

# Reservation Energy Bonds and Structural Stability of Series of Multihydrated ( $n_{\text{H}_2\text{O}} = 1-10$ ) Glycine- $\text{H}^+\text{M}^+$ ( $\text{M} = \text{Li}, \text{Na}, \text{or K}$ ) Complexes

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A systematic study with the density functional theory (DFT/B3LYP) on the reservation energy bonds (REBs) and structural stability of series of biologically relevant multihydrated ( $n_{\text{H}_2\text{O}} = 1-10$ ) glycine- $\text{H}^+\text{M}^+$  ( $\text{M} = \text{Li}, \text{Na}, \text{or K}$ ) complexes in the gas phase has been presented. The results show that the bonds (REBs) of  $(\text{H}_2\text{O})_{n-x}\text{-glycine-H}^+\dots\text{M}^+(\text{H}_2\text{O})_x$  (for  $x = 0-4$ ,  $x < n$ ) can release energy if they are broken. Calculations confirm that each of these REBs can be yielded without suffering any energy barrier only when the carboxyl oxygen of the glycine molecule is first bound by the metal ion  $\text{M}^+$  (or its hydrate) and then its amino nitrogen is protonated. Stepwise multiple hydrations can reduce charge distributions on the glycine- $\text{H}^+\text{M}^+$  gradually and, thus, increase the binding strength of REBs and favor the stability of the entire hydrated system. Consequently, the effects of reservation energy of these REBs are weakened. However, their energy decreases are not limitless. The lower limit of decrease, being not less than 20.0 kcal/mol for these complexes, is first predicted in the paper. In other words, the formation of a similar REB in a biological system can reserve energy of  $\geq 20$  kcal/mol. All the dissociation free energies are more than their corresponding electronic dissociation energies. Different from those dissociation energies, calculations show that these dissociation free energies change less with the addition of attached water molecules. All these reveal that two free reactants in a hydrate system finally become a regular complex and involve more energy in the new complex. These are very consistent with the real biological phenomenon. The small activation energy barrier ( $< 3.0$  kcal/mol) in the process of cleavage of the REB is also revealed in the present study, which also is in good agreement with behavior of a real biological system. Correspondingly, the affinity strength of the latter attached water molecules becomes weaker and weaker with further hydration, however, ultimately showing a tendency toward invariableness. The ordering of affinity strengths between these different ions (or ion hydrates) and its corresponding ligand is  $\text{Li}-n\text{W} > \text{Na}-n\text{W} > \text{K}-n\text{W}$  (where  $n = 1-10$  and W represents a water molecule), whereas the magnitude ordering of REB energies of these different metal-ion-involved glycine- $\text{H}^+$  hydrates reverses. In general, these different  $\text{M}^+$ -involved complexes do not present very large differences in regard to the effect on reservation energy; however, they behave differently in coupling water molecules, using their various active sites. For example, only a water molecule can be attached to the  $\text{Na}^+$  end of the glycine- $\text{H}^+$ - $\text{Na}^+$  complex (GHNa) to form the most-stable  $\text{Na}-\text{W}_1$  monohydrate among its isomers, whereas the most-stable  $\text{K}-\text{W}_1$  monohydrate is generated by attaching a water molecule to the frontal amino hydrogen of the glycine- $\text{H}^+\text{K}^+$  complex (GHK). Comparisons of the relative energies of these  $\text{Li}^+/\text{Na}^+$ -involved glycine- $\text{H}^+$  hydrates indicate that the carboxyl moiety that is coupling the  $\text{M}^+$  of the glycine is preferred to be hydrated, whereas the relative energies of the isomers of the  $\text{K}^+$ -involved glycine- $\text{H}^+$  hydrates imply that the protonated amino moiety of the glycine is the optimal combination site.

## 1. Introduction

Amino acid chemistry in aqueous solution has been the subject of many studies, because of its importance in the understanding of protein behavior.<sup>1-8</sup> Since 1970, many efforts have been devoted to interpreting why amino acids exist as neutral forms in the gas phase, while the zwitterionic ones are predominant in aqueous solution.<sup>1,2</sup> Because of the computational convenience and the availability of experimental data, glycine usually has been chosen as the prototype of amino acids in theoretical investigations.

Because of the complexity of the surroundings of an amino acid or protein molecule in a real life system, it is impossible

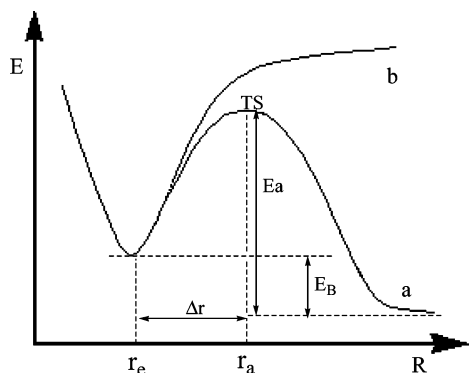
for these biological molecules to exist in a pure water solution. For example, the pH of extracellular fluid is 7.35–7.45, whereas estimates of intracellular pH suggest values of  $\sim 6.9$ .<sup>9</sup> Thus, these results suggest that an amino acid molecule may exist in surroundings with weak acidity or basicity. Moreover, dipeptides and tripeptides that contain amino acids with nonacidic side chains can bond with transition metals to release three protons in the gas phase;<sup>10</sup> therefore, protons that exist in a real biological system would be numerous. A considerable number of papers have reported the protonation of amino acids in theory or experiment;<sup>11</sup> Zhang et al.<sup>11e</sup> concluded that the amino nitrogen of the glycine is the best combination site for protonation.

In contrast to the proton, alkali-metal cations also participate in many functions of life systems, such as the osmotic equilibrium of cells, the electrical excitability of nerves and

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**Figure 1.** Binding or dissociating potential energy surface (PES) plots for the reactions described by glycine- $\text{H}^+ + \text{M}^+ \rightarrow \text{products}$  ( $\text{M} = \text{Li}, \text{Na}, \text{or K}$ ) (curve a) and glycine- $\text{M}^+ + \text{H}^+ \rightarrow \text{products}$  (curve b). The variables  $r_e$  and  $r_a$  denote the corresponding contact distances in equilibrium and transition states (TS), respectively;  $E_a$  and  $E_B$  represent the activity energy barrier and released energy of REB of equilibrium complex, respectively.

muscles, the active transport of glucides and amino acids, and so forth, through their binding to protein.<sup>12</sup> Therefore, the alkali-metal cation/amino acid (peptide) interactions have received much interest, and many studies have been devoted to such systems, both in experiment and theory.<sup>11g,13</sup> However, most of these studies have focused only on the properties of such interactions of a bare alkali-metal ion with an amino acid molecule or peptide in the gas phase. To approach and simulate those real biological systems, the Williams group<sup>14</sup> studied such interactions, including some hydration effects, and had some interesting conclusions. Indeed, the ionic environment in a water solution is indispensable for making the ideal structure or conformation of biological macromolecules including ionic groups (e.g., DNA, RNA, and proteins<sup>15</sup>); however, such an assemblage is also complicated, because these alkali-metal ions and protons may coexist in some biological molecule. A biological molecule interacts not only with one ion but also with two or even more other ions, because of its more-active sites. Interactions with two ions per biological molecule have been observed in experiments. For example, Shields et al.<sup>16</sup> observed an unusual dipeptide complex, which covered both proton transfer and  $\text{Cu}^+$  binding, in their mass spectrometry experiment. It has potential for use as a biological molecule that has more-active sites to bind more ions, which is still an unknown field, to our best knowledge. On the basis of the experimental observations and the importance of the interactions between these ions and biological molecules in life systems, we have probed the interactions using the simplest amino acid (glycine) and  $\text{H}^+$ ,  $\text{Li}^+$ , or  $\text{Na}^+$  as a model in the gas phase in our recent works,<sup>17</sup> and an interesting biologically relevant phenomenon has been observed. In those studies, we first presented an observation of positive bonding energy. Namely, the sum of the energies of two free dissociated (reactant) species was lower than that of their complexes, whereas the frequency calculations confirmed that all these complexes were still genuine minima in their potential energy surfaces (PESs), as shown in Figure 1. The observations are reminiscent of the ingestion of energy and growth of organisms, which are hackneyed phenomena in nature, however, that are in dire need of investigation, in regard to their micromechanisms. Our studies elementarily revealed that two free reactants finally become a regular complex and involve more energy in the new complex. However, these studies are only limited to a glycine molecule and two bare alkali-metal ions, and there is greater distance from the real biological surroundings. Because of the indispensability of the water

solution, a hydrated system would be more similar to the real cases. However, how would the different number of water molecules influence the three different glycine- $\text{H}^+\text{M}^+$  complexes, and which pathways would be best for the interactions between 1–10 water molecules and these different glycine- $\text{H}^+\text{M}^+$  complexes? Most importantly, do the reservation energy bonds (REBs) still exist under these different hydrated surroundings, and, if so, what are the results? To probe these interesting questions, a series of multihydrated ( $n_{\text{H}_2\text{O}} = 1-10$ ) glycine- $\text{H}^+\text{M}^+$  ( $\text{M} = \text{Li}, \text{Na}, \text{or K}$ ) clusters in the gas phase are investigated. The selection of the number of water molecules is based on how the first water shell, which is the most important environment to influence the REB and other properties of its covering complex, can be formed. Using systematic study, we expect to provide some helpful information in regard to understanding and revealing the mystery of life growth.

## 2. Calculation Schemes

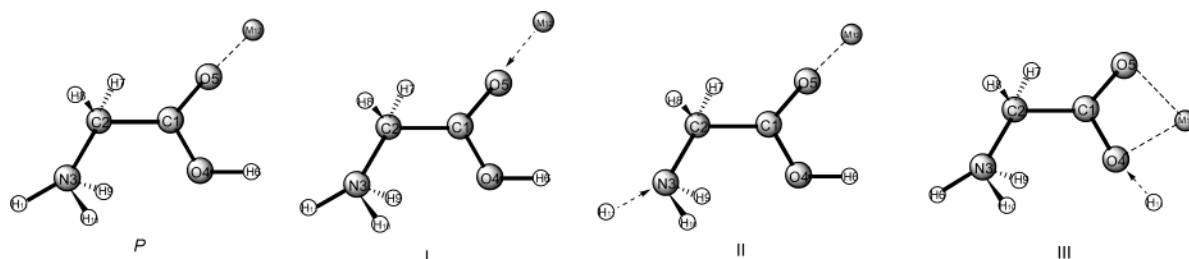
The starting structures for the monohydrated glycine- $\text{H}^+\text{M}^+$  are generated using one  $\text{H}_2\text{O}$  molecule to attack the C1, C2, N3, O4, and O5 sites and the metal-ion end of the most-stable glycine- $\text{H}^+\text{M}^+$  complex<sup>17</sup> (see product P in Figure 2). The ground-state monohydrate then can be obtained and taken as a model to bind the second water molecule, based on the principle that the second water molecule interacts with one site of the glycine- $\text{H}^+\text{M}^+$  directly, instead of with the first water molecule, to form the second shell. Similar schemes have also been used for the addition of the third, fourth, and up to tenth water molecule, so that the optimal affinity pathway (the minimum-energy pathway) can be obtained for these water molecules. Note that some hydrates of the glycine- $\text{H}^+\text{Li}^+$  complex adopt the optimal combination mode of their glycine- $\text{H}^+\text{Na}^+$  counterparts, because of their similarity in structure.

