Interaction of Alkali Metal Cations ($Li^+ - Cs^+$) with Glycine in the Gas Phase: A Theoretical Study

Sophie Hoyau and Gilles Ohanessian*

Abstract: The complexes formed by alkali metal cations (Cat^+) and glycine (Gly) were studied by means of ab initio quantum chemical methods. Seven types of $Gly - Cat^+$ interaction were considered in each case. It was found that in the most stable forms of $Gly - Li^+$ and $Gly - Na^+$ the metal ion is chelated between the carbonyl oxygen and nitrogen ends of glycine. For $Gly - K^+$ an isomer involving complexation with both oxygens of the carboxylic function is found to be degenerate with the above chelate, and becomes slightly more stable for $Gly - Rb^+$ and $Gly - Cs^+$. In all cases, interaction of the ion with the carboxylate group of zwitterionic glycine is also low in energy. Computed binding energies (ΔH_{298}, kcal mol^{-1}) are 54.5 ($Gly - Li^+$), 36.3 ($Gly - Na^+$), 26.5 ($Gly - K^+$), 24.1 ($Gly - Rb^+$) and 21.4 ($Gly - Cs^+$). The values for $Gly - Na^+$ and $Gly - K^+$ are in good agreement with recent experimental determinations. For $Gly - Li^+$, a revised experimental value of 54.0 kcal mol^{-1} is obtained, based on the computed complexation enthalpy and entropy of Li^+ with *N,N*-dimethylformamide (51.7 kcal mol^{-1} and 23.8 cal mol^{-1} K^{-1}, respectively). Three isomers among the most stable of the lithiated dimer $Gly - Li^+ - Gly$ have been determined and found to involve local $Gly - Li^+$ interactions analogous to those in the monomer. However, the relative energies of the various isomers show nonnegligible differences between the monomer and the dimer, implying that the kinetic method must be used with care for the determination of cation affinities of larger molecules. Finally, the fluxionality of the $Gly - Na^+$ complex has been considered by locating the transition states for interconversion of the lowest energy isomers. In particular it is found that the lowest isomer can be transformed into the one involving zwitterionic Gly with a rate-determining barrier of 20.4 kcal mol^{-1}.

Keywords: ab initio calculations · alkali metals · cations · gas-phase chemistry · glycine

Introduction

Alkali cations participate in many functions of living systems, such as the osmotic equilibrium of cells, the electrical excitability of nerves and muscles, the active transport of glucides and amino acids, and so forth, through their binding to proteins.[1] Modeling alkali – protein complexes requires that the local interactions between the cation and amino acids be accurately known.[2] Such knowledge is also important for the interpretation of the mass spectra of alkali ion – peptide complexes. Fragmentations of such complexes, either metastable or under collisional activation, may provide valuable information on the primary structure of peptides. Interpretation of mass spectra has invoked many possibilities for the attachment site(s) of alkali ions on peptides, including the carboxylate end of zwitterionic peptides,[3] the N-terminal amino group,[4] carbonyl oxygens,[5] basic sites (if any) on lateral chains,[4] and various combinations thereof. In fact, it has also been recognized that mixtures of isomers involving different types of ion complexation are likely to exist in many cases.[4d, 5b, 5c] From this breadth of hypotheses it is clear that the details of cation – peptide interactions remain largely unknown.

In the last few years, both experimental and theoretical techniques have been employed to quantify the interactions in simpler systems, cationized amino acids in the gas phase. Experimental affinities of glycine for the lithium,[6] sodium[6, 7] and potassium[7] cations have been determined. There have also been several papers devoted to the theoretical description of such systems.[8, 9] The present work aims at providing a comprehensive and consistent description of the interaction of all five alkali metal cations ($Li^+ - Cs^+$) with glycine. Several attachment modes have been considered for each of the five alkali metal ions, leading to accurate affinities involving the most stable isomer, and to a quantitative description of several other, low-lying isomers. We use computations on *N,N*-dimethylformamide (DMF) and $DMF - Li^+$ to derive accurate corrections to the published experimental affinity of glycine for Li^+. Overall there is very good agreement between the experimental and computational affinities of glycine for

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Li^+, Na^+, and K^+. The present work provides the first affinities of Gly for Rb^+ and Cs^+. We have also considered the cationized dimer Gly – Li^+ – Gly in order to see if the attachment mode and energetics are similar to those in the monomer, an assumption underlying the kinetic method[10] which has been used to determine relative affinity scales of amino acids to several atomic ions. Finally, we have studied in detail the isomerization pathways connecting some of the isomers of Gly – Na^+ in order to see the extent to which the attached ion can sample the various binding sites available in an amino acid.

Computational Methods

There is a fairly abundant literature, both experimental[11] and computational,[12] on the conformations of glycine. The most accurate computations show that the MP2(full)/6-31G* level is a good compromise for obtaining satisfactory relative energies for the lowest conformers at reasonable computational cost. This is also true for ligand – Na^+ structures in small complexes.[13] Therefore all Gly – Cat^+ geometry optimizations have been performed at this level. The standard 6-31G* basis exists for H, C, N, O, Li and Na. For the heavier alkali ions of K, Rb and Cs, similar bases had to be determined.

Abstract in French: *Les complexes constitués d'un cation alcalin (Cat^+) et de la glycine (Gly) ont été étudiés en utilisant des méthodes de chimie quantique ab initio. Sept types d'interaction Gly – Cat^+ ont été considérés dans chaque cas. La structure la plus stable pour Gly – Li^+ et Gly – Na^+ implique une chélation de l'ion métallique entre l'oxygène du carbonyle et l'azote de la glycine. Pour Gly – K^+, un isomère impliquant une complexation sur les deux oxygènes de la fonction carboxylique est de même énergie que le chélate précédent, et devient un peu plus stable pour Gly – Rb^+ et Gly – Cs^+. Dans tous les cas, l'interaction de l'ion avec la fonction carboxylate de la glycine zwitterionique conduit également à une structure de basse énergie. Les énergies de liaison calculées (ΔH_{298}) valent 54,5 kcal mol^{-1} (Gly – Li^+), 36,3 kcal mol^{-1} (Gly – Na^+), 26,5 kcal mol^{-1} (Gly – K^+), 24,1 kcal mol^{-1} (Gly – Rb^+) et 21,4 kcal mol^{-1} (Gly – Cs^+). Les valeurs pour Gly – Na^+ et Gly – K^+ sont en bon accord avec deux mesures expérimentales récentes. Pour Gly – Li^+, une valeur expérimentale révisée de 54,0 kcal mol^{-1} a été obtenue en utilisant les valeurs calculées de l'enthalpie et l'entropie de complexation de Li^+ avec le N,N-diméthyle formamide (51,7 kcal mol^{-1} et 23,8 cal mol^{-1} K^{-1} respectivement). Trois isomères parmi les plus stables du dimère lithié Gly – Li^+ – Gly ont été calculés; ils impliquent des interactions locales Gly – Li^+ analogues à celles des monomères. Cependant les énergies relatives des différents isomères présentent des différences non négligeables entre le monomère et le dimère. La méthode cinétique doit donc être utilisée avec précaution pour déterminer les affinités cationiques de molécules plus grandes. Finalement, la fluxionnalité du complexe Gly – Na^+ a été étudiée en localisant les états de transition de l'interconversion des isomères de basse énergie. En particulier, la plus haute barrière au cours de la transformation de l'isomère le plus bas en celui impliquant la glycine zwitterionique est de 20,4 kcal mol^{-1}.*

