the complex by hydrogen bonds of non-ionic structures or by electrostatic interactions of zwitterionic structures. For the complex of M_5, a substantial entropic contribution of the long and highly flexible side chain must be considered, possibly the reason for the reduced complex stability of the $[2M_5 + Na]^+$ ion. The maximum stability of the three examined complexes was found for the M_3 complex. Here, the M_3 molecules combine the ability to generate zwitterionic structures with a sufficient flexibility and length of the side chain that provides the conformational prerequisites for a strong stabi-

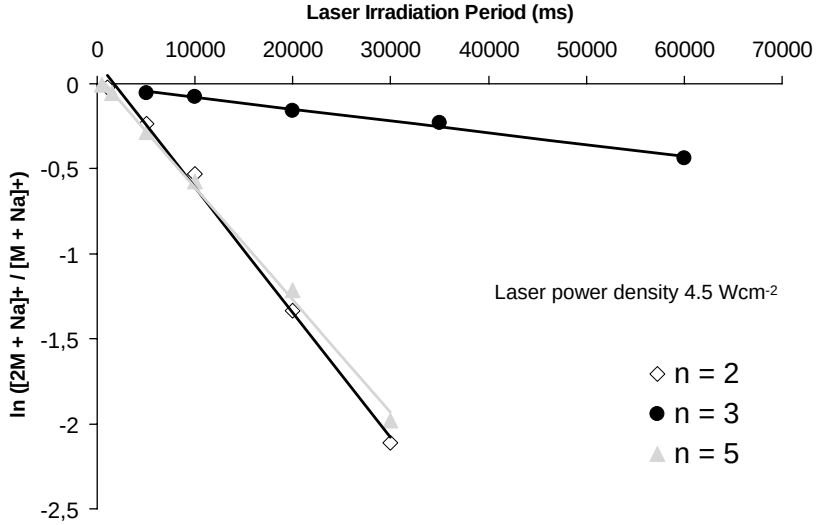

Fig. 10. Natural logarithm of the abundance for each of three precursor ions $[2M_n + Na]^+$ (n = 2, 3, 5) relative to the abundance of the respective primary fragment $[M_n + Na]^+$, vs. irradiation period of the laser at 4.5 $W\,cm^{-2}$ power density.

lization of the $[2M_3 + Na]^+$ complex by formation of salt bridges.

4. Conclusion

The gas-phase structures of the structurally related compounds M_n $[Gua^+–NH–(CH_2)_n–COO^-]$ with $n = 1, 2, 3, 5$ and Gua = guanidiniocarbonyl pyrrole have been determined from a series of MS/MS experiments. The strong tendency of these species to aggregate in polar solvents and the formation of zwitterionic structures was analogously found in the gas phase, at least for the compounds M_3 and M_5. In particular, the QIT MS^2 fragmentation behavior of sodium adduct ions $[M_n + Na]^+$ ions ($n = 1, 2, 3, 5$) provides evidence for the change in structure varying from predominantly charge solvation of non-ionic molecules (M_1, M_2 and arginine) to salt-bridge interactions of zwitterionic structures of M_3 and M_5. This assumption was additionally confirmed by the determination of a maximum sodium affinity for M_5 relative to the reference value of arginine.

Dimeric sodium adduct ions $[2M_n + Na]^+$ of M_2, M_3 and M_5 dissociate upon IR activation in an FT-ICR mass spectrometer exclusively into the respective monomeric sodium adduct ion $[M_n + Na]^+$. Hence, a relative ordering of the gas-phase dissociation energy barriers E_a^{laser} for the selective disruption of the non-covalent interaction in these complexes could be established by IRMPD-FT-ICR MS/MS. We find the complex ion $[2M_3 + Na]^+$ to be more stable than the respective complexes of M_2 and M_5. Obviously, the stability of the examined complex ions $[2M_n + Na]^+$ seems to be controlled more by various electrostatic interactions of the two molecules M_n than by the sodium affinity of the monomer compound M_n. Thus, the stability of the gas-phase complex ions $[2M_n + Na]^+$ depends on the occurrence and the influence of steric hindrance, of Coulombic attraction in the case of zwitterionic structures as well as the stabilization by multiple hydrogen bonds for non-ionic structures. Moreover, a destabilizing contribution of entropy has to be considered, especially for the complex $[2M_5 + Na]^+$ connected with the high flexibility of the long side chain, making the formation of a stable complex conformation energetically difficult. A conformational analysis by molecular mechanics/dynamics calculations of gas-phase $[2M_n + Na]^+$ ions with energy minimization of theoretically proposed structures is currently being attempted but will be protracted due to the complexity of the problem.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijims.2004.07.001.