For all these reactions of glycine- $\text{H}^+\text{M}^+ + n\text{H}_2\text{O} \rightarrow \text{products}$  (for  $n = 1-10$ ), the geometrical optimizations, frequency calculations, and zero-point vibrational energy (ZPVE) corrections are performed at the 6-31G\* basis set (basis1) level, and then the single-point energy calculations (relative energies, dissociation electronic energies, dissociation free energies, etc.) with the 6-311+G\* basis set (basis2) are made at the basis1-optimized geometry. The basis set superposition error (BSSE) correction<sup>18</sup> has been used with basis2 to estimate the error levels for all the binding energies. Hybrid density functional theory (DFT/B3LYP) calculations<sup>19</sup> on these selected structures are performed thoroughly, because the hybrid methods of DFT can give very similar structural parameters, compared to those from MP2, and the density functional vibrational frequencies and intensities are in excellent agreement with the experimental data for glycine and other amino acid systems<sup>20</sup> and also because of its successful treatments on the similar systems.<sup>14,21</sup>

Both the dissociation electronic energy and the dissociation free energy (REB energies)  $E_B$  of the  $(\text{H}_2\text{O})_{n-x}\cdot\text{glycine}-\text{H}^+\dots\text{M}^+(\text{H}_2\text{O})_x$  (or rewritten as glycine- $\text{H}^+\text{M}^+(\text{H}_2\text{O})_n$ ) (for  $x = 0-4$ ,  $x < n$ ) complex in a given coordination geometry is defined as

$$E_B = E_c - E_g - E_M \quad (1)$$

where  $E_g$ ,  $E_M$ , and  $E_c$  denote the energies of the optimized  $(\text{H}_2\text{O})_{n-x}\cdot\text{glycine}-\text{H}^+$  species, the  $\text{M}^+(\text{H}_2\text{O})_x$  species (or two reactants), and the corresponding  $(\text{H}_2\text{O})_{n-x}\cdot\text{glycine}-\text{H}^+\dots\text{M}^+(\text{H}_2\text{O})_x$  complex, respectively. The electronic affinity energy ( $E_A$ ) of the last attached water molecule in a glycine- $\text{H}^+\text{M}^+(\text{H}_2\text{O})_n$



**Figure 2.** Three different attacking modes (I, II, and III) for two ions  $\text{H}11^+$  and  $\text{M}12^+$  ( $\text{M} = \text{Li}, \text{Na}, \text{or K}$ ) to form product P.

complex is obtained using the following formula:

$$E_A = E_c - E_g - E_W \quad (2)$$

where  $E_g$  and  $E_W$  denote the energies of the optimized glycine- $\text{H}^+\text{M}^+(\text{H}_2\text{O})_{n-1}$  species and the last attached water molecule, respectively.

All calculations have been performed with the GAUSSIAN 98 package of program Version A.9,<sup>22</sup> using Origin 300 and Pentium IV/2.4 GHz computers.

### 3. Results and Discussions

**3.1. Bound Ordering of Two Ions ( $\text{H}^+$  and  $\text{M}^+$ ).** For the two ions  $\text{H}^+$  and  $\text{M}^+$ , one should be preferred for binding at the active site of the glycine- $\text{M}^+(\text{H}^+)$  to yield the product (P in Figure 2), because of the possible different activation energy barriers ( $E_a$ ) in the reaction process (see Figure 1). Although the dissociation free energies, dissociation free-energy barrier, binding free-energy barrier, etc. may be more important in a biological system, the electronic results, except those specified, are used in the following discussions, because of its more theoretical and experimental references. The binding energy (i.e.,  $-E_b$ ) and  $E_a$  for the reaction i (which is denoted as glycine- $\text{H}^+ + \text{Na}^+ \rightarrow$  product) are  $-38.6$  and  $39.4$  kcal/mol, respectively (Figure 1, curve a), whereas the binding energy for reaction ii (which is denoted as glycine- $\text{Na}^+ + \text{H}^+ \rightarrow$  product) is  $150.8$  kcal/mol and no energy barrier is observed in the reaction process (see Figure 1, curve b) that is obtained at the B3P86/6-31+G\*\*/B3P86/6-31G\* level.<sup>17a</sup> Moreover, it was reported that the binding energies between the free glycine and  $\text{M}^+$  were positive,<sup>11g,13b</sup> which indicated that the binding of the carboxyl oxygen of a free glycine and a metal ion was easy. Optimized results have shown that there also was no energy barrier for the interaction between the proton ( $\text{H}11$ ) and the amino nitrogen site of glycine- $\text{M}^+$ , although the binding strength has decreased somewhat in value ( $141.8$  kcal/mol for  $\text{H}^+ +$  glycine- $\text{Li}^+$  and  $150.8$  kcal/mol for  $\text{H}^+ +$  glycine- $\text{Na}^+$ ),<sup>17a</sup> relative to the binding strength between the proton and the nitrogen site of the free glycine ( $211.1$  kcal/mol).<sup>11e</sup> Therefore, reaction ii is preferred and also easily proceeds in the system, because no extra energy needs to be input when a REB is generated. Experiments have confirmed that free  $\text{Na}^+$  and  $\text{K}^+$  ions could exist in the water solutions, although their monohydrated and dihydrated alkali-metal ion forms predominate the density ratio of water solution.<sup>15</sup> The dissociation energy barriers ( $E_d$ ) and bonding energy barriers ( $E_b$ ) have been calculated and are listed in Table 1 for reactions iii (where is denoted by glycine- $\text{H}^+ + \text{Na}^+ - \text{H}_2\text{O} \rightarrow$  glycine- $\text{H}^+\text{Na}^+ - \text{H}_2\text{O}$  ( $\text{Na}-\text{W}_1$ )) and iv (where is denoted by glycine- $\text{H}^+ + \text{Na}^+ - 2\text{H}_2\text{O} \rightarrow$  glycine- $\text{H}^+\text{Na}^+ - (\text{H}_2\text{O})_2$  ( $\text{Na}-2\text{W}_1$ )). The results of the same terms of reaction i are included in Table 1 so that a convenient comparison can be made. The table shows that the present reaction energy barrier for reaction i is consistent with that of ref 17a. The minor deviation stems only from the different levels

**TABLE 1: Dissociation Electronic Energy and Dissociation-Free Barriers ( $E_d$ ), and Binding Energy and Binding-Free-Energy Barriers ( $E_b$ ) of Three Selected  $\text{Na}^+$ -Involved Complexes Obtained at the B3LYP Level<sup>a</sup>**

complex	$E_d^a$ (kcal/mol)		$E_b^b$ (kcal/mol)	
	basis1	basis2	basis1	basis2
GHNa	3.6 (2.9)	2.1 (1.4)	38.7, 39.4 <sup>c</sup> (45.7)	29.1 (46.1)
Na-W <sub>1</sub>	2.2 (5.7)	2.9 (6.4)	36.4 (48.1)	29.0 (40.7)
Na-2W <sub>1</sub>	3.4 (2.3)	2.4 (1.3)	35.6 (47.8)	38.4 (50.6)

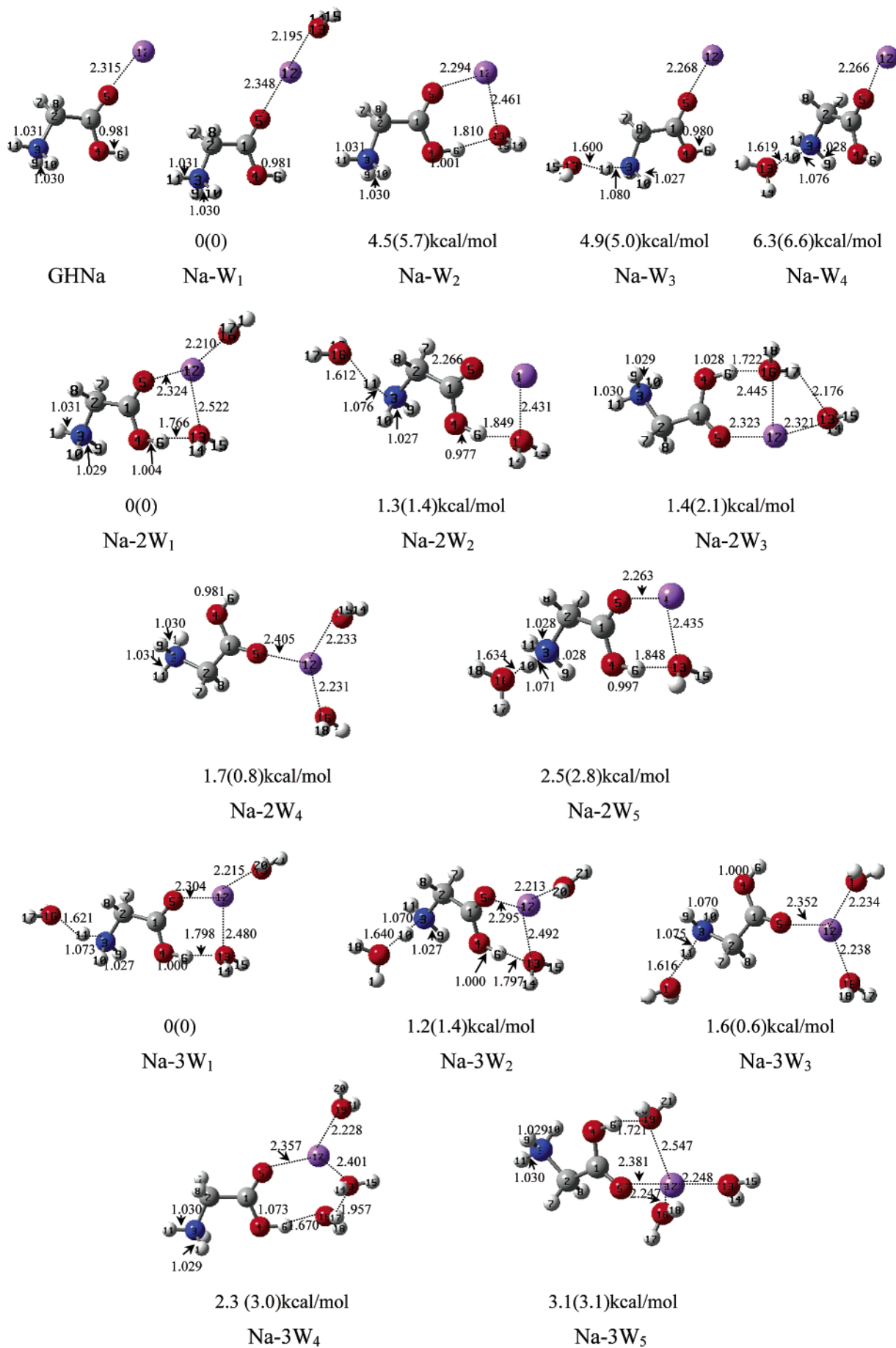
<sup>a</sup> Values given in parentheses represent dissociation-free barriers (in units of kcal/mol). <sup>b</sup> Values given in parentheses represent binding-free-energy barriers (in units of kcal/mol). <sup>c</sup> From ref 17a.

and basis sets that have been used. Results obtained with two different basis sets in the table reveal that all three complexes have lower dissociation energy barriers ( $2.1$ – $2.9$  kcal/mol). From the viewpoint of free energy, these values (except that of  $\text{Na}-\text{W}_1$ ) decrease further, which indicates that it is very easy for all of these REBs to release energy, without or with hydration effects, which is similar to the phenomenon observed in the life system. In contrast, the bonding processes have larger energy barriers for both reactions iii and iv, as reaction i does. According to the aforementioned observations, we have reason to believe that monohydrated or dihydrated alkali-metal ions ( $\text{Li}^+$ ,  $\text{Na}^+$ , or  $\text{K}^+$ ) first react with the carboxyl oxygen of the free glycine readily and then the proton attachment follows at the amino group without any activation energy barriers, just as reaction ii does. The reaction modes of reactions i and ii can also be simplified as I and II, respectively, in Figure 2. Another probable mode, which can also yield the product, is mode III in Figure 2. However, an obvious barrier to opening the four-membered ring indicates that the reaction pathway to form a REB also is difficult, although the zwitterionic glycine dominates in water solution. Thus, only reaction ii is preferred.