For K we started with the basis of Schäfer et al.[14] and adapted the valence basis for the cation. The geometric mean of the outer two s exponents was optimized so as to minimize the total Hartree – Fock energy of NH_3 – K^+ while maintaining their internal ratio. This led to $\zeta_s = 0.0436$ and 0.0182. A single p gaussian was optimized for Gly – K^+ and then split into two: the optimized exponent was taken as the geometric mean, and the internal ratio was the same as that of the above s gaussians, leading to $\zeta_p = 0.0831$ and 0.0346. Finally a d-type gaussian was optimized for Gly – K^+ ($\zeta_d = 0.36$). Since the heavier alkali metals require the use of relativistic effective core potentials (RECP), the all-electron and RECP approaches were compared in the case of Gly – K^+. In the latter case the RECP of Hurley et al.[15] was used, with their recommended 3s and 3p contractions and the above optimized split-valence plus polarization basis. The structures of the **1** and **3** isomers (vide infra) were optimized by both methods at the MP2 level, and found to differ negligibly from one another (by less than 0.01 Å and 0.1° in bond lengths and angles, respectively). In both cases **3** was found to be lower in energy, with **1** lying higher by 0.3 kcal mol^{-1} (all-electron) and 0.1 kcal mol^{-1} (RECP). The absolute complexation energy is 33.4 and 33.2 kcal mol^{-1} at the all-electron and RECP levels, respectively. Therefore the RECP approach can be used with confidence for this type of complexes. For Rb and Cs, we used the recommended contractions for the 4s,4p and 5s,5p gaussians provided with the RECP of LaJohn et al.[16] and Ross et al.,[17] respectively, and the valence bases were reoptimized following the same procedure as for K. Overall these bases and 6-31G* for the lighter atoms will be denoted basis1. In all cases involving a RECP, the outer core s and p electrons were included in the MP2 correlation calculations. Entropies and thermal corrections were based on MP2/basis1 vibrational frequencies for Gly – Li^+ and Gly – Na^+. The analytical calculation of frequencies is not yet available at the MP2 level if an ECP is involved. Therefore HF/6-31G* frequencies were used for the heavier alkali metals. Test calculations for Gly – K^+ showed that both sets of frequencies lead to very similar thermal corrections to binding energies, but that $T\Delta S$ terms at 298 K can be affected by ca. 1 kcal mol^{-1}.

Extensive test calculations on Gly – Li^+ showed that the use of 6-31G* leads to significant basis set superposition errors (BSSE) and that diffuse functions are essential to reduce BSSE to typically 4% of the binding energy. We found that a good compromise between accuracy and tractability is obtained with 6-311 + G(2d,2p).[18a–c] MP4 calculations for the lowest isomer of each complex showed negligible differences in binding energies with MP2. Therefore final energetics were computed at the level MP2/6-311 + G(2d,2p)//MP2/basis1. Again the extended basis does not exist for heavier alkali metals, and similar sets had to be determined. For Li and Na the 2d polarization set is such that the geometric mean is the 1d exponent[18d] and the internal ratio is 4.[18e] The same was applied to generate the 2d sets for K, Rb, and Cs from basis1. Diffuse s and p functions were also added. The ratios between the outer s and p valence exponents and that of the diffuse ones in 6-311 + G(2d,2p) for the lighter atoms were averaged, yielding a value of 3.15, which was applied to the outer valence exponents of basis1 to generate diffuse gaussians for K, Rb, and Cs. These bases and 6-311 + G(2d,2p) for the lighter atoms are herein denoted basis2. BSSE corrections were computed at the MP2/basis2 level with the standard counterpoise method. Although this method is known to slightly overestimate the corrections, the use of an extended basis leads to corrections small enough that the counterpoise approximation is satisfactory.

For Gly – Li^+ – Gly the HF/6-31G* level was used for geometry optimization in order to keep computations tractable. Given the close similarity between geometries obtained at the H[9] and MP2 (this work) levels for Gly – Li^+, this should not be too severe an approximation. Final energetics were obtained at the MP2/6-31G*//HF/6-31G* level.

Results and Discussion

Structure and binding energies of Gly – Cat^+ (Cat = Li – Cs):

1. Selection of starting structures: Our choice of starting structures for geometry optimization is analogous to that of our previous study of copper complexes of glycine,[19] and benefitted from the extensive exploration of the potential energy surface by Jensen[9] and by Bouchonnet and Hoppil-

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liard.[8] It is based on the combination of two criteria. On the one hand, binding of Cat^+ to a neutral molecule mainly involves electrostatic interactions. The most favorable binding sites are therefore the electron-rich, negatively charged nitrogen and oxygens. Thus the first criterion is the maximization of such interactions through polydentate binding. On the other hand, the lowest conformations of the free amino acids are partly determined by the strength of intramolecular hydrogen bonds. Therefore the second criterion is to retain hydrogen bonding as much as possible.

Eight types of initial structures have been chosen. Most involve complexation to the neutral form of glycine because of its stability relative to the zwitterion.[20] However, the strong ionic interaction between Cat^+ and the negatively charged end of the latter could be competitive and was therefore also considered.

As shown in Figure 1, the selected structures involve five different modes of complexation:

1) On all three basic sites of neutral glycine. If Cat^+ is placed above the OCO plane, it can interact with both oxygens and at the same time with nitrogen.
2) On nitrogen and one of the oxygens of neutral glycine, either the carbonyl or hydroxyl one.
3) On both oxygens of neutral glycine, with two possible orientations of the amino group.
4) On both oxygens of zwitterionic glycine. If a N–H···O bond is maintained, this corresponds to a single structure.
5) On a single basic site: if on nitrogen, by attaching Cat^+ to N in the most stable conformation of glycine. If on oxygen, this was done by attaching Cat^+ to the carbonyl oxygen in the most stable conformation which does not bear a O–H···O bond.

