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On the question of salt bridges of cationized amino acids in the gas phase: glycine and arginine

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Abstract

The geometrical shapes of the sodiated and cesiated amino acids glycine and arginine were probed in the gas phase by using the ion mobility based ion chromatography method. The data were compared to those obtained for alkali cationized methyl esters and for all the protonated species. Molecular mechanics, semiempirical, and ab initio/density functional theory (DFT) calculations were carried out to generate model structures for comparison with experiment and to determine the relative energies of different structures. For alkali cationized glycine the experimental cross sections agreed with charge solvation structures which were found by calculation to be the most stable forms as well. Both experiment and theory indicated that sodium is solvated by both the amino and the carbonyl groups, while cesium is solvated by one or both oxygen(s) of the carboxyl group. Alkali cationized arginine was found to form a salt bridge structure. The carboxylate group is stabilized by both the charged guanidinium group and the alkali ion. High level (6-311++G** and DZVP) ab initio/DFT calculations carried out on sodiated and rubidiated N amidinoglycine, which contains a guanidino group and which was used as a model for the larger arginine molecule, indicated that the salt bridge structures are ~10 kcal/mol more stable than the charge solvation forms for both alkali ions. The structure of protonated arginine, i.e. salt bridge or charge solvation, could not be unambiguously determined. (Int J Mass Spectrom 182/183 (1999) 243–252) © 1999 Elsevier Science B.V.

Keywords: Ion mobility; Ion chromatography; Salt bridge; Ion structure

1. Introduction

Amino acids are known to exist as zwitterions both in crystals and in aqueous solution. In the gas phase, however, in the absence of solvating intermolecular interactions zwitterions are far less stable than in the condensed phase. Glycine, for instance, is not a zwitterion in the gas phase as has been shown unambiguously by experiment [1–5] and theory [6–10]. The N terminus is not basic enough to deprotonate the carboxylic acid on the C terminus. The much more basic amino acid arginine, on the other hand, could potentially form a stable zwitterion in the gas phase, a hypothesis that is supported by some indirect experimental evidence[†] and some preliminary theoretical studies [11]. Zwitterions are

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Dedicated to the memory of Ben Freiser.

[†] Experimental results reported by Price et al. [11] are based on kinetic studies, which are sensitive to transition states rather than equilibrium geometries; however, because transition states often (but not necessarily) mirror equilibrium structures, kinetic data can be considered as an indirect probe of equilibrium geometries.

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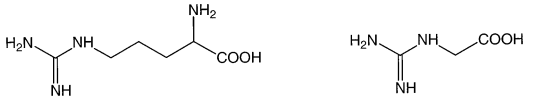
greatly stabilized by the addition of counter ions. Thus, although the glycine zwitterion is unstable by 15–20 kcal/mol [6–10], the sodiated glycine zwitterion is only ~3 kcal/mol less stable than the charge solvation structure [12]. Based on this result and on the fact that the proton affinity of arginine is >30 kcal/mol larger than that of glycine [13], sodiated arginine is expected to form a salt bridged zwitterion.

An open and important question is: "How does the stability of the salt bridge structure depend on the choice of the cation?" In this work, we will address this question and report results on the stability of cationized amino acids (glycine, arginine) as a function of choice of cation (sodium, rubidium, cesium).

Experimental data are obtained via the ion mobility based ion chromatography method [14], which yields information on the shape of ions. In this fashion, the shape of a protonated species can be compared to that of an alkali ion cationized species. Theory often predicts distinctly different shapes for protonated, cationized charge solvation, and cationized salt bridge structures [12]. Comparing the theoretical data with experiment often allows unambiguous interpretation of the experimental data. Theory is also used to calculate relative energies of different selected structures.

Arginine (Scheme 1, left) is a very flexible molecule with six rotatable bonds. It can assume many different conformations, which jeopardizes a thorough theoretical investigation. Therefore, the much smaller molecule N amidinoglycine (NAG) (see Scheme 1, right) has extensively been used in this work as a model molecule for arginine.



Scheme 1

2. Experimental

Experimental details and instrumentation have been previously described [15,16]. Briefly, ions were formed by MALDI [17] (matrix assisted laser desorption ionization) using 2,5-dihydroxybenzoic acid (DHB) as a matrix and an excimer laser running as a nitrogen laser at 100 Hz as a photon source. For this study the strongest ion signals were obtained with an unusually high sample-to-matrix ratio of ~1:100. Ions of interest were mass selected in a reverse geometry double focusing mass spectrometer. A packet of ions of 1–5 μs duration was injected at 10 eV (lab) into a drift cell filled with ~3 Torr of helium typically at 300 K and pulled through the cell by a weak electric field of 2.5–25 V/cm. After exiting the drift cell, the ions passed through a quadrupole mass filter and an ion arrival time distribution (ATD) was obtained at the detector. Using kinetic theory [18], an experimental value of the ion–He collision cross section could readily be calculated from the ATD data. Experimental cross sections were reproducible within 1%. Systematic errors are expected to be in the same range.

