A Theoretical Study of Potassium Cation Binding to Glycylglycine (GG) and Alanylalanine (AA) Dipeptides

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Abstract: By combining Monte Carlo conformational search technique with high-level density functional calculations, the geometry and energetics of K^+ interaction with glycylglycine (GG) and alanylalanine (AA) were obtained for the first time. The most stable K^+-GG and K^+-AA complexes are in the charge-solvated (CS) form with K^+ bound to the carbonyl oxygens of the peptide backbone, and the estimated 0 K binding affinities (ΔH_0) are 152 and 157 kJ mol^{-1}, respectively. The K^+ ion is in close alignment with the molecular dipole moment vector of the bound ligand, that is, electrostatic ion–dipole interaction is the key stabilizing factor in these complexes. Furthermore, the strong ion–dipole interaction between K^+ and the amide carbonyl oxygen atom of the peptide bond is important in determining the relative stabilities of different CS binding modes. The most stable zwitterionic (ZW) complex involves protonation at the amide carbonyl oxygen atom and is approximately 48 kJ mol^{-1} less stable than the most stable CS form. The usefulness of proton affinity (PA) as a criterion for estimating the relative stability of ZW versus CS binding modes is examined. The effect of chain length and the nature of metal cations on cation–dipeptide interactions are discussed. Based on results of this study, the interaction of K^+ with longer peptides consisting of aliphatic amino acids are rationalized.

Keywords: cations · density functional calculations · peptides · potassium · zwitterions

Introduction

As one of the most abundant alkali metal cations in living systems, K^+ has numerous biochemical functions such as osmotic equilibrium of cells,[1, 2] stabilization of protein structures,[1] and activation of enzyme functions.[3] Recent studies have shown that K^+ and the other alkali metal cations can induce conformational changes when they bind to proteins.[4, 5] The trans-membrane movement of potassium ions underlies many fundamental biological processes, including biological (electrical) signaling in the nervous system, generation of rhythmic signals by the heart, and unceasing sifting of toxic solutes in the blood by the kidney.[6] X-ray crystallographic data suggest that the interaction between K^+ and the main chain carbonyl oxygen atoms of the potassium ion channel (protein) is the molecular basis that accounts for the selective transport of K^+ across cell membranes.[7] Hence, detailed knowledge of the structural and energetic aspects of the local interaction between K^+ and prototypical amino acid residues and peptides is essential for understanding these processes. Such knowledge is of practical importance in interpreting the mass spectra of K^+-peptide/protein complexes, from which sequencing information can be obtained.[8–11]

The interactions of alkali metal cations with simple amino acids are reasonably well-studied theoretically.[12–16] In the gas phase, experimental evidence indicates that binding of K^+ to arginine stabilizes the zwitterionic (ZW) form of the amino acid,[17–19] and the stability of the metal-cationized ZW structure relative to the charge-solvated (CS) structure is postulated to be enhanced by increase in the proton affinity of the amino acid.[20] To extrapolate from these small model amino acid systems to metal cation–protein systems, the interaction between metal cation and the simplest dipeptide glycylglycine (GG) and alanylalanine (AA) is a vital bridge. With no functionalized side chain, the interaction between metal cations and GG/AA are restricted to O/N binding sites

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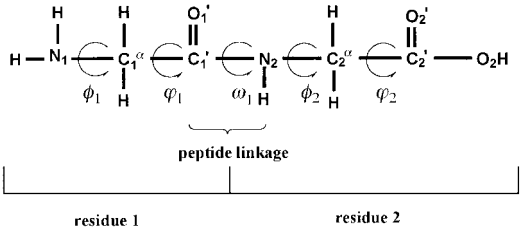
at the amide oxygen of the peptide bond, the amino nitrogen at the N-terminal, and the carboxyl oxygen atoms at the C-terminal, and this provides valuable information on the individual metal-cation-binding sites on the peptide/protein backbone. Because of the large molecular sizes, only three high-level theoretical studies on peptide complexes have been reported: Na^+-GG[21, 22] and Cu^+/Ag^+-GG[23].

To gain a better understanding of how different metal ions interact with the peptide backbone, we carried out DFT studies on K^+-GG and K^+-AA. The Monte Carlo conformational search technique is used to generate plausible conformers for further ab initio/DFT studies. Using this combined approach, we located several modes of metal-cation binding on the GG and AA backbones that were not reported in previous studies. In the case of K^+-GG/K^+-AA complexes, some of these binding modes are fairly low lying and hence may be energetically accessible under laboratory conditions. Factors affecting the relative K^+ affinities of these different ZW versus CS binding modes will be discussed.

Computational Methods

For the free ligand GG, the stability of its various conformers has been the subject of several theoretical publications.[21, 23–26] We re-optimized the four most stable conformers presented in refs. [21, 23] at the HF/6-31G(d) level, and refined their geometries at the B3-LYP/6-31G(d) level. Single-point energy calculations were performed at the B3-LYP/6-311+G(3df,2p) level based on the B3-LYP/6-31G(d) geometries.

There are no prior high level theoretical studies on the interaction of K^+ and GG ligands. The K^+ may interact with the dipeptide ligand in charge-solvated (CS) or zwitterionic (ZW) forms. The zwitterionic form of the ligand can in turn be classified into three types in which the proton is attached to the terminal amino nitrogen atom (N_1), the nitrogen in the peptide linkage (N_2) or the amido carbonyl oxygen atom of the peptide bond (O_1') as depicted in Scheme 1.[27]



Scheme 1. Systematic naming of atoms and dihedral angles of the glycylglycine ligands according to the IUPAC recommendation.[27] Atoms and their positions in the peptide backbone are indicated by letters/symbols/numbers in bold fonts).

Because of the flexibility of the peptide backbone by single bond rotation, the K^+-GG complex can exist as many isomers/conformers. Given the complexity, we first obtained plausible geometries of these species using the Monte Carlo multiple minimum (MCMM) conformational searching technique,[28] with AMBER* force field[29] implemented in the Macromodel 7.0 package.[30]

In the MCMM search, the K^+ was not covalently bound to any atoms of glycylglycine so that the ion could move freely to interact with any part of the ligand. Point charges and distant-dependent dielectric constant were used in the electrostatic interaction treatment.[30] Extended cutoff bond lengths of 20 Å were employed for plausible van der Waals and electrostatic interactions, and hydrogen-bonding distances. For the interaction of K^+-GG in CS and ZW forms, n_{conf} Monte Carlo steps (where $n_{conf} = 1500 \times$ number of rotatable torsional angles of the GG ligand in CS or ZW form, that is, n_{conf} is 9000 for CS and **ZW(O_1')**, and 7500 for **ZW(N_1)** and **ZW(N_2)**) were carried out to locate the low energy structures for K^+-GG. Thus, more than 130 isomers/conformers were obtained within an energy window of 50 kJ mol^{-1}. Any complexes generated from the MCMM step without intramolecular hydrogen bondings were discarded.