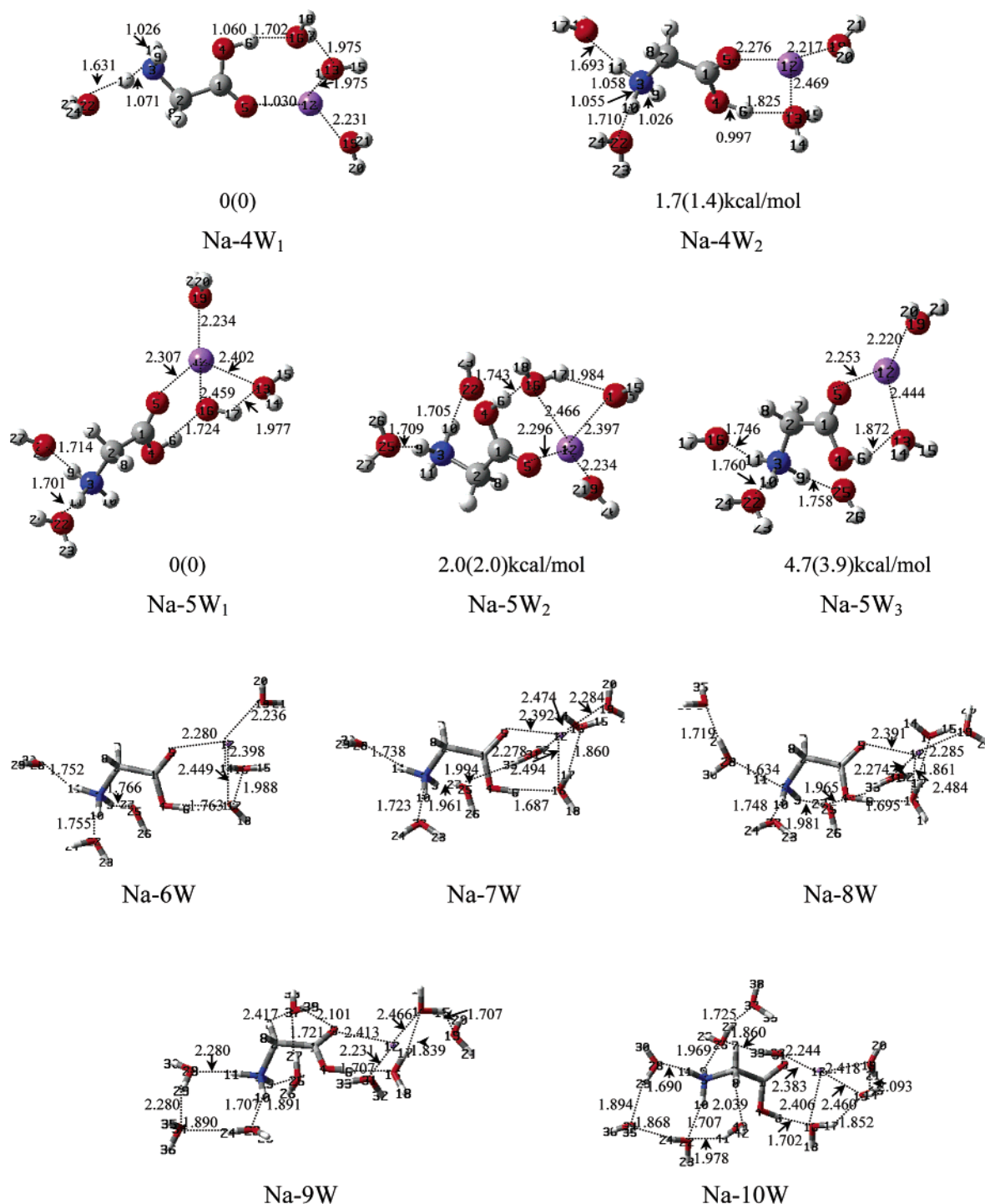
For the multihydration effects in these systems, the aforementioned conclusion of reaction ii should also be suitable, because the  $\text{O}5-\text{M}^+$  REBs still exist in these multihydrated glycine- $\text{H}^+\text{Na}^+(\text{H}_2\text{O})_n$  products, which will be discussed in detail in the following sections.

### 3.2. Series of Hydrated Glycine- $\text{H}^+\text{Na}^+$ Complexes.

Figure 3 displays the geometries of the series of hydrated glycine- $\text{H}^+\text{Na}^+$  (GHNa) complexes, i.e., monohydrated, dihydrated, up to decahydrated complexes. In Figure 3, ball-and-stick structures are used to represent monohydrated to pentahydrated ( $n = 1$ – $5$ ) complexes and tube structures are used to represent hexahydrated to decahydrated ( $n = 6$ – $10$ ) complexes, so that legible parameter descriptions can be given. The relative energy of each of the hydrate isomers is also listed under its corresponding geometry in the figure, which are obtained at the B3LYP/basis2//B3LYP/basis1 level without and with ZPVE corrections (given in parentheses in the figure) from the B3LYP/basis1 frequency calculations. Because  $\text{Na}^+$  is between  $\text{Li}^+$  and  $\text{K}^+$  on the periodic table, and because it is the most familiar ion in the body, the complexes that involve the  $\text{Na}^+$  cation are







**Figure 3.** B3LYP/6-31G\*-optimized multihydrated complexes (glycine- $\text{H}^+\text{Na}^+-n\text{H}_2\text{O}$ , where  $n = 0-10$ ) with a reservation energy bond (REB). Values shown in the figure are distances, given in angstroms. Relative energies for the various isomers of these different hydrates are obtained at the B3LYP/basis2//B3LYP/basis1 level without and with ZPVE corrections (values with ZPVE correction are shown in parentheses).

discussed first. As a frame of reference, the unhydrated GHNa complex is also shown in Figure 3. The reserved energies of the O5- $\text{Na}^+$  bonds of these different complexes are shown in Table 2. To describe the calculation schemes conveniently, geometries of those hydrated alkali-metal ion clusters are drawn in Figure 4. The charge populations on the glycine- $\text{H}^+$  ligands (GH) and metal ions ( $\text{M}^+$ ) of these hydrates are shown in Figures 5 and 6, respectively. Figure 7 shows the change tendencies of dissociation energies and dissociation free energies with the number of attached water molecules.

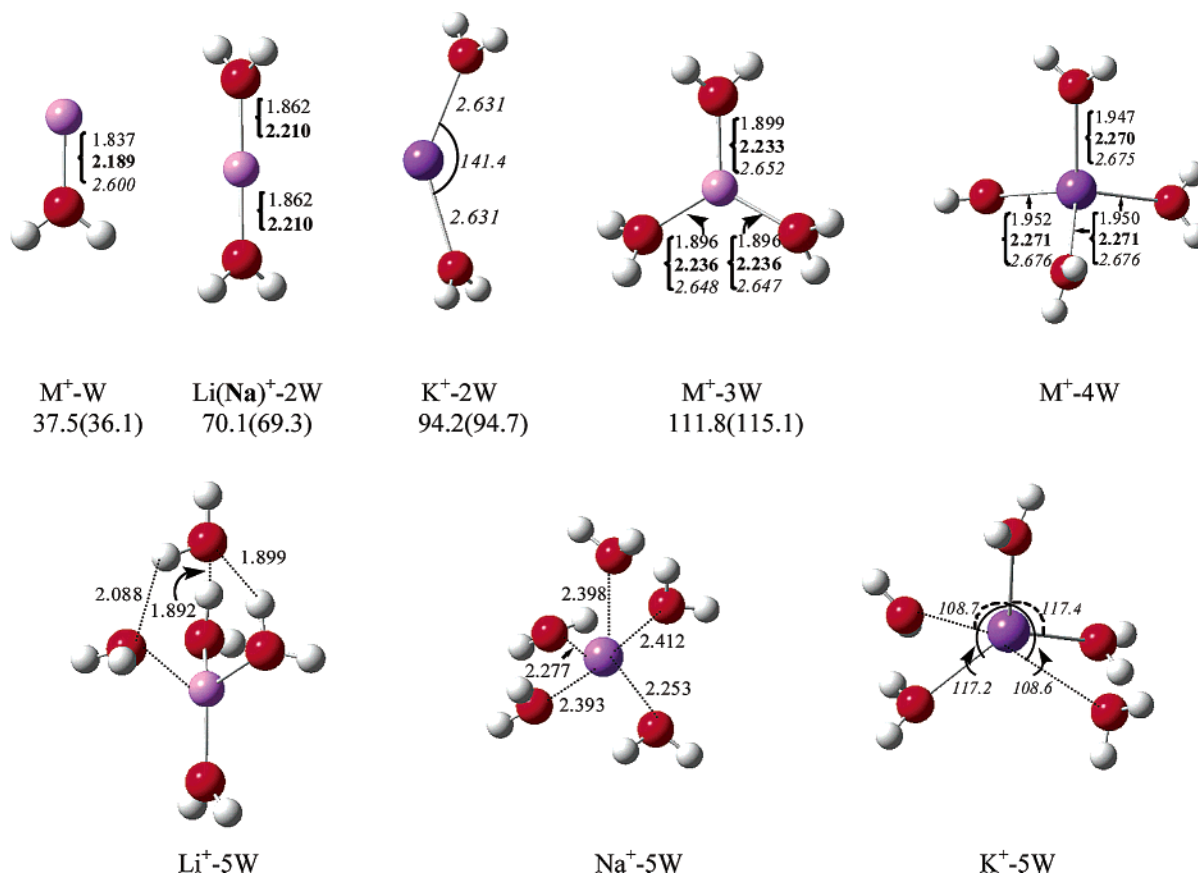
**3.2.1. Monohydrated Glycine- $\text{H}^+\text{Na}^+$  ( $\text{Na}-\text{W}_1$ -4) Complexes.** Because of the possibilities for a water molecule to attach

to the GHNa ligand from different directions, various hydrates can be obtained. The optimized results show that  $\text{Na}-\text{W}_1$  is the most stable, in regard to energy; in this hydrate form, the water molecule is bound at the metal-ion end, with a ligand-water distance of 2.195 Å. The hydration makes the O5- $\text{Na}^+$  bond become elongated by 0.033 Å, relative to that of the nonhydrated GHNa compound. For the  $\text{Na}^+-\text{O5}$  bond in GHNa, the calculated energy results with basis1 and basis2 are 35.0 and 37.1 kcal/mol, respectively, which is in good agreement with that from ref 17a (38.6 kcal/mol obtained at the B3P86 level). The hydration effect in  $\text{Na}-\text{W}_1$  makes its REB energy decrease by 1.0 kcal/mol at the B3LYP/basis2 level. The

**TABLE 2: Energies of Reservation Bond Energy (B) and Affinity Energy (A) of the Last Affiliated Water in Each Na<sup>+</sup>-Involved Hydrate Obtained at the B3LYP/Basis1 Level and by Single-Point Calculation with B3LYP/Basis2 Level on the B3LYP/Basis1-Optimized Geometry<sup>a</sup>**

