Structure of Cationized Glycine, Gly·M^{2+} (M = Be, Mg, Ca, Sr, Ba), in the Gas Phase: Intrinsic Effect of Cation Size on Zwitterion Stability

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Interactions between divalent metal ions and biomolecules are common both in solution and in the gas phase. Here, the intrinsic effect of divalent alkaline earth metal ions (Be, Mg, Ca, Sr, Ba) on the structure of glycine in the absence of solvent is examined. Results from both density functional and Moller−Plesset theories indicate that for all metal ions except beryllium, the salt-bridge form of the ion, in which glycine is a zwitterion, is between 5 and 12 kcal/mol more stable than the charge-solvated structure in which glycine is in its neutral form. For beryllium, the charge-solvated structure is 5−8 kcal/mol more stable than the salt-bridge structure. Thus, there is a dramatic change in the structure of glycine with increased metal cation size. Using a Hartree−Fock-based partitioning method, the interaction between the metal ion and glycine is separated into electrostatic, charge transfer and deformation components. The charge transfer interactions are more important for stabilizing the charge-solvated structure of glycine with beryllium relative to magnesium. In contrast, the difference in stability between the charge-solvated and salt-bridge structure for magnesium is mostly due to electrostatic interactions that favor formation of the salt-bridge structure. These results indicate that divalent metal ions dramatically influence the structure of this simplest amino acid in the gas phase.

Introduction

Amino acids exist as zwitterions in aqueous solution within a wide range of pH. In contrast, all experimental evidence indicates that the ground-state structure of amino acids in the gas phase is the neutral form. In the gas phase, the zwitterion form of glycine is 18 kcal/mol higher in energy than the neutral form, and there is no barrier to isomerization.[1] For the more basic amino acid, arginine, the neutral and zwitterionic forms are comparable in energy,[2] although both experiment[3] and high level theory[2b] indicate that the neutral form is still more stable. The differences between the gas-phase and solution-phase structures of amino acids are due to solvent preferentially stabilizing the zwitterion form. Electric fields, such as those generated by a nearby counterion, can also stabilize a zwitterion relative to its neutral form.[2,4,5] Glycine bound to alkali metal cations form charge-solvated complexes, but the salt-bridge or ion-zwitterion form is only 1−3 kcal/mol less stable.[5] In contrast, both experiment and theory indicate that arginine is a zwitterion when bound to protonated arginine[2a] or large alkali metal cations.[6] However, the difference in energy between the neutral and zwitterion forms is small (∼3 kcal/mol for Arg·Cs^+).[5a,6] Evidence for the zwitterion form of other amino acids in the presence of a charge has also been reported.[7,8]

In contrast to the extensive attention that amino acids bound to singly charged ions have received, the intrinsic structure of amino acids in the presence of multivalent ions is almost completely unknown despite the importance of divalent metal ion interactions with biomolecules both in solution and in the gas phase. The formation of polypeptides from amino acids is enhanced by the presence of divalent metal ions.[9] It has been suggested that compounds vital to the early life forms on primordial earth may have been formed through a salt-bridge mechanism involving Cu^{2+}.[10] A very recent report compared the difference in structure when glycine is bound to mono vs divalent copper ions.[8] In the former, glycine was found to be neutral, whereas in the latter, the zwitterion form was reported to be 4−6 kcal/mol more stable. Here, we systematically investigate the effects of divalent cation size on the structure of glycine by examining the structure and energies of glycine bound to alkaline earth divalent cations. These ions can be readily produced from aqueous solutions using electrospray ionization, and the structure of these ions can be investigated by a variety of experimental methods.