The remaining 60 isomers/conformers were re-optimized at the HF/6-31G(d) level by using the Gaussian 98 package.[31] Within each CS/ZW series, only stable isomers/conformers (80 kJ mol^{-1} above the most stable K^+-GG complex calculated at the HF/6-31G(d) level) were retained for further calculations in which the geometries were refined at the B3-LYP/6-31G(d) level.[32] These structures were used for single-point energy calculations at the B3-LYP/6-311+G(3df,2p) level to yield the theoretical affinities at 0 K (ΔH_0) given in Equation (1):

$$\Delta H_0 = [(E_{K^+} + E_{GG}) - E_{K^+\text{-}GG}] + [ZPE_{GG} - ZPE_{K^+\text{-}GG}] \times 0.8929 \quad (1)$$

where E_{K^+}, E_{GG}, $E_{K^+\text{-}GG}$ are the electronic energies (calculated at the B3-LYP/6-311+G(3df,2p)//B3-LYP/6-31G(d) level) of the potassium cation, the glycylglycine ligand (in this case, the energy of conformer **GG1**; see below) and the K^+–glycylglycine complex, respectively; and ZPE is the zero-point energy of the various species, calculated at the HF/6-31G(d) level and scaled by 0.8929.[33] For simplicity of expression, we abbreviate this protocol as "EP(K^+)" for "Energetic Protocol for estimating K^+ binding affinity". We calibrated this protocol against the computationally more expensive ab initio G2(MP2,SVP) method for 13 small organic ligands, and found that the K^+ affinities (ΔH_0) are comparable,[34] and the mean-absolute-deviation (MAD) is only 4 kJ mol^{-1}. Furthermore, we have applied EP(K^+) to obtain the theoretical K^+ affinities for all five aliphatic amino acids,[35] and found that they are in excellent agreement with the absolute affinities determined by the mass spectrometric kinetic method to within 2 kJ mol^{-1}, which is well within the estimated experimental uncertainty of about 10 kJ mol^{-1}.[35, 36]

The affinities at 0 K (ΔH_0) for all K^+-GG complexes calculated with the EP(K^+) protocol are summarized in Table 1. Standard thermodynamic relations[37] were applied to obtain the affinities ΔH_{298} and basicity ΔG_{298} of various modes of binding at 298 K (Table 1). As expected, for a given mode of binding, ΔH_{298} is larger than ΔH_0 (by av 1.4 kJ mol^{-1}). Moreover, as entropy is expected to increase when the cation is released from the complexes, ΔG_{298} is smaller than ΔH_{298} (by av 31.5 kJ mol^{-1}). Nevertheless, the relative affinity and basicity scales are essentially parallel, and this suggests that entropy effects are not important in determining the preferred interaction of K^+ with the GG ligand.

To understand how metal-cation binding affects the structural and electronic energy of the ligand, we also calculated the deformation energy

Table 1. The theoretical energetics of potassiated glycylglycine (K^+-GG) complexes [kJ mol^{-1}].

Species	Binding site	ΔH_0[a]	ΔH_{298}[b]	ΔG_{298}[b]	E_{def}[c]	$E_{stabilization}$[d]
CS1	O_1', O_2'	151.8	153.0	120.7	31.1	182.9
CS2	O_1', O_2', N_1	145.3	147.2	110.6	45.4	190.7
CS3	O_1', O_2	122.5	123.4	92.6	21.5	144.0
CS4	O_1', O_2, O_2'	106.1	107.0	75.9	63.6	169.7
CS5	O_2', O_2	137.4	139.1	105.8	9.3	146.7
CS6	O_1', N_1	137.2	138.6	106.9	33.3	170.5
CS7	O_1'	127.3	127.8	100.1	6.7	134.0
CS8	N_1	80.2	80.7	54.9	14.0	94.2
ZW(O_1')	carboxylate COO^-	103.8	105.5	74.6	149.5	253.3
ZW(N_1)	carboxylate COO^-	90.4	93.5	55.9	88.8	179.2
ZW(N_2)	carboxylate COO^-	46.1	47.8	18.6	156.1	202.2

[a] Calculated by the EP(K^+) protocol. [b] Standard thermodynamic relations[37] were applied to obtain the affinities (ΔH_{298}) and basicity (ΔG_{298}) at 298 K. [c] Calculated at the B3-LYP/6-31G(d) level of theory. We found that the deformation energy calculated at the B3-LYP/6-311+G(3df,2p) and B3-LYP/6-31G(d) levels using the B3-LYP/6-31G(d) geometries differs by no more than 1 kJ mol^{-1} for a few test cases. Hence, the deformation energies calculated at the B3-LYP/6-31G(d) level are considered to be sufficient. [d] Sum of ΔH_0 and E_{def}, representing the raw interaction energy between the cation and the ligand.

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E_{def} at the B3-LYP/6-31G(d) level of theory, where E_{def} (listed in Table 1) is given by Equation (2).[16, 35, 38]

$$E_{def} = E(\text{GG in the } K^+\text{-GG complex}) - E(\text{GG in the uncomplexed form}) \quad (2)$$

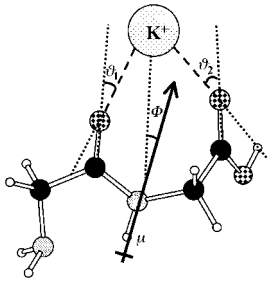
Physically, E_{def} represents the destabilization energy arising from structural distortion, disruption of intramolecular hydrogen bonding, and intramolecular electrostatic repulsion among electron-rich functional groups of the ligand when the ligand deforms itself to accommodate the metal cation. As the deformed ligand in the complexed form is always less stable than the free ligand, E_{def} is always positive. The total favorable (stabilizing) interaction energy at 0 K is then given by $E_{stabilization}$, which is the sum of ΔH_0 and E_{def}.

By replacing one hydrogen atom on each of C_1^{α} and C_2^{α} with a methyl group (without performing the MCMM conformation search again), we carried out EP(K^+) calculations to obtain ΔH_0 for the K^+-AA system at the B3LYP/6-311 + G(3df,2p)//B3LYP/6-31G(d) level. Also, the same computational procedures were applied to obtain ΔH_{298}, ΔG_{298}, E_{def} and $E_{stabilization}$ of the K^+-AA complexes (Table 2).

Table 2. The theoretical energetics of K^+-AA complexes [kJ mol^{-1}].