All chemicals were purchased from SIGMA (St. Louis, MO) with the exception of NAG methyl ester, which was synthesized as the trifluoroacetate salt by mixing 1 part of NAG with ~1.5 parts of trifluoroacetic acid and ~10 parts of methanol and by letting esterification occur overnight at room temperature. Mass spectra indicated that yields were between 50% and 90%. One synthesis was carried out with CD_3OH in order to obtain an ion with mass $m/z = 157$ for $[(NAG)OCD_3]Na^+$, which could be distinguished from a small peak at $m/z = 154$ due to the matrix.

3. Calculations

Theoretical molecular structures were obtained from molecular mechanics, semiempirical (AM1, PM3), Hartree–Fock ab initio, and density functional theory (DFT) calculations. The programs used include AMBER [19], GAMESS [20], and GAUSSIAN94 [21]. Molecular mechanics was generally used to search the potential surface for possible minima via a simulated annealing procedure [22]. On the most promising minima, semiempirical (for molecules without alkali ions), ab initio and/or DFT calculations were carried out in order to get as accurate a set of relative energies as possible. Full geometry optimizations were carried out at each level. The standard basis sets used for the

Table 1
Relative (electronic[a]) energies (kcal/mol) for selected structures of sodiated and rubidiated glycine and NAG calculated at the level indicated

		B3LYP/6-311++G**	B3LYP/DZVP	
	Structure[b]	Na^+	Na^+	Rb^+
Gly	B_G	−2.8	−2.5	−2.3
	C_1	—	+1.2	−3.6
	C_2	—	+1.4	−3.7
	X_G	0	0	0
NAG	B_1	+8.8	+9.4	+10.1
	B_2	+8.0	—	—
	X	0	0	0

[a] Without zero point energy correction.
[b] See Figs. 2 and 3 for schematic structures corresponding to these labels.

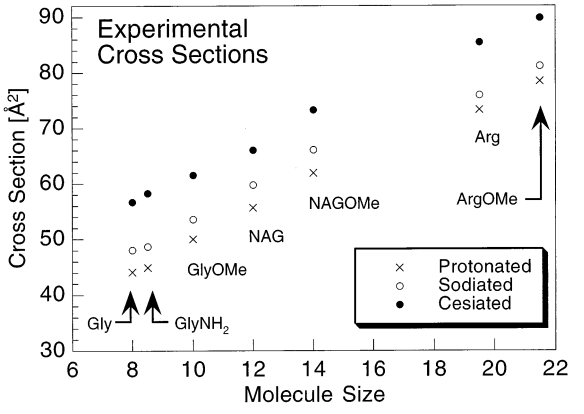


Fig. 1. Experimental cross sections for glycine (Gly), N-amidinoglycine (NAG), arginine (Arg), their methyl esters (. . . OMe), and glycine amide ($GlyNH_2$). Molecular size, which increases from left to right, is indicated in units of $n_{C,N,O} + 1/2\, n_{H,\,alkali}$ (n_i = number atoms i; see the text).

ab initio and DFT (B3LYP) [23] calculations are indicated with the results. For calculations on rubidium containing molecules, the DZVP basis set [24] has been used. We found that B3LYP/DZVP results obtained for sodiated species compare very favorably with B3LYP/6-311++G** results (see Table 1).

For each model structure, an orientation averaged cross section was calculated using the projection approximation [25]. Atomic collision radii were calculated based on a (12,6,4) potential [26] employing Lennard-Jones parameters* that were scaled by the molecular size as previously described [12]. Errors of calculated cross sections resulting from numerical integrations are <1%.

4. Results

4.1. Experimental data

All of the ion ATDs recorded at 300 K showed one single peak, as expected, for all species that have the same shape or for species that interconvert rapidly between different structures on the experimental time scale. The collision cross sections obtained from the ATD data for the protonated, sodiated, and cesiated forms of glycine (Gly), N-amidinoglycine (NAG), L-arginine (Arg), their methyl esters (GlyOMe, etc.), and glycine amide ($GlyNH_2$) are shown in Fig. 1. As the number of atoms in a system increases, the cross section increases as well. For instance, replacing a hydroxy group by a methoxy group adds 3 atoms and increases the cross section by ∼6 Å^2. It is found empirically that if the number of carbon, nitrogen, and oxygen atoms plus half the number of hydrogen (and alkali) atoms is used as a measure of molecular size, a reasonable correlation between size and cross section is obtained within the protonated, sodiated, and cesiated species, respectively. The choice of these size units has no consequences for the data analysis that follows other than to say that adding two hydrogens to a molecule accounts for about the same cross section increase as adding one carbon, nitrogen, or oxygen atom. From the data shown in Fig. 1, it can further be concluded that replacing a hydrogen atom by a sodium increases the cross section by ∼4 Å^2 and replacing a hydrogen by cesium by ∼12 Å^2. Geometric differences between the different species are subtle and hidden below these large molecular size effects.

* The following He–X Lennard-Jones parameters have been used for the atoms X indicated in brackets: $r_{LJ(60)}$ = 2.38 Å (H), 2.70 (Na), 3.02 (C, N, O), 3.34 (Rb, Cs), $\epsilon_{LJ(60)}$ = 0.34 kcal/mol (H), 0.36 (Na), 0.37 (C, N, O, Rb, Cs); the values $r_{LJ(60)}$ and $\epsilon_{LJ(60)}$ were scaled by the molecule size as described in the appendix of [12] before collision radii were calculated as described in [26].

