

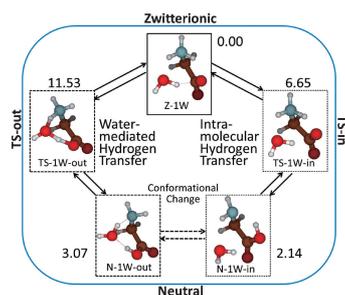
Ab Initio QM/MM-MC Study on Hydrogen Transfer of Glycine Tautomerization in Aqueous Solution: Helmholtz Energy Changes along Water-mediated and Direct Processes

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Routes of hydrogen transfer of glycine with one water (QM complex). The QM complex is surrounded by 100 MM water molecules to calculate Helmholtz free-energy changes in aqueous solution. Helmholtz energy changes (in kcal mol⁻¹) are indicated relative to Z-1W.

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Helmholtz free-energy changes in aqueous solution along two paths of glycine tautomerization were investigated by using an ab initio QM/MM-MC method with the thermodynamic perturbation theory. Glycine and one water molecule were treated at the quantum mechanics (QM) level of MP2/6-31G(d); these were surrounded by 100 water molecules at the molecular mechanics (MM) level of TIP3P. AIM (atoms-in-molecules) analysis showed that the transfer of two hydrogen atoms occurs simultaneously along the water-mediated path. The free-energy barrier height of this path in aqueous solution was higher than that of the direct path.

Glycine is the smallest amino acid; it exists as the neutral (N) form in the gas phase, and as the zwitterionic (Z) form in the aqueous and crystalline states. Although the tautomerization of glycine between the N and Z forms has been studied extensively, the process of hydrogen transfer in glycine tautomerization is not yet understood completely.¹⁻⁷ For micro-hydrated glycine complexes with two to five water molecules, it was shown that the energy barrier for hydrogen transfer via the direct path was lower than those for water-mediated paths.⁷ The ab initio QM/MM-FEG method was applied to glycine tautomerization via direct hydrogen transfer surrounded by MM water molecules,⁴ where QM energy calculations were performed at the theoretical level of MP2/6-31+G(d), with the glycine structural parameters optimized at the HF/6-31+G(d) level, and it was demonstrated that the computational free-energy difference between the N and Z forms compared well with experimentally observed values. On the other hand, a ReaxFF (reactive force field) MD simulation was applied to glycine tautomerization (direct as well as mediated by one or two water molecules), and it was suggested that the hydrogen transfer of glycine was mediated by a single water molecule in the aqueous environment.⁵ Thus, the hydrogen-transfer path in glycine tautomerization is still controversial.

In this letter, we aim to present the probable route starting from the same complex in aqueous solution: one route is a single-water-mediated hydrogen-transfer path, and the other is a direct hydrogen-transfer path. To this end, we treat a complex of glycine with one water molecule, gly-1W, at the QM level (ab initio molecular orbital (MO) method with MP2/6-31G(d)). The Helmholtz free-energy differences along the tautomerization routes are calculated on the basis of thermodynamic perturbation theory with Monte Carlo (MC) sampling of 100 MM water molecules surrounding the QM complex. Furthermore, we decompose the processes of single and double hydrogen transfers to neutral, transition state (TS), and zwitterionic regions on the basis of the AIM analysis, and we show that it is not a “proton” but a “hydrogen atom” that is transferred.

First, the ab initio MO method was employed for the complex gly-1W in the gas phase. We obtained the stationary structures of intra- and intermolecular hydrogen-transfer reactions by using the MP2/6-31G(d) level of theory. Since it is probable that the N/Z energy differences, the TS structures, and the barrier heights are strongly sensitive to the electron correlation effect, we adopted the MP2 level of theory for ab initio MO calculations throughout this study. All stationary structures were confirmed by means of vibrational frequency calculations. We found one Z-form structure (Z-1W), two N forms (N-1W-out and N-1W-in), and two TS structures (TS-1W-out and TS-1W-in). These structures are shown in Figure 1A, and the coordinates are given in the Supporting Information.⁸ The intrinsic reaction coordinates (IRCs) along the two paths (water-mediated and direct paths) were calculated at the same level of theory, and we confirmed that TS-1W-out is a TS connecting Z-1W and N-1W-out, and TS-1W-in is a TS connecting Z-1W and N-1W-in. Ab initio MO calculations were performed with Gaussian 03⁹ and GAMESS.¹⁰

Along the two IRC paths, we performed the atoms-in-molecules (AIM)¹¹ analysis of electron density at the MP2 level, using the program packages of Gaussian 09¹² and AIM2000.¹³ For each of the selected points along the two IRC paths, the electron density and its Laplacian at each of the bond critical points (BCPs) on (a) O_{carboxy}-H₁, (b) H₁-O_{water}, (c) O_{water}-H₂, (d) H₂-N, (e) N-H₃, and (f) H₃-O_{carboxy} were calculated. These plots are shown in Figure 2; the numbering of hydrogen atoms and the bonds considered are indicated in Figure 1A.