Species	Binding site	ΔH_0[a]	ΔH_{298}[b]	ΔG_{298}[b]	E_{def}[c]	$E_{stabilization}$[d]
CS1	O_1', O_2'	157.2	158.1	127.0	28.8	186.0
CS2	O_1', O_2', N_1	145.9	147.5	112.3	50.2	196.1
CS3	O_1', O_2	130.1	130.8	100.0	19.6	149.7
CS4	O_1', O_2, O_2'	113.1	113.6	83.6	64.9	178.0
CS5	O_2', O_2	140.6	142.2	109.7	14.0	154.6
CS6	O_1', N_1	135.8	137.1	106.0	40.3	176.1
CS7	O_1'	123.8	124.1	98.0	6.4	130.2
CS8	N_1	83.7	84.1	58.1	15.3	99.0
ZW(O_1')	carboxylate COO^-	110.6	112.2	79.8	143.5	254.1
ZW(N_1)	carboxylate COO^-	104.0	106.4	71.2	79.7	183.8
ZW(N_2)	carboxylate COO^-	59.6	60.8	31.7	148.5	208.1

In the present study, the dipole moment (μ in Debye) of the deformed GG and AA in the complexed states were calculated by standard Mulliken population analysis in the Gaussian 98 package.[31] Classically, the strength of the ion–dipole interaction is directly proportional to the molecular dipole moment of the *deformed* ligand in the complexed state, the cosine of the angle of deviation between the cation and the dipole moment vector (Φ in degrees), and inversely proportional to the square of the distance between the cation and the centre of the dipole moment vector (r_μ in Å), with the origin at centre of charge of the deformed ligand (Scheme 2).[39] The ion–dipole interactions are strongest when the metal ion is in perfect alignment ($\Phi = 0°$) with the dipole-moment vector. The alignment of K^+ with the amide O=C, carboxyl O=C and OH bonds at the individual O/N heteroatom binding sites are represented by the angles of deviation ϑ_1, ϑ_2, and ϑ_3, respectively (only ϑ_1 and ϑ_2 are shown in Scheme 2).



Scheme 2. Representation of the ion–dipole interactions in the K^+-glycylglycine complex in which the angle of deviation between the cation and the dipole moment vector is Φ (in °), and the distance between the cation and the centre of the dipole moment vector is r_μ, (in Å, with origin at centre of charge of the *deformed* ligand). The alignment of K^+ with the O=C bond axis is represented by the angles of deviation ϑ_1 and ϑ_2.

Results and Discussion

Glycylglycine ligand: Four glycylglycine conformers, **GG1** to **GG4** (Figure 1) were investigated. The most stable conformer we obtained, **GG1**, is stabilized by three intramolecular hydrogen bonds.

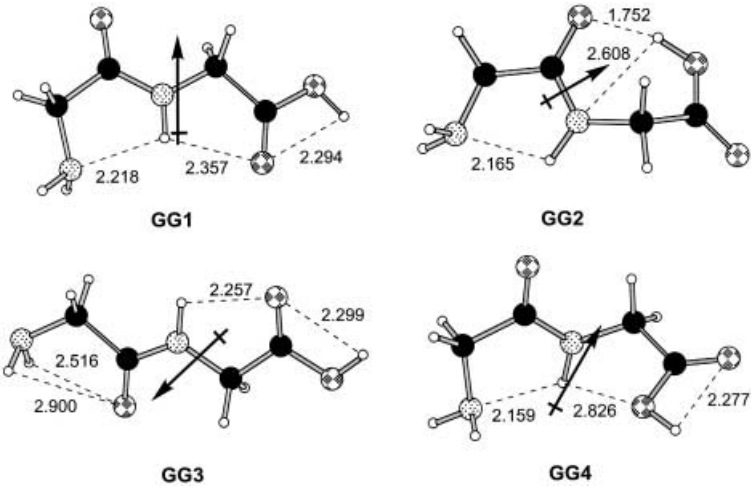


Figure 1. The geometries of four conformers of the glycylglycine (GG) ligand, optimized at the B3-LYP/6-31G(d) level. The intramolecular hydrogen bonds are indicated by dotted lines.

Our findings are in agreement with Cerda et al (species **X** in ref. [21]) but in contrast to those of Cassady et al.[26] and Siu et al.[23] Without electron correlation (at the HF/6-31G(d) level), Cassady et al.[26] suggested that **GG3** is most stable, while Siu et al.[23] reported that conformer **GG2** (species **5N** in ref. [23]) is the global minimum. At our current level of theory, the energy difference between these three conformers is within 4.0 kJ mol^{-1} and such minor energy differences can easily be the result of using a different theoretical treatment or protocol. Given such small energy differences among these "low-lying" GG conformers, the final theoretical K^+ binding affinity of glycylglycine will not be significantly affected even if GG1 is not found to be the most stable conformer at another level of theory.

The most stable K^+-GG complex: Using the MCMM and the EP(K^+) protocols, we have located 27 complexes (18 in CS forms and nine in ZW forms) within 140 kJ mol^{-1} from the most stable K^+-GG complex. These 27 species can be further classified according to their modes of binding into eight CS (Figure 2) and three ZW (Figure 3) forms; the remaining 16 low-lying CS and ZW forms are shown in the Supporting Information, Figure S1. The structures shown in Figure S-1 are less stable although they have the same K^+ binding modes as the structures shown in Figures 2 and 3.

We found that the most stable mode of interaction between K^+ and GG is one in which the ligand is in the charge-solvated CS form. This mode of binding, denoted **CS1** here (Figure 2), involves binding of K^+ to the two carbonyl O=C oxygen atoms (one at the peptide amide bond, and one at the carboxyl group) on both amino acid residues. The K^+ ion is in very close alignment ($\Phi = 9°$) with the molecular dipole moment vector of *deformed* GG (Scheme 2), and this suggests that the ion–dipole interaction is important in stabilizing the **CS1** mode of binding. We note that **CS1** was also identified as the most stable binding mode in the Na^+-GG complex.[21]

