

Effects of Microsolvation on the Structures and Reactions of Neutral and Zwitterion Alanine: Computational Study

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Calculations are presented for the structures and the reactions of various conformers of the bare alanine, neutral alanine-(H₂O)_n, and alanine zwitterion-(H₂O)_n ($n = 1$ and 2) clusters. The effects of the binding water molecules on the relative thermodynamic stability and the isomerization reaction of alanine are examined. Hydrogen bonding between alanine and the water molecule(s) may significantly affect the thermodynamic stability of conformers of the neutral alanine-(H₂O)_n ($n = 1$ and 2) clusters. Detailed analysis is presented on the isomerization (proton transfer) pathways between the neutral alanine-(H₂O)₂ and the alanine zwitterion-(H₂O)₂ clusters including the structures of the transition states by carrying out the intrinsic reaction coordinate analysis. We find that at least two water molecules need to bind to produce the stable alanine zwitterion-water cluster in the gas phase. The isomerization reaction for the alanine-(H₂O)₂ cluster proceeds by the concerted double proton-transfer mechanism via the binding water molecules. The effects of continuum water are also examined by employing the supramolecule/continuum type model to study the properties of alanine in aqueous solution. The water continuum is predicted to considerably alter the thermodynamic and kinetic stability of the neutral and zwitterionic alanine-(H₂O)_n ($n = 0-2$) clusters.

I. Introduction

The effects of solvation are one of the fundamental issues of chemistry, because the structure and reactivity of free molecules are very different from those in the solvent environment. The interactions with the solvent molecules may alter the relative stability of various conformers of the solute, or they may profoundly influence the reaction path of molecules, affecting the properties of the solute molecule both thermodynamically and kinetically. Studying the effects of solvation is usually quite difficult, because the solute-solvent interactions are of a molecular nature, and thus, they must be treated on the molecular level. The cluster or the supramolecule model will be quite useful in this regard, because it may help examine the effects of "microsolvation" by analyzing the detailed interactions between the solute and the solvent molecule(s) affecting the structure and reaction of the solute.

Because most biochemical reactions of interest occur in the solution phase, the effects of solvation on biomolecules will be very important. Detailed interactions between the functional groups both in the biomolecule and in the solvent molecules may efficiently be probed by systematically examining the structures of the clusters consisting of the biomolecule and a number of solvent molecules.¹⁻⁸ If one is interested in obtaining more realistic descriptions for the biomolecule in the solution phase, one may also employ the continuum model^{9,10} for the other infinite number of solvent molecules around the cluster (supramolecule/continuum model). Because the difference in energy between the conformers is usually rather small (usually at most 5 kcal/mol), many biomolecules may exist in solution as a number of conformers, and thermal energies of the

molecules may easily transform from one conformer to another in room-temperature solution phase. In clusters formed by cooling in the supersonic expansion experiments at very low temperature, however, the magnitude of barrier for isomerization between conformers is very important, because they determine the kinetic stability (that is, the rate constant of isomerization) of the conformers. Amino acid, which is the building block of protein, is an ideal system to study the effects of solvation on the biochemical activity due to their moderate size. Thus, a systematic study was carried out recently on the amino acid-water clusters, notably by Tomasi and co-workers,¹¹ and by Silla and co-workers¹²⁻¹⁴ theoretically, and experimentally by Simons and co-workers,⁴ and by Kim's group.^{15,16} Both the structures of neutral amino acids¹⁵⁻²⁴ and the corresponding zwitterions^{11,19,25-31} in clusters and in solution are of fundamental interest. It is well-known that amino acids exist in the neutral form in the gas phase, whereas in aqueous solution the zwitterion is the predominant form.^{12,29,32-34} However, studying the structures of the neutral and zwitterionic amino acid and the transformation between them in solution is extremely difficult, and thus, as an efficient approach to addressing these interesting questions concerning the effects of solvation, one may take the cluster model, monitoring the influence of microsolvation on the structures and reactions between the neutral and zwitterionic forms as a function of the number and the arrangement of solvent molecules near the functional groups in the solute. The influence of solvent molecules on the reactivity of biomolecules and their role in the reaction mechanism is not understood on the molecular level very well. In this regard, the cluster (supramolecular) approach is again very useful, yielding invaluable information for the reaction path (mechanism) for isomerization between the neutral amino acid and the corresponding zwitterionic clusters that is of fundamental importance in relation