There are other low-lying isomers for such complexes, as shown by the somewhat more extensive exploration of the PES of Gly–Li^+ and Gly–Na^+ by Jensen at the HF/6-31G* level.[9] We have not tried to characterize all minima thoroughly, as this turned out to be a rather tedious task. We made sure that several (at least three) lowest isomers were included in the present study. In only one case, Gly–Na^+, did we locate more minima as part of a study of the interconversion between these isomers (see Interconversion of Gly–Na^+ isomers).

No attempt was made to insert the ions into one of the covalent bonds of glycine, based on the neutralization–reionisation results of Polce et al. for Gly–Na^+,[21] and on the exclusive loss of Cat^+ in the metastable decompositions of Gly–Cat^+ with all five alkali metals,[8] which is most consistent with a simple complexation, whereas an intense formation of $CH_2NH_2^+$ is observed with Gly–H^+, indicating that some form of bond activation has occurred.

2. Structures and relative energies of Gly–Cat⁺ isomers: Optimized structures for several low-lying isomers of Gly–Li^+ and Gly–Na^+ have been previously reported at the HF/6-31G*[9] and HF/3-21G[8] levels. Our MP2/6-31G* structures are close enough to these that only the structures of the lowest isomers are provided in Figure 2 (relative energies of all isomers are reported in Table 1). Both correspond to chelation of the cation between the amino end and the carbonyl

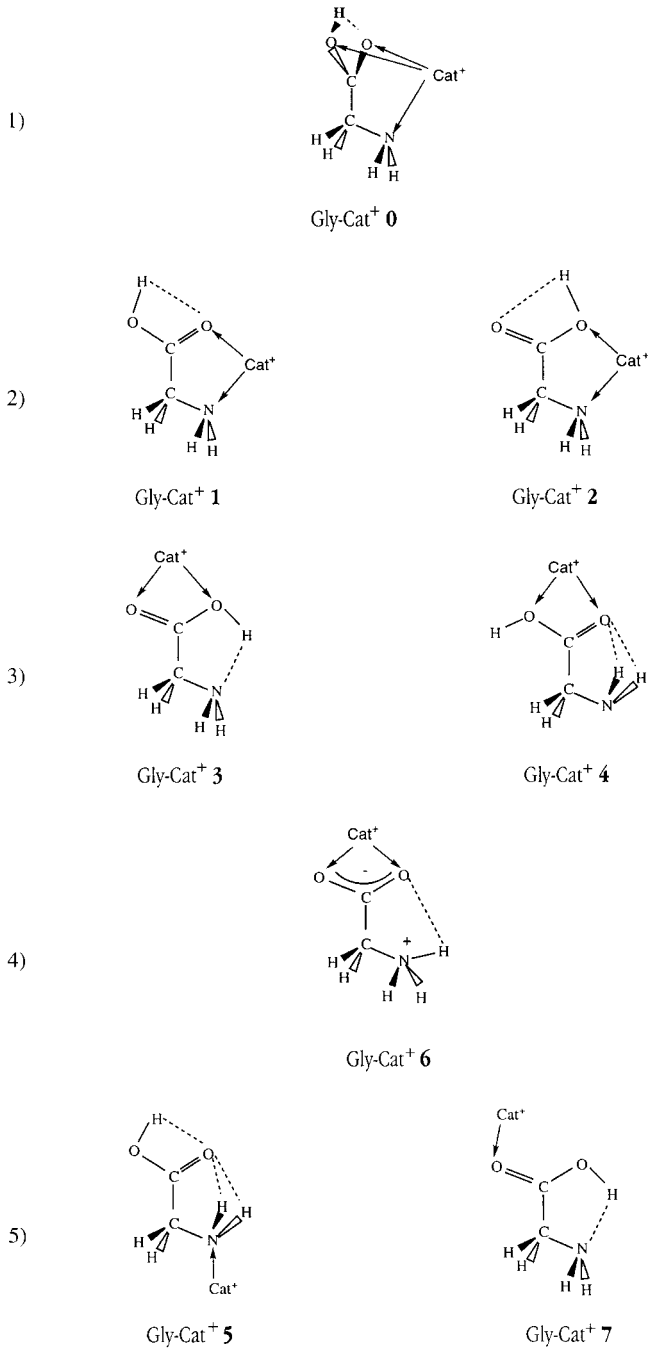

Figure 1. Isomers of Gly–Cat^+ considered.

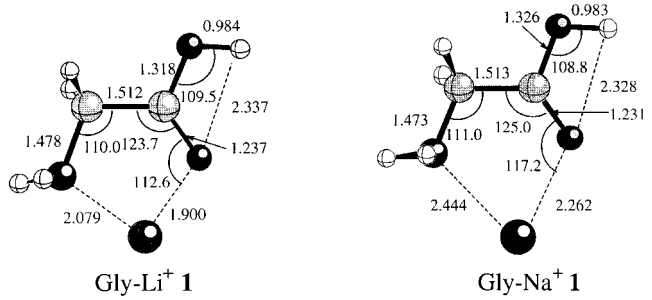

Figure 2. Optimized structures for the most stable isomer of Gly–Li^+ and Gly–Na^+ at the MP2/basis1 level.

Table 1. Relative energies of Gly–Cat$^+$ (Cat = Li–Cs) isomers at the MP2/basis1 level.

Isomer	Gly–Li$^+$	Gly–Na$^+$	Gly–K$^+$	Gly–Rb$^+$	Gly–Cs$^+$
1	**0.0**	**0.0**	0.1 (0.9)[a]	0.8	1.6
2	9.0	8.3	7.9	8.2	8.9
3	10.8	3.8	**0.0**	**0.0**	**0.0**
4	24.7	16.8	12.1	12.0	11.7
5	22.4	15.9	11.9	11.3	11.1
6	3.3	1.4 (2.7)[a]	1.9	2.3	3.3
7	11.3	NS[b]	NS[b]	NS[b]	NS[b]

[a] Relative energy at the MP2/basis2 level including correction for BSSE. [b] No minimum of this type was obtained.

oxygen. They are quite similar to the HF/6-31G* structures reported by Jensen:[9] in Gly–Li$^+$ **1** the Li–O distances are 1.864 and 1.900 Å at the HF and MP2 levels, respectively, while the Li–N distances are 2.086 and 2.079 Å. In isomer **3**, where Cat$^+$ is bound to both oxygens of the acid function, the short Li–O distances are 1.788 and 1.821 Å at the same levels. In fact the motivation for our use of MP2 wavefunctions for geometry optimization is the desire for high accuracy in Gly–Cat$^+$ binding energies (see next section).