Appendix B. Supplementary material

References

[1] C.V. Robinson, E.W. Chung, B.B. Kragelund, J. Knudsen, R.T. Aplin, F.M. Poulsen, C.M. Dobson, J. Am. Chem. Soc. 118 (1996) 8646.
[2] N. Potier, L.J. Donald, I. Chernushevich, A. Ayed, W. Ens, C.H. Arrowsmith, K.G. Standing, Protein Sci. 7 (1998) 1388.
[3] T.D. Wood, R.A. Chorush, F.M. Wampler, D.P. Little, P.B. Oconnor, F.W. McLafferty, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 2451.
[4] Y. Dotsikas, Y.L. Loukas, J. Am. Soc. Mass Spectrom. 14 (2003) 1123.
[5] V. Gabelica, N. Nives Galic, F. Rosu, C. Houssier, E. De Pauw, J. Mass Spectrom. 38 (2003) 491.
[6] V. Ryzhov, R.C. Dunbar, J. Am. Soc. Mass Spectrom. 10 (1999) 862.
[7] M.J. Greig, H. Gaus, L.L. Cummins, H. Sasmor, R.H. Griffey, J. Am. Chem. Soc. 117 (1995) 10765.
[8] K. Eckart, J. Spiess, J. Am. Soc. Mass Spectrom. 6 (1995) 912.
[9] H.-K. Lim, Y.L. Hsieh, B. Ganem, J. Henion, J. Mass Spectrom. 30 (1995) 708.
[10] S.D. Friess, J.M. Daniel, R. Hartmann, R. Zenobi, Int. J. Mass Spectrom. 219 (2002) 269.
[11] M.C. Crowe, J.S. Brodbelt, J. Am. Soc. Mass Spectrom. 14 (2003) 1148.
[12] M. Schäfer, Angew. Chem. Int. Ed. 42 (2003) 1896.
[13] C.A. Schalley, Int. J. Mass Spectrom. 194 (2000) 11.
[14] C.A. Schalley, Mass Spectrom. Rev. 20 (2001) 253.
[15] J.L.P. Benesch, F. Sobott, C.V. Robinson, Anal. Chem. 75 (2003) 2208.
[16] J.A. Loo, Mass Spectrom. Rev. 16 (1997) 1.
[17] J.A. Loo, Int. J. Mass Spectrom. 200 (2000) 175.
[18] J.M. Daniel, G. McCombie, S. Wendt, R. Zenobi, J. Am. Soc. Mass Spectrom. 14 (2003) 442.
[19] J.M. Daniel, S.D. Friess, S. Rajagopalan, S. Wendt, R. Zenobi, Int. J. Mass Spectrom. 216 (2002) 1.
[20] V.J. Nesatyy, Int. J. Mass Spectrom. 221 (2002) 147.
[21] V.J. Nesatyy, J. Mass Spectrom. 36 (2001) 950.
[22] J.A. Loo, J.X. He, W.L. Cody, J. Am. Chem. Soc. 120 (1998) 4542.
[23] D.T. Kaleta, M.F. Jarrold, J. Phys. Chem. A 106 (2002) 9655.
[24] A.M. Cappalonga Bunn, R.S. Alexander, D.W. Christianson, J. Am. Chem. Soc. 116 (1994) 5063.
[25] K.A. Sannes-Lowery, H.Y. Mei, J.A. Loo, Int. J. Mass Spectrom. 193 (1999) 115.
[26] Q. Wu, J. Gao, D. Joseph-McCarthy, G.B. Sigal, J.E. Bruce, G.M. Whitesides, R.D. Smith, J. Am. Chem. Soc. 119 (1997) 1157.
[27] H. Rogniaux, A. Van Dorsselaer, P. Barth, J.F. Biellmann, J. Barbanton, M. van Zandt, B. Chevrier, E. Howard, A. Mitschlere, N. Potier, L. Urzhumtseva, D. Moras, A. Podjarny, J. Am. Soc. Mass Spectrom. 10 (1999) 635.
[28] F.W. McLafferty, N.L. Kelleher, T.P. Begley, E.K. Fridriksson, R.A. Zubarev, D.M. Horn, Curr. Opin. Chem. Biol. 2 (1998) 571.
[29] P.G. Wolynes, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 2426.
[30] M.J. Locke, R.T. McIver, J. Am. Chem. Soc. 105 (1983) 4226.
[31] M.J. Locke, R.L. Hunter, R.T. McIver, J. Am. Chem. Soc. 101 (1979) 272.