In the AIM analysis, the hydrogen-bonding interaction is characterized by a positive value of the density Laplacian at the BCP of the bonding, and a covalent bond by a negative value.¹¹

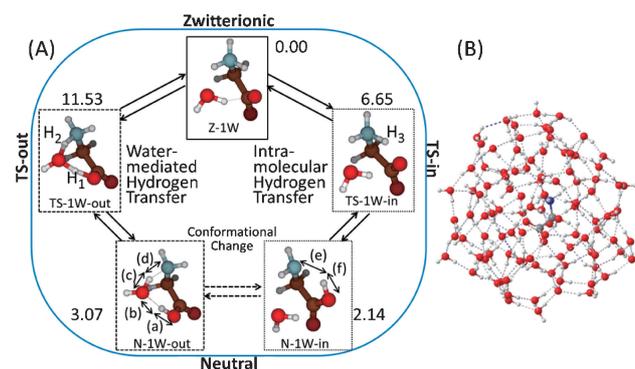


Figure 1. (A) Routes of hydrogen transfer of glycine with one water (QM complex). Helmholtz energy changes (in kcal mol⁻¹) are indicated relative to Z-1W. (B) 100 MM water molecules surrounding the QM complex.

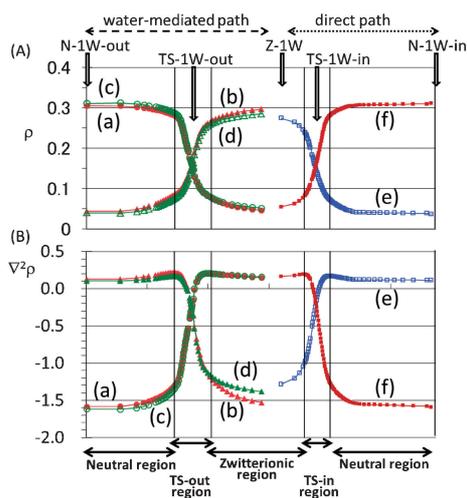


Figure 2. (A) Electron density ρ and (B) its Laplacian $\nabla^2\rho$ of bond critical points of the bonds (a)–(f) along two IRC paths of gly-1W tautomerization. See Figure 1A for the bond indices.

Along each IRC path of hydrogen transfer, the formation and cleavage of a covalent bond and hydrogen bonding are seen clearly in Figure 2. We used the change in the density Laplacian at the BCP of a hydrogen bond to define the borders between TS and N regions and between TS and Z regions. We consider a point as a border where the density Laplacian at the BCP of a hydrogen bond turns to decrease, which indicates that the hydrogen bond starts to change toward a covalent bond, and that a hydrogen atom starts to transfer. In Figure 2, the four borders between the regions are indicated.

Along the water-mediated tautomerization process, two hydrogen atoms (H_1 and H_2) transfer. As shown in Figure 2A, in the transfer process of H_1 , the change in the density at the BCP on (a) is concerted with that at the BCP on (b), and the crossing of the two changes occurs near TS-1W-out. In the transfer process of H_2 , the change in the density at the BCP on (c) is concerted with that at the BCP on (d), and the crossing of the two changes occurs near TS-1W-out. It is noteworthy that the two crossings (one for (a) and (b), and the other for (c) and (d)) occur near TS-1W-out. The same characteristics are found for the changes in the density Laplacian (Figure 2B). This coincidence indicates that two hydrogen atoms (H_1 and H_2) transfer simultaneously.

Along the direct tautomerization process, one hydrogen atom (H_3) transfers. In the transfer process of H_3 , the change in density (Figure 2A) at the BCP on (e) is concerted with that at the BCP on (f), and the crossing of the two changes occur near TS-1W-in. The change in the density Laplacian (Figure 2B) at the BCP on (e) is concerted with that at the BCP on (f), and the crossing of the two changes occur near TS-1W-in. Two crossings (one for the density and the other for the density Laplacian) occur near TS-1W-in.