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to the mechanism of chemical reactions in clusters or in the solution phase. In some instances, the proton transfer, which is a very important biochemical³⁵ process, may occur directly from the ammonium to the carboxylate group in the zwitterionic cluster without intervention of solvent molecules. In others, the proton(s) may move via the water molecules either in concerted motion or in stepwise fashion. The actual mechanism of the transformation would, of course, depend on the structures (conformations) of the neutral amino acid clusters and the zwitterionic clusters and also on the properties of the transition states connecting them. A systematic study on this extremely interesting subject, however, has been very rare due to the difficulty of locating the transition states and of carrying out the rigorous intrinsic reaction coordinate (IRC) analysis for the dynamic pathways.

In this work, we present calculations for the bare alanine, neutral alanine-(H₂O)_n, and alanine zwitterion (AlaZW)-(H₂O)_n, (*n* = 1 and 2) clusters. A variety of conformers are predicted, and their relative energies are compared to estimate their relative thermodynamic stability. A detailed analysis is presented for the isomerization between the neutral alanine-(H₂O)_n and the corresponding AlaZW-(H₂O)_n (*n* = 1 and 2) clusters as an example of *solvent-mediated chemical reaction* to determine the kinetic stability of the zwitterionic clusters. The transition states and the reaction (proton transfer) paths are obtained by carrying out the IRC analysis. At least two binding water molecules are needed to produce the stable AlaZW in the gas phase. We show that the isomerization reaction between the neutral alanine-(H₂O)₂ and the AlaZW-(H₂O)₂ clusters proceeds by the concerted double proton-transfer mechanism. We also employ the supramolecule/continuum approach by adopting techniques based on Tomasi's polarizable continuum model^{9,36} (PCM) to study the effects of infinite number of water-molecules surrounding the bare alanine or the alanine-water cluster. We demonstrate that the presence of the water continuum may also profoundly affect the structures, thermodynamic stability, and the reactivity of the alanine molecule.

II. Computational Methods

In this study, all of the calculations are carried out using the Gaussian 94 and the Gaussian 98W set of programs.³⁷ The stationary structures are found by ascertaining that all of the harmonic frequencies are real. The structures of the transition states are obtained by verifying that one of the harmonic frequencies is imaginary and by carrying out the IRC analysis for the reaction pathways. The MP2 and the density functional theory methods (BLYP^{38,39} and B3PW91^{40–42}) are employed with various basis sets. Barrier heights are computed by subtracting the energies of the reactants and products from those of the transition states, correcting for zero-point energies (ZPE). Default criteria are employed for the optimization processes. The self-consistent reaction field (SCRf) method is employed to study the effects of water continuum. There exist various methods for the SCRf approach, but we employ several versions of Tomasi's very useful polarizable continuum model (PCM),³⁶ which converts the quantum mechanical SCRf equations into the boundary element problem with apparent surface charges on the solute cavity surface, available in the Gaussian set of programs in all of the calculations including the water continuum.