	GHNa	Na-W <sub>1</sub>	Na-W <sub>2</sub>	Na-W <sub>3</sub>	Na-W <sub>4</sub>	Na-2W <sub>1</sub>	Na-2W <sub>2</sub>	Na-2W <sub>3</sub>	Na-2W <sub>4</sub>	Na-2W <sub>5</sub>
B <sub>basis1</sub>	35.0 (42.8)	34.2 (42.4)	34.3	28.9	29.6	25.4 (35.7)	18.2	26.6	35.0	19.6
B <sub>basis2</sub>	37.1 (44.9)	36.1 (47.9)	37.9	31.6	32.2	29.2 (39.5)	22.3	30.6	37.7	23.5
A <sub>basis1</sub>		-31.9	-31.8	-29.0	-28.1	-30.2				
A <sub>basis2</sub>		-29.4	-27.6	-27.2	-26.3	-27.6				
	Na-3W <sub>1</sub>	Na-3W <sub>2</sub>	Na-3W <sub>3</sub>	Na-3W <sub>4</sub>	Na-3W <sub>5</sub>	Na-4W <sub>1</sub>	Na-4W <sub>2</sub>			
B <sub>basis1</sub>	27.7 (39.1)	28.7	30.2	29.7	30.5	26.1 (40.5)	24.8			
B <sub>basis2</sub>	32.0 (43.4)	33.0	33.5	33.8	34.6	30.5 (44.9)	29.4			
A <sub>basis1</sub>	-27.3					-26.4	-22.2			
A <sub>basis2</sub>	-25.8					-25.0	-21.0			
	Na-5W <sub>1</sub>	Na-5W <sub>2</sub>	Na-5W <sub>3</sub>	Na-6W	Na-7W	Na-8W	Na-9W	Na-10W		
B <sub>basis1</sub>	23.7 (40.4)	25.0	22.6	22.2 (38.1)	23.0 (42.5)	20.8 (40.6)	18.2 (40.2)	24.5 (43.1)		
B <sub>basis2</sub>	28.4 (45.1)	29.5	27.3	27.0 (42.9)	28.6 (48.1)	25.3 (45.1)	24.7 (46.7)	27.9 (46.5)		
A <sub>basis1</sub>	-21.7	-19.8	-18.5	-17.8	-18.7	-17.4	-23.2	-11.6		
A <sub>basis2</sub>	-20.5	-18.5	-17.5	-16.9	-15.7	-16.7	-18.2	-10.5		

<sup>a</sup> Values given in parentheses are dissociation free energies of the most-stable isomer of each hydrated series. Results given in italic are from the ground state of each of the corresponding hydrates.



**Figure 4.** Optimized geometries for the monohydrated, dihydrated, trihydrated, and quadhydrated alkali-metal (M = Li, Na, or K) ion clusters (W represents water). Parameters in normal typeface are those for hydrated-Li<sup>+</sup> clusters, whereas parameters in boldface type are those for hydrated-Na<sup>+</sup> clusters and parameters in italic typeface are those for hydrated-K<sup>+</sup> clusters. Distances are given in angstroms and angles are given in degrees. Calculated binding energies (in units of kcal/mol) for the Li ion clusters (Li(H<sub>2</sub>O)<sub>n</sub>) ( $-E_1(n) = E[\text{Li}((\text{H}_2\text{O})_n)] - E[\text{Li}] - nE[\text{H}_2\text{O}]$ ) are given under the corresponding complexes. Energies shown without parentheses were obtained by the B3LYP/6-311+G\*\*/B3LYP/6-31G\* level in the present paper, and energies given within parentheses were obtained by the MP2/6-31+G\*\*//HF/6-31+G\*\* level from ref 23.

following discussions will also use those results obtained at the latter level, because of its greater reliability. The affinity energy

of the water is -29.4 kcal/mol. Different from Na-W<sub>1</sub>, two complexes (Na-W<sub>2</sub> and Na-W<sub>3</sub>) are obtained when the water

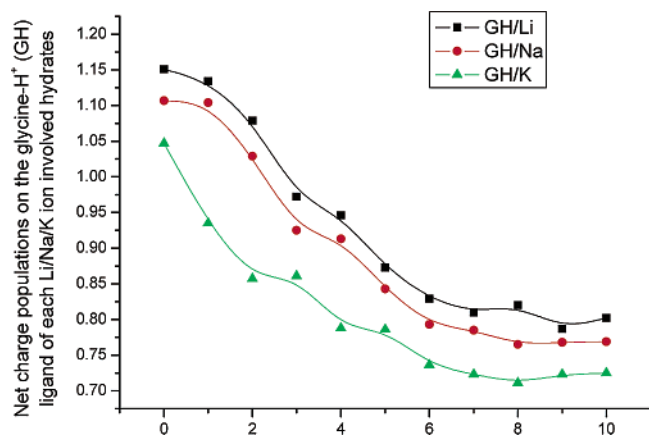


Figure 5. Plot of net charge population on the GH ligand versus the number of attached water molecules.

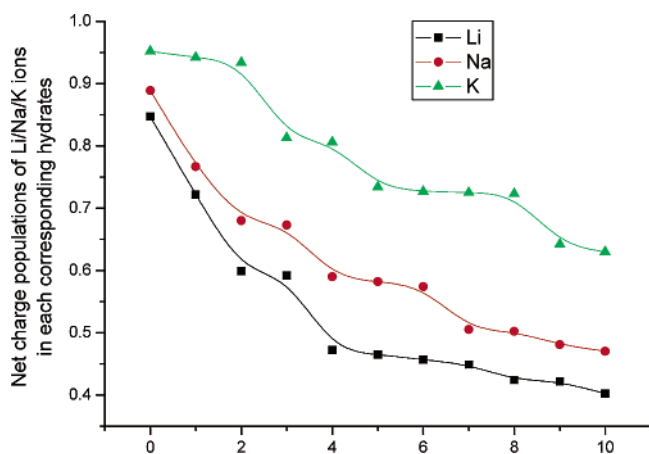


Figure 6. Plot of net charge population on the metal ion versus the number of attached water molecules.

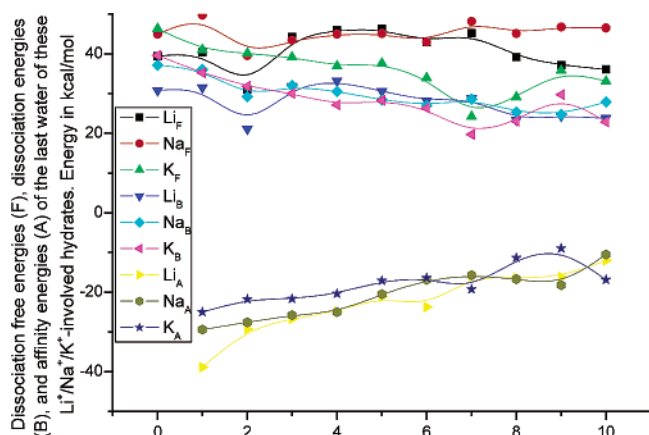


Figure 7. Plot of the dissociation energies and affinity energies versus the number of attached water molecules.

molecule attacks the ligand from its H6(O4) and H11(N3) sites, respectively. For the former, the water molecule interacts not only with the metal end of the ligand but also with the hydroxyl hydrogen to form a hydrogen bond. Calculations show that their energies are 4.5 and 4.9 kcal/mol, respectively, greater than that of Na-W<sub>1</sub>. However, the ZPVE-corrected results reveal that Na-W<sub>3</sub> seems more stable than Na-W<sub>2</sub>, with an energy difference of 0.7 kcal/mol, which indicates that ZPVE correction is indispensable for identifying their relative stabilities. The REB energies in the two complexes are 37.9 and 31.6 kcal/mol,

respectively. The energy of the former is larger and that of the latter is smaller than that of Na-W<sub>1</sub>, by 1.8 and 4.5 kcal/mol, respectively. Note that the REB energy (obtained by eq 1) of Na-W<sub>2</sub> is calculated by dividing the complex into a protonated glycine and a free Na-H<sub>2</sub>O complex (see Figure 4, M<sup>+</sup>-W), which would result in some deviations, because the hydrogen-bond effect between the water and H6(O4) is omitted arbitrarily. Including the hydrogen effects, a smaller REB energy (28.4 kcal/mol) of Na-W<sub>2</sub> can be obtained if only the metal ion is separated from the complex. The energy difference of REB obtained by two different dissociation modes stems from the hydrogen-bonding effect between H6 and the attached water. Taking into account the hydrogen-bond effect of Na-W<sub>2</sub> and relative REB distances in Na-W<sub>2</sub> and Na-W<sub>3</sub>, we estimate that the energy magnitudes in the two complexes should be similar. On the other hand, the case of Na-W<sub>2</sub> also indicates that the hydrogen bond can reduce the effect of the REB energy, to some extent. In comparison to Na-W<sub>3</sub>, the reservation energy behavior and affinity of the first water molecule in Na-W<sub>4</sub> only exhibit slight changes when the water molecule attaches to the ligand from the side-hydrogen site of the amino group (H10-(N3)). The differences are <1.0 kcal/mol both for the REB energies and water affinity of two modes. In general, the bonding strength in the Na-W<sub>4</sub> complex is weaker somewhat than that of Na-W<sub>3</sub>, which is consistent with their relative stabilities. Hydrates that have a water molecule attached to one of the two H(C2) sites are not observed.

Because the electronic effect predominates their bonding strength,<sup>17b</sup> analysis of the net charge populations over each species can help us understand these different bonding behaviors. Calculations show that the monohydration effect can alleviate the population of net charges over the GH ligand and the metal ion, consequently decreasing the electrostatic repulsion in the system. Therefore, the values of reservation energy also are decreased. The analysis of net charge populations also reveals that direct hydration of the metal-ion end can markedly decrease the charge populations of metal ion, whereas direct hydration at the amino group decreases the population less.

**3.2.2. Dihydrated Glycine- $\text{H}^+\text{Na}^+$  Complexes.** On the basis of the monohydrates, five dihydrates have been obtained by attaching the second water molecule to other potential active sites of monohydrated glycine- $\text{H}^+\text{Na}^+$  complexes. The most-stable dihydrate (Na-2W<sub>1</sub>) is yielded by the combination between the second water molecule and the metal-ion end of Na-W<sub>2</sub>; i.e., both the hydration at the metal-ion end and the hydrogen-bond effect at the H6(O4) site are included in the complex. Because of the existence of more isomers for each type of hydrate, only the most-stable isomer (such as Na-W<sub>1</sub>, Na-2W<sub>1</sub>, to Na-6W) in each series is discussed in detail in the following sections. The calculated affinity energy of the second water molecule in Na-2W<sub>1</sub> is -27.6 kcal/mol, which is 1.8 kcal/mol less than that of the monohydrated compound (Na-W<sub>1</sub>). Meanwhile, its REB energy is reduced by 6.9 kcal/mol, because some of the charges on the metal ion transfer to the second attached water molecules (see Figure 6).