Second, we put the complex gly-1W in an aqueous environment. For the aqueous solution, we used a mixed Hamiltonian QM/MM model, with the complex gly-1W treated at the QM level of theory (MP2/6-31G(d)) and the other 100 solvent water molecules treated at the MM level of theory (TIP3P¹⁴). We used the QM/MM protocol, which is the same as that used previously.^{15,16} In our protocol, the potential operator acting on the electron density of the QM part due to the effective charges of solvent MM molecules is included in the one-electron Hamiltonian operator of the QM subsystem. The total QM/MM energy of a system can be expressed as follows:

$$E^{\text{QM/MM}} = E_{\text{QM}} + E_{\text{QM-MM}} + E_{\text{MM}} \quad (1)$$

E_{QM} is the energy of the QM subsystem (i.e., gly-1W complex) in the presence of the effective charges of solvent MM molecules. $E_{\text{QM-MM}}$ is the interaction energy between the QM subsystem and MM molecules, including electrostatic and van der Waals terms. E_{MM} is the interaction energy between MM molecules. The parameters¹⁷ used for the Z and N forms in the QM/MM calculation are listed in the Supporting Information.⁸ The parameters of N and O of the Z form are different from those of the N form. Along the hydrogen-transfer processes, the parameters of N and O of glycine must change. Thus, we used a different value that changes linearly according to the variation in the distance between transferring H and N for the parameter of N or between transferring H and O for the parameter of O in glycine.

We calculated the Helmholtz free-energy differences in the aqueous QM/MM system along the selected points on the IRC paths of gly-1W by means of the thermodynamic perturbation theory. The difference in Helmholtz energy between gly-1W structures i and j in the aqueous QM/MM system is given by

$$\Delta A_{ij} = -kT[\ln\langle \exp\{-(E_j^{\text{QM/MM}} - E_i^{\text{QM/MM}})/kT\} \rangle_{(i)}] \quad (2)$$

The bracket $\langle \dots \rangle_{(i)}$ denotes the ensemble average of the difference in total QM/MM energies of gly-1W (in structures i and j) with the solvent molecules in configurations originating from a Monte Carlo random walk around gly-1W in structure i in a solvation sphere with a radius of 9.32 Å (Figure 1B), in which the water density is 1 g cm⁻³.

To sample the solvent configurations, we adopted the double perturbation approach of Dupuis et al.¹⁸ In this approach, an intermediate MM/MM level of theory is used for the random walk, with the gly-1W subsystem treated at the MM level of theory, using the same Lennard-Jones parameters as in the QM/MM model and with atomic partial charges extracted from a fit to the electrostatic potential of the solute in the gas phase (CHELPG charges).¹⁹ In the present work, for each gly-1W structure i along the selected IRC points, 20000000 solvent configurations were generated at the MM/MM level of theory, at a constant temperature $T = 298$ K and constant volume (NVT ensemble), of which 2000 configurations were selected randomly to be used for QM/MM calculations. The total QM/MM energies of gly-1W structures i and j with the same solvent configuration based on gly-1W structure i were calculated, and their differences were averaged. The numbers of points used for calculations along the IRC paths were 43 between N-1W-out and TS-1W-out, 18 between TS-1W-out and Z-1W, 10 between Z-1W and TS-1W-in, and 69 between TS-1W-in and N-1W-in. The free-energy calculations were performed using HONDO.²⁰

It is informative to express the change in Helmholtz free energy ΔA of a given QM/MM aqueous system relative to a reference state and as the sum of two terms,²¹ one being the change in QM energy ΔE_{QM} and the other the change in solvation free energy $\Delta\Delta A_{\text{solv}}$ as follows:

$$\Delta A = \Delta E_{\text{QM}} + \Delta\Delta A_{\text{solv}} \quad (3)$$

From the free-energy perturbation theory, it is the sequence of ΔA that is calculated. Since we know the ΔE_{QM} values along the IRC paths, we can obtain the sequence of solvation free energy $\Delta\Delta A_{\text{solv}}$. Note that these are relative values, and any point can be taken as a reference state. In Figure 3, the change in Helmholtz free energy ΔA is plotted relative to Z-1W along the IRC steps (black filled circles). The change in solvation free energy $\Delta\Delta A_{\text{solv}}$