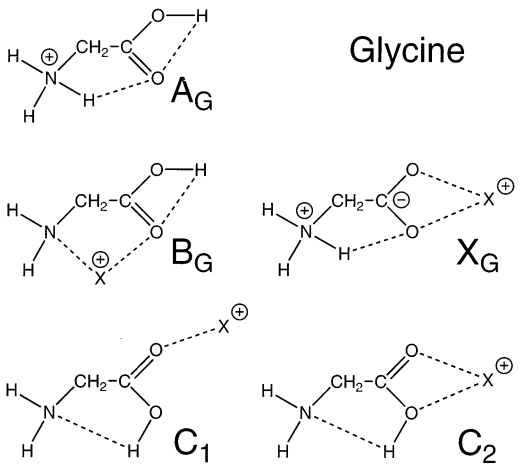


Fig. 2. Schematic representation of calculated glycine structures (X^+ = alkali ion).

However, the systematic study of a series of analogous compounds allows extraction of information about the shape of a given compound. Such an approach has been proven useful in a number of previous studies on carbon clusters [25,27], synthetic polymers [16], and polypeptides [12]. In this work, a set of compounds M = Gly, NAG, Arg is measured and compared within the series $X^+ = H^+$, Na^+ Cs^+ for both MX^+ and the methyl esters thereof.

4.2. Calculated structures

The most studied and best known of the structures considered in this work is that of protonated glycine [28–30]. The most basic site, the N-terminus, is protonated and there exists a relatively strong hydrogen bond between the N- and C-terminus ($–N^+H_3 \ldots O{=}C<$) as schematically shown in Fig. 2, structure A_G. A weaker hydrogen bond ties the –OH and the >C═O of the carboxyl group together.

For sodiated glycine the only two reasonable candidate structures are B_G and X_G (Fig. 2). It is fairly well established that the charge solvation form B_G is energetically more stable than the salt bridge form X_G by several kilocalories per mole, and corresponds to the dominant isomer present at 300 K [12]. Thus, the Gly "backbone" assumes very much the same conformation in both $GlyH^+$ and $GlyNa^+$. The only difference is that the $GlyH^+$ molecule contains a covalent bond/hydrogen bond (–H . . .) system ($–H_2N^+–H \ldots O{=}C<$) while the sodium in $GlyNa^+$ is bound electrostatically ($–H_2N \ldots Na^+ \ldots O{=}C<$).

Table 2
Differences between (electronic [a]) energies of structures C_2 and those of B_G calculated at the level indicated (in kcal/mol)[b]

	$E(C_2)–E(B_G)$	
	HF/STO-3G	B3LYP/DZVP
$GlyNa^+$	+6.9	+3.9
$GlyRb^+$	−0.1	−1.4
$[GlyOMe]Na^+$	+15.9	—
$[GlyOMe]Rb^+$	+9.3	—

[a] Without zero point energy correction.

[b] Structures C_2 and B_G are shown in Fig. 2. Energies of structures C_1 are comparable to those of C_2 at the B3LYP/DZVP level (Table 1), whereas C_1 is not a minimum at the HF/STO-3G level.

The structures of glycine cationized by larger alkali ions like Rb^+ and Cs^+ are less well studied. DFT calculations on $GlyRb^+$ indicate that structures C_1 and C_2 shown in Fig. 2 with the Rb^+ ion coordinated to the carboxylate end of the molecule are the most stable. Both C_1 and C_2 are calculated to be very close in energy and there appears to be no substantial barrier between them. The results summarized in Table 1 show that these rubidiated type C charge solvation structures are 3–4 kcal/mol more stable than the salt bridge structure X_G. In contrast, the B_G structure for $GlyNa^+$ is calculated to be the most stable with the X_G salt bridge structure next and the C_1 and C_2 structures highest in energy. Thus, replacing a sodium ion by a rubidium ion reduces the relative stability of the salt bridge structure. Although the C structures of $[GlyNH_2]Rb^+$ are expected to be similarly stable, the corresponding C structures for $[GlyOMe]Rb^+$ are considerably less stable because of the lack of the hydrogen bond between the N- and the C-terminus (see Table 2).

AMBER molecular mechanics calculations carried out for $GlyCs^+$ agree very well with the higher level calculations for $GlyRb^+$ and indicate that the structure C_1 is 1.6 kcal/mol more stable than B_G. Because the structure C_1 is more extended than structures B_G and X_G, it will have a larger cross section. The calculated

Table 3
Relative (electronic[a]) energies (kcal/mol) of protonated N-amidinoglycine conformations A_1 and A_2[b]

	Structure A_1	Structure A_2
AMBER	3.0	0
AM1	3.4	0
PM3	3.4	0
B3LYP/6-31G*	2.0	0

[a] Without zero point energy correction.
[b] Structures A_1 and A_2 are given in Fig. 3.