For the isomers of these dihydrates, the next-most-stable dihydrate (Na-2W<sub>2</sub>) can be regarded as the combination of hydrations in Na-W<sub>2</sub> and Na-W<sub>3</sub>. The energy increase of 1.4 kcal/mol for Na-2W<sub>2</sub> over Na-2W<sub>1</sub> indicates that the metal site is still preferred for the affinity of the second water molecule. For its REB, the demarcation and calculation undergo some difficulties, i.e., if the calculations should consider the continuous but relatively distant water molecules. The cases are different for only the metal ion or the monohydrated metal

ion being separated from the corresponding complex in the treatment of REB energy calculations, as shown for Na–W<sub>2</sub>. Both cases seem to cover some rationality. For the sake of convenience of comparison, both cases are applied in the following calculations according to the idiographic combination mode and will be specified individually. As done for Na–W<sub>2</sub>, the REB energy of Na–2W<sub>2</sub> is 22.3 kcal/mol, obtained by only separating the metal ion from the dihydrate. A similar hydration effect on the REB is observed for the second water attacking the amino hydrogen or the metal ion, as evidenced by the parallel differences of dissociation energies between Na–2W<sub>1</sub> and Na–W<sub>1</sub>, and between Na–2W<sub>2</sub> and Na–W<sub>2</sub> (6.9 kcal/mol versus 6.1 kcal/mol). However, the differences of net charge over the respective metal ions in the two complex pairs are 0.087 e versus 0.008 e, which are 10 times different from each other. The charge difference (0.087 e) between respective metal ions in the most-stable monohydrate (Na–W<sub>1</sub>) and dihydrate (Na–2W<sub>1</sub>) is greater than that of other counterpart pairs, such as the charge difference of the Na–W<sub>2</sub> and Na–2W<sub>2</sub> pairs (0.008 e). These indicate that the greater decrease of net charge population of the metal ion favors the stability of the corresponding complex. The third-most-stable dihydrate is Na–2W<sub>3</sub>, in which the second water molecule interacts with the metal-ion end and the first water molecule of the Na–W<sub>2</sub>, respectively, through the electrostatic effect and hydrogen-bond effect, and the electrostatic-interaction and hydrogen-bond distances are 2.321 and 2.176 Å, respectively. The fourth-most-stable complex (Na–2W<sub>4</sub>) has a structure in which both water molecules are bound to the metal ion of GHNa with an O16–Na–O13 angle of 124.5°. Its REB distance (2.405 Å) is the longest among its isomers. The calculated dissociation energy is also the largest (37.7 kcal/mol, obtained by separating the dihydrated metal ion from the complex; see Na<sup>+</sup>–2W in Figure 4), which indicates that the structure is the best candidate among the dihydrates for the reservation energy, also because of its relatively strong stability (its energy is only 0.8 kcal/mol greater than that of the most-stable dihydrate if the ZPVE correction is included). The fifth-most-stable dihydrate is Na–2W<sub>5</sub>, which has a geometry that is comparable to that of Na–2W<sub>2</sub>. The structural difference between them is similar to that between Na–W<sub>3</sub> and Na–W<sub>4</sub>. The energy calculations for the REBs of Na–2W<sub>3</sub> and Na–2W<sub>5</sub> adopt the methodology of dissociating the monohydrated metal ion and free ion from each of the corresponding complexes, respectively. In contrast to the energy magnitude of these REBs, we can observe that the dihydrated Na–2W<sub>4</sub> mode has the greatest energy among GHNa and its all monohydrates and dihydrates.

**3.2.3. Trihydrated Glycine–H<sup>+</sup>Na<sup>+</sup> Complexes.** Also, on the basis of the geometries of these dihydrates, five trihydrated GHNa complexes are obtained. For the first three most-stable complexes, the REB energies are obtained by removing the dihydrated metal ion from their corresponding complexes according to the need of convenient comparison between the first two complexes and the geometric character of the third one. The energies for the last two complexes are obtained by two ways: (i) by removing the dihydrated metal ion and (ii) by removing the trihydrated metal ion (see Figure 4, Na<sup>+</sup>–2W and M<sup>+</sup>–3W, respectively), to observe if the trihydration effect is as strong as the monohydration or dihydration effects on the REBs.

The relative energies displayed in Figure 3 show Na–3W<sub>1</sub> to be the ground-state trihydrate among its five isomers. There are two choices for the affinity of the third water molecule to form Na–3W<sub>1</sub>: one is the H atom of amino nitrogen and

another is the metal-ion end of Na–2W<sub>1</sub>. Results reveal that the H11(N3) site is preferred. The next optimal candidate is the side-hydrogen site of the amino group of Na–2W<sub>1</sub>, in which the third water molecule can be attached and then the second-most-stable isomer (Na–3W<sub>2</sub>) is produced. The energy of the newly produced trihydrate is only 1.2 kcal/mol higher than that of the ground-state trihydrate. The third-most-stable complex stems from the attachment of the third water molecule at the H11(N3) site of the Na–2W<sub>4</sub> complex. Interestingly, the energy of the trihydrate is 1.6 kcal/mol greater than that of the ground state but is only 0.6 kcal/mol higher than the ground-state trihydrate if the ZPVE correction is included, similar to the behavior exhibited with its corresponding dihydrate. For these three similar isomers, in regard to both energy and structure (e.g., two water molecules are attached at the metal ion), their REB calculations can be done in the same manner. Therefore, REBs in these complexes should be the most comparable. Table 2 shows that their REB energies increase as their stability decreases, which is in agreement with the decreasing charge populations on their corresponding metal ions. The stability of Na–3W<sub>4</sub> and Na–3W<sub>5</sub> lying in the fourth and fifth positions among their isomers reveals that it is disadvantageous for the stability of the system if the charges over the metal ions are distributed excessively. The REB energies obtained by way of reaction ii of the two complexes are similar to those of the first three most-stable isomers. A decrease of ~2.0 kcal/mol, obtained by way of reaction i, for both of the complexes reveals that the hydration effect from a water molecule attaching at the H6-(O4) site on the REB of the corresponding complex has been weakened.

**3.2.4. Tetrahydrate Glycine–H<sup>+</sup>Na<sup>+</sup> Complexes.** Using the changing trend of stability and the corresponding geometries considered for trihydrates as a reference, only two tetrahydrates (Na–4W<sub>1</sub> and Na–4W<sub>2</sub>) are optimized, including the ground state. Calculated results reveal that the fourth water molecule prefers binding the metal ion to the amino hydrogen of Na–3W<sub>1</sub>, as evidenced by the fact that the energy difference between Na–4W<sub>1</sub> and Na–4W<sub>2</sub> is only 1.4 kcal/mol. The Na–4W<sub>1</sub> complex is a metal-ion-site-hydrated Na–3W<sub>1</sub>, whereas the Na–4W<sub>2</sub> complex is the amino-hydrogen-hydrated compound. Their REB energies are 30.5 and 29.4 kcal/mol, respectively, obtained by removing the trihydrated and dihydrated metal clusters (see M<sup>+</sup>–3W and Na<sup>+</sup>–2W, respectively, in Figure 4) from each corresponding tetrahydrate. The results are almost parallel to those of the Na–(2–3)W<sub>1</sub> complexes.

**3.2.5. Pentahydrated Glycine–H<sup>+</sup>Na<sup>+</sup> Complexes.** Further hydration is possible at the remaining active sites of those tetrahydrated complexes; the three most-stable candidates are displayed in Figure 3. Na–5W<sub>1</sub> is the most stable in energy; it is obtained by the attachment of the fifth water molecule at the side-hydrogen site of the amino group of Na–4W<sub>1</sub>. Different from Na–5W<sub>1</sub>, slight geometric changes for the second-most-stable Na–5W<sub>2</sub> complex lie in that two water molecules are attached at two side-hydrogen sites of the amino group. The geometric distinctions result in its 2.0 kcal/mol promotion in energy, relative to the most-stable isomer. The least-stable complex (Na–5W<sub>3</sub>) is obtained by binding the fifth water molecule at the third amino-hydrogen site of the Na–4W<sub>2</sub> complex. The decreasing trend of affinity of the fifth water molecule in the three different complexes is consistent with their relative stability. Because of more similarities for Na–5W<sub>1</sub> and Na–5W<sub>2</sub>, both in geometry and energy, the REB energies of the two complexes obtained by removing the M<sup>+</sup>–3W species from each corresponding complex are compared. Results



indicate that two hydrations at both side-hydrogen sites favor the energy reservation of REB ( $\text{Na}-5\text{W}_2$ ) slightly, with an energy promotion of 1.1 kcal/mol, compared to that of  $\text{Na}-5\text{W}_1$ . According to the characters of the geometry of  $\text{Na}-5\text{W}_3$ , its REB energy is obtained by removing the  $\text{Na}^+-2\text{W}$  from the complex. Results show that, in regard to energy, there are also no changes in magnitude that are larger than those of its two isomers.

**3.2.6.  $(\text{H}_2\text{O})_6-(\text{H}_2\text{O})_{10}$ -Hydrated Glycine- $\text{H}^+\text{Na}^+$  Complexes.** After the five ground-state complexes ( $\text{Na}-(1-5)\text{W}_1$ ) are identified, we expect  $\text{Na}-6\text{W}$  to be the most-stable structure of the  $(\text{H}_2\text{O})_6$ -hydrated complexes, in which the sixth water molecule is attached at the third amino-hydrogen site of the  $\text{Na}-5\text{W}_1$ , which is the only promising position that can be bound directly by the latter water molecule. There are too many possibilities for the seventh water molecule to interact with the  $(\text{H}_2\text{O})_6$ -hydrate. Several attempts of attaching the seventh water molecule to the two H(C2) sites have failed. One of the  $(\text{H}_2\text{O})_7$ -hydrated complexes ( $\text{Na}-7\text{W}$ ) is displayed in Figure 3, in which the seventh water is attached to  $\text{Na}^+$ , with a contact distance of 2.278 Å, and also to the O25 site of an attached water molecule (with a distance of 1.994 Å) by its H atom.  $\text{Na}-8\text{W}$  is obtained by the interaction of the hydrogen bond of the eighth water molecule with the water attaching at the frontal-hydrogen site of the amino group of the  $\text{Na}-7\text{W}$  complex. The ninth attached water molecule interacts with the H(C2) weakly, because of the longer H7-O37 distance (2.417 Å) in  $\text{Na}-9\text{W}$ . The interaction between the water molecule and the  $\text{Na}-8\text{W}$  complex is mainly dependent on two hydrogen bonds between them, i.e., O5-H39 (2.101 Å) and O37-H27 (1.721 Å), which further confirms that the two H(C2) sites hardly bind with the water molecules around it. Our principle of additions of these water molecules is to make any water molecules interact with the GHNa ligand directly and form the first shell around it as best as possible. Therefore, the attack of the tenth water molecule is designed to occur at the opposite site of the ninth water molecule. As expected, the optimized geometry of  $\text{Na}-10\text{W}$  is obtained. In comparison with the ninth water molecule, the tenth water molecule added approaches the H(C2) site of the ligand somewhat, at a distance of 2.039 Å (H8-O40). However, we still prefer to regard the interaction between the water molecule and the ligand as the major contribution of the hydrogen-bond effect formed between its H41 and O32 sites of the fourth water, because of their shorter distance (1.978 Å). In fact, there has been a water molecule interacting outside the first shell in the last three multihydrated complexes ( $\text{Na}-8\text{W}$ ,  $\text{Na}-9\text{W}$ , and  $\text{Na}-10\text{W}$ ). The water just lies around the amino group, which indicates that the amino group has been saturated and cannot be bound directly by the fourth approaching water molecule. On the other hand, the carboxyl group ( $\text{Na}^+$ ) seems to have also been saturated by four water molecules and one O5- $\text{Na}$  bonding. Optimized hydrated  $\text{Na}^+$  clusters displayed in Figure 4 show that five water molecules are distributed over the surroundings of the  $\text{Na}^+$  species symmetrically, as evidenced by the five almost-equivalent  $\text{Na}^+-\text{O}$  distances (see Figure 4,  $\text{Na}^+-5\text{W}$ ). Therefore, the ninth and tenth attached water molecules in these multihydrates must interact with the metal ion indirectly. This is consistent with the observations of Wakisaka et al.,<sup>15</sup> that the  $\text{Na}^+$  species can form its hydrated clusters with less than six water molecules. Further hydration can continue and it would be more similar to a real biological system. However, our interest focuses on the REB behavior of these systems, which mainly stem from the electronic effects.<sup>17</sup> The charge populations of each composing species of  $\text{Na}-10\text{W}$

have offered us sufficient information to understand the relationship of electronic effect and REB. Calculations show that the tenth water molecule and the metal ion only have net charge populations of 0.008 and 0.470 e, respectively. These imply that further hydration would share the net charges of the metal ion less. Now the charge population of the metal ion (0.470 e) can approximately match that of neighboring other heavy atoms with positive charges, which indicates that the charge distributions among the heavy atoms of the complex have attained an equipoise. In other words, further hydration would affect the REB less, as evidenced by the smaller energy changes of the REBs in these different hydrates.