III. Results

III.1. Alanine. Although the conformers of the bare alanine have been studied computationally in several investigations, there

still exists some uncertainty concerning which conformer is of the lowest energy in the gas phase. Cao et al.,⁴² for example, carried out extensive calculations for numerous conformers of alanine, but the results depended on the methods and the basis sets employed. Cao et al. predicted that the three conformers, whose characteristic torsional angles (N1–C2–C3–O4, C3–C2–N1–H7, and C2–C3–O4–H10) are given in Figure 1, were of the lowest-energy but that the electronic energies (without the ZPE correction) were very similar (to within 0.05 kcal/mol) at the MP2/6–311G** level of approximation. When the zero-point energies are computed by the same method and added to the electronic energies of the conformers, we find that the conformer (N-1) is of a lower energy than the conformers (N-2) and (N-3) by 0.38 and 0.52 kcal/mol, respectively, as listed in Table 1. Because the relative energies of the conformers of bare alanine are very relevant to the discussions for the alanine-water clusters below, we further check them in detail. We employ the MP2 method employing 5 different basis sets (6–311G**, 6–31++G**, 6–311++G**, cc-PVDZ, and aug-cc-PVDZ). As listed in Table 1, all of the calculations indicate that the conformer (N-1) is of the lowest energy. The relative energies of conformers (N-2) and (N-3), however, depend on the basis sets employed: in calculations using only the polarization functions in the basis set (6–311G** and cc-PVDZ), conformer (N-3) is calculated to be of lower energy, whereas the energy of the conformer (N-2) is predicted to be lower when the diffuse functions are included (6–31++G**, 6–311++G**, and aug-cc-PVDZ). Therefore, the diffuse functions in the basis sets produce a marked difference in the ordering of the relative energies of conformers (N-2) and (N-3), and therefore, we predict that the conformer (N-2) is of a lower energy than the conformer (N-3). The difference in the structures of conformers (N-1)–(N-3) lies mainly in the torsional angle with respect to the central C–C bond. In (N-1), the C–N and the O–H bonds are in trans-like positions, whereas in (N-2) and (N-3) they are in cis-like positions with the intramolecular hydrogen bonding between the amino and the OH groups. This prediction seems to be in agreement with the identification of the conformers (N-1) and (N-2) (in 8:1 ratio) by Godfrey et al.²⁴ in the microwave spectroscopic experiment, and the observation of (N-1) by Powis et al.⁴⁴ in the synchrotron radiation photoelectron spectroscopy experiment. However, the differences between the ZPE-corrected energies of the conformers are small, mostly within 1 kcal/mol. Also, because the basis sets 6–31++G**, 6–311++G**, aug-cc-PVDZ, and aug-cc-PVTZ give consistent ordering of the energies of the conformers (N-1), (N-2), and (N-3), we employ the basis sets 6–31++G** or 6–311++G** for the alanine-water clusters as discussed below.

III.2. Alanine–H₂O Cluster. Based on the three lowest energy conformers of the bare alanine shown in Figure 1, we calculate the structures of the alanine–H₂O clusters by employing the MP2/6–311++G** method and obtain the conformers depicted in Figure 2. The other conformers of alanine–H₂O clusters, in which the water molecule binds to the higher energy conformers of the bare alanine (conformers 4–13 in Cao et al.'s paper⁴³), are not discussed here. The conformers (N1-1)–(N1-3), (N1-4)–(N1-6), and (N1-7)–(N1-8) may be considered, respectively, as being formed by binding of a water molecule to the conformer of the bare alanine (N-1), (N-2), and (N-3). The ZPE corrected relative energies of the alanine–H₂O clusters are also given in Figure 2, taking the energy of the global minimum energy conformer (N1-1) as zero. The dihedral angles of the alanine moiety in the cluster (N1-1) change little from those of the bare alanine (N-1), ∠(N1–C2–C3–O4) from