[32] C.J. Chapo, J.B. Paul, R.A. Provencal, K. Roth, R.J. Saykally, J. Am. Chem. Soc. 120 (1998) 12956.
[33] O. Geller, C. Lifshitz, J. Phys. Chem. A 107 (2003) 5654.
[34] M.A. Freitas, A.G. Marshall, Int. J. Mass Spectrom. 183 (1999) 221.
[35] S. Campbell, M.T. Rodgers, E.M. Marzluff, J.L. Beauchamp, J. Am. Chem. Soc. 117 (1995) 12840.
[36] J.L. Beauchamp,?, in: P.B. Armentrout (Ed.), Encyclopedia of Mass Spectrometry, 1, Elsevier, Amsterdam, New York, Tokyo, 2003, p. 843.
[37] T. Wyttenbach, M. Witt, M.T. Bowers, J. Am. Chem. Soc. (2000) 122.
[38] T. Wyttenbach, M. Witt, M.T. Bowers, Int. J. Mass Spectrom. 183 (1999) 243.
[39] M.M. Kish, G. Ohanessian, C. Wesdemiotis, Int. J. Mass Spectrom. 227 (2003) 509.
[40] J.M. Talley, B.A. Cerda, G. Ohanessian, C. Wesdemiotis, Chem. Eur. J. 8 (2002) 1377.
[41] W.D. Price, R.A. Jockusch, E.R. Williams, J. Am. Chem. Soc. 120 (1998) 3474.
[42] S. Hoyau, K. Norrman, T.B. McMahon, G. Ohanessian, J. Am. Chem. Soc. 121 (1999) 8864.
[43] W.D. Price, P.D. Schnier, E.R. Williams, J. Phys. Chem. B 101 (1997) 664.
[44] W.D. Price, R.A. Jockusch, E.R. Williams, J. Am. Chem. Soc. 119 (1997) 11988.
[45] R.A. Jockusch, A.S. Lemoff, E.R. Williams, J. Am. Chem. Soc. 123 (2001) 12255.
[46] R.C. Dunbar, Mass Spectrom. Rev. 23 (2004) 127.
[47] D.S. Gross, E.R. Williams, Int. J. Mass Spectrom. Ion Process. 158 (1996) 305.
[48] V. Ryzhov, R.C. Dunbar, B. Cerda, C. Wesdemiotis, J. Am. Soc. Mass Spectrom. 11 (2000) 1037.
[49] R.C. Dunbar, J. Phys. Chem. A 104 (2000) 8067.
[50] E.F. Strittmatter, E.R. Williams, J. Phys. Chem. A 104 (2000) 6069.
[51] R.R. Julian, J.L. Beauchamp, W.A. Goddard, J. Phys. Chem. A 106 (2002) 32.
[52] R.R. Julian, R. Hodyss, J.L. Beauchamp, J. Am. Chem. Soc. 123 (2001) 3577.
[53] C.A. Schalley, P. Weis, Int. J. Mass Spectrom. 221 (2002) 9.
[54] P. Skurski, M. Gutowski, R. Barrios, J. Simons, Chem. Phys. Lett. 337 (2001) 143.
[55] M. Meot-Ner, in: P.B. Armentrout (Ed.), Encyclopedia of Mass Spectrometry, 1, Elsevier, Amsterdam, New York, Tokyo, 2003, p. 687.
[56] J.H. Jensen, M.S. Gordon, J. Am. Chem. Soc. 117 (1995) 8159.
[57] R.A. Jockusch, A.S. Lemoff, E.R. Williams, J. Phys. Chem. A 105 (2001) 10929.
[58] P.D. Schnier, W.D. Price, R.A. Jockusch, E.R. Williams, J. Am. Chem. Soc. 118 (1996) 7178.
[59] N.P. Ewing, C.J. Cassady, J. Am. Soc. Mass Spectrom. 10 (1999) 928.
[60] K.A. Cox, S.J. Gaskell, M. Morris, A. Whiting, J. Am. Soc. Mass Spectrom. 7 (1996) 522.
[61] R.A. Jockusch, W.D. Price, E.R. Williams, J. Phys. Chem. A 103 (1999) 9266.
[62] R.G. Cooks, J.S. Patrick, T. Kotiaho, S.A. McLuckey, Mass Spectrom. Rev. 13 (1994) 287.
[63] R.G. Cooks, P.S.H. Wong, Acc. Chem. Res. 31 (1998) 379.
[64] R.G. Cooks, J.T. Koskinen, P.D. Thomas, J. Mass Spectrom. 34 (1999) 85.
[65] C. Schmuck, L. Geiger, T. Rehm, Org. Lett., submitted for publication.