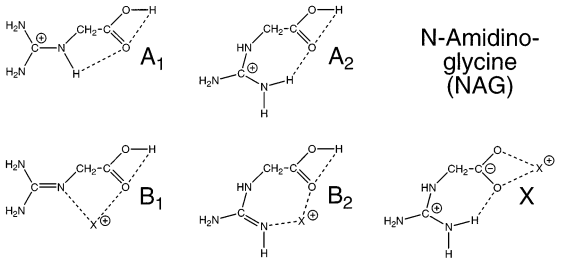


Fig. 3. Schematic representation of calculated NAG structures (X^+ = alkali ion).

values for $GlyRb^+$ C_1 and B_G are 57.1 and 54.6 Å^2, respectively.

Like for $GlyH^+$, the structure of protonated NAG is straightforward to predict. The molecule is protonated on the only basic site, the guanidino group. A salt bridge form is not possible due to the lack of a second basic site. From the very few conformations this molecule can assume, all levels of theory (see Table 3) indicate that the fairly planar conformation A_2 is the most stable. Similar to $GlyH^+$, the structure of $[NAG]H^+$ is a good standard for comparison with any data obtained for alkali cationized NAG.

A search for possible structures of sodiated NAG indicates that there are three or four (depending on the level of theory) different significant minima on the potential surface including two (or three) charge solvation structures and one salt bridge structure. At the highest level of theory employed here (B3LYP/6-311++G**), the charge solvation structures B_1 and B_2 and the salt bridge are the only significant stable structures found.* It can be seen in Fig. 3 that the neutral guanidino group in B_1 is an $(NH_2)_2C{=}N–$ unit while in B_2 it is of the form $HN{=}C(NH_2)–NH–$. The salt bridge structure X exhibits a protonated guanidinium group. Structures B_1 and B_2 are about equally stable but ~8 kcal/mol less stable than salt bridge X (Table 1). The cross sections calculated for the B3LYP/6-311++G** structures B_1, B_2, and X are virtually identical at 61.8, 61.2, and 61.3 Å^2, and that of structure A_2 of protonated NAG is 58.5 Å^2.

The calculations for rubidiated NAG indicate that the situation is very much the same as for $[NAG]Na^+$. The salt bridge X is much more stable (by ~10 kcal/mol) than the charge solvation forms B (Table 1). It should be pointed out that, like for glycine, the relative energies between the B and the X structures are about the same for both the sodiated and the rubidiated forms. Although it has been shown previously that for $GlyRb^+$ and $GlyCs^+$ the charge solvation structures of type C are more stable than the B structure, AMBER calculations carried out on $[NAG]Cs^+$ indicate that the analogous C structures are somewhat (~1 kcal/mol) less stable than the B structures. The $[NAG]Cs^+$ type C structures are, however, relatively much more stable than in the sodiated species.

Typical examples of calculated structures obtained by molecular mechanics for protonated and sodiated arginine are shown in Fig. 4. Arginine is a fairly large and flexible molecule that can assume a range of conformations, rather than just one or two. A number of these structures are clustered at the lowest energies. The range of cross sections calculated for structures with energies within the lowest 2 kcal/mol for both the charge solvation and salt bridge species are indicated by the bars in Fig. 5(a). Errors in the cross section calculations (<1%) are also included in the bars. The global minima located by molecular mechanics are marked with open symbols for charge solvation forms and with closed symbols for salt bridges. It can be seen that the charge solvation

* It is interesting that in lower level calculations, a minimum B_3 is found corresponding to a charge solvation structure with a threefold Na^+ coordination; atoms underlined are those bound to the sodium ion: $H\underline{N}{=}C(NH_2)–\underline{N}H–CH_2–C\underline{O}OH$; at the HF/STO-3G level structure B_3 is even the global minimum being ~4 kcal/mol below B_1 and B_2, whereas the salt bridge X is not a minimum.

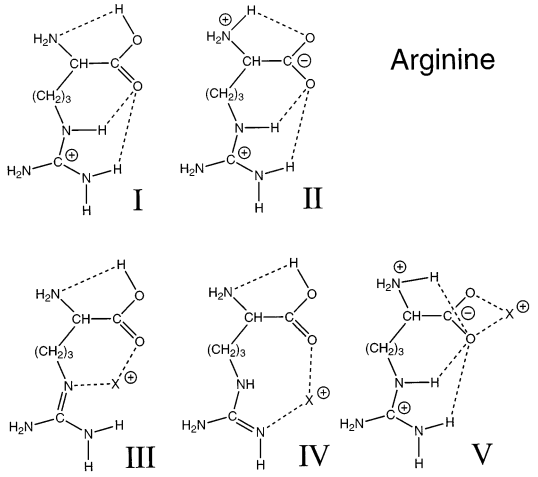


Fig. 4. Schematic representation of calculated arginine structures (X^+ = alkali ion).

conformers cover a relatively large range of cross sections including both compact and more extended structures. Structures with a guanidino group such as the one in structure III (Fig. 4) are marked with squares, those with a guanidino group like structure IV with a circle. The salt bridge structures of $ArgX^+$ ($X^+ = H^+$, Na^+, Cs^+) have a more distinct shape and tend to be rather compact [Fig. 5(a)].

