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On the tautomerization process of glycine in aqueous solution

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Abstract

The experimental activation energy for the tautomerization of glycine (zwitterion → neutral form) has been reported to be 14.6 kcal/mol. It has been generally assumed that this energy barrier is needed for proton transfer to occur. However, previous theoretical results do not support this interpretation. In the present work, we examine this question using density functional calculations, extended basis sets and a polarizable continuum solvent model. Our results suggest that the limiting step for the tautomerization process corresponds basically to H-atom reorientation in the −COOH group. This could be a general feature in the tautomerization of amino acids.

1. Introduction

Tautomeric equilibrium between neutral and zwitterionic forms of amino acids is of fundamental importance in biochemistry. Such an equilibrium is extremely sensitive to the medium effect. For instance, in the gas phase, glycine exists in the neutral (NE) tautomeric form only [1,2] whereas, in aqueous solution and the solid phase, the zwitterion (ZW) is the predominant form of this amino acid [3,4] (see Scheme 1). This may be easily explained by the fact that interactions with the medium are much more stabilizing in the case of the zwitterionic tautomer.

Some experimental and theoretical studies have been devoted to investigating the tautomerization reaction in aqueous solution. On the one hand, thermodynamic [5–7] measurements for the ZW → NE process predict 7.3 [5,6] and 7.7 [7] kcal/mol for the reaction free energy (at 298 K) and between 9.9 and 11.5 kcal/mol for the reaction enthalpy. These values were obtained from measurements of acidity constants [5,6] or using thermodynamic cycles [7]. The reaction entropy is thus deduced to range between 7.5 and 14.1 cal/mol K. The positive sign of the reaction entropy is expected from chemical intuition because the zwitterionic structure generates a considerable solvent organization around it. The measured activation free energy for the ZW → NE process is 14.6 kcal/mol, obtained by using a temperature modulated chemical relaxation method [8]. By measurements of the rate constant at several temperatures, an activation entropy of −48.9 cal/mol K was deduced by the same authors, suggesting that the origin of the barrier in water is essentially due to the entropic contribution. Using the reaction and activation free energies for the

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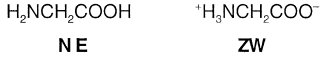

Scheme 1.

ZW → NE process, the activation free energy for the reverse process NE → ZW is estimated to be roughly 7 kcal/mol.

Theoretical computations of the tautomerization process have focused on the proton transfer step between the zwitterion and the neutral form which has the amino and the acid group correctly oriented for a direct proton transfer. Such a conformer has been shown to be the most stable one for neutral glycine in aqueous solution, in contrast to the gas phase [4,9]. A recent empirical valence bond (EVB) molecular dynamics (MD) simulation of the intramolecular proton transfer process [10] yielded an activation free energy of 16.9 kcal/mol for the ZW → NE process and 8.4 kcal/mol for the NE → ZW one, in very good agreement with the experimental values. By analyzing the temperature dependence of the free energy change, the authors showed that the enthalpy contribution to the activation free energy is larger than the entropic term [11], in contrast to the conclusions reached experimentally by Slifkin and Ali [8]. These results were obtained by fitting EVB parameters to HF energies, which severely overestimate, the activation barrier. Thus, the computed NE → ZW activation barrier is 11.0, 2.4 and 1.9 kcal/mol at the Hartree–Fock (HF), second-order Møller–Plesset (MP2) and density functional theory (DFT) levels respectively (using the 6-31 + G** basis set and a continuum solvent model in all cases) [4,9]. Remarkably, the activation energy is about five times higher at the HF level than in correlated methods. Very close values have been obtained for alanine [9]. Combined density functional-molecular mechanics (DF/MM) simulations have predicted that, in aqueous solution, neutral glycine undergoes a very fast and exothermic conversion to its zwitterionic form [12], suggesting a very small energy barrier for the proton transfer step.

The results of theoretical calculations at correlated levels for the proton transfer process and the thermodynamic data for the tautomerization process are therefore in conflict. It seems that this disagreement cannot be ascribed to computational level limitations, which suggests that the tautomerization mechanism is probably more complex than previously believed. A possible reason could be the coupling between the tautomerization process and the conformational equilibrium of neutral glycine in water. In this Letter, we show that the neutral conformer exhibiting a suitable conformation for proton transfer has a very short lifetime so that interpretation of experimental data requires to be taken into account several neutral conformations. In fact, the limiting step appears to be H-atom reorientation in the –COOH group that must occur after proton transfer. This was not envisaged in the recent computer simulation reported in Ref. [10] and to the best of our knowledge such an interpretation is proposed here for the first time. We use density functional calculations and a continuum model for the solvent. The resulting scheme is compatible with experimental thermodynamic measurements.