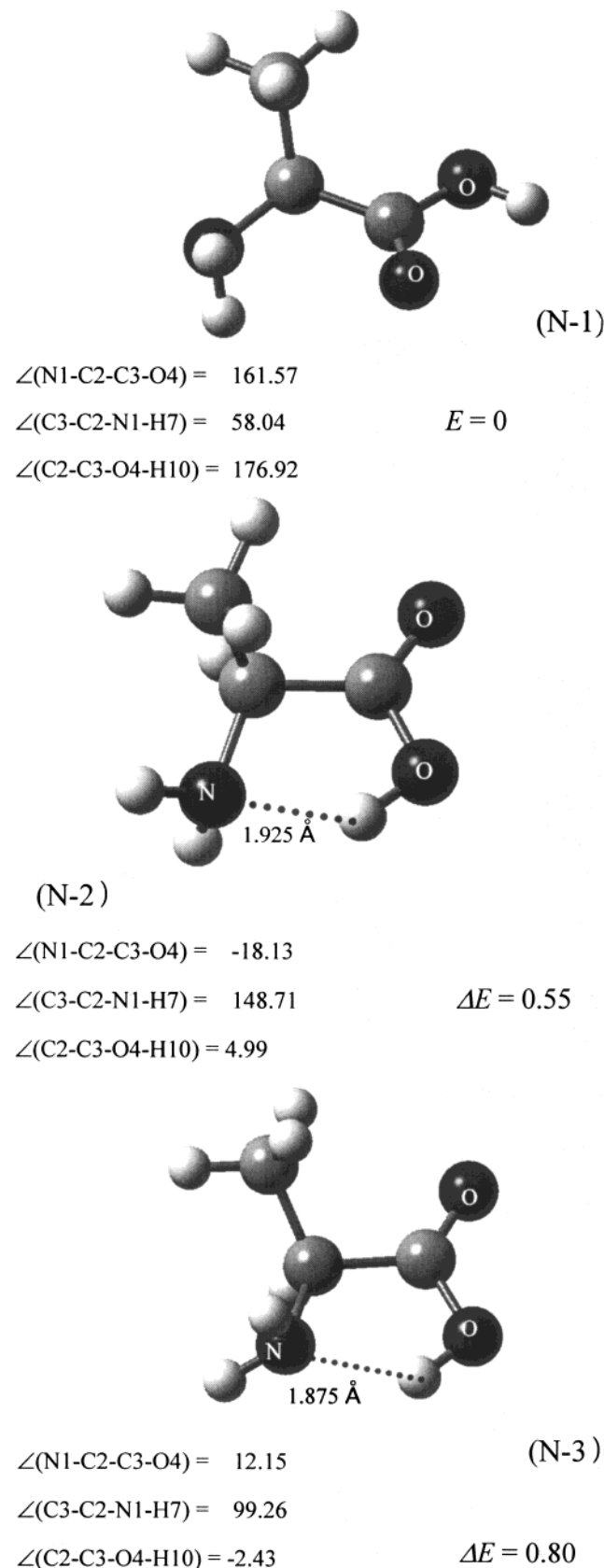


Figure 1. Three lowest energy conformers of the bare alanine computed by the MP2/6-311++G* method (relative energies in kcal/mol).

162.1° to 159.4°, $\angle(\text{C3-C2-N1-H7})$ from 56.4° to 56.0°, and $\angle(\text{C2-C3-O4-H})$ from 177.2° to 177.4°.

The two lowest-energy conformers (N1-1) and (N1-2), in which the water molecule interacts with the carboxyl group of

TABLE 1: Calculated Electronic Energies and Zero-Point Energies of the Three Lowest Energy Conformers of the Bare Alanine

	<i>E</i> (Hartree)	ZPE (kcal/mol)	ΔE (kcal/mol)
MP2/6-311G**			
(N-1)	-322.97249	68.75	0
(N-2)	-322.97237	69.20	0.52
(N-3)	-322.97255	69.17	0.38
MP2/cc-PVDZ			
(N-1)	-322.85252	68.55	0
(N-2)	-322.85202	68.95	0.72
(N-3)	-322.85257	68.93	0.35
MP2/6-31++G**			
(N-1)	-322.85781	69.10	0
(N-2)	-322.85727	69.53	0.77
(N-3)	-322.85676	69.46	1.02
MP2/6-31++G**			
(N-1)	-322.98887	68.51	0
(N-2)	-322.98860	68.89	0.55
(N-3)	-322.98812	68.84	0.80
MP2/aug-cc-PVDZ			
(N-1)	-322.92839	67.75	0
(N-2)	-322.92832	68.10	0.39
(N-3)	-322.92770	68.02	0.71
MP2(full)/6-311++G** ^a			
	-323.103014		0
	-323.102783		0.15
	-323.102256		0.48