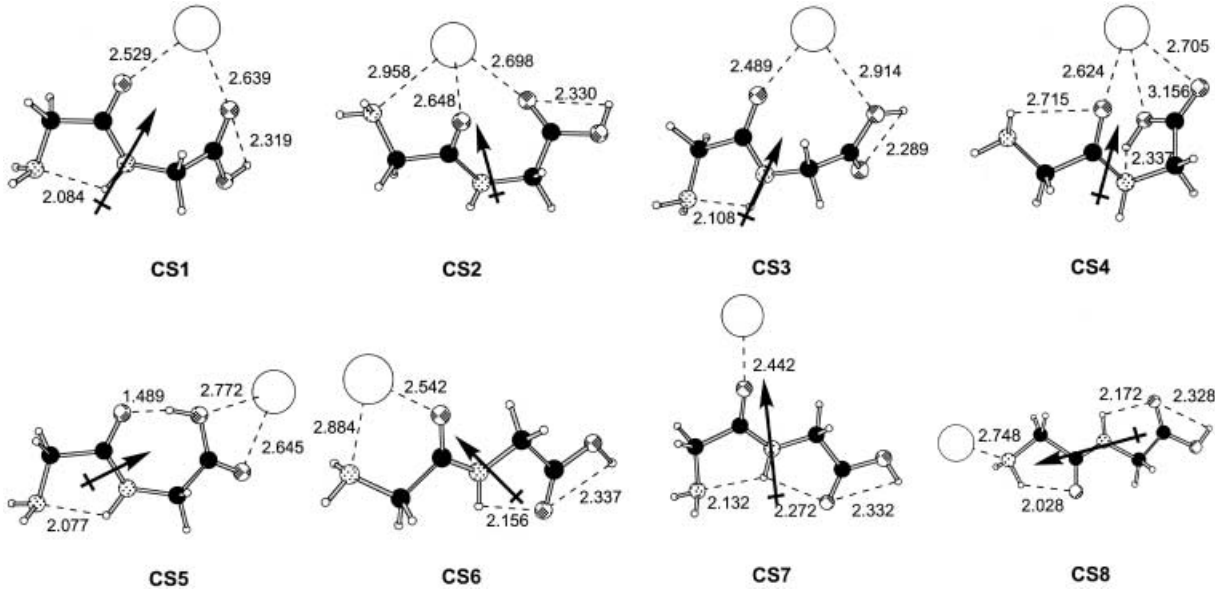


Figure 2. The geometries of eight CS binding modes of K^+-GG, optimized at the B3-LYP/6-31G(d) level. The intramolecular hydrogen bond and the interaction between K^+ and the binding site of the ligand are indicated by dotted lines. The molecular dipole moment vector of the *deformed* GG ligand is indicated by an arrow (not to scale).

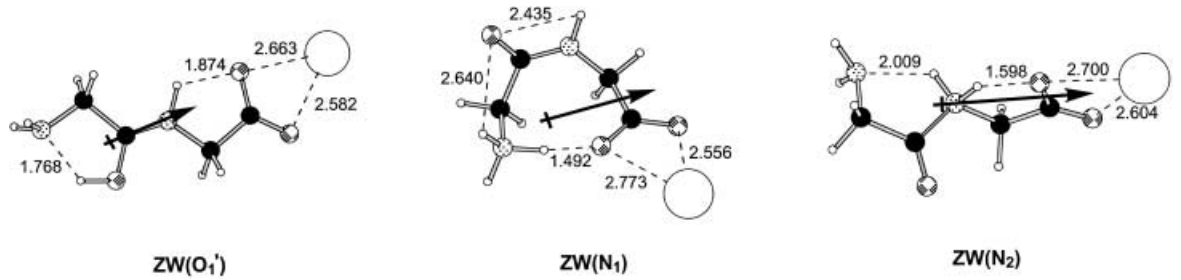


Figure 3. The geometries of three ZW binding modes of K^+-GG, optimized at the B3-LYP/6-31G(d) level. The intramolecular hydrogen bonds and the interaction between K^+ and the binding site of the ligand are indicated by dotted lines. The molecular dipole moment vector of the *deformed* GG ligand is indicated by an arrow (not to scale).

Other charge-solvated (CS) complexes: We found eight complexes (Figure 2) in which K^+ binds to GG in the CS form; the least stable CS isomer **CS8** has a binding affinity 71.6 kJ mol^{-1} above the **CS1** complex (Table 1). Complexes **CS1**–**CS8** can be classified into two groups in terms of how K^+ interacts with the GG ligand: those that involve binding to O/N sites of *both* amino acid residues and those that involve binding to only *one* residue. As in the case of **CS1**, we generally found good alignment of K^+ with the dipole moment vector ($\Phi = 7$ to 24°) in the **CS2** to **CS8** modes of binding.

In **CS2**, **CS3** and **CS4** complexes, K^+ is attached to O/N heteroatoms of both residues. One can view these complexes as "derivatives" of the **CS1** mode of binding. Relative to **CS1**, the tridentate **CS2** complex is stabilized by an additional interaction between K^+ and the N-terminal amino nitrogen (N_1). While this additional interaction stabilizes the complex, it also leads to greater deformation of the GG ligand (E_{def} is 14.3 kJ mol^{-1} greater than that of **CS1**), hence decreases the overall stability of the **CS2** mode of binding.

The binding affinity of **CS3** is comparable to that of **CS1**: in **CS1**, K^+ interacts with two carbonyl oxygen atoms, while in **CS3**, the cation interacts with one O=C and one OH. The interaction of K^+ with the hydroxyl group is known to be weaker than with O=C,[34] and this accounts for the decrease in stability of the **CS3** mode relative to **CS1**.

The **CS4** mode differs from the **CS1** mode in two aspects. Firstly, the N-terminal amino group adopts a "*cis*" ($\varphi_1 = 29°$), rather than a "*trans*" conformation ($\varphi_1 = -173°$) in **CS1**. Secondly, K^+ interacts with an additional OH group in the **CS4** mode. Although these changes might be considered minor, it is interesting to note that the E_{def} of **CS4** is more than twice that estimated for **CS1**. We attribute this large E_{def} to intraligand repulsion. In the **CS4** mode, simultaneous binding of the three negative sites to K^+ requires that the three oxygen atoms be very close to each other. The distance between O_1' and O_2 is only 3.04 Å in **CS4** mode, which is 1.41 Å shorter than that in **CS1**. Since the $K^+ \cdots$ OH interaction is weaker, the **CS4** mode of binding is less stable than **CS1** by 46 kJ mol^{-1}.

Complexes **CS5**–**CS8** are charge-solvated complexes in which the K^+ interacts with the O/N heteroatom sites of one amino acid residue only. Hence, it is of interest to compare these species with the corresponding modes of binding in K^+-Gly[16] so that the effect of the additional glycyl residue can be

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elucidated. Firstly, the ΔH_0 of K^+-GG is at least 13 kJ mol^{-1} higher than the corresponding modes of binding in K^+-Gly, and the largest difference (43 kJ mol^{-1}) found in **CS7**.[16] Secondly, the order of relative affinity for these modes for binding (**CS5** > **CS6** > **CS7** > **CS8**) of K^+-GG is identical to that of K^+-Gly.[16] This implies that for the same mode of binding, the role of the additional spectator glycyl group in GG is simply to enhance the affinity of K^+, presumably due to the increase of permanent dipole moment and polarizability in the presence of the glycyl group. In other words, when the peptide backbone is extended from GG to GGG and longer, and K^+ only binds to one glycine residue, the relative stabilities of the binding modes should have the following order: O=C + OH ≈ O=C + NH_2 > O=C > NH_2.