For Gly–K$^+$ computational results have been obtained previously at the MP2/6-31G*//HF/3-21G level,[8b] but few structural details were reported. For Gly–Rb$^+$ and Gly–Cs$^+$, we are not aware of any previous study. Therefore MP2/basis1 structural parameters of all isomers optimized for Gly–K$^+$, Gly–Rb$^+$, and Gly–Cs$^+$ are reported in Figures 3, 4, and 5,

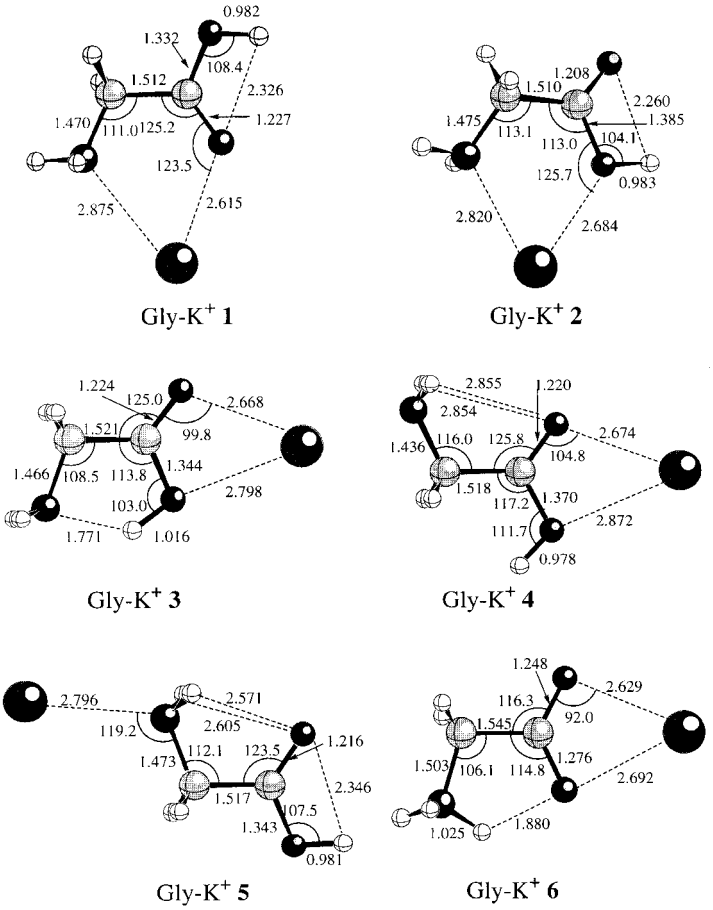

Figure 3. Optimized structures for the isomers of Gly–K$^+$ at the MP2/basis1 level.

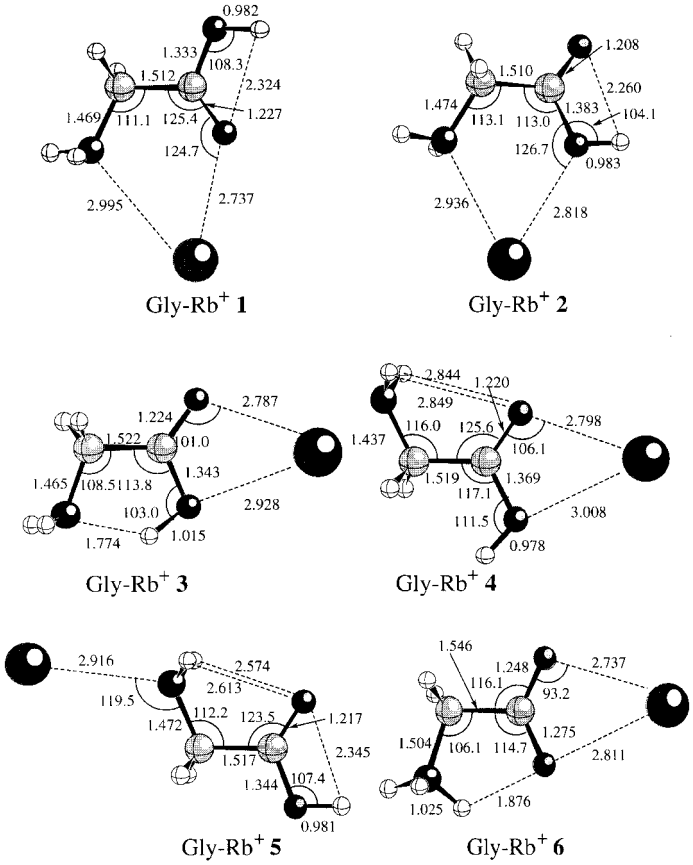

Figure 4. Optimized structures for the isomers of Gly–Rb$^+$ at the MP2/basis1 level.

respectively, and the corresponding relative energies are gathered in Table 1. A low-energy isomer in all cases is **1**, which involves chelation of the metal ion between the amino and carbonyl ends of glycine. This structure is well known for many metal–peptide complexes in solution.[22] Here glycine behaves as a cation clip, provided that the cation remains relatively small. However, the average cation-binding site distances increase sharply down the periodic table, from ca. 2 Å (Li$^+$) to 3 Å (Cs$^+$). This destabilizes the five-membered ring formed in **1** relative to other isomers in which little structural distortion of glycine is necessary to accommodate bigger ions, such as isomer **3**. In this case, the ion interacts with the carboxylic acid function. While structure **3** lies 10.8 and 3.8 kcal mol^{-1} higher in energy than **1** for Gly–Li$^+$ and Gly–Na$^+$ respectively, the structures are degenerate for Gly–K$^+$ and **3** becomes marginally more stable for Gly–Rb$^+$ and Gly–Cs$^+$. In fact the interaction of Cat$^+$ with the acid function generates two energy minima, **3** and **4**. Because the H-bonding scheme in **4** requires inversion at ammonia, the global dipole moment of glycine has a smaller magnitude and different orientation than in **3**, leading to a much less favorable interaction with Cat$^+$. The relative energies of **3** and **4** remain fairly similar across the whole alkali metal series. This similarity also holds for the two structures in which a pseudo-five-membered ring is formed, **1** and **2**. In the latter the ion is attached to the amino and hydroxy ends of glycine, rather than the amino and carbonyl in **1**. It lies 7–9 kcal mol^{-1}