^a Reference 43.

alanine, are very similar in energy, the difference being only 0.02 kcal/mol. The difference is in the position of the water molecule with respect to the carboxyl group of the alanine moiety. In the conformer (N1-3), the water molecule binds to the amino group with the nitrogen atom acting as a proton acceptor. The conformers (N1-4) and (N1-7), which are the lowest-energy conformers formed by the binding of a water molecule to the second and the third lowest energy conformer (N-2) and (N-3) of the bare alanine, respectively, are also of very similar energy, lying 2.47–2.48 kcal/mol above (N1-1). This finding indicates that the binding of a water molecule to the higher energy alanine conformer (N-3) is more favorable energetically than to the more stable alanine conformer (N-2), easily neutralizing the energy difference of 0.25–0.35 kcal/mol of the alanine conformers.

It is interesting note that the conformer (N1-5), in which the water molecule interacts with the two oxygen atoms of the carboxyl group, is not of the lowest energy among the conformers formed from the binding of the water molecule to the alanine conformer (N-2), whereas the corresponding conformers (N1-1) and (N1-2) are of the lowest energy among the conformers formed from the binding of the water molecule to the alanine conformer (N-1). This difference may be due to the fact that in (N1-5) the two oxygen atoms act as proton acceptor, whereas those of (N1-1) and (N1-2) are proton donor and acceptor. In (N1-6) the binding water molecule bridges the carboxyl and the amino groups of the alanine moiety with the N...H distance of 2.535 Å. The conformers (N1-7)–(N1-8) are the two lowest-energy stationary structures derived from the alanine conformer (N-3). In (N1-8), the binding water molecule bridges the carboxyl and the amino group. We also obtain the alanine (H₂O) conformer deriving from the bare alanine (N-3) in which the water molecule interacts with the two proton-accepting oxygen atoms of the carboxylic group (corresponding to (N1-6)) but find it to be much higher in energy above (N1-7)–(N1-8), and thus, we do not describe it here. The relative energies of the conformers derived from the bare