[66] M.M. Kish, C. Wesdemiotis, Int. J. Mass Spectrom. 227 (2003) 191.
[67] W.Y. Feng, C. Gronert, K.A. Fletcher, A. Warres, C.B. Lebrilla, Int. J. Mass Spectrom. 222 (2003) 117.
[68] D.G. Morgan, M.M. Bursey, J. Mass Spectrom. 30 (1995) 473.
[69] S. Alves, F. Fournier, J.C. Tabet, M. Quelquejeu, J.M. Valery, Eur. J. Mass Spectrom. 6 (2000) 451.
[70] M. Schäfer, C. Schmuck, M. Heil, H.J. Cooper, C.L. Hendrickson, M.J. Chalmers, A.G. Marshall, J. Am. Soc. Mass Spectrom. 14 (2003) 1282.
[71] M.W. Senko, C.L. Hendrickson, L. Pasa-Tolic, J.A. Marto, F.M. White, S. Guan, A.G. Marshall, Rapid Commun. Mass Spectrom. 10 (1996) 1824.
[72] K. Hakansson, M.J. Chalmers, J.P. Quinn, M.A. McFarland, C.L. Hendrickson, A.G. Marshall, Anal. Chem. 75 (2003) 3256.
[73] J.P. Quinn, M.R. Emmett, A.G. Marshall, in: Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, 1998, p. 1388.
[74] S.K. Chowdhury, V. Katta, B.T. Chait, Rapid Commun. Mass Spectrom. 4 (1990) 81.
[75] M.W. Senko, C.L. Hendrickson, M.R. Emmett, S.D.H. Shi, A.G. Marshall, J. Am. Soc. Mass Spectrom. 8 (1997) 970.
[76] S.C. Beu, D.A. Laude Jr., Int. J. Mass Spectrom. Ion Process. 112 (1992) 215.
[77] A.G. Marshall, T.-C.L. Wang, T.L. Ricca, J. Am. Chem. Soc. 107 (1985) 7893.
[78] S. Guan, A.G. Marshall, Int. J. Mass Spectrom. Ion Process. 157/158 (1996) 5.
[79] M.B. Comisarow, A.G. Marshall, Chem. Phys. Lett. 26 (1974) 489.
[80] A.G. Marshall, D.C. Roe, J. Chem. Phys. 73 (1980) 1581.
[81] M.W. Senko, J.D. Canterbury, S. Guan, A.G. Marshall, Rapid Commun. Mass Spectrom. 10 (1996) 1839.
[82] R.G. Cooks, in: P.B. Armentrout (Ed.), Encyclopedia of Mass Spectrometry, 1, Elsevier, Amsterdam, New York, Tokyo, 2003, p. 350.
[83] P.B. Armentrout, J. Mass Spectrom. 34 (1999) 74.
[84] R.C. Dunbar, J. Chem. Phys. 95 (1991) 2537.
[85] K. Paech, R.A. Jockusch, E.R. Williams, J. Phys. Chem. A 106 (2002) 9761.
[86] M.A. Freitas, C.L. Hendrickson, A.G. Marshall, Rapid Commun. Mass Spectrom. 13 (1999) 1639.
[87] M.A. Freitas, C.L. Hendrickson, A.G. Marshall, J. Am. Chem. Soc. 122 (2000) 7768.
[88] J.W. Flora, D.C. Muddiman, J. Am. Soc. Mass Spectrom. 15 (2004) 121.
[89] J.W. Flora, D.C. Muddiman, J. Am. Chem. Soc. 124 (2002) 6546.
[90] J.C. Hannis, D.C. Muddiman, Int. J. Mass Spectrom. 219 (2002) 139.
[91] E.E. Jellen, A.M. Chappell, V. Ryzhov, Rapid Commun. Mass Spectrom. 16 (2002) 1799.
[92] V. Gabelica, E. De Pauw, J. Mass Spectrom. 36 (2001) 397.
[93] P. Burk, I.A. Koppel, I. Koppel, R. Kurg, J.F. Gal, P.C. Maria, M. Herreros, R. Notario, J.L.M. Abboud, F. Anvia, R.W. Taft, J. Phys. Chem. A 104 (2000) 2824.
[94] J.M. Farrugia, R.A.J. O'Hair, Int. J. Mass Spectrom. 222 (2003) 229.
[95] S.W. Lee, H.S. Kim, J.L. Beauchamp, J. Am. Chem. Soc. 120 (1998) 3188.
[96] V.H. Wysocki, G. Tsaprailis, L.L. Smith, L.A. Breci, J. Mass Spectrom. 35 (2000) 1399.