Methods

Experimental Methods. Experiments were performed on a 2.7 T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer.[11] Hydrated ions are formed by nanoelectrospray ionization from aqueous solutions containing 1 mM concentrations of glycine and M^{2+} in water. Nanospray needles are made from 1.0 mm o.d. (0.68 mm i.d.) borosilicate capillaries that are pulled to an i.d. of 3−6 μm using a micropipet puller (Sutter Instruments, Novato, CA). A pulse of N_2 gas at a pressure of 10^{-6} Torr is used to assist in trapping and thermalizing of the ions. The base pressure of the instrument is between 3 and 7 × 10^{-9} Torr for the rest of the experiment. For hydrogen/deuterium exchange experiments, CD_3OD was introduced into the FT-ICR mass spectrometer at a pressure of 5 × 10^{-7} Torr for a minimum of 5 h prior to conducting the exchange experiment. A static gas pressure of 7 × 10^{-8} Torr of the exchange reagent, CD_3OD, was used during these experiments. Extensive clustering with deuterated methanol and the divalent metal-glycine complex occurs at higher reagent pressure.

Calculations. Calculations using effective core potentials of Hay and Wadt were done for M = Ca, Sr, Ba. The three parameter hybrid density functional of Becke, B3LYP, and local MP2 calculations were done using Jaguar v 3.5 (Schrodinger, Inc., Portland, OR). For glycine bound to Be^{2+} and Mg^{2+}, both density functional and MP2 (full) calculations were also done

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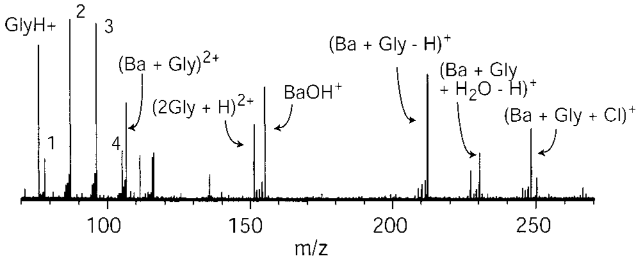

Figure 1. Electrospray ionization Fourier transform mass spectrum of an aqueous solution of $BaCl_2$ and glycine (both 10^{-3} M) obtained at a cell temperature of 135 °C. The numbers correspond to the peaks for $Ba^{2+}(H_2O)_n$.

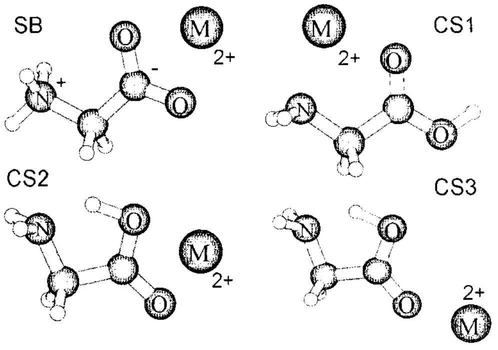

Figure 2. Four structural isomers of $Gly{\cdot}M^{2+}$.

using the 6-311++G(d,p) and the 6-311(2+,2+)G(2df,2pd) basis sets using Q-Chem v 1.1 (Q-Chem Inc., Pittsburgh, PA). Zero-point energies were determined using frequencies calculated at the LACVP* level (frequencies scaled by 0.895).

Results and Discussion

Electrospray Ionization Mass Spectrometry. A typical electrospray mass spectrum obtained from an aqueous solution containing $BaCl_2$ and glycine is shown in Figure 1. Several barium-attached glycine ions are observed in high abundance, including $(Ba + Gly - H)^+$ and $(Ba + Gly)^{2+}$, each of these ions with one water molecule attached, and $(Ba + Gly + Cl)^+$. The relative abundances of $(Ba + Gly - H)^+$ and $(Ba + Gly)^{2+}$ are not strongly dependent on the source conditions, but the hydrates of these ions decrease under more energetic source conditions. The abundance of $(Ba + Gly)^{2+}$ is reduced when nonaqueous solvents are used. Abundant hydrated divalent barium ions, $Ba(H_2O)_n{}^{2+}$, are also formed. The abundances of these ions remain essentially unchanged when the ions are stored for 20 s at a cell temperature of 135 °C, indicating that all these ions are very stable. Similar results are obtained for solutions containing glycine with the other alkaline earth metal ions, except for beryllium which results in $Gly{\cdot}Be(OH)^+$. Hydroxide formation from aqueous beryllium solutions has been observed previously.[12]