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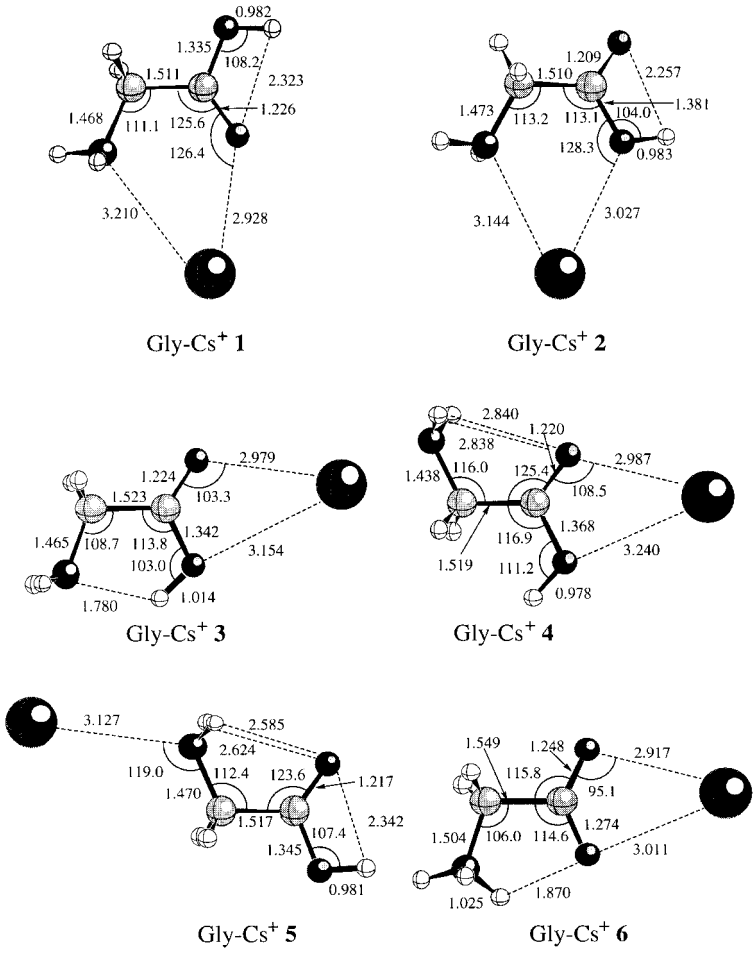

Figure 5. Optimized structures for the isomers of Gly–Cs^+ at the MP2/basis1 level.

higher than **1** for the whole series. If the interaction of Cat^+ is restricted to the most basic site of glycine, the amino end as in **5**, the results in Table 1 show that a much less favorable binding energy is obtained. At the other extreme, complexation on nitrogen and both oxygens requires a 90° rotation around the C–C bond of glycine; no minimum was found for this type of structure, and geometry optimization collapsed onto isomer **1**. These results confirm that multidentate binding is preferred as long as it does not require extensive structural distorsions of glycine.

It can be seen that isomer **6**, which involves binding of Cat^+ to the carboxylate group of zwitterionic glycine, is of low energy in all cases. In fact, it is so close to **1** for Gly–Na^+ that the accuracy of MP2/basis1 relative energies may be questioned. Therefore we have recalculated the energy of Gly–Na^+ **6** relative to **1** at the MP2/basis2 level. It is found to be 2.7 kcal mol^{-1}, somewhat larger than with MP2/basis1, and remains unchanged after correction for BSSE. Thus it is likely that the true 0 K structure is of type **1**. This conclusion appears to be at variance with that drawn from recent H/D exchange experiments involving Gly–Na^+ and ND_3[26] or D_2O.[27] In fact the contradiction may only be apparent, as discussed in detail below in Interconversion of Gly–Na^+ isomers.

3. Computation of glycine affinity for Cat$^+$ (Cat = Li–Cs): The isomer of lowest energy was considered in each case, that is, Gly–Li^+ **1**, Gly–Na^+ **1** and **6**, Gly–K^+ **1** and **3**, Gly–Rb^+ **3**, and Gly–Cs^+ **3**. It is well known that computations of binding energies using intermediate-sized basis sets are plagued with basis set superposition errors (BSSE). Our MP2/basis1 results fully support this trend, with counterpoise BSSE values computed at the MP2 level as large as 7.0 kcal mol^{-1} for Gly–Li^+. We have chosen to use significantly larger bases for the final computation of binding energies (see the description of basis2 in Computational Methods), but even with the latter, BSSE corrections must be considered (2.0 kcal mol^{-1} for Gly–Li^+). Zero-point vibrational energy (ZPVE) or thermal corrections at 298 K may then be used to obtain 0 K or 298 K affinities. Typical values for the thermal corrections at 298 K are 1.9 and 1.5 kcal mol^{-1} for Gly–Li^+ and Gly–Na^+, respectively. Complexation entropies (see Table 2) are also calculated from the vibrational frequencies.

For Gly–K^+ computations were carried out for both isomers **1** and **3**. MP2/basis2 results corrected for BSSE and thermal contributions led to **3** being more stable than **1** by 0.9 kcal mol^{-1}. Although such a small difference is not significant, we use structure **3** to derive a computed affinity of Gly for K^+.

4. Comparison with experimental affinities: Two different methods have been used to derive experimental Gly–Cat^+ binding energies. Kebarle et al. formed cationized Gly–Na^+ and Gly–K^+ in an electrospray source and used a triple quadrupole instrument to determine the appearance threshold of Cat^+ under collisional activation of Gly–Cat^+.[7] This method requires theoretical modeling of the variation of the cross-section for dissociation to $Cat^+ + Gly$ as a function of kinetic energy.[23] A completely different approach was used by Bojesen et al.[6] who used the kinetic method of Cooks and coworkers.[10] A cationized dimer of two molecules M_1 and M_2 is formed and its metastable or collision-induced dissociations are used to obtain relative affinities of M_1 and M_2 for Cat^+. In order to obtain an absolute affinity of Gly for Cat^+, a cationized dimer with a reference molecule of known affinity for Cat^+ must be generated. This was obtained with Li^+, with *N,N*-dimethylformamide (DMF) as the reference base.

Table 2. Binding energies (D_e, D_0, and D_{298}) in kcal mol^{-1}, entropies in cal mol^{-1} K^{-1}, and free energies in kcal mol^{-1} of Gly–Cat^+ (Cat = Li–Cs) at the MP2/basis2//MP2/basis1 level including BSSE.

Isomer	Gly–Li^+ **1**	Gly–Na^+ **1**	Gly–K^+ **3**	Gly–Rb^+ **3**	Gly–Cs^+ **3**
D_e	56.4	37.8	27.4	25.2	22.5
D_0	54.0	36.2	26.7	24.6	21.9
ΔH_{298}	54.5	36.3	26.5	24.1	21.4
Exptl ΔH_{298}	54.0[a]	40.1[a]	–	–	–
	–	36.6 ± 2.3[b]	30.0 ± 2.3[b]	–	–
ΔS	28.2[c]	27.8[c]	24.8,[c] 21.8[d]	20.8[d]	19.5[d]
ΔG_{298}	46.1	28.0	19.1	17.9	15.6

[a] Revised value based on ref. [6] and computations of DMF and DMF–Li^+ (see text). [b] Ref. [7]. [c] Based on MP2/6-31G* frequencies. [d] Based on HF/6-31G* frequencies.