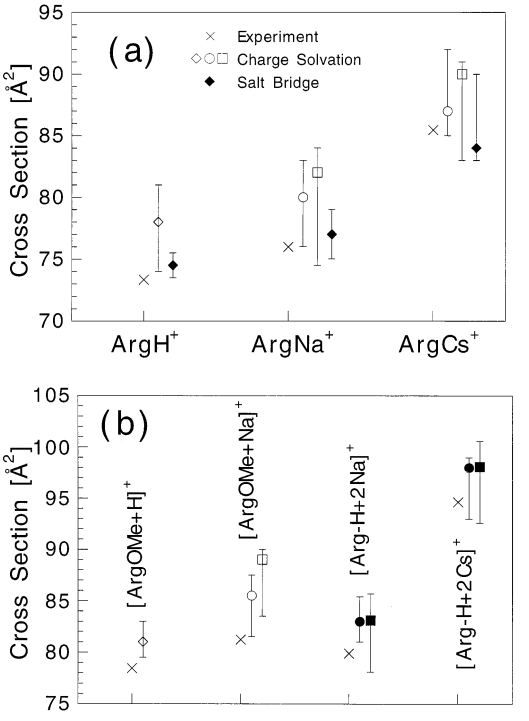


Fig. 5. Experimental and theoretical cross sections of arginine species. Experimental data are indicated with a cross, theoretical charge solvation data with open, and salt bridge with closed symbols. Diamonds are used for theoretical structures featuring a charged guanidinium group (e.g. structures I, II, V in Fig. 4), circles a neutral guanidino group of type HN═C(NH_2)–NHR (e.g. structure IV), squares (NH_2)$_2$C═NR (e.g. structure III). Bars cover structures within 2 kcal/mol AMBER energy.

5. Discussion

5.1. Glycine

Previous experiments and theory [12] have shown that the most stable $GlyNa^+$ structure is the charge solvation structure B_G schematically depicted in Fig. 2. As mentioned in Sec. 4, theoretical work on $GlyRb^+$ and $GlyCs^+$ indicates that the relatively extended charge solvation forms C_1 and C_2 with relatively large cross sections are the most stable structures in these cases. For $[GlyOMe]Rb^+$ and $[GlyOMe]Cs^+$, on the other hand, theory unambiguously predicts the more compact structures of type B to be the ground states. The experimental data indicate indeed that the cross sections of $GlyCs^+$ and $[GlyNH_2]Cs^+$ are relatively large (in line with C type structures), whereas the $[GlyOMe]Cs^+$ cross section is relatively small (in line with a B type structure). To verify that this is the case the data have to be normalized by the cross sections of the protonated species since the acid, the amide, and the methyl ester have different numbers of atoms (i.e. intrinsic sizes). In order to be able to compare data for both sodiated and cesiated species and to account for the different sizes of the two ions the data are further normalized by the methyl ester data. Any other system (acid or amide) would have worked as well as a normalizing agent, but the methyl ester was chosen because theory indicates that all of its alkali cationized species assume structure B_G. The result shown in Fig. 6(a) demonstrates that the $GlyCs^+$ and $[GlyNH_2]Cs^+$ cross sections after normalization are about 5% larger

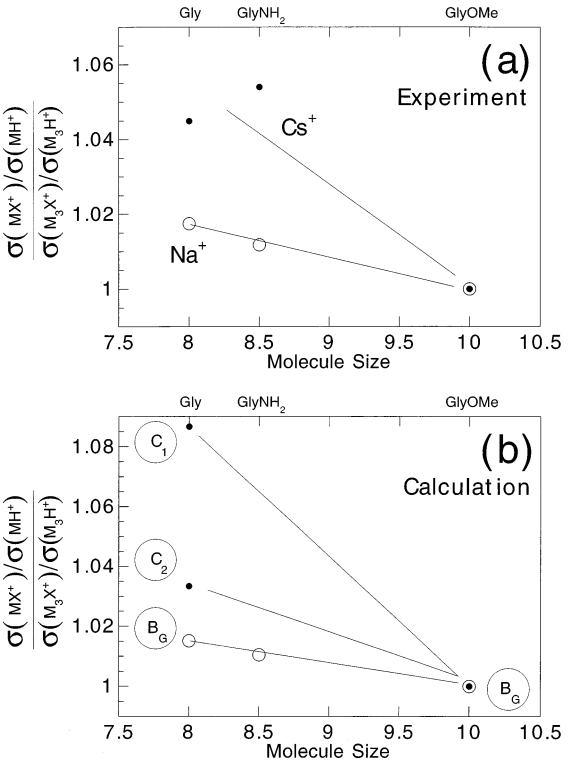


Fig. 6. (a) Experimental and (b) theoretical (AMBER) cross sections of the sodiated ($X^+ = Na^+$, circles) and cesiated (Cs^+, dots) glycine derivatives (M = Gly, $GlyNH_2$, GlyOMe) normalized by the data of the protonated species (MH^+) and by the methyl ester (M_3) data. The symbols B_G, C_1, C_2 in (b) refer to the corresponding structures in Fig. 2. See caption of Fig. 1 for units.