$Gly{\cdot}M^{2+}$ Structure. The ion, $Gly{\cdot}M^{2+}$, could be either a charge-solvated structure in which glycine is neutral or a salt-bridge structure in which glycine is a zwitterion. Three conformations of the charge-solvated structures (CS1−3, Figure 2) and one salt-bridge structure (SB, Figure 2) were examined. These structures were found by others to be lowest-energy structures identified for glycine bound to alkali metal (1+)[5] and copper ions (2+).[8] In the charge-solvated structures, the metal ion is coordinated to either the amine nitrogen and carbonyl oxygen (CS1), both oxygens of the carboxylic acid group (CS2), or just the carbonyl oxygen (CS3). In the salt-bridge structure (SB), the metal ion is coordinated to both carboxylate oxygens.

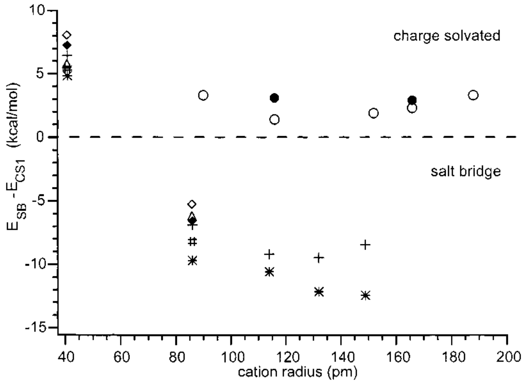

Figure 3. Plot of the difference in energy between the salt-bridge (SB) and charge-solvated (CS1) forms of $Gly{\cdot}M^{2+}$ as a function of ionic radii of the metal ion. Values were calculated using the B3LYP functional using the LACVP+(d,p) (+), basis 6-311(2+,2+)G(2df,2pd) (◇), basis 6-311++G(d,p) (◆) and using MP2 LACVP+(d,p)(*), basis 6-311(2+,2+)G(2df,2pd) (#) and basis 6-311G++G(d,p) (△). Values for $Gly{\cdot}M+$ (M = alkali metal) from refs 5a (●) and 5b (○) are also indicated.

TABLE 1: Effect of Basis Set on the Difference in Energy between the Lowest Energy Charge-solvated (CS1) and Salt-Bridge (SB) Structures of $Gly{\cdot}M^{2+}$ [a]

	R	B3LYP			MP2		
M	(pm)	LACVP+(d,p)	**1**	**2**	LACVP+(d,p)	**1**	**2**
Be	41	6.5	8.0	7.2	4.8	5.7	5.4
Mg	86	−6.9	−5.3	−6.6	−9.7	−6.2	−8.2
Ca	114	−9.2			−10.6		
Sr	132	−9.5			−12.1		
Ba	149	−8.4			−12.4		

[a] The values are calculated using both the B3LYP and the local MP2 level using the 6-311++G(d,p), abbreviated **1**, and the 6-311(2+,2+)G(2df,2pd)//6-311++G(d,p), abbreviated **2**, basis sets. These values include zero-point energy corrections.

The most stable charge-solvated structure at the LACVP+(d,p) level is CS1 for all metal ions. With CS2, the proton from the carboxylic acid group transfers to the amine group forming SB for all metal ions. CS3 is less stable than CS1 by ~25 kcal/mol for all metal ions. The energy difference between the SB and CS1 structures for each $Gly{\cdot}M^{2+}$ complex at different levels of theory is shown in Figure 3 and in Table 1. In contrast to previous calculations for $Gly{\cdot}M^+$ (M = Li, Na, K, Rb, Cs),[5] there is a clear change in the relative stability of the charge-solvated and salt-bridge structures of $Gly{\cdot}M^{2+}$ (M = Be, Mg, Ca, Sr, Ba) with increased cation size (Figure 3). For Be^{2+}, CS1 is more stable than SB by 4−8 kcal/mol depending on the level of theory. In contrast, SB is 5−10 kcal/mol more stable than CS1 for Mg^{2+}. The energy differences between CS1 and SB at the B3LYP level are consistently higher than those obtained by MP2 with a given basis set, although this difference is small (<3 kcal/mol). The addition of more diffuse and angular momentum functions (6-311++G(d,p) vs 6-311(2+,2+)G(2df, 2pd)) increases the stability of the SB relative to CS1 by 0.3 to 2.0 kcal/mol for both B3LYP and MP2. Effective core potentials are required for calculations with Ca, Sr, and Ba. With both B3LYP LACVP+(d,p) and LACVP+(d,p) methods, SB is more stable than CS1 by 8 kcal/mol or more. For Be^{2+} and Mg^{2+}, energies calculated with effective core potentials are similar to those obtained using both B3LYP and MP2 methods with standard basis sets.