2. Calculations

Geometry optimization calculations have been done using Density Functional theory at the B3LYP/6-31 + G** level [13–15]. The solvent effect is accounted for with the help of a continuum model ($\varepsilon = 78.4$) using the self-consistent reaction field approach. For the geometry optimization, we simply use an ellipsoidal cavity model [16,17] because it allows the analytical energy derivatives and thus zero-point energies and thermal contributions to the free energy to be calculated more easily [18]. For the energy, we preferred to choose a general cavity shape because it is expected to provide more accurate solvation energies. In fact, the solvation energy of the zwitterionic form is highly dependent on the cavity shape and size [19]. Thus, the final solute–solvent interaction is computed using the PCM method [20,21] including non-electrostatic contributions to the free energy (dispersion-repulsion and cavitation) on the previously optimized geometries. Calculations were made using the GAUSSIAN 94 package of programs [22].

One should note that the solvent free energy is obtained in the calculations using the continuum approach. If one neglects the cavitation contribution (that is expected to remain nearly constant along the

reaction path), the solvent free energy is roughly given by $-1/2\ E_{int}$, where E_{int} is the solute–solvent electrostatic interaction energy. However, it is not possible, formally, to evaluate separately the enthalpic and entropic contributions. Hence, in this work, we shall not attempt to discuss the enthalpic or entropic origin of the activation barrier although, as we show below, our results suggest that this is not the capital question in the glycine tautomerization process.

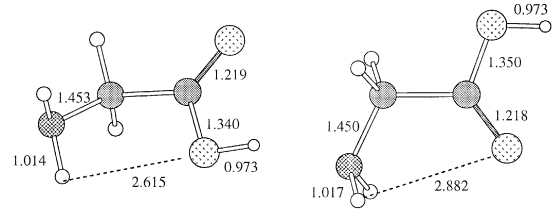

Fig. 2. Other structures of neutral glycine considered in this work. Values in Å.

3. Results

The stationary structures involved in the proton transfer step of the tautomerization process are presented in Fig. 1. The free energies, relative to the zwitterion (ZW), of the transition structure (TS1) and the neutral conformer (A) are 5.42 and 4.77 kcal/mol. Thus, the free energy barrier for the A → ZW conversion is of only 0.65 kcal/mol, i.e. of the order of kT. Therefore, in agreement with previous calculations [12], structure A is expected to have a very small lifetime and direct comparison between experimental measurements and ZW/A conversion seems not to be adequate. For the neutral form to be detected, conformation A needs to reorganize into a more kinetically stable structure.

A detailed study of neutral glycine conformations is a complex problem that is beyond the aim of the present communication and will be reported elsewhere [23]. Here we focus on two structures, B and C in Fig. 2, that have been obtained through full geometry optimization starting from A after varying some dihedral angles. Thus, B is obtained from A by rotation of the acidic hydrogen around the C–O bond that represents the easiest process allowing a favorable reorganization of the neutral form, as far as this makes proton transfer infeasible. C is obtained from B by rotation around the CC bond and corresponds to the most stable structure in the gas phase [24]. One could also imagine a process starting from A in which the acid group rotation around the C–C bond precedes the H-atom reorientation. Exploratory calculations indicate that the corresponding neutral intermediate conformer is significantly higher in energy compared to A, B and C and the associated energy barrier is also larger. The transition states for A → B and B → C conversion are shown in Fig. 3. The energy profile for the whole process is plotted in Fig. 4.

In the A → B step, the inversion of the $-NH_2$ moiety and rotation of the –OH proceed in a concerted way although, at the transition structure (TS_2), the inversion of the amino group is practically achieved. Once structure B is formed, a rotation around the C–C bond becomes possible allowing it to reach the more stable structure C through TS_3.

The ZW tautomer is significantly more stable than the neutral forms, the relative energies of A, B and C

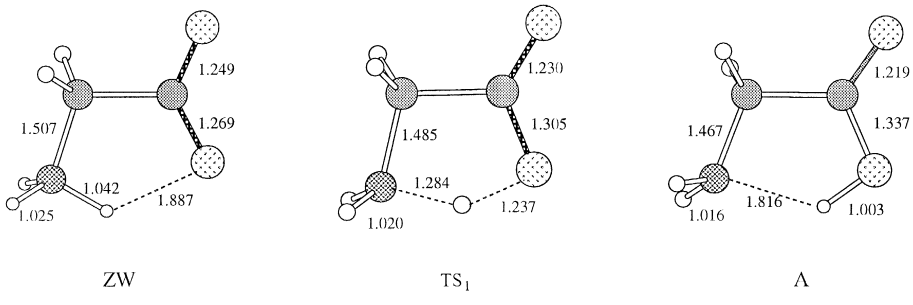

Fig. 1. Optimized structures involved in the intramolecular proton transfer reaction of glycine. Zwitterion (ZW), transition state (TS_1), and neutral structure (NE) in conformation A. Values in Å.

with respect to ZW being 4.77, 8.41 and 5.51 kcal/mol, respectively. Thus, in agreement with experimental investigations, the structure of glycine amino acid in water is predicted to be essentially zwitterionic. As in previous theoretical studies [4,9], we predict A to be the most energetically stable neutral structure in aqueous solution, but the C structure lies only 0.74 kcal/mol above. Our calculations predict a free energy of activation equal to 5.42 kcal/mol for the process ZW → A, 14.0 kcal/mol for the process A → B and 1.31 kcal/mol for the process B → C. Note that conformation B, as noted above for conformation A, represents a shallow energy minimum in the energy surface.