It is also interesting to compare the stability of **CS1** to that of **CS5** and **CS6**. When K^+ binds to small ligands,[34] the raw interaction energy $E_{stabilization}$ for formamide is especially large: formamide (115) ≫ formic acid (83) ≈ ammonia (74) ≈ water (68 kJ mol^{-1}). This is in line with the much larger theoretical dipole moment of *deformed* formamide (≈4 D) in the complexed state compared with the other three ligands (≈2 D). The binding of K^+ to these small organic ligands can be viewed as model interactions between K^+ and the individual O/N heteroatom binding site in peptides: K^+ binding to the amide C=O oxygen atoms (formamide), carboxyl C=O oxygen atoms (formic acid), N-terminal NH_2 (ammonia) and the C-terminal OH groups (water).[34] Here, we found that the $E_{stabilization}$ term (Table 1) for these bidentate modes of binding is in the order of: **CS1** (amide C=O + carboxyl C=O) > **CS6** (amide C=O + N-terminal NH_2) > **CS3** (amide C=O + carboxyl OH), in line with the greater $E_{stabilization}$ derived from binding of K^+ to the amide C=O oxygen atom. The only exception is **CS5** (carboxyl C=O and OH), which shows comparable $E_{stabilization}$ to **CS3**, presumably because **CS5** is stabilized by a particularly strong intramolecular hydrogen bond (≈1.5 Å, Figure 2). Furthermore, the $K^+ \cdots$ O=C interaction is enhanced by better alignment of K^+ with the bond axis of the binding sites (and presumably the "local" dipole moment vector of the C=O binding sites) in **CS1** (with angles of deviation $\vartheta_1 = 33$ and $\vartheta_2 = 47^\circ$ for the amide and carboxyl C=O bonds, respectively) than that in K^+-Gly, in which K^+ binds to the carboxyl C=O and OH oxygen atoms (with angles of deviation $\vartheta_2 = 77$ and $\vartheta_3 = 91^\circ$, respectively).[16] Thus, the stability of **CS1** is also related to the strength of the local ion–dipole interaction between K^+ and the carbonyl oxygens, especially binding of K^+ to the amide carbonyl oxygen atom of the peptide bond, which is energetically favored. In fact, the highest $E_{stabilization}$ values (Table 1) are found for the four CS forms (**CS1**, **CS2**, **CS4** and **CS6**) which involve binding of K^+ to the amide carbonyl oxygen atom of the dipeptide (Figure 2). Our findings are in line with previous postulates that $M^+ \cdots$ O=C ion–dipole interactions (M = Na or K) are important sources of attractive interaction that contribute to the stability of M^+-formamide/acetamide and Na^+-GG complexes.[40–42]

Zwitterionic (ZW) modes of binding: We have identified three zwitterionic (ZW) forms of the K^+-GG complex in which the K^+ interacts in a bidentate fashion with the two carboxylate oxygen atoms COO^-, but differ in the site of attachment of the carboxyl proton (Figure 3): i) at the amide carbonyl oxygen atom (O_1') of the peptide bond, ii) at the N-terminal amino nitrogen (N_1), or iii) at the amide nitrogen (N_2) of the peptide linkage.

The binding affinities of the ZW complexes in K^+-GG system are at least 48 kJ mol^{-1} lower than the **CS1** mode. It is interesting to compare this difference with our previous study of the K^+-Gly system.[16] In the case of K^+-Gly, the lowest energy ZW mode is only 13 kJ mol^{-1} less stable compared to the most stable CS complex.[16] As the site of K^+ binding is identical (at the carboxylate COO^-) in both K^+-Gly and K^+-GG, the relative instability of ZW modes of binding in K^+-GG arises from the more unfavorable (greater) charge separation (additional coulombic energy required to maintain the separation of the *positive* proton charge and the *negative* carboxylate charge) in the zwitterionic dipeptide backbone. A similar conclusion has been drawn in the corresponding Na^+ system.[22]

Previously studies[24–25, 43–45] suggested that, when an external proton is attached to GG and tripeptide GGG, the relative stability and basicity of different sites is in the order of:
amino nitrogen N_1 ≈ amide carbonyl O_1' ≫ peptide amide N_2.

In the case of ZW K^+-GG complexes, the preference for intramolecular proton transfer from the carboxyl acid group to the three basic sites is in the order (Table 1): amide carbonyl O_1' > amino nitrogen N_1 ≫ peptide amide N_2. Thus, it can be concluded that on complexation with K^+ the amide nitrogen (N_2) remains the least favorable site of protonation. This could be attributed to the loss of resonance stabilization of the peptide bond after protonation at the amide nitrogen atom[43] which destabilizes the **ZW(N_2)** structure. Hence, the **ZW(N_2)** complex is approximately 44 kJ mol^{-1} less stable than **ZW(N_1)**, which has the protonation site at the N-terminal amino nitrogen N_1.

For the GG ligand, it has been estimated that in terms of proton affinity at 0 K (ΔH_0), protonation at the N-terminal amino site N_1 is only favored by 3.7 kJ mol^{-1} over that at the amide carbonyl oxygen site O_1' (Table IV of Ref. [25]). Recent high-level DFT calculations on the GGG ligand also indicate that the proton affinity (ΔH_0) of the N-terminal amide carbonyl oxygen atom is only marginally smaller (by 0.9 kJ mol^{-1}) than that of the amino site (Supplementary Information of ref. [45]). In both cases, protonation at the N-terminal amide C=O group is stabilized by internal hydrogen bonding between the additional proton and the amino nitrogen NH_2 atom; this leads to very similar proton affinities for these two proton-binding sites at the N-terminal glycyl residue. However, in the case of the zwitterionic K^+-GG complexes, protonation at the N-terminal amide carbonyl oxygen site O_1' is preferred by 13.4 kJ mol^{-1} (in terms of ΔH_0, Table 1) over protonation at the N-terminal amino site N_1. We attribute the greater stability of the **ZW(O_1')** binding mode of K^+-GG to three factors: i) the enhanced resonance stabilization (partial double bond character) in the peptide linkage O_1'-C_1'-N_2-H, ii) the formation of a stable hydrogen-bonding interaction $O_1'H^+ \cdots N_1$ (1.77 Å), analogous to the "internal proton solvation" found at the N-terminal glycyl residue of protonated GGG; and iii) the better alignment of the

molecular dipole moment with K^+ in the **ZW(O_1')** mode of binding (Φ of 15° in **ZW(O_1')** versus 35° in **ZW(N_1)**).

The ZW complex with protonation site at the N-terminal N_1, **ZW(N_1)**, deserves further attention. Its deformation energy is ≈40% smaller than that of the **ZW(O_1')** and **ZW(N_2)**, and quite comparable to that found in some CS modes of binding (e.g. **CS4**). The relatively small E_{def} in **ZW(N_1)** probably arises from an extraordinarily strong hydrogen bond between the hydrogen of the positively charged amino group and the oxygen of the negatively charged carboxylate group ($N_1H^+ \cdots {}^-OOC$ 1.49 Å). Such strong hydrogen bond could even compensate the two destabilizing factors arising from N_1 protonation: the preference for a more stable "*cis*" conformation in the peptide bond,[46] and the charge separation in a ZW structure. This interaction is so stabilizing that upon metal complexation, the dipeptide is driven into a compact "cyclic" configuration in **ZW(N_1)** as opposed to an "extended" conformation found in the other zwitterionic K^+-GG complexes (Figure 2).