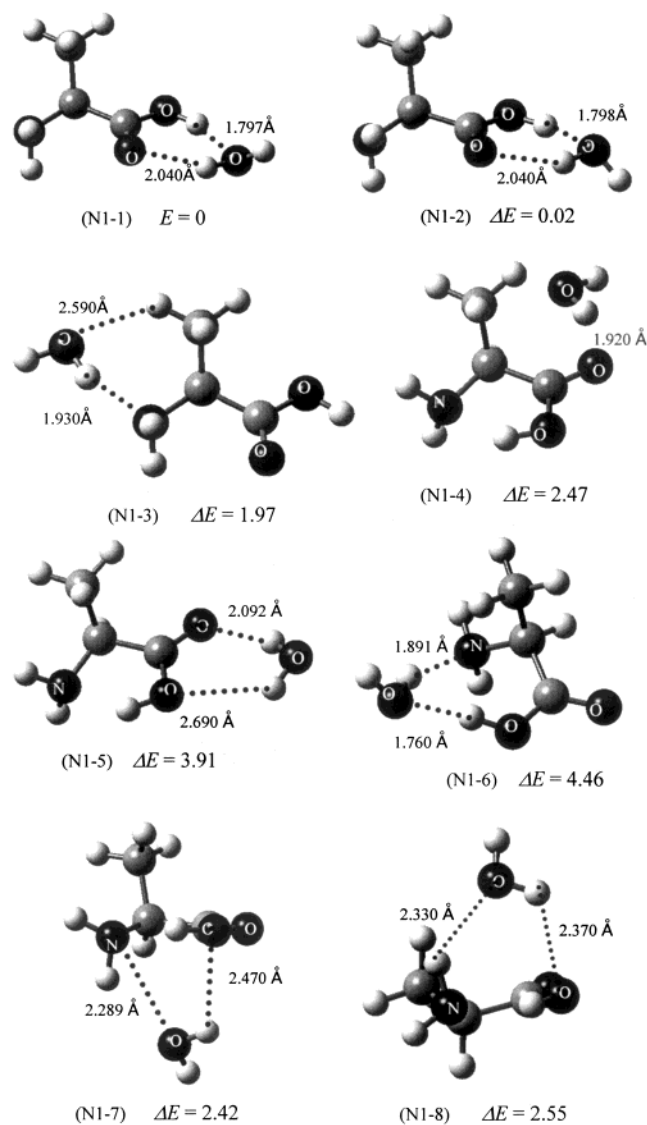


Figure 2. Lowest energy conformers of the neutral alanine-(H₂O)₂ cluster computed by the MP2/6-311++G** method (relative energies in kcal/mol).

alanine (N-2) and (N-3) also display a very interesting pattern: The conformer (N1-8) formed from the higher energy conformer (N-3) of the bare alanine is lower in energy than (N1-5) and (N1-6) derived from the lower energy alanine conformer (N-2). This clearly suggests that the binding of a water molecule may reverse the thermodynamic stability of the bare alanine conformers.

III.3. Alanine-(H₂O)₂ Cluster. The computed structures of the alanine-(H₂O)₂ clusters are presented in Figure 3, in which the relative energies of the conformers are also given. We find that the global minimum energy conformer (N2-1) of the alanine-(H₂O)₂ cluster derives from the minimum energy conformer (N-1) of the bare alanine, indicating that the binding of the two water molecules does not significantly change the lowest energy structure of the alanine moiety in the cluster. In (N2-1), the two water molecules bind to the carboxyl group with the four oxygen atoms forming a ring. There is no intramolecular hydrogen bonding between the amino and the OH group of the alanine moiety, as in bare alanine (N-1). The dihedral angles of the alanine moiety in the cluster (N2-1) slightly change from those of the bare alanine (N-1), $\angle(\text{N1}-\text{C2}-\text{C3}-\text{O4})$ from 162.1° to 158.6°, $\angle(\text{C3}-\text{C2}-\text{N1}-\text{H7})$ from 56.4° to 54.8°, and $\angle(\text{C2}-\text{C3}-\text{O4}-\text{H})$ from 177.2° to 176.5°.

The energies of the two other conformers (N2-2) and (N2-3), also derived from (N-1), are calculated to be substantially higher in energy (3.21 and 4.55 kcal/mol, respectively) than (N2-1). In these two conformers, one of the two water molecules binds to the carboxyl group, while the other interacts with the amino group. The difference between (N2-2) and (N2-3) is that the amino group in (N2-2) acts a proton acceptor, while it donates proton to the binding water in (N2-3). Considering that the energies of the two alanine-H₂O conformers ((N1-1) and (N1-2)) based on (N-1) are very similar (see Table 2), this finding indicates that the energies of the alanine-(H₂O)₂ conformers tend to split due to the additional interaction with a water molecule.

The conformer (N2-4) is of the lowest energy among those derived from the second lowest energy alanine (N-2), lying 2.53 kcal/mol above (N2-1). In this conformer, the two oxygen atoms of the binding water molecules form a ring with the nitrogen and the oxygen atoms of the amino and the carbonyl group, respectively. The conformer (N2-5) also corresponds to the bare alanine (N-2), lying 2.64 kcal/mol above (N2-1). Compared with (N-2), the bond lengths of N...OH of (N2-4) and (N2-5), 1.872 Å and 1.885 Å, respectively, are shorter than that (1.925 Å) of the bare alanine (N-2). The conformer (N2-6) is of the lowest energy based on (N-3), 2.86 kcal/mol above (N2-1).