The REB energies for the five multihydrated complexes are 27.0, 28.6, 25.3, 24.7, and 27.9 kcal/mol, which show a relatively stable trend. These results are calculated by the removal of the tetrahydrated cluster of  $\text{Na}^+$  ions from these complexes, except for the  $\text{Na}-6\text{W}$  complex, which occurs by removal of the trihydrated cluster, because only three water molecules are attached at the metal ions of the latter. In comparison to the REBs in  $\text{Na}-6\text{W}$  and  $\text{Na}-7\text{W}$ , it can be observed that more hydration at the carboxyl group (including the  $\text{Na}^+$ ) would favor the energy promotion to some extent, but the promotion is not quite obvious. Further hydration trends to link the amino section and the carboxyl section, such as that in the cases for  $\text{Na}-9\text{W}$  and  $\text{Na}-10\text{W}$ . Calculated REB energies for these further hydration cases almost remain stable, which implies that the hydration of more than six water molecules would have less effect on the REBs. Moreover, the affinity energies of the last attached water molecule in these complexes also has a tendency to decrease, however, by a slow speed.

Now let us go back to Figures 5 and 6, in which the net charges of the GH ligand and  $\text{Na}^+$  species that constitute these complexes are drawn. Results show that the populations both on the glycine- $\text{H}^+$  and  $\text{Na}^+$  decrease as the addition of the attached water molecules increases. However, the decrease in tendency becomes inconspicuous when the ninth and tenth water molecules are affiliated. We can also observe the phenomenon by comparing the charge populations over those water molecules with same attachment order, such as 0.106 e versus 0.106 e and 0.117 e versus 0.116 e populations on the first and second attached water molecules in the two complexes  $\text{Na}-9\text{W}$  and  $\text{Na}-10\text{W}$ . Similarly, the populations are 0.056 e versus 0.053 e and 0.045 e versus 0.043 e, respectively, in the eighth and ninth attached water molecules of the two complexes. All these pairs have almost negligible distribution differences and the charge population of the last attached water molecule in  $\text{Na}-10\text{W}$  only shows a difference of 0.007 e. Therefore, these observations can also confirm that the reservation energy behavior of the REB would exist stably in a biological system when the hydration reaches some extent and does not decrease limitlessly as the number of surrounding water molecules increases. The initial hydration can greatly reduce and share the repulsion effect induced by  $\text{H}^{11+}$  and  $\text{Na}^+$  and favor the stability of the entire system, whereas a greater amount of hydration can keep the system stable.

### 3.3. Series of Hydrated Glycine- $\text{H}^+\text{Li}^+/\text{K}^+$ Complexes.

Using the GHNa series of hydrates as a reference, the hydrated counterparts of glycine- $\text{H}^+\text{Li}^+/\text{K}^+$  complexes are obtained. Their geometries and relative energies are displayed in Figures S1 and S2 of the Supporting Information, respectively. The corresponding REB energies and net charge populations of GH ligands and  $\text{Li}^+/\text{K}^+$  species of these hydrates are displayed in Tables S1 and S2 of the Supporting Information, and Figures 5 and 6, respectively. The change tendencies of their REB

energies, relative to the number of attached water molecules, are exhibited in Figure 7.

**3.3.1. Geometric Characteristics of the Multihydrated Glycine- $H^+Li^+/K^+$  Complexes.** According to the schemes used for the monohydrated GHNa complexes, four glycine- $H^+Li^+$  (GHLi) and four glycine- $H^+K^+$  (GHK) counterparts are obtained, respectively. In contrast to their differences in geometry, it can be observed that the distance of each REB has been greatly shortened, by  $\sim 0.305$ – $0.404$  Å, in these GHLi hydrates, because of the smaller radius and higher charge density of the  $Li^+$  ion, relative to the  $Na^+$  ion, whereas the REB distance in each GHK monohydrate has been elongated. Being compact, only the most-stable isomer of the  $Li^+$ - or  $K^+$ -involved monohydrate is discussed. Those monohydrates with higher energies and their relative energies are shown in the Supporting Information; data for each of their corresponding multihydrates are also given.

Relative energies show that the  $Li-W_1$  complex, which has a similar geometry to the  $Na-W_1$  complex, is also the most stable among its four isomers ( $Li-W_{1-4}$ ). For the monohydrated GHK complexes, their geometries present other behaviors. Optimizations reveal that the first two most-stable monohydrates are formed by the water molecule attaching to the frontal-hydrogen site (H11) and the side-hydrogen site (H9) of the amino group of the GHK complex (product P in Figure 2), respectively. Relative energy comparisons show that the frontal-hydrogen site is preferred. These behaviors are very different from those of their counterparts that involve the  $Li^+$  or  $Na^+$  ion. The difference of energies of the two most-stable monohydrates is 1.1 kcal/mol. These phenomena indicate that the GHK complex prefers hydration at its amino group, rather than at the carboxyl group.

Further hydrations show that the most-stable  $Li-2W_1$  complex also has a structure that is similar to that of its  $Na-2W_1$  counterpart, except that the former has more-compact structural parameters, relative to the latter. Taking into account the great distinctions in geometries presented in the monohydrated GHK complexes, more geometric probabilities may also be presented again in their dihydrates; therefore, seven combination modes are designed. Results show that the most-stable dihydrate is formed by attaching two water molecules at the frontal-hydrogen site H11(N3) and side-hydrogen site (H9 or H10) of the amino group of the GHK complex (see product P in Figure 2), respectively, with H11-W distances of 1.686 Å and H10-W distances of 1.699 Å. The structures of (3–6)-hydrated GHLi complexes are also similar to each of the corresponding GHNa counterparts. The minor distinctions mainly lie in that the former has a shorter binding distance.  $Li-7W$ , however, exhibits structures that the seventh water molecule is attached by two water molecules, which are binding at both H atoms of the amino group of the glycine. Although the addition of the seventh water molecule gives some measure of randomness, the same addition schemes used for the water in  $Na-7W$  lead to a completely different geometry; i.e., the water molecule binds to the metal-ion end and also links with an amino-coupled water molecule through a hydrogen bond. The phenomenon can be easily understood by analyzing the hydrated clusters yielded by the two different metal ions. Figure 4 shows that both tetrahydrated clusters of the  $Li^+$  and  $Na^+$  species seem similar to tetrahedron structures ( $M^+-4W$ ). However, their pentahydrated ion clusters assume two types of completely different structures: the fifth water molecule can also interact with the  $Na^+$  ion directly as the other four do in the  $Na^+-5W$  complex, whereas that in the  $Li^+-5W$  complex only links with the

tetrahydrate ( $Li^+-4W$ ) through three hydrogen bonds. This indicates that four water molecules are sufficient to form the first shell to surround a  $Li^+$  ion and the remaining water molecules only interact with the cluster in the second shell. The result is in good agreement with that of Hashimoto et al.,<sup>23</sup> and the calculated binding energies for these hydrated  $Li^+$  clusters also agree with those of Hashimoto et al. very well (see Figure 4), which imply the reliability of our results. This is why the seventh water molecule in the  $Li-7W$  cannot directly bind to the  $Li^+$  anymore and cannot bind as the seventh water molecule does in the  $Na-7W$  complex. In fact, the three complexes  $Li-8W$ ,  $Li-9W$ , and  $Li-10W$  also confirm the viewpoint, because no fourth water molecule interacts directly with the  $Li^+$  ion in these complexes. In these complexes, the  $Li^+$  ion binds to the carboxyl oxygen of the glycine and further links three surrounding water molecules by the electrostatic interaction, which also reach a state of saturation, just as  $Li^+-4W$  does. The addition of the eighth water molecule makes two respective water molecules, at the ends of the carboxyl group and the amino group of the cationized amino acid, which are linked by two hydrogen bonds (see  $Li-8W$  in Figure S1 in the Supporting Information). Optimized geometries for the  $Li-9W$  and  $Li-10W$  complexes show that the additions of the ninth and tenth water molecules all lie in the outer shell, i.e., they only interact with the inner water molecules (the first shell). The tenth water molecule in  $Li-10W$  binds the H8(C2) of the ligand, with a distance of 2.357 Å. However, the hydrogen-bond (2.015 Å) interaction between the water molecule and its neighboring water molecule should have a more important role. Therefore, it can be judged that H(C2) still hardly interacts with its surrounding water molecules. Although we cannot ensure that ten water molecules have formed a sufficient shell to cover the cationized amino acid (GHLi), calculations have shown that the net charge population on the tenth water molecule reaches a value of 0.007 e, which is a negligible value, which indicates that further hydration would hardly affect the REB, as discussed in the case of  $Na-10W$ .

For the series of multihydrated ( $n > 2$ ) GHK complexes, the most-stable trihydrate is  $K-3W_1$ , which has a structure with two water molecules attached at the moiety of the carboxyl group ( $K^+$ ), whereas only a single water molecule is attached at the moiety of the amino group. This ground-state structure is the first whose geometry matches that of its two counterparts, which involve the  $Li^+$  and  $K^+$  species, respectively. The most-stable tetrahydrated complex is  $K-4W_1$ , in which the fourth water molecule is bound to the side-hydrogen site of the amino group of  $K-3W_1$ . Based on the geometries of the tetrahydrated complexes, the most-stable pentahydrate ( $K-5W_1$ ) does not go beyond the range of the further hydration at the site of the remaining amino hydrogen or at the metal-ion end of  $K-4W_1$ . The relative energy shows that the metal-ion end is preferred for the fifth water molecule to attach. Because there is still a free amino hydrogen in the most-stable  $K-5W_1$  and the  $K^+$ -cationized glycine- $H^+$  hydrates prefer to be further hydrated at the amino hydrogens, the sixth water molecule is designed to couple the free amino hydrogen of  $K-5W_1$  so that the most-stable ( $H_2O$ )<sub>6</sub>-hydrate can be obtained. The seventh hydration is designed to occur at the sites of two H(C2) sites, as done for the  $Li-7W$  and  $Na-7W$  complexes, so that the first water shell can be formed. Similar to the case of  $Li-7W$ , the optimized geometry for  $K-7W$  shows that the seventh water molecule is attached by two water molecules at two amino-hydrogen sites of  $K-6W$  in two hydrogen-bond forms. However, their formation mechanisms are different. For  $K-7W$ , its metal ion still