Nature of cation on metal cation-glycylglycine (M^+-GG) interactions: Two high-level theoretical studies on Na^+-GG were independently reported by Bowers et al.[22] and Cerda et al.[21] independently. Bowers et al.[22] reported the relative energies of three Na^+-GG isomers, which are also included in a more comprehensive study on Na^+-GG interaction by Cerda et al., with qualitatively the same results.[21] Thus, our comparison is only against the results of Cerda et al. More recently, the interaction between GG and Cu^+/Ag^+ was also reported.[23] As Cu^+ is a substantially smaller cation than K^+, and the similarities and differences between Cu^+-GG and Ag^+-GG interactions have been extensively discussed,[23] we focus only on the interaction of GG with K^+, as opposed to Na^+ and Ag^+ here.

By geometry search we have located some fairly stable modes of binding (e.g. **CS3**, **CS4**, **CS5**, **CS7**, **CS8**, **ZW(O_1') and ZW(N_2)**) previously not reported in the Na^+-, Cu^+-, and Ag^+-GG systems.[21, 23] Certain modes of binding previously reported (e.g. species **V** in ref. [21]) are less stable conformers on the K^+-GG potential energy surface that share the same mode of cation binding in Figures 2 and 3 here, and the structure and energetics of these low-lying confomers are summarized in the Supporting Information (Figure S-1). We note in passing that the relative stabilities of GG conformers sharing the same mode of binding are in fact governed by the hydrogen-bonding patterns adopted by the GG ligand. The results on this aspect of K^+-GG binding will be reported elsewhere.[47]

Here, we focus on how the nature of the metal cation affects the relative affinity of various modes of binding (Figure 4). Relative to Na^+-GG[21] the relative affinity scale of K^+-GG is compressed. Most of the analogues of Na^+-GG isomers are much less stable relative to the **CS1** mode of binding (species **II** in ref. [21]), except for **CS2** (species **I** in ref. [21]) which is of comparable stability (0.4 kJ mol^{-1}) to that of **CS1**.

On the other hand, the K^+-GG relative affinity scale is expanded compared to that of the Ag^+-GG system (Figure 4). Moreover, the most stable mode of binding also differs. In the most stable charge-solvated Ag^+-GG conformer (species **3** in ref. [23], corresponding to our **CS6**), Ag^+ interacts with O_1' and N_1, while K^+ prefers to bind to O_1' and O_2' of the GG ligand (**CS1** mode of binding). One can rationalize this difference in terms of the hard-soft-acid-base (HSAB) principle.[48] The silver cation is a softer acid (hence more

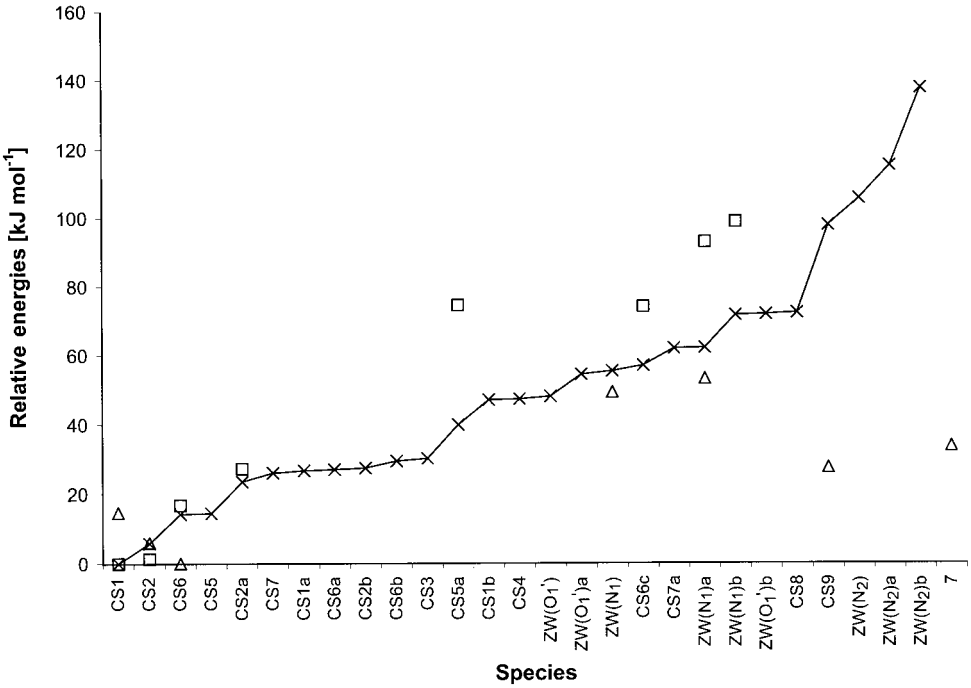

Figure 4. The relative energies (at 0 K) of different modes of binding of glycylglycine for various cations: K^+ (this work at the EP(K^+) level, × with connecting lines for ease of visualization), Na^+ (ref. [21] at the HF/6-31G(d) level, indicated by □) and Ag^+ (ref. [23] at the B3LYP/DZVP, indicated by △). The effect of zero-point energies are not included in the comparison as it was not reported in ref. [21]. Species **7** in ref. [23] for the Ag^+-GG could not be located on the K^+-GG potential energy surface here.

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polarizable) than Na^+/K^+. Therefore Ag^+ is a better electron acceptor and prefers to bind to the softer nitrogen binding sites than the harder oxygen donor sites of a ligand. The preferred binding of Ag^+ to nitrogen sites is also exemplified in the **CS2** mode of binding. As the **CS2** binding mode is already of comparable stability to the **CS1** binding mode in the case of Na^+, the preference of Ag^+ for the softer nitrogen site further stabilizes the **CS2** mode of binding. A further example can be found in species **6** in ref. [23] in which the Ag^+ binds to carboxyl C=O and N-terminal NH_2. Relative to the corresponding **CS1** mode, this mode of binding is relatively stable in Ag^+-GG ($\approx$15 kJ mol^{-1}), but very unstable (the **CS9** binding mode, $\approx$100 kJ mol^{-1}, see Supporting Information Figure S-1) in K^+-GG.[49]

We have failed to locate the corresponding zwitterionic species **7** from ref. [23] on the K^+-GG potential energy surface. Starting from sensible trial K^+-GG structures, these complexes invariably optimized to **CS5** with K^+ bound to carboxyl oxygen atoms (Figure 2) at both HF/6-31G(d) and B3-LYP/6-31G(d) levels. Species **7** (ref. [23]) and the **CS5** complex presented here are isomers that differ in the site of protonation: **CS5** is charge-solvated in nature, while species **7** is a zwitterion in which the carboxyl proton has been transferred from the C-terminal to the N-terminal amide oxygen atom O_1'. We carried out additional geometry optimization with larger 6-31+G(d), 6-31G(d,p) and 6-31+G(d,p) basis sets with the B3-LYP function. As these more flexible basis sets also failed to yield stable complex similar to that of species **7**[23] found in the Ag^+-GG system, it appears that such mode of binding is in fact unstable on the K^+-GG potential energy surface.