DMF – Li^+ – Ala and Ala – Li^+ – Gly dimers were used to generate an affinity of Gly relative to that of DMF. However the affinity of DMF for Li^+ (50 kcal mol^{-1}) has never been measured directly, but is the conjunction of an affinity of 34 kcal mol^{-1} of H_2O and several equilibrium measurements of the type described by reaction (1).[24] Since this is a fairly

$$M_1 - Li^+ + M_2 \rightleftharpoons M_2 - Li^+ + M_1 \quad (1)$$

indirect determination, the experimental error bar is difficult to evaluate but might be expected to be large. We have therefore computed DMF and DMF – Li^+ at the MP2/basis2// MP2/basis1 level (including BSSE at the MP2/basis2 level) in order to obtain an improved affinity of DMF for Li^+. The optimized structures of DMF and DMF – Li^+ are shown in Figure 6. The 298 K affinity so obtained is 51.7 kcal mol^{-1}. This

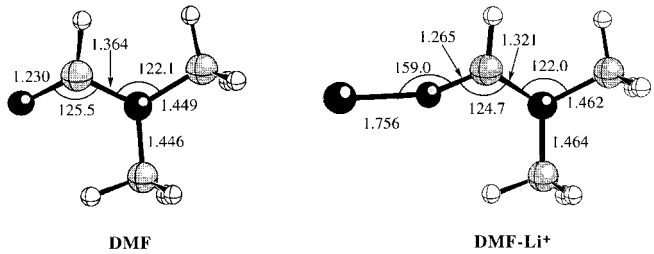

Figure 6. Optimized structures of DMF and DMF – Li^+ at the MP2/basis1 level.

is 1.7 kcal mol^{-1} larger than the previously assumed value, leading in turn to an upward revision of the affinity of Gly for Li^+. There is another incentive for these calculations. The kinetic method assumes that the binding characteristics of M_1 and M_2 to Cat^+ are sufficiently similar that no relative dissociation entropy need be taken into account. In other words, the measured differences of free energies of binding are considered to be equal to the differences of binding enthalpies. It is not obvious, however, that complexation on a carbonyl function only (as in DMF – Li^+, see Figure 6) involves an entropy similar to bidentate complexation between amino and carbonyl groups (as in Gly – Li^+, see Figure 2). Cerda and Wesdemiotis[25] have recently derived a modified kinetic method to take such differences into account. Using the formulae obtained by these authors, we have used the computed $T\Delta S$ terms for bonding of Gly and DMF to Li^+ (-8.4 and -7.1 kcal mol^{-1} at 298 K, respectively) to transform the measured relative free energies into relative enthalpies. With both of these corrections, the revised affinity of glycine for Li^+ is 54.0 instead of 51.0 kcal mol^{-1}. There is very good agreement between this revised value and our direct computations. It should be noted that this assumes an experimental temperature of 298 K, while the ions do not have a defined temperature in the conditions used. In order to quantify the impact of temperature on our entropy corrections, we also calculated them at 500 K and 800 K, in the range of effective temperatures determined by Cerda and Wesdemiotis for nucleobases studied in analogous conditions.[25] The resulting revised affinity of glycine for Li^+ is 54.9 kcal mol^{-1} at 500 K and 56.2 kcal mol^{-1} at 800 K.

The MP2/6-31 + G*//HF/6-31G* complexation energy of Jensen is slightly higher than ours, 58.4 kcal mol^{-1}. The probable reason for this overestimation is the lack of BSSE correction. Based on our computed affinity of Gly and the relative affinities obtained by Bojesen et al.,[6] affinities of alanine (Ala) and valine (Val) for Li^+ are 55.5 and 57.5 kcal mol^{-1} at 298 K, respectively.

For sodium, no cationized dimer with a reference base of known sodium affinity could be obtained.[6] The authors circumvented this problem by noting that several molecule – Na^+ binding energies appear to be close to 75% of the corresponding molecule – Li^+ binding energies, and used 75% of their affinity of Ala for Li^+ as the affinity of Ala for Na^+. Using the relative sodium affinities of Ala and Gly determined by the kinetic method, this yields an affinity of Gly for Na^+ of 37.9 kcal mol^{-1}. With our revised affinity of Gly for Li^+, this value becomes 40.1 kcal mol^{-1}, in moderately good agreement with our computations. However, this experimental value is only approximate, so that the computed value is probably more reliable. This conclusion is supported by the very good agreement of our computational results with the threshold dissociation energy value of Kebarle et al.[7] of 36.6 kcal mol^{-1}. The MP2/6-31 + G*//HF/6-31G* value of Jensen is slightly higher, 38.5 kcal mol^{-1}, again because no BSSE correction was included.

It should be noted that the error bar of the latter experimental value, 0.1 eV or 2.3 kcal mol^{-1}, is a theoretical one due to the uncertainty in the curve-fitting procedure. Another uncertainty arises from the treatment of metastable ions. From the results in ref. [7] (see Table 1 of this paper), this uncertainty amounts to ca. ± 0.5 kcal mol^{-1}. Finally, purely experimental uncertainties such as the energy spread of parent ions should be added.[26] Therefore the nearly perfect agreement with our computed value, for which an uncertainty of about 2 kcal mol^{-1} may be hypothesized, is partly fortuitous.

These authors also derived an affinity of Gly for K^+ of 30.0 kcal mol^{-1}, in somewhat less impressive agreement with our computed value of 26.5 kcal mol^{-1}. Given the uncertainties in both the computed and experimental values recalled above, these values are still in reasonable agreement with each other.

5. Structure of cationized dimer Gly – Li$^+$ – Gly: Together with threshold collision-induced dissociation, the kinetic method[10] is one of the most widely used ways to determine binding enthalpies. As discussed above for Gly – Li^+ and Gly – Na^+, one of the prerequisites for this method to yield accurate enthalpies is that either complexation of both molecules involved in the cationized heterodimer have similar entropies, or else that the entropy difference be evaluated with respect to a reference molecule of known complexation entropy. However, there is another requirement for the kinetic method, which is that the binding mode of the molecule of interest be the same in the cationized monomer and heterodimer. For large cases, binding in the heterodimer may lead to steric repulsions between the two molecules, which could be relieved by a different, less polydentate binding of one or both of the molecules. In such cases, the binding enthalpy derived from the kinetic method would

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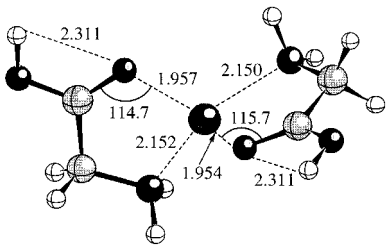
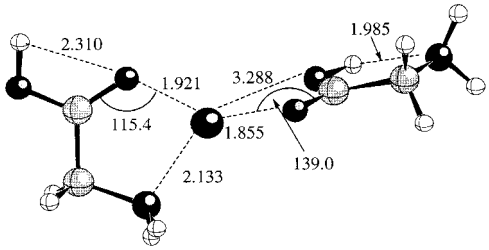
pertain to a "high"-energy isomer of the cationized monomer, and might therefore be flawed. We have addressed this issue in the case of two amino acids attached to an alkali cation, taking lithium as the smallest, therefore leading to closer interaction between the two molecules. As a first approach to the problem, the homodimer Gly–Li^+–Gly was considered. One should bear in mind that this may not be the ultimate test of the kinetic method, since lateral chains of larger amino acids are able to fold toward the metal ion for tridentate binding, leading to higher steric congestion than bidentate binding as with glycine.