It is interesting to note that the conformers (N2-4) and (N2-5), formed from the binding of two water molecules to the bare alanine (N-2), are of lower energy than (N2-2) and (N2-3) based on the lower energy alanine conformer (N-1). Similarly, the conformer (N2-6), corresponding to the third-lowest energy alanine conformer (N-3), is of lower energy than (N2-2) or (N2-3). Thus, although the global minimum energy conformer the alanine-(H₂O)₂ cluster retains the lowest energy structure of the alanine moiety, the interactions with the two water molecules may render the alanine conformer of higher energy to be more stable.

Some of these conformers of neutral alanine-(H₂O)₂ clusters may be quite stable or amenable to isomerization (proton transfer) to the corresponding zwitterionic cluster. Thus, it will be extremely interesting to examine the thermodynamic and the kinetic stability of these conformers with respect to the neutral ↔ zwitterion isomerization reaction by calculating the detailed mechanism of the proton-transfer processes. The proton transfer may be direct, from the carboxylate to the amino group, or it may be mediated by the binding water molecules, providing an excellent example of the solvent-mediated chemical reaction. This very important biochemical process of alanine is discussed in the next section.

III.4. Isomerization between AlaZW-(H₂O)₂ and Neutral Alanine-(H₂O)₂ Clusters. It is well-known that amino acids exist as zwitterions in aqueous solution, whereas the neutral form is much more stable in the gas phase.^{12,29,32-34} Thus, one may expect the neutral alanine to be more stable than its zwitterion form in the gas phase. The stability of the neutral alanine vs the zwitterion form in the gas phase, however, must be understood in terms of both thermodynamic and kinetic aspects. Even when the energy of the zwitterion form is higher than that of the neutral form, the former form of alanine may be kinetically stable at least in the low-temperature gas-phase environment if the barrier for the transformation between the two forms is rather large. Thus, both the relative energy of the two forms and the barriers for isomerization must be known to estimate the stability of the AlaZW. It will also be very interesting to examine their relative stability as a function of the number and the arrangement of the binding water molecules