Natural Energy Decomposition Analysis. These calculations show that the stability of the salt-bridge vs charge-solvated structures changes with increased alkaline earth cation size. The

TABLE 2: NEDA for Gly·M^{2+} at the Restricted Hartree−Fock 6-31+G Level[a]**

	M	ES	CT	DEF (Gly)	DEF (M^{2+})	Δ*E*	DIS	*E*$_o$	*R* (bohr)
SB	Be	−409.0	−111.9	152.2	43.7	−325.0	28.0	−297.0	3.96
CS1	Be	−404.9	−128.7	180.4	52.7	−300.3	20.1	−280.2	3.22
SB	Mg	−304.9	−34.8	101.4	33.1	−205.2	13.4	−191.8	4.02
CS1	Mg	−292.6	−37.6	123.6	36.5	−170.8	9.9	−160.9	3.51

[a] Electrostatic, charge transfer, and deformation contributions (in kcal/mol) are indicated by ES, CT, and DEF, respectively. DIS is the energy difference between the equilibrium geometries of Gly as a neutral and when complexed with a metal ion. The total interaction energy (Δ*E*) and the heterolytic binding energy (E$_o$) for Gly·M^{2+} is also indicated (kcal/mol). The value of *E*(SB) − *E*(CS1) at the RHF 6-31+G** is 2.1 kcal/mol for Gly·Be^{2+} (CS more stable) and -11.8 kcal/mol for Gly·Mg^{2+} (SB more stable). *R* is the distance between the center of mass of Gly·M^{2+} and M^{2+}.

structure of Gly·Be^{2+} is unique in that neutral glycine prefers to solvate Be^{2+}, despite the strong electrostatic interaction between the metal and carboxylate group that occurs in SB. To determine which interactions contribute to the stability of these two forms of the ion, a breakdown of the noncovalent interactions was done using the Natural Energy Decomposition Analysis program (NEDA)[13,14] for both Gly·Be^{2+} and Gly·Mg^{2+}. These calculations separate interactions between noncovalently bound molecules into electrostatic (ES) and charge transfer (CT) terms that contribute to the stability of the dimer, and a deformation term (DEF) that destabilizes the dimer.[14] The calculations are based on the Hartree−Fock wave functions of the interacting and noninteracting molecular units at the equilibrium geometry of Gly·M^{2+}. The distortion energy, DIS, which accounts for the energy change due to the perturbation in geometry of the glycine molecule when the metal ion is present, was also calculated.

The results of the NEDA calculations are given in Table 2. For Gly·Be^{2+}, charge-transfer stabilization in CS1 is 16.8 kcal/mol larger than that in SB. In CS1, donation of the electron density from the lone pairs of the amine nitrogen and carbonyl oxygen to the vacant 2s orbital in Be^{2+} occurs to a greater extent than from the carboxylate oxygens in SB. This is mostly due to the better electron pair donating ability of the nitrogen relative to the oxygen. In contrast, the charge transfer term is only 2.8 kcal/mol higher in CS1 relative to SB for Gly·Mg^{2+}. Thus, it appears that charge transfer interactions play a significant role in the stabilization of the CS1 structure of Gly·Be^{2+} relative to Gly·Mg^{2+}. The apparent reason for the larger contribution of CT in Gly·Be^{2+} is due to the high charge density of Be^{2+} relative to Mg^{2+}. The high charge density enhances the ability of Be^{2+} to become a good electron pair acceptor, and this produces larger CT energies for both Gly·Be^{2+} forms.