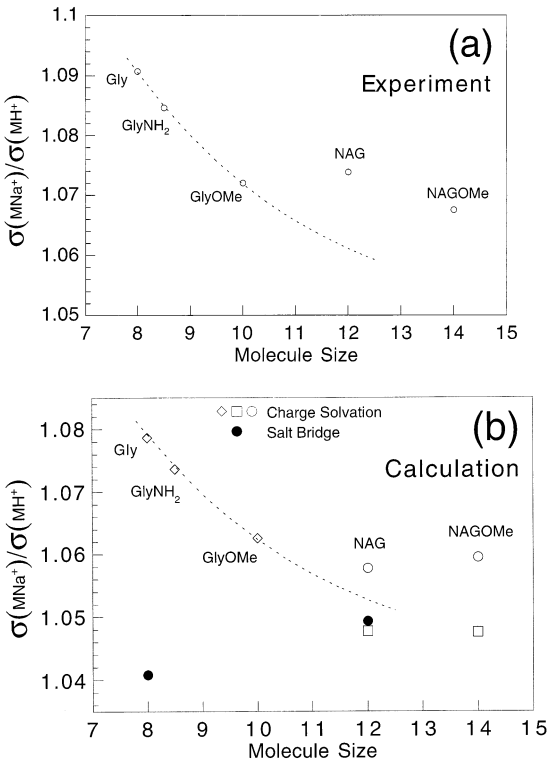


Fig. 7. (a) Experimental and (b) theoretical cross section data for glycine and NAG derivatives. Diamonds in (b) refer to charge solvation structure B_G (Fig. 2), circles to B_1 (Fig. 3), squares to B_2, and dots to salt bridge structures. See caption of Fig. 1 for units.

than the $[GlyOMe]Cs^+$ cross section supporting their assignment as C type structures. In contrast to the cesiated species, Fig. 6 shows that the sodiated ions $GlyNa^+$, $[GlyNH_2]Na^+$, and $[GlyOMe]Na^+$ have very similar shapes, in line with theory that favors the type B_G structures for all sodiated species.

5.2. NAG

The experimental data indicate that both $[NAG]Na^+$ and its methyl ester exhibit large cross sections compared to the respective protonated forms and compared to the glycine systems. This can be seen in Fig. 7(a), where the system size (related to the number of atoms per system) increases from left to right. Assuming that the protonated and sodiated forms exhibit the same structures, as is the case for Gly, $GlyNH_2$, and GlyOMe (structures A_G for protonated and B_G for sodiated forms, respectively), the cross section ratio of sodiated to protonated forms should asymptotically reach a value of 1 as the system size increases. For the glycine system, there is indeed a steady drop (dashed line), but as the system increases further to NAG, the Na^+/H^+ cross section ratio shows a discontinuity. Thus, replacing a proton by a sodium has a stronger effect in the NAG systems than it has in the glycine systems. Because the effect is present for both NAG and its methyl ester, it cannot be considered a direct indication for formation of a salt bridge structure of the sodiated NAG system. (The NAG methyl ester cannot form a salt bridge due to the lack of an acidic hydrogen.)

Theory, on the other hand, clearly favors formation of a salt bridge for $[NAG]Na^+$ (Table 1). The salt

bridge structure X is relatively compact with a small cross section compared to structure A_2 of $[NAG]H^+$ [Fig. 7(b)]. For the methyl ester theory predicts the charge solvation structures, both B_1 and B_2, to be the preferred geometries. While B_1 is nearly planar B_2 is folded similarly to X. Of the calculated cross section ratios B_1/A_2, B_2/A_2, and X/A_2, that for B_1 matches experiment best (Fig. 7). In an attempt to check for the presence of multiple structures, some experiments were carried out at 80 K. Reducing the temperature has two effects. First, structures which interconvert rapidly at room temperature might be frozen out at lower temperatures [31]. Second, the resolution of the experiment increases by lowering the temperature [18]. Structures with cross sections that differ by ~5% are expected to be baseline resolved in our experimental setup [22], whereas structures with ~1% different cross sections should be observed as a broadened peak at 80 K. In this case, however, only one narrow peak is observed in the 80 K ATDs of both $[NAG]Na^+$ and $[(NAG)OMe]Na^+$.

While structure B_1 is a reasonable candidate for sodiated (NAG)OMe there appears to be a conflict between experiment and calculation for sodiated NAG. Theory clearly favors the salt bridge X and experiment the charge solvation form B_1. One possible reason for this discrepancy could be the cross section calculation algorithm [26,32,33].* In any case, at this point no definitive conclusion can be made about the structure of $[NAG]Na^+$.

For $[NAG]Rb^+$, theory indicates that the salt bridge form is also the most stable structure by ~10 kcal/mol. Among the charge solvation structures, the extended type C structures, which are most stable for $GlyRb^+$ and $GlyCs^+$, are somewhat less stable than the B structures in the NAG systems. This result is in line with the experimental data, which do not support any structures with large cross sections. The experimental value of 66.1 $Å^2$ for $[NAG]Cs^+$ is small (in line with B and X structures) as normalization analogous to that used in Fig. 6 yields a ratio of 1.003. Thus, $[NAG]Cs^+$ is only 1.003 times as large as $[(NAG)OMe]Cs^+$ after correction for the fact that the methoxy group is larger than the hydroxy group. For glycine, the value is 1.045 (Fig. 6). Hence, C structures can be ruled out for rubidiated and cesiated NAG and (NAG)OMe systems, but unfortunately, neither a salt bridge nor a B type charge solvation structure can be unambiguously identified.