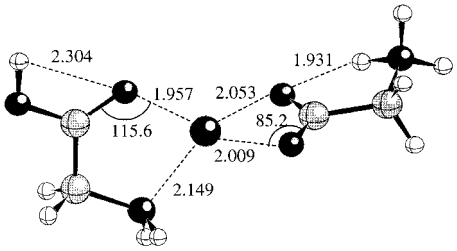
Computations were carried out on three Gly–Li^+–Gly isomers (see Figure 7). The first, denoted **1_1**, involves two



[Gly-Li^+-Gly] **1_1**



[Gly-Li^+-Gly] **1_3**



[Gly-Li^+-Gly] **1_6**

Figure 7. Optimized structures for the isomers of Gly–Li^+–Gly at the HF/basis1 level.

glycine molecules interacting with Li^+ as in the most stable isomer of Gly–Li^+ **1**, through chelation between nitrogen and the carbonyl oxygen. In the second, denoted **1_3**, one of the glycines has the same binding mode as above, while the second interacts with the cation through both oxygens, as in Gly–Li^+ **3**. The latter structure should be less sterically demanding than the cyclic structure of **1**. The third isomer, **1_6**, again has one of the glycines attached to Li^+ as in **1**, while the second is in the zwitterion form. Given the size of the dimers, calculations were carried out at the MP2/6-31G*//HF/6-31G* level. The optimized geometries displayed in Figure 7 show that the binding of both glycines in **1_1** is very similar, and also similar to that in the monomer. Li–O and Li–N bonds are slightly longer in the dimer than in the monomer. The structure is pseudotetrahedral around Li^+. In **1_3** one of the glycines binds to lithium with a geometry very similar to the above, while the second is attached only through the carbonyl oxygen, corresponding to Gly–Li^+ **7**. It should be remembered that at the HF/6-31G* level, **7** is a minimum while **3** is not in the monomer (both minima exist at the MP2 level only, with **3** being 0.5 kcal mol^{-1} more stable than **7**). In **1_6** the structure is again nearly tetrahedral around the lithium, and the zwitterionic Gly has slightly larger Li–O distances than in the monomer. The energies of **1_6** and **1_3** relative to **1_1** are 6.1 and 7.7 kcal mol^{-1} respectively at the MP2/6-31G*//HF/6-31G* level, while those of **6** and **7** relative to **1** are 4.1 and 11.8 kcal mol^{-1} in the monomer.[9] This shows that relative energies are significantly different in the monomer and in the dimer, with **6** being disfavored and **7** favored relative to **1** in the dimer. These differences are due at least in part to differences in the amount of charge transfer from Gly to Li^+ in **6**, in which it is larger than in **1**, in which in turn it is larger than in **7**. The larger the charge transfer, the larger the decrease in binding energy induced by the second glycine ligand. The computed differences between the monomer and the dimer are such that one can anticipate an energy reversal between isomers if they have different electronic and/or steric demands and lie close in energy in the monomer. This may well happen for amino acids with long side chains, for which strong tridentate binding can be anticipated. If, however, the dimer ions have enough internal energy to isomerize prior to or during dissociation, the monomer fragments may be formed in their lowest energy isomers, and relative binding energies determined by the kinetic method are correct.

Interconversion of Gly–Na^+ isomers: There is a wealth of results in the literature on the fragmentation of sodiated peptides under collisional activation.[3–5] In many cases the observed dissociation pathways have been associated with the postulated binding site of Na^+. In fact, it has been often assumed that the cation binding site could be inferred from the observed fragmentations. This implies that the most stable and most reactive (under collision) isomers be the same, an assumption known to be often wrong in solution phase organic chemistry, or else that interconversion between both is unfeasible. In order to evaluate the validity of this assumption, we have undertaken a study of the interconversion of Gly–Na^+ isomers.

Another impetus for this study comes from recent experiments which have suggested that when sodiated glycine oligopeptides Gly_n–Na^+ ions ($n = 1-5$) undergo reaction with deuterating agents such as D_2O[26] or ND_3,[27] the numbers of fast and slow H/D exchanges observed are characteristic of certain types of structures for the initial Gly_n–Na^+ ions. If, for example, Gly–Na^+ undergoes three fast exchanges with D_2O or ND_3, it might be anticipated that all three exchangeable hydrogens of Gly are equivalent and easily available, as in the zwitterion. This suggests that the isomer involved (thus the most stable one) is Gly–Na^+ **6**. If, on the other hand, two fast and one slow H/D exchange are

observed, then neutral glycine should be involved, most probably in structure Gly–Na^+ **1**. Experiments show that three fast exchanges occur in the Gly–Na^+/D_2O or Gly–Na^+/ND_3 system, consistent with structure **6**. Our computations are consistent with the reverse ordering of Gly–Na^+ **1** and **6**, but with a rather small energy difference. Although such small differences might lead to long debate about the reliability of computations, we would like to show that the experimental methods used may not provide structural information in such cases. Our point is that if interconversion of Gly–Na^+ isomers, particularly between **1** and **6**, does not involve high energy barriers, then the necessary energy for isomerization may well be provided by the initial complexation energy between Gly–Na^+ and D_2O or ND_3.

We have determined an energy profile connecting Gly–Na^+ **1** to **6**. Calculations were run at the MP2/6-31G* level. Optimized structures are shown in Figure 8 and the energy profile in Figure 9. The path starts from **1** by a 180° rotation around the C–OH bond, leading to Gly–Na^+ **8** through the transition state **TS1–8**. It is followed by migration of Na^+ from one side of the carbonyl group to the other, forming Gly–Na^+ **9** via **TS8–9**. The next step is inversion of nitrogen pyramidalization from **9** to **TS 9–4** to **4**. A 180° rotation around the C–C bond links **4** to **3** via **TS 4–3**. Finally, proton transfer can occur from oxygen to nitrogen, yielding the zwitterion in **6** through **TS3–6**. While the energy difference between **3** and **TS3–6** is 1.9 kcal mol^{-1} on the electronic surface, it goes to zero when ZPVE corrections are taken into account (see Figure 9). As could be expected, the highest two energy barriers relate to **4**, the highest lying minimum along the way. The highest of the transition states, **TS4–3**, lies 18.7 kcal mol^{-1} higher than the global minimum **1**.