Examination of species **7**[23] reveals that the proton bridges between the carboxylate oxygen atom and amide oxygen atom O_1' are short (at 1.40 and 1.07 Å, respectively),[23] and hence the proton would be expected to be quite mobile between these two alternative protonation sites. While cation binding could stabilize a ZW complex (through strong interactions between positively charged metal cations and the negatively charged carboxylate COO^- group), it also introduces instability into the ligand because of the charge-separation effect. It appears that as the smaller Ag^+ binds more strongly and closely to the ligand, the stabilization factor outweighs the charge-separation destabilization effect. As the interaction between the larger K^+ and GG is generally weaker, the stabilizing effect of the $K^+ \cdots COO^-$ interaction is not strong enough to overcome the instability arising from the charge separation effect.

Effect of alkyl side chain and proton affinity: The modes of binding in K^+-AA is very similar to that of K^+-GG, with the more stable CS and ZW modes of binding depicted in Figure 5. Generally speaking, the relative affinities of analogous binding modes in K^+-AA is parallel to that of K^+-GG.

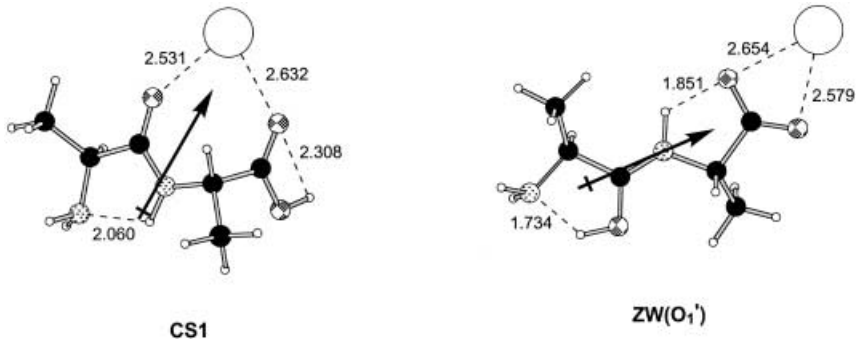

Figure 5. The geometries of the most stable conformer of alanylalanine ligand (AA), charge-solvated complex (**CS1**), zwitterionic forms (protonated at O_1', **ZW(O_1')**, optimized at B3-LYP/6-31G(d) level of theory. The intramolecular hydrogen bonds and the interaction between K^+ and the binding sites of the ligand are indicated by dotted lines. The molecular dipole moment vector of the *deformed* AA ligand is indicated by an arrow (not to scale)

Presumably, because of the increase in polarizability, the raw interaction energy ($E_{stabilization}$) is increased; this leads to a general increase in K^+ binding affinities of approximately 5 kJ mol^{-1} from GG to AA, which is identical to the change observed in going from K^+-Gly to K^+-Ala.[16] Hence, it seems that the effect of increasing the alkyl chain length (polarizability) on relative stability of CS versus ZW in K^+ binding modes is the same for both aliphatic amino acids and dipeptides.

A greater proton affinity (PA) would favor intramolecular proton transfer from the C-terminal carboxyl OH group to the N-terminal NH_2 group, and is expected to confer greater stability to ZW forms of the M^+–amino acid complex.[15, 20, 35] Here, we would like to investigate how this criterion can be extended to metal complexes of the dipeptides GG and AA. The proton affinities of glycine, glycylglycine, alanine and alanylalanine are summarized in Table 3, along with the relative stability of the CS/ZW forms in these K^+–ligand systems.

Table 3. A comparison of proton affinities [kJ mol^{-1}] of glycine (Gly), glycylglycine (GG), alanine (Ala), alanylalanine (AA) and the relative stabilities ($E_{zw\text{-}cs}$, in kJ mol^{-1}) of K^+ bound CS/ZW forms

Species	Gly	GG	Ala	AA
PA[a]	902.5	934.7	912.5	946.8
$E_{zw\text{-}cs}$[b]	13.2	48.0	8.0	46.6

[a] Experimental data at 298 K and 1 atm from ref. [26]. [b] The ΔH_0 of the most stable K^+–ligand complex in the ZW form, relative to the most stable CS complex of the same system.

The PA of Ala is greater than that of Gly by 10 kJ mol^{-1}, and this suggests that the more basic N-terminal site in Ala is more prone to proton attachment. Accordingly, formation of the ZW complex is more favorable for Ala than Gly. A similar trend is observed when AA is compared against GG: the

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energy difference between the most stable CS and ZW forms is smaller for K^+-AA. Hence, the proton affinity affects the relative stabilities of CS and ZW complexes of aliphatic amino acids and dipeptides in the same way.

However, this does not hold for K^+-Gly/K^+-GG or K^+-Ala/K^+-AA pairs. While GG and AA have greater proton affinities than Gly and Ala, the most stable ZW complex of the dipeptide ligand is much less stable than the most stable **CS1** complexes (by 47–48 kJ mol^{-1}, Table 3), a difference significantly greater than the corresponding 8–13 kJ mol^{-1} for K^+-Gly and K^+-Ala complexes.[16] This is because the most stable CS binding mode in the aliphatic amino acids[16] differs from that of the dipeptides. Moreover, the most stable ZW complex for aliphatic amino acids[16] also has a different protonation site to that of the dipeptides. Thus, the PA criterion can only be applied to systems that have the same CS or ZW modes of metal-cation binding, and their corresponding ZW forms should have the same protonation sites.

Interaction of K^+ with peptide backbones: Comparing our present results on the factors governing the relative stability of the CS and ZW modes of binding in K^+-GG/K^+-AA with the intrinsic K^+–peptide backbone interactions in the gas phase,[4, 5, 22] and biological systems,[7, 50–52] provided new insights into the interactions of these and related systems.

Results from the present study illustrate the importance of "local" $K^+ \cdots O{=}C$ ion–dipole interactions between K^+ and GG/AA, so that the cation prefers to bind to two carbonyl oxygen atoms of the dipeptide backbone in the CS mode. Therefore, in longer peptides, the increased flexibility of the backbone should allow the ligand to align its various O=C groups more closely with the cation and this maximize the number of $K^+ \cdots O{=}C$ interactions. At the same time, the ZW binding modes of K^+ become much less stable (by at least about 47 kJ mol^{-1}) in the aliphatic dipeptides GG and AA than in the amino acids glycine and alanine. This is due to i) the enhanced stability conferred by the strong $K^+ \cdots O{=}C$ interaction associated with the peptide bond in the most stable **CS1** structure, and ii) the instability of the ZW structures that arise from the greater charge-separation effect in the dipeptide backbone.