5.3. Arginine

As can be seen in Fig. 5(a), theory indicates that protonated and alkali cationized arginine salt bridge structures are on average more compact than charge solvation structures, even though some overlap occurs when a 2 kcal/mol range of structures is included. It can also be seen that the experimental cross sections (shown as crosses) are in line with compact structures for all species, protonated, sodiated, and cesiated. This agreement is very suggestive of salt bridge structures for all three systems.

The experimental and theoretical results for the arginine methyl ester and for the doubly cationized ions $[Arg–H + 2Na]^+$ and $[Arg–H + 2Cs]^+$ are given in Fig. 5(b). Agreement is acceptable. However, it should be noted that experiment is somewhat smaller than theory in all cases. Because the methyl ester systems must have charge solvation structures and the doubly cationized ions salt bridges, this result suggests some caution must be employed in strongly interpreting absolute cross section comparisons.

On the basis of the ab initio/DFT calculations carried out on alkali cationized NAG, which is a good model for alkali cationized arginine, it is expected that the latter will assume a salt bridge structure. In fact,

* The presence of three significantly charged centers (2+ and 1−) in salt bridge ions could potentially be responsible for an enhanced He–ion interaction, therefore increasing the measured collision cross section due to a stronger interaction rather than a larger geometry. In fact, projection cross sections have to be considered approximations to true collision cross sections anyway. For instance, it is known that projection cross sections calculated for planar systems are typically an overestimation for the true collision cross section [26], whereas the reverse is true for cup-shaped structures [32,33]. For the glycine systems such effects would cancel (when comparing Na^+/H^+ ratios) because A_G and B_G have basically the same shape. For NAG, however, the shape of the lowest energy protonated structure A_2 is somewhat different than any of the sodiated structures. B_1 and B_3 are obviously different from A_2 while B_2 and X have up to 20° different dihedral angles compared to A_2.

arginine is more likely to form salt bridges than NAG as the guanidinium group sits at the end of a rather long and flexible chain, making stabilization of a salt bridge easier. For example, the model structures shown in Fig. 4 indicate that the cationized arginine salt bridge structures exhibit two hydrogen bonds from the guanidinium to the carboxylate group. Furthermore, if neutral arginine is a zwitterion as previously suggested [11], then cationized arginine is most definitively a salt bridge. As mentioned in Sec. 1, the sodium affinity for a zwitterion is much larger than that for the corresponding neutral form (for glycine by 12–17 kcal/mol [12]).

For protonated arginine, on the other hand, $[NAG]H^+$ is not a good model because arginine has two basic sites, as opposed to NAG, which has only one. $ArgH^+$ is therefore much more likely to form a salt bridge than $[NAG]H^+$. However, the glycine studies indicate that the $H_3N^+–CH_2–COO^-$ zwitterion cannot be stabilized enough by the addition of an alkali ion to have it become more stable than the charge solvation structure. It is therefore not obvious that the bulkier and less flexible guanidinium group is able to do a better job stabilizing the $H_3N^+–CHR–COO^-$ zwitterion than an alkali ion. One factor favoring the guanidinium group is the formation of strong hydrogen bonds (see Fig. 4) as opposed to purely electrostatic interactions in the case of alkali ions. At this point, although no definite conclusions can be drawn about the structure of protonated arginine, the data are most consistent with a salt bridge structure.

6. Conclusions

Calculations and experiment indicate that alkali cationized glycine, glycine amide, and glycine methyl ester assume exclusively charge solvation structures. For structures including smaller alkali ions like sodium and for methyl ester structures the alkali ion preferably binds to both the N and the C terminus (structure B_G). On the other hand, for glycine and glycine amide structures larger alkali ions like rubidium and cesium bind preferentially to the C terminus (structures C_1, C_2). The stabilization of the charge solvation structure compared to that of the salt bridge is larger for $GlyCs^+$ than for $GlyNa^+$.

For alkali cationized N-amidinoglycine (NAG), theory predicts that salt bridges are 9–10 kcal/mol more stable than any charge solvation forms independent of the alkali ion. Experiments indicate that cationized NAG and (NAG)OMe have similar and relatively extended shapes. This similarity can best be explained by invoking charge solvation structures (structure B_1 in particular) for both NAG and (NAG)OMe. Thus, a disagreement between theory and experiment exists for alkali ion cationized NAG and further work will be required to sort it out.

The experimental data for protonated, sodiated, and cesiated arginine agrees best with salt bridge structures. Salt bridges are expected for the cationized species on the basis of calculations performed on the NAG system, a good model for arginine. However, a salt bridge structure may be less likely for protonated arginine than for sodiated or cesiated arginine and depends on the relative ability of a guanidinium group or an alkali ion to stabilize the zwitterion. Hence, compact charge solvation structures cannot be absolutely ruled out, especially for protonated arginine. Higher level theory on this system is needed.

Finally, the experimental data indicate that for all glycine, NAG, and arginine species studied, only a single isomer is evident at 300 K.