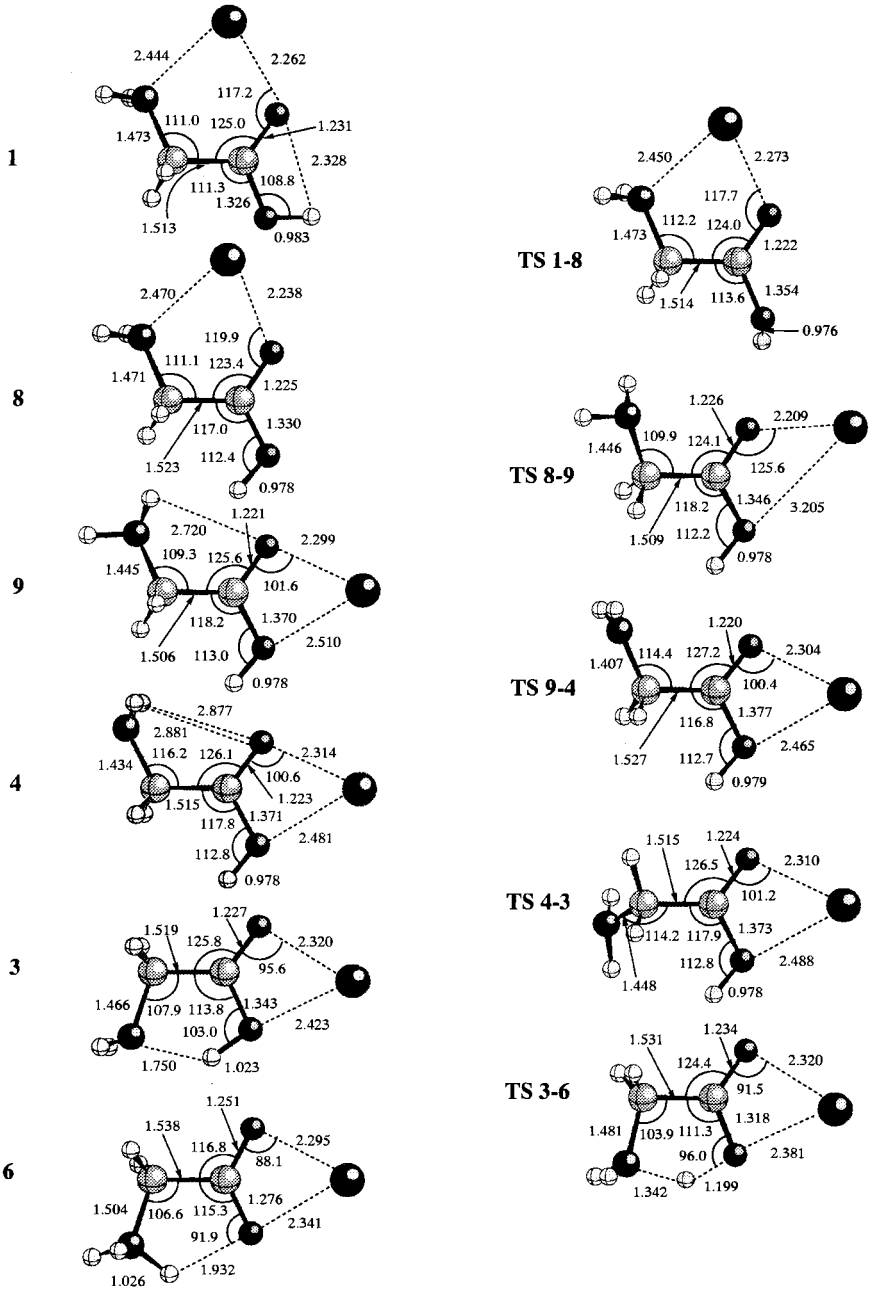


Figure 8. Optimized structures for the minima and transition states on the Gly–Na^+ potential energy surface at the MP2/basis1 level.

Other paths from **1** to **6** are conceivable. For instance rotation around the C–OH and C–N bonds and migration of Na^+ could occur concertedly, leading directly from **1** to **4**. All attempts to locate the corresponding transition state failed. Since the potential energy surface for complexation bears many local minima, it is difficult to identify such a TS without ambiguity. In any case the highest energy region is in the vicinity of **4**, and a direct path from **1** to **4** would not change the rate-determining barrier significantly. Yet another possibility would be to avoid sampling this high-energy region by a rotation around the C–C bond occurring concertedly with cation migration. Attempts in this direction were also unsuccessful.

Our own computations of the complexation energies of water and ammonia with sodium[13] yield 30.5 and 34.4 kcal mol^{-1} at the same level (more accurate calculations converge to 23.9 and 27.5 kcal mol^{-1}, respectively). It it likely that this enthalpy is slightly smaller when Gly is also attached to Na^+, based on the recent determinations of successive binding energies of ethers to Na^+,[28] and of water to Li^+.[29] However it remains likely that the interaction energy between Gly–Na^+ and either D_2O or ND_3 is large enough to induce fast isomerization between Gly–Na^+ isomers. It remains to be determined how the competition of H/D exchange and isomerization of Gly–Na^+ takes place. If the latter is fast

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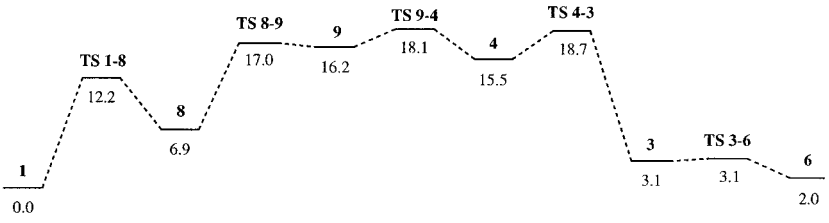

Figure 9. Potential energy profile on the Gly–Na^+ potential energy surface at the MP2/basis1 level.

enough, the observation of three equivalent H/D exchanges does not discriminate between **1** and **6**. Therefore we conclude that the above experiments do not necessarily provide specific information regarding the lowest energy structure of Gly–Na^+. This is all the more true as complexation of Gly–Na^+ by either D_2O or ND_3 may easily change the relative energies of the lowest lying isomers. In contrast, this method should be useful in cases where the energy difference between isomers involving neutral and zwitterionic isomers is larger.

The calculations described herein emphasize the difficulty in connecting directly the fragmentations observed under collisional activation of sodiated amino acids and peptides with the initial, most stable site of sodium fixation to the peptide. Indeed, collisional activation provides much more energy than needed to isomerize Gly–Na^+. It is even likely that selected ions have enough internal energy *before* collisional activation to exist in a mixture of isomers. If, for instance, a zwitterionic isomer is initially formed, easy proton transfer may occur back and forth between the initially protonated site and the carboxylate end, provided that they can readily come close to one another as in Gly or if long, basic side chains are involved.

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