Both factors suggest that the K^+ is likely to be encapsulated inside the peptide chain in a macrocyclic CS conformation with multidentate binding to mostly amide O=C sites of the peptide backbone. Support for this is provided by the potassium complex of valinomycin, a dodecadepsipeptide in which the K^+ binds to the six valine carbonyl oxygen atoms in a near octahedral arrangement in the gas phase.[50] Moreover, given the similarity between the mode of binding between Na^+ and K^+ shown here for GG and AA, and previous works for smaller ligands,[53] our results are consistent with previous reports that macrocyclic CS modes of Na^+ binding to backbone carbonyl oxygen atoms in sodium oligoglycine complexes (Na^+-Gly$_n$, $n = 2-6$) and oligoalanines (Na^+-Ala$_n$, $n = 10$, 15, 20, and $[Ala_n + 3Na]^{3+}$, $n = 18-36$).[4, 5, 22] In biological systems, K^+ binding to backbone carbonyl oxygen atoms in a macrocyclic pattern are found in the X-ray protein structures of K^+ channels,[7] tryptophanase[51] and pyruvate kinase,[52] and suggest that the importance of local $K^+ \cdots O{=}C$ ion–dipole interaction can be extrapolated from the gas phase to solution phase.

Finally, we found that the most stable ZW binding mode **ZW(O_1')** for K^+-GG and K^+-AA is protonated at the amide carbonyl oxygen atom O_1' of the N-terminal glycyl residue (Scheme 1), which has proton affinity very similar to that of the amino N_1 site. Despite stabilization by very strong hydrogen bonding (as indicated by the very short bond length of 1.77 Å) between the $O_1'H$ and N_1 within the N-terminal glycyl residue, this potassiated **ZW(O_1')** structure of GG and AA remains much less stable than the most stable conformer **CS1**. For longer aliphatic peptides, the ZW conformers are expected to become even less stable due to the greater charge-separation effect. Drawing on the similarity in binding modes between K^+ and Na^+ again, our results on the relative instability of ZW structures **ZW(O_1')** and **ZW(N_1)** for the dipeptides GG and AA are in line with a previous report that a longer helical ZW Na^+-Ala$_{15}$ structure collapsed to a random globular structure (presumably in the CS form) in numerical simulations.[4] Furthermore, a helix with a salt bridge from the deprotonated C-terminus (zwitterionic $COO^- \cdots Na^+$) and protonation of the backbone C=O near to the C-terminus (to minimize charge-separation effect) appears to be a stable structure in the numerical simulation. Hence, the larger aliphatic peptides are most likely to remain in the CS form when sodiated or potassiated.

Conclusion

To our knowledge, this is the first high-level ab initio/density functional study on K^+ interaction with dipeptides. In this study, we have located 18 charge-solvated (CS) and nine zwitterionic (ZW) stable isomers/conformers for the K^+-GG/AA dipeptide complexes at the B3-LYP/6-311+G(3df,2p)//B3-LYP/6-31G(d) (abbreviated at EP(K^+)) level of calculations, which can be classified into eight CS and three ZW K^+ binding modes to different O/N heteroatom sites of the peptide. Several of these binding modes are not found in previous studies of Na^+, Cu^+ and Ag^+ binding to the dipeptide GG.

The most stable K^+-GG and K^+-AA complex involve a bidentate interaction in which the K^+ coordinates to two carbonyl oxygen atoms of two amino acid residues in the CS form. We found good general alignment of K^+ with the dipole moment vector of the *complexed (deformed)* aliphatic dipeptides in all of the CS modes of binding, and this suggests that ion–dipole interaction is the key electrostatic interaction contributing to the stability of the K^+-GG/AA complexes. Among the different O/N heteroatom binding sites on the peptide backbone, K^+ binding to the amide carbonyl oxygen is energetically very much preferred, and this is attributed to the very strong *local* ion-dipole interaction between K^+ and the peptide amide C=O bond. Consequently, the more stable CS forms are those that involve K^+ binding to and in close alignment with the amide C=O.

Since the most stable ZW form (with K^+ binding to two carboxylate oxygens) is 48 kJ mol^{-1} less stable than the most stable CS form (with K^+ binding to two amide carbonyl

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oxygens), it appears that the $K^+ \cdots C{=}O$ (amide) interaction can confer greater stability to K^+-GG/AA complex than $K^+ \cdots COO^-$ (carboxylate) interaction. By definition, in ZW K^+–peptide structures K^+ is bound to the two carboxylate oxygen atoms at the C-terminus. While K^+ binding to backbone amide carbonyl oxygen atoms in the ZW structures of larger helical or globular peptide chains is still possible, the CS form always has the advantage of being able to bind to more amide C=O binding sites than the ZW form, and the former thus gains additional stability. Protonation and internal proton solvation at the basic amide carbonyl oxygen atom of the N-terminal glycyl/alanyl residue in GG and AA cannot compensate for the destabilizing charge-separation effect even for the lowest energy (most stable) **ZW(O_1')** structure involving protonation and strong hydrogen bonding at the more basic N-terminal glycyl/alanyl residue. Since the charge-separation effect is expected to be amplified in ZW structures of longer peptides (due to greater separation distances between the postive proton charge and the negative carboxylate charge), and coupling with the lesser probability of K^+ binding to amide C=O of the peptide backbone for ZW structures, it is very likely that the most stable K^+ stabilized aliphatic peptide complexes have charge-solvated conformations in the gas phase.

The stability of the lowest energy ZW binding mode of GG/AA (relative to their respective most stable CS forms) increases slightly with the proton affinity of the dipeptide. However, the proton affinity criterion fails when cross comparisons are made between the dipeptides and the aliphatic amino acids (i.e., compare GG/Gly pair or AA/Ala pair). Hence, we would suggest caution in extending the proton-affinity criterion to compare or predict the relative stability of different ZW structures of amino acids or peptides having different metal cation binding modes or sites of proton attachment.

While the CS and ZW binding modes and the trend of relative stabilities are similar for Na^+/K^+–dipeptide complexes, we found the most stable CS form of K^+-GG and Ag^+-GG complex to have different O/N heteroatom binding sites, and other stable CS conformers also show significant differences in their relative stabilities. The origin of these differences may reflect the different nature of bonding and ionic sizes of Ag^+ and K^+. Hence, differences in the mass spectral fragmentation patterns between Ag^+–peptides and K^+/Na^+–peptides are expected. Mass spectra of Na^+-,[9–11, 54] K^+-[8–11] and Ag^+-[55] cationized peptides have been used to identify peptides and provide sequence information. It may be of practical interest to examine whether such differences can be exploited to provide complementary mass spectral information on peptide sequence, which has become an important issue in proteomic analysis today.

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