possesses the ability to accept further hydration, although three water molecules have been attached. This can be observed from  $\text{K}^+-5\text{W}$  in Figure 4. The geometry shows that  $\text{K}^+-5\text{W}$  takes on the shape of a pentahedron and four side faces are almost equal. In other words, the distribution of water molecules around the  $\text{K}^+$  ion is highly asymmetric and further hydration from other directions would be very easy, because there are still larger free spaces for other water molecules to attach. Therefore, the seventh water molecule should be attached at the carboxyl moiety; however, the actual result reverses, because the  $\text{K}^+$ -cationized glycine- $\text{H}^+$  system prefers to hydrate at its amino moiety. Because the surroundings of the  $\text{Li}^+$  ion have reached the state of saturation, the seventh hydration occurs at the amino moiety in  $\text{Li}-7\text{W}$ , although the relative stability of those oligohydrated isomers has implied the carboxyl moiety is preferred to be hydrated in these complexes, as mentioned previously. The relative stability of those hydrated GHNa complexes has also shown that their carboxyl moieties are preferred to be hydrated. The  $\text{Na}^+$  ion is in the middle of the  $\text{Li}^+$  ion and the  $\text{K}^+$  ion on the periodic table; thus, there should be more than four water molecules that can interact with the ion directly. In fact, optimization has shown that all five water molecules can interact with it directly, as evidenced by the five almost-equivalent  $\text{Na}-\text{O}$  bonds (2.253–2.412 Å) (see  $\text{Na}^+-5\text{W}$  in Figure 4). Therefore, the seventh water molecule is attached at the carboxyl moiety ( $\text{Na}^+$ ) with a  $\text{Na}-\text{O}31$  distance of 2.278 Å in  $\text{Na}-7\text{W}$ . The eighth hydration also leans toward the amino moiety of the complex (see  $\text{K}-8\text{W}$  in Figure S2 of the Supporting Information), for the same reason as that of the seventh hydration ( $\text{K}-7\text{W}$ ). Because of the lesser charge density of the  $\text{K}^+$  ion<sup>15</sup> and longer bonding distance between the GHK complex and each attached water molecule, the ninth and tenth hydrations have a tendency to make the multihydrates become spherical (see  $\text{K}-9\text{W}$  and  $\text{K}-10\text{W}$  in Figure S2 in the Supporting Information), similar to the shapes of their counterparts,  $\text{Li}-9\text{W}$  and  $\text{Li}-10\text{W}$ , respectively. However, the formation of the latter two should be ascribed to the opposite effects. In other words, the higher ion charge density and shorter bonding distance can also make the amino moiety and the carboxyl moiety link if the number of attached water molecules is sufficient. In contrast to them, each of the multihydrated GHNa complexes has a long and narrow shape (see  $\text{Na}-8\text{W}$ ,  $\text{Na}-9\text{W}$ , and  $\text{Na}-10\text{W}$  in Figure 3). Note that all the subsequent water molecules (after the seventh hydration) link with the glycine- $\text{H}^+\text{M}^+$  ligand, mainly by interacting with H atoms of the first water shell. Interestingly, the latter water molecules, together with those of the amino and carboxyl moieties, almost surround the ligand to form a water ball, which is exactly our expectation. Therefore, in view of the computational conveniences and the great importance of the first shell for the property of complexes,<sup>15</sup> only those before  $(\text{H}_2\text{O})_{10}$ -hydrated complexes are utilized in the present paper.

**3.3.2. REB Characteristics of the Multihydrated Glycine- $\text{H}^+\text{Li}^+/\text{K}^+$  Complexes.** Comparison of the REB energy in  $\text{Li}-\text{W}_1$  (31.5 kcal/mol) obtained by the same calculation methods as that in the corresponding GHNa counterparts ( $\text{Na}-\text{W}_1$ ) (36.1 kcal/mol) shows that the effect of reservation energy of the  $\text{Li}^+$ -chelated complexes decreases, while the affinity strength of the water molecule increases (−38.9 kcal/mol versus −29.4 kcal/mol). The occurrence of these phenomena stems from the higher charge density of the  $\text{Li}^+$  ion than that of the  $\text{Na}^+$  ion, whereas the higher charge density makes the  $\text{Li}^+$  ions distribute more charges to the surrounding ligands, such as the attached water molecules, and consequently strengthens the interactions be-

tween each species (see Figures 5 and 6 in this work and Figure S1 in the Supporting Information). The REB value (35.2 kcal/mol) of the  $\text{K}-\text{W}_1$  complex, however, is obtained by removing the single ion from its corresponding complexes, because of its structural character.  $\text{K}-\text{W}_4$  (see Figure S2 in the Supporting Information), which has a similar structure to that of the  $\text{Na}-\text{W}_1$  and  $\text{Li}-\text{W}_1$  complexes, is used to probe the ionic effect. Its REB energy of 38.1 kcal/mol indicates that the reservation energy effect of these monohydrates increases as the augmentation of the ion radius increases, i.e.,  $E_B^K > E_B^{\text{Na}} > E_B^{\text{Li}}$ . On the other hand, the external factors, which can compete efficiently with the carboxyl oxygen to interact with these ions and weaken the  $\text{M}^+-\text{O}5$  bond, would favor the effect of reservation energy of the corresponding complex. The lesser value of the reservation energy of the  $\text{K}^+-\text{O}5$  bond in  $\text{K}-\text{W}_1$  than that in  $\text{K}-\text{W}_4$  confirms this observation, although the relative energy shows that  $\text{K}-\text{W}_1$  is the most-stable complex among its isomers. However, the competition effect should be limited to the range of keeping the REB stable, i.e., not breaking the bond.

For the five dihydrates, as shown in Figure S1 in the Supporting Information, the energies of their corresponding REBs are all obtained by removal of the monohydrated  $\text{Li}^+$  cluster (see  $\text{M}^+-\text{W}$  in Figure 4). Results show that a second hydration makes the effect of the reservation energy of the most-stable hydrate decrease by 10.4 kcal/mol, relative to that of the monohydrated  $\text{Li}-\text{W}_1$  complex. Correspondingly, the affinity strength of the second water molecule decreases, relative to the first water molecule. For example, the affinity of the second water molecule in  $\text{Li}-2\text{W}_1$  is −29.3 kcal/mol, which is 9.6 kcal/mol less than that of the first water molecule in  $\text{Li}-\text{W}_1$ . Similarly, calculations also show that the second hydration at the side-hydrogen sites of the amino group of  $\text{K}-\text{W}_1$  to form  $\text{K}-2\text{W}_1$  would greatly shorten the REBs, with a difference of 0.074 Å, and decrease the effect of reservation energy. These phenomena are consistent with their different net charge populations of the  $\text{M}^+$  ion (0.722 e for  $\text{Li}-\text{W}_1$  versus 0.600 e for  $\text{Li}-2\text{W}_1$ , and 0.942 e for  $\text{K}-\text{W}_1$  versus 0.934 e for  $\text{K}-2\text{W}_1$ ; see Figure 5). The decrease of electronic effect in  $\text{Li}/\text{K}-2\text{W}_1$  contributes to the decrease of effect of its reservation energy. Calculated energy results for the REBs in the multihydrated complexes indicate that the further hydration effect on the corresponding complex (GHLi) has become smaller and smaller as the number of attached water molecules has increased. For example, the REB energies in  $\text{Li}-3\text{W}_1$ ,  $\text{Li}-4\text{W}_1$ , and  $\text{Li}-5\text{W}_1$  are 31.8, 33.2, and 30.6 kcal/mol, respectively. Only some minor energy fluctuations are observed as the number of added water molecules increases. The REB calculations of  $\text{Li}-3\text{W}_1$  are dependent on the manner in which the dihydrated ion clusters (see  $\text{Li}^+-2\text{W}$  in Figure 4) are removed, whereas the calculations for the remaining complexes adopt the one of the trihydrated ion clusters that are being removed ( $\text{M}^+-3\text{W}$ ), because only three water molecules are attached at the end of the metal ion in all these remaining complexes. The results reveal that the energy range is within 28.8–23.4 kcal/mol, only with a smaller decrease in charge. The net charge of the  $\text{Li}^+$  ion in the most-stable pentahydrate ( $\text{Li}-5\text{W}_1$ ) decreases by 0.262 e, relative to that in the most-stable monohydrate ( $\text{Li}-\text{W}_1$ ), and the corresponding decrease in REB energy is 10.4 kcal/mol. In contrast, the net charge of the  $\text{Li}^+$  ion in the  $(\text{H}_2\text{O})_{10}$ -hydrates ( $\text{Li}-10\text{W}$ ) decreases by only 0.061e, compared to that of the most-stable  $(\text{H}_2\text{O})_6$ -hydrate ( $\text{Li}-6\text{W}$ ), where the corresponding decrease in REB energy is 5.4 kcal/mol. The smallest REB energy in the multihydrated  $\text{Li}-8\text{W}$  is 23.4 kcal/mol, which is only 8.1 kcal/mol less than that in the most-stable monohydrated complex



(Li–W<sub>1</sub>). Thus, it can be concluded that the REB that involves a Li<sup>+</sup> ion could exist in the real solution surroundings, as well as in a real biological system. Further hydration would hardly decrease their reservation energy effects.

The REB energy in K–3W<sub>1</sub> (30.0 kcal/mol) is obtained by removing a monohydrated ion complex. For the most-stable tetrahydrated complex (K–4W<sub>1</sub>) and its two isomers (K–4W<sub>2</sub> and K–4W<sub>3</sub>), their REB energies are 27.1, 28.5, and 30.8 kcal/mol, respectively, obtained by removing K<sup>+</sup>–W<sub>1</sub> from K–4W<sub>1</sub> and K–4W<sub>2</sub> and by removing K<sup>+</sup>–2W from K–4W<sub>3</sub>, respectively. The energy fluctuations of the three REBs are smaller. *These fluctuations may stem from their intrinsic difference, or from the different calculated schemes. Therefore, some indefinite factors exist to weigh their relative effects of reservation energy. However, the results are sufficient to reflect their general effects.* For four monohydrated complexes, the fluctuation is in the range of 34.6–38.1 kcal/mol. Similar ranges of the five dihydrated complexes and trihydrated complexes are, respectively, 31.8–34.9 and 30.0–34.9 kcal/mol. Lengthwise comparisons among the ground-state complexes in the four different hydrates can reflect the effect of hydration on the REBs intuitively. For example, the energy order of 39.6 kcal/mol (GHK) > 35.2 kcal/mol (K–W<sub>1</sub>) > 31.8 kcal/mol (K–2W<sub>1</sub>) > 30.0 kcal/mol (K–3W<sub>1</sub>) > 27.1 kcal/mol (K–4W<sub>1</sub>) indicates that further hydration can gradually decrease the effect of reservation energy of these K<sup>+</sup> complexes, as observed for the corresponding Li<sup>+</sup> and Na<sup>+</sup> complexes (see Figure 7). A similar pattern can also be observed for the affinity energies of the last attached water molecule, together with the net charge populations of both the GH ligand and the metal ion of these complexes. The calculated REB energy of K–5W<sub>1</sub> is 28.3 kcal/mol, which is greater than that of K–4W<sub>1</sub>, because of its fifth hydration at the metal-ion end. On the other hand, the REB energy of K–5W<sub>2</sub> (24.7 kcal/mol), which is 2.4 kcal/mol less than that of K–4W<sub>1</sub>, again reveals that further hydration at the hydrogen sites of the amino group would disfavor the effect of the reservation energy of the complex. Note that the same schemes of calculating the REB energies of K–4W<sub>3</sub> and K–4W<sub>1</sub> have again been used to calculate the REB energies of K–5W<sub>1</sub> and K–5W<sub>2</sub>, respectively.