According to these results, the experimentally determined activation free energy cannot be assigned to the proton transfer process. In order to interpret the experimental data, one must derive equations for the effective rate constants. The tautomerization process could be roughly represented by the following kinetic scheme:

$$\mathrm{ZW} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{A} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{B} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{C} \rightleftharpoons \ldots . \qquad (1)$$

The species A can be considered as a reaction intermediate in equilibrium with ZW, since its rate of formation or decay back ZW is much faster than the rate of formation of B which, in turn, is in equilibrium with C and other neutral forms not considered here explicitly. Under these approximations (steady state approximation for A and equilibrium between B and C species) and using standard rate laws, one deduces the following expression:

$$\frac{\mathrm{d}[ZW]}{\mathrm{d}t} = \frac{k_{-2}}{1+K_3}[NE] - k_2 K_1 [ZW] \qquad (2)$$

where K_1 and K_3 are the equilibrium constants for ZW/A and C/B, respectively, and [NE] = [B] + [C].

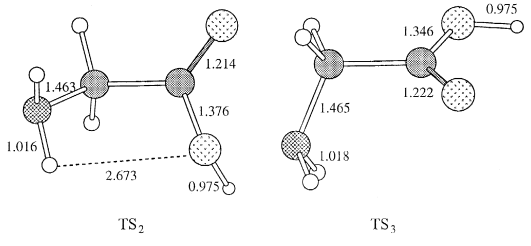

Fig. 3. Transition states for A → B (TS_2) and B → C (TS_3) conversion. Values in Å.

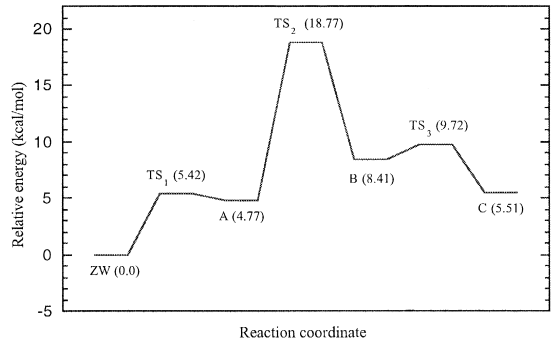

Fig. 4. Computed free energy profile for the tautomerization process of glycine in water.

Thus, the previous scheme suggests that the experimental measured activation energy for the ZW → NE process must roughly correspond to the energy difference TS_2–ZW (18.77 kcal/mol). The activation energy for the reverse process should range between the energy difference TS_2–C (13.26 kcal/mol) and TS_2–B (10.36 kcal/mol) depending on the value of the equilibrium constant K_3. Besides, the reaction free energy should range between C–ZW and B–ZW (8.41–5.51 kcal/mol). The agreement between the experimental values and our results is reasonably good considering the approximations made, particularly for the solvent modeling. Therefore, the proposed tautomerization process seems to be realistic.

The proposed kinetic scheme could be extended to account for other neutral conformations by changing K_3 by the appropriate summatory of equilibrium constants. Note finally, that for other amino acids, the relative value of the kinetic constants may change and some of the approximations made above should be reconsidered. However, the main conclusion, i.e. the relatively low importance of proton transfer to explain the measured tautomerization activation barrier, should still be valid.

4. Conclusions

In this Letter, we have studied the tautomerization of glycine in solution by means of density functional calculations and continuum solvent models. From our results, it can be concluded that glycine in water solution may be basically described as a zwitterionic

species. By means of a proton transfer this zwitterionic species gives the neutral form A, although the equilibrium is substantially shifted towards the former. The lifetime of A should be extremely small. The neutral tautomer of glycine displays a complex conformational equilibrium. It would exist in two forms, B and C, that are separated by a small energy barrier although other conformations may be imagined. C is more stable than B by 2.90 kcal/mol and corresponds to the most stable conformation in the gas phase. Conformation C is, however, less stable than A by 0.74 kcal/mol. The tautomerization mechanism proposed in this work (see Eq. (1)) is consistent with available experimental thermodynamic data. It stresses the fact that proton transfer is not the rate-limiting step in the tautomerization process. That role would be played by reorientation of the acid OH group. Our conclusions can be extended for the consideration of a more complex configurational equilibrium. Finally, one may remark that previous controversy concerning the enthalpic or entropic origin of the activation barrier can be explained by the complexity of the tautomerization mechanism proposed here. A definite answer to this question will deserve further experimental work.

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