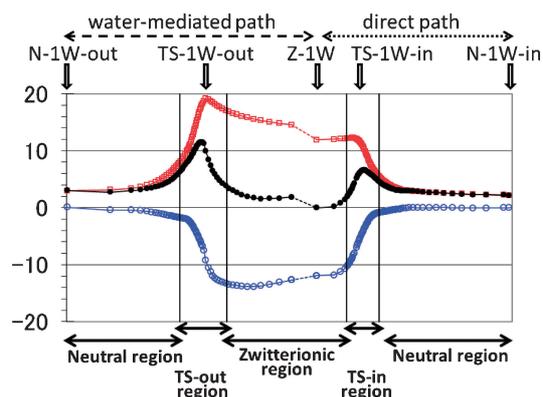


Figure 3. Changes in Helmholtz free energy ΔA (black full circle), QM energy ΔE_{QM} (red open square), and solvation free energy $\Delta\Delta A_{\text{solv}}$ (blue open circle) are plotted along IRC steps. The values are in kcal mol^{-1} .

is plotted (blue open circles) relative to N-1W-in. The change in QM energy ΔE_{QM} is plotted (red open squares) such that the relation of eq 3 is satisfied using the values of ΔA and $\Delta\Delta A_{\text{solv}}$ at each step.

As shown in Figure 3, Z-1W is the most stable in aqueous solution from the viewpoint of the Helmholtz free energy. In the Z region, the QM energy is high, while it is partly compensated by the solvation free energy. Along hydrogen transfer, the solvation free energy changes drastically. The stabilization by the solvation free energy is larger in the water-mediated double hydrogen-transfer path than that in the direct hydrogen-transfer path, while the barrier height of the QM part is higher in the double hydrogen-transfer path than that in the direct transfer path. As a result, the total Helmholtz free-energy barrier height for the direct transfer path is lower than that for the water-mediated transfer path. The QM calculations for micro-hydrated glycine complexes had shown that the energy barrier increased with an increasing number of water molecules in the transfer bridge.⁷ It is probable that the increase in the number of water molecules in the transfer path does not result in the decrease in the free-energy barrier height in the aqueous environment.

For the direct transfer path, the free-energy barrier height from the Z to N forms in the current work is $6.65 \text{ kcal mol}^{-1}$, which is lower than that found in the previous work,⁴ where the HF/6-31+G(d) level of theory was used for optimization of the TS structure.

The changes in the group charges (from CHELPG atomic charges of the QM part) along both tautomerization routes are plotted in Figure 4. The CH_2NH_2 and COO group charges change drastically in the TS regions. In the zwitterionic region, the CH_2NH_2 group charge is highly positive, and the COO group charge is highly negative. This results in the large stabilization of the solvation free energy in the Z region, as shown in Figure 3. It should be noted that the group charge of the transferring group (W + H in the double transfer path, and H₃ in the direct path) is almost constant along both IRC paths and that it is a “hydrogen atom” that transfers. The characteristic that the charge of the transferring atom in the tautomerization is almost constant is also seen in the AIM charges (see Supporting Information⁸).

Starting from the QM complex of glycine with one water molecule in aqueous solution, we found that the Helmholtz free-energy barrier of the water-mediated hydrogen-transfer path is

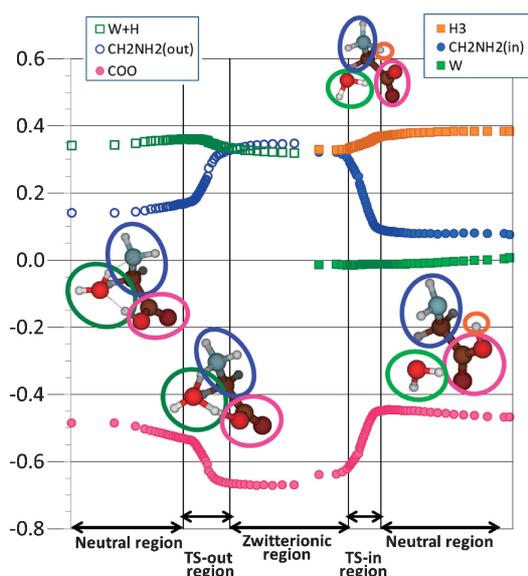


Figure 4. Changes of group charges along two IRC paths.

higher than that of the direct path. We conclude that the tautomerization of glycine occurs via a direct path in the aqueous environment.

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