For these multihydrated ( $n > 5$ ) complexes that involve the K<sup>+</sup> ion, the calculations for their REB energies apply the same dissociation schemes arbitrarily, i.e., the removal of a dihydrated K<sup>+</sup> cluster (see K<sup>+</sup>–2W in Figure 4). This phenomenon is because the distance between the ion and the third-closest attached water molecule is similar to, and even larger than, 3.0 Å, which is much longer than the distances between the ion and the first two closest water molecules that would be removed together with the ion when the REB energy is calculated. Moreover, the effects of the calculation schemes of removing dihydrated or trihydrated ion clusters on the REB energies have become weaker and weaker as the addition of hydrated water molecules increased. For example, the REB energies of K–9W are 29.7 and 29.9 kcal/mol, obtained by removing dihydrated (K<sup>+</sup>–2W) and trihydrated (K<sup>+</sup>–3W) K<sup>+</sup> clusters, respectively, from the parental complex (K–9W). For K–6W to K–10W, the REB energies and affinity energies of each of their last attached water molecules, together with the net charge populations of each of their involved ions and the GH ligand, have a tendency to decrease as the addition of water molecules increases. However, the decreasing tendencies of the REB energies and affinity energies of the last water molecule have become milder and milder, and some undulations are present even after the seventh water molecule is attached. Thus, these

tendencies could be deemed to become invariable when the further hydration reaches a certain extent, as Figure 7 has shown. The tendency in net charge populations of the GH ligands becomes more obvious, whereas that in the net charge populations of metal ions becomes relatively poor but also has a tendency to be smooth. Therefore, the seventh hydration would be a boundary.

*3.3.3. Comparisons of a Series of Multihydrated GHLi/Na/K Complexes and Several Thoughts.* As previously described, Figures 5 and 6 exhibit the change in the net charge populations on the GH ligand and the metal ions, respectively, relative to the addition of water molecules. Figure 6 indicates that four water molecules can effectively reduce the charge density of the Li<sup>+</sup> ion. The subsequent portion of the curve has become smooth. All these observations indicate that a plentiful amount of charge from the Li<sup>+</sup> ion has been transferred to the surrounding attached water molecules. Different from the series of multihydrated GHLi complexes, those hydrated GHNa complexes transfer less ionic charge, relative to their GHLi counterparts, and the relationship curve becomes smooth only after the seventh hydration. The tendency is more obvious for their GHK counterparts. For example, a charge of 0.632 e is still distributed over the K<sup>+</sup> ion after the tenth hydration, which implies that the charge transfer of the K<sup>+</sup> ion to its surrounding water molecules is the least; this is simply consistent with the fact that, among the three ions (Na<sup>+</sup>, Li<sup>+</sup>, and K<sup>+</sup>), the K<sup>+</sup> ion has the smallest charge density.

The changes in the charge of the GH ligand show the opposite regularity, compared to their corresponding metal ions (see Figure 5). For example, the K<sup>+</sup> ion transfers fewer charges to the attached water molecules, relative to the Na<sup>+</sup> ion, whereas its corresponding GH ligand transfers more charge, compared to the GH ligand of the Na<sup>+</sup> ion. The figure shows that all these ligands in the three different hydrate systems would further import few charges to its surrounding water molecules after the sixth water molecule ((H<sub>2</sub>O)<sub>6</sub>-hydration) is bound. For the metal ion and the GH ligand (two charge donors in each hydration), the charge transfer to the attached water molecule is different. For example, the charges of water molecules in the monohydrate K–W<sub>1</sub> and dihydrate K–2W<sub>1</sub> are mainly from the contributions of the GH ligands (subsidiarily from the K<sup>+</sup> ion). Oppositely, the charges of the water molecule in the monohydrates Li–W<sub>1</sub> and Na–W<sub>1</sub> are mainly from the contribution of their corresponding metal ion (Li<sup>+</sup>/Na<sup>+</sup>), whereas the charges of the second attached water molecule in the dihydrates Li–2W<sub>1</sub> and Na–2W<sub>1</sub> are mainly from contributions from both the corresponding ligand GH and the metal ion (Li<sup>+</sup>/Na<sup>+</sup>). As observed from Figures 5 and 6, the charge distributions on the ligands GH of these multihydrates (after (H<sub>2</sub>O)<sub>6</sub>-hydration) tend toward invariableness. These results indicate that the charge populations of the ligands (GH) in these multihydrates have been reduced to the minima. As a result, the electronic repulsion between the GH ligand and the metal ion would greatly decrease as the addition of attached water molecules increased and, consequently, greatly favor the stability of the entire system.

Together with the decrease until finally attaining invariableness of the charge populations over the ligand GH and metal ion, the affinity strengths of later attached water molecules have also become weaker and weaker, as Figure 7 shows. Generally, the ordering of affinity strengths of these different ion hydrates is Li– $n$ W > Na– $n$ W > K– $n$ W ( $n = 1-10$  and W represents water), whereas the ordering of REB energies of these different ion hydrates reverses. Most important to us is the fact that the REB energies in the three different ion-involved multihydrates



generally remain constant after the seventh hydration, and most of these values are  $>20$  kcal/mol. The calculated corresponding hydration free energies are significantly larger than the electronic dissociation energies and are almost unchanged as the number of water molecules changes, further confirming that the hydration hardly affects the reservation energy of the three different systems. Therefore, a conclusion can be drawn a reservation energy behavior really does exist in these biologically relevant systems and the values should be  $>20$  kcal/mol if the REB involves an alkali-metal ion, judging from the change tendency of Figure 7.

The conclusion can easily extend to a real biological system by the following hypothesis:

(1) The water molecule in  $\text{K}-\text{W}_1$ , for example, can be regarded as being another amino acid molecule or a simple peptide, and then the complex is a real linear peptide molecule. Furthermore,  $\text{K}-10\text{W}$  can become a larger stereo peptide if other amino acids substitute for the water molecules to attach the  $\text{K}^+$  ends. Anyway, it can be believed that there are, indeed, REBs in real biological systems.

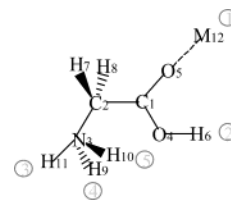
(2) No BSSE corrections are included in the aforementioned calculations of the binding energies, because they are minor and would not affect our conclusions qualitatively. For example, the BSSEs of REBs in  $\text{Na}-\text{W}_1$  and  $\text{Na}-2\text{W}_1$  are  $-0.48$  and  $-1.4$  kcal/mol, respectively, whereas that of the affinity energy of the water in  $\text{Na}-\text{W}_1$  is  $-2.31$  kcal/mol. Similarly, the BSSEs of the affinity energies of the first and second attached water molecules in  $\text{Na}-2\text{W}_1$  are  $-2.33$  and  $-2.82$  kcal/mol, respectively, obtained at the B3LYP/basis2 level, based on the B3LYP/basis1-optimized geometries. Therefore, it can be roughly predicted that the BSSEs for REBs and affinity energies of the attached water molecules in all these complexes should not go beyond the values of  $-2.0$  and  $-3.0$  kcal/mol, respectively.

## Conclusions

We have performed a comprehensive density functional theory (DFT) study of the hydration effect on the structures and reservation energy behaviors of the biologically relevant complexes glycine- $\text{H}^+\text{M}^+$  (where M is Li, Na, or K) in the gas phase. Results show that the formation (reservation energy) or break (release energy) of these reservation energy bonds (REBs) would suffer an energy barrier if the amino-nitrogen site is first protonated. In contrast, if the carboxyl oxygen is first cationized by a metal ion or its hydrate, and then the amino nitrogen is protonated, there would be no energy barrier to hinder the formation of the REBs in these complexes.

There are several active sites in the glycine- $\text{H}^+\text{M}^+$  complexes that can be hydrated. However, only those which meet the energy minimum of the corresponding systems are preferred. Calculations show that the hydration energy minimum pathways are different for the different ion-involved hydrated systems. The preference ordering, in turn, of these sites in both glycine- $\text{H}^+\text{Li}^+$  (GHLi) and glycine- $\text{H}^+\text{Na}^+$  (GHNa) complexes for the first to sixth water molecules to attach would be as follows: site 1  $>$  site 2  $>$  site 3  $>$  site 1  $>$  site 4  $>$  site 5. The following hydration positions would also be preferable to the carboxyl moiety for the two types of different complexes. However, the GHLi complex would choose its amino moiety as the following hydration sites (after sixth hydration), because its  $\text{Li}^+$  ion has been saturated by four interactions (three water molecules and the carboxyl oxygen of glycine). Different from the GHLi and GHNa complexes, the preferred order for the hydrated glycine- $\text{H}^+\text{K}^+$  (GHK) complex would be as follows: site 3  $>$  site 1  $>$

site 2  $>$  site 4  $>$  site 1  $>$  site 5. The subsequent preferential hydration sites would also be in the amino moiety. In fact, sites 1 and 4 are mutually competitive when the second water molecule is attached. Similar phenomena have also been observed for sites 2 and 4 in the process of the third hydration. Note that sites 4 and 5 are equivalent and can be exchangeable during the hydration processes.



Because the electronic effect predominates the binding (or dissociation) behaviors and the stability of these glycine- $\text{H}^+\text{M}^+$  complexes, hydration can effectively decrease the charge populations of these systems and thus favor their stability. Calculations show that the stepwise hydration can gradually transfer the charges of the glycine- $\text{H}^+\text{M}^+$  complex to its surrounding water molecules. The net charges distributed on the tenth attached water molecules are only 0.007 and 0.008 e, respectively, in the  $\text{Li}-10\text{W}$  and  $\text{Na}-10\text{W}$  hydrates. These indicate that further hydration (after the tenth hydration) would hardly affect these systems. Because of the larger ion radius and less charge density of the  $\text{K}^+$  ion, the net charge distribution on the tenth attached water molecule of  $\text{K}-10\text{W}$  (0.018 e) also reaches a minimum value. In fact, optimized geometries show that the subsequent water molecules (after the seventh hydration) link with the inner ligand mainly through the interaction with the water molecules directly attached to the glycine- $\text{H}^+\text{M}^+$  complex. The approximate invariableness of the net charge distributions confirms this viewpoint. The direct effects of stepwise hydration make the binding strengths between the attached water molecules and the glycine- $\text{H}^+\text{M}^+$  complex become smaller and smaller, whereas the binding strengths of the REBs become stronger and stronger with further hydration; consequently, the effects of reservation energy become gradually weaker. However, these tendencies are not limitless. Their variation curves become smooth and flat after the seventh hydration. Further hydration would hardly affect the reservation energy behaviors of these REBs. The calculated corresponding hydration free energies are significantly larger than the electronic dissociation energies and are almost unchanged, relative to the number of water molecules, further confirming that the hydration hardly affects the reservation energy of the three different systems, from the viewpoint of free energy. A conclusion can be drawn that (i) the REBs indeed exist in these biologically relevant systems and (ii) the reservation energy values in these systems would be  $>20$  kcal/mol. These energies would be indispensable sources for organism activity.

The  $\text{K}^+$  ion, as well as the  $\text{Na}^+$  ion, is one of the most biologically abundant alkali-metal cations.<sup>24</sup> However, its cases of coordinating a glycine molecule are very different from those of  $\text{Li}^+$  and  $\text{Na}^+$  ions but are similar to those of  $\text{Rb}^+$  and  $\text{Cs}^+$  ions.<sup>13b</sup> Therefore, we expect to provide some useful insights into the properties of the  $\text{Rb}^+$  and  $\text{Cs}^+$  ions involved in similar biological systems by the investigation of the series of hydrated glycine- $\text{H}^+\text{K}^+$  complexes in the present paper.

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**Supporting Information Available:** Optimized structures for glycine- $\text{H}^+\text{Li}^+(\text{H}_2\text{O})_n$  and glycine- $\text{H}^+\text{K}^+(\text{H}_2\text{O})_n$  ( $n = 1-10$ ), and calculated relative energies of each series of isomers obtained at various levels (Figures S1 and S2), and REB energies in these hydrates (Table S1 (glycine- $\text{H}^+\text{Li}^+(\text{H}_2\text{O})_n$ ) and Table S2 (glycine- $\text{H}^+\text{K}^+(\text{H}_2\text{O})_n$ )). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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