The difference in stability between CS1 and SB for Gly·Mg^{2+} is primarily due to electrostatic interactions that favor formation of SB. The ES component in Table 2 represents the sum of classical static and induced electrostatic interactions.[15] The value of ES in both Gly·Be^{2+} structures is larger than in either Gly·Mg^{2+} structure due to the high charge density of Be^{2+} which induces a stronger dipole than does Mg^{2+}. The values of ES for the SB forms of Gly·M^{2+} are larger than those of the corresponding CS1 forms. This is due to the higher static dipole of the Gly zwitterion in the salt-bridge structures, creating a favorable charge−dipole interaction. However, the difference in the ES term between the SB and the CS1 structures ($\mathrm{ES_{SB}} - \mathrm{ES_{CS1}}$) is larger for Mg^{2+} than for Be^{2+}. $\mathrm{ES_{SB}} - \mathrm{ES_{CS1}}$ is 12.3 kcal/mol for Mg^{2+}, but only for 4.1 kcal/mol for Be^{2+}. For the salt-bridge structure, the distance between the metal ion and the center of mass of Gly·M^{2+}, R, is similar for the two metal ions. For the charge-solvated structures, this value is much lower for Be^{2+} than for Mg^{2+}. Thus, the smaller value of $\mathrm{ES_{SB}} - \mathrm{ES_{CS1}}$ for Be^{2+} is primarily due to increased ES interactions in CS1 due to the closer metal ion to glycine distance. It should be noted that the location of the center of mass will change slightly solely due to the heavier mass of Mg compared to Be, but has only a minor effect on the distances mentioned above.

The total repulsion energy from both DEFs and DIS is higher for the CS1 form of Gly·M^{2+} for both Gly·Be^{2+} and Gly·Mg^{2+}. The repulsive interactions (sum of DEFs and DIS) favor the formation of SB form by 29.3 and 22.1 kcal/mol for Be and Mg, respectively. Since the repulsion interactions favor the formation of SB by 7.2 kcal/mol more for Be^{2+} relative to that determined for Mg^{2+}, DEF and DIS do not account for the preference of Gly·Be^{2+} to form a charge-solvated structure.

H/D exchange. The structure of Gly·Ba^{2+} was investigated by H/D exchange using CD_3OD as the exchange reagent. Under conditions where (Gly + H)$^+$ undergoes rapid exchange of one hydrogen for deuterium (with two subsequent slower exchanges), Gly·Ba^{2+} undergoes no measurable exchange. Beauchamp and co-workers have measured H/D exchange rates for both (Gly + H)$^{+}$ [16] and Gly·Na^{+} [17,18] and found that the latter ion undergoes three fast exchanges with ND_3. The corresponding methyl ester of glycine, which has no acidic hydrogen, does not undergo H/D exchange.[18] Both experiment and theory indicate that Gly·Na$^+$ is a charge-solvated structure.[17] The lack of any observable exchange for Gly·Ba^{2+} is consistent with the absence of a carboxylic hydrogen that would occur in a salt-bridge structure. However, the structure of the ion in an H/D exchange experiment can be perturbed by the presence of the exchange reagent, and these experiments do not necessarily reflect ground-state structures. Thus, these results are only suggestive of the structure of the original ion.

Conclusions

The difference in energies between the CS1 and SB structures is large, and these results are consistent at all levels of theory used. These ions are stable and can be formed experimentally. For divalent alkaline earth glycine complexes, except beryllium, the SB form is the most stable due to larger electrostatic interactions. For Gly·Ba^{2+}, H/D exchange experiments are consistent with a SB structure. Other probes of structure, such as ion mobility or infrared spectroscopy could be used to obtain more conclusive experimental verification of these results. Divalent metal ions are ubiquitous in biological environments, and are often attached to gas-phase proteins produced by electrospray ionization. The results presented here indicate that, in the presence of larger multivalent ions, stable zwitterion and salt-bridge structures will almost certainly play an important role in the gas-phase structure and chemistry of these ions.

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