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References

[1] R.D. Suenram, F.J. Lovas, J. Mol. Spectrosc. 72 (1978) 372.
[2] R.D. Brown, P.D. Godfrey, J.W.V. Storey, M.-P. Bassez, J. Chem. Soc., Chem. Commun. (1978) 547.
[3] M.J. Locke, R.T. McIver Jr., J. Am. Chem. Soc. 105 (1983) 4226.
[4] K. Iijima, K. Tanaka, S. Onuma, J. Mol. Struct. 246 (1991) 257.
[5] F.J. Lovas, Y. Kawashima, J.-U. Grabow, R.D. Suenram, G.T. Fraser, E. Hirota, Astrophys. J. 455 (1995) L201.
[6] W.R. Oegerle, J.R. Sabin, J. Mol. Struct. 15 (1973) 131.
[7] Y.-C. Tse, M.D. Newton, S. Vishveshwara, J.A. Pople, J. Am. Chem. Soc. 100 (1978) 4329.
[8] D. Yu, D.A. Armstrong, A. Rauk, Can. J. Chem. 70 (1992) 1762.
[9] Y. Ding, K. Krogh-Jespersen, Chem. Phys. Lett. 199 (1992) 261.
[10] J.H. Jensen, M.S. Gordon, J. Am. Chem. Soc. 117 (1995) 8159.
[11] W.D. Price, R.A. Jockusch, E.R. Williams, J. Am. Chem. Soc. 119 (1997) 11988.
[12] T. Wyttenbach, J.E. Bushnell, M.T. Bowers, J. Am. Chem. Soc. 120 (1998) 5098.
[13] E.P. Hunter, S.G. Lias, J. Phys. Chem. Ref. Data, to be published (http://webbook.nist.gov/chemistry).
[14] P.R. Kemper, M.T. Bowers, J. Phys. Chem. 95 (1991) 5134.
[15] P.R. Kemper, M.T. Bowers, J. Am. Soc. Mass Spectrom. 1 (1990) 197.
[16] G. von Helden, T. Wyttenbach, M.T. Bowers, Int. J. Mass Spectrom. Ion Processes 146/147 (1995) 349.
[17] F. Hillenkamp, M. Karas, R.C. Beavis, B.T. Chait, Anal. Chem. 63 (1991) A1193.
[18] E.A. Mason, E.W. McDaniel, Transport Properties of Ions in Gases, Wiley, New York, 1988.
[19] D.A. Pearlman, D.A. Case, J.C. Caldwell, G.L. Seibel, U.C. Singh, P. Weiner, and P.A. Kollman, AMBER 4.0, University of California, San Francisco, 1991.
[20] M.W. Schmidt, K.K. Baldridge, J.A. Boatz, S.T. Elbert, M.S. Gordon, J.J. Jensen, S. Koseki, N. Matsunaga, K.A. Nguyen, S. Su, T.L. Windus, M. Dupuis, J.A. Montgomery, J. Comput. Chem. 14 (1993) 1347.
[21] M.J. Frisch, G.W. Trucks, H.B. Schlegel, P.M.W. Gill, B.G. Johnson, M.A. Robb, J.R. Cheeseman, T. Keith, G.A. Petersson, J.A. Montgomery, K. Raghavachari, M.A. Al-Laham, V.G. Zakrzewski, J.V. Ortiz, J.B. Foresman, J. Cioslowski, B.B. Stefanov, A. Nanayakkara, M. Challacombe, C.Y. Peng, P.Y. Ayala, W. Chen, M.W. Wong, J.L. Andres, E.S. Replogle, R. Gomperts, R.L. Martin, D.J. Fox, J.S. Binkley, D.J. Defrees, J. Baker, J.P. Stewart, M. Head-Gordon, C. Gonzalez, and J.A. Pople, GAUSSIAN94, Revision C.2, Gaussian, Inc., Pittsburgh, PA, 1995.
[22] T. Wyttenbach, G. von Helden, M.T. Bowers, J. Am. Chem. Soc. 118 (1996) 8355.
[23] A.D. Becke, J. Chem. Phys. 98 (1993) 5648.
[24] N. Godbout, D.R. Salahub, J. Andzelm, E. Wimmer, Can. J. Chem. 70 (1992) 560.
[25] G. von Helden, M.-T. Hsu, N.G. Gotts, M.T. Bowers, J. Phys. Chem. 97 (1993) 8182.
[26] T. Wyttenbach, G. von Helden, J.J. Batka Jr., D. Carlat, M.T. Bowers, J. Am. Soc. Mass Spectrom. 8 (1997) 275.
[27] G. von Helden, M.-T. Hsu, P.R. Kemper, M.T. Bowers, J. Chem. Phys. 95 (1991) 3835.
[28] S. Bouchonnet, Y. Hoppilliard, Org. Mass Spectrom. 27 (1992) 71.
[29] F. Jensen, J. Am. Chem. Soc. 114 (1992) 9533.
[30] D. Yu, A. Rauk, D.A. Armstrong, J. Am. Chem. Soc. 117 (1995) 1789.
[31] J. Gidden, T. Wyttenbach, J.J. Batka, P. Weis, A.T. Jackson, J.H. Scrivens, and M.T. Bowers, J. Am. Chem. Soc., submitted.
[32] A.A. Shvartsburg, M.F. Jarrold, Chem. Phys. Lett. 261 (1996) 86.
[33] A.A. Shvartsburg, G.C. Schatz, M.F. Jarrold, J. Chem. Phys. 108 (1998) 2416.