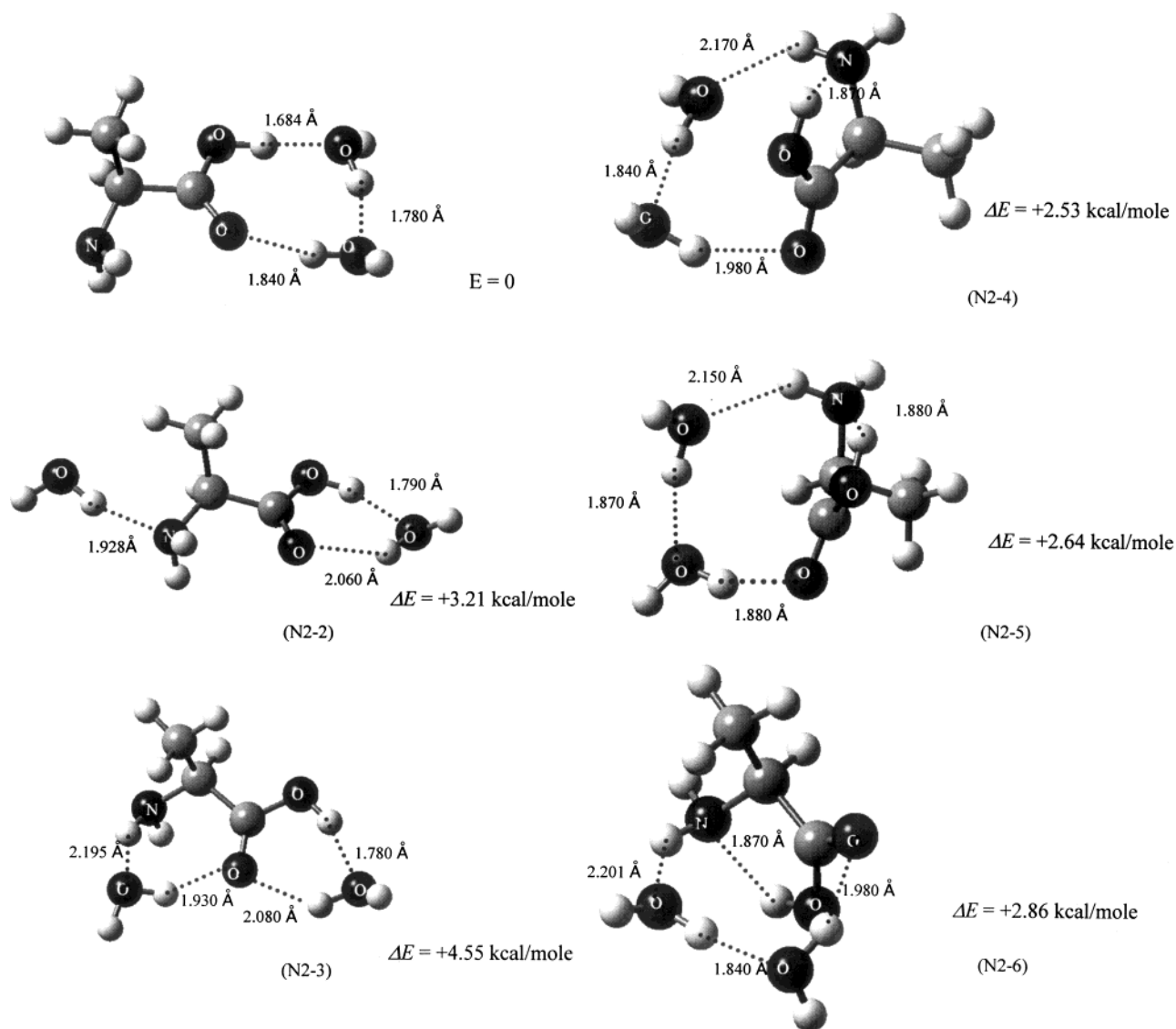


Figure 3. Lowest energy conformers of the neutral alanine-(H₂O)₂ cluster computed by the MP2/6-31++G** method (relative energies in kcal/mol).

TABLE 2: Calculated Electronic Energies and Zero-Point Energies of the Neutral Alanine-H₂O Clusters

MP2/6-311G**	<i>E</i> (Hartree)	ZPE (kcal/mol)	Δ <i>E</i> (kcal/mol)	corresponding bare alanine
(N1-1)	-399.28056	84.47	0	(N-1)
(N1-2)	-399.28053	84.47	0.02	(N-1)
(N1-3)	-399.27714	84.29	1.97	(N-1)
(N1-4)	-399.27642	84.34	2.47	(N-2)
(N1-5)	-399.27368	84.07	3.91	(N-2)
(N1-6)	-399.27401	84.82	4.46	(N-2)
(N1-7)	-399.27615	84.12	2.42	(N-3)
(N1-8)	-399.27589	84.09	2.55	(N-3)

to see how many water molecules would need to interact with the alanine molecule to yield thermodynamic and/or kinetic stability of the zwitterion isomer, and in what fashion.

Therefore, we seek to obtain the stable structures of the bare AlaZW- and the AlaZW-(H₂O)_{*n*} clusters by calculating their stationary structures. We find that there exists no stationary structure for the bare AlaZW in gas phase, which indicates that the bare AlaZW is not stable and readily isomerizes to the neutral form in the gas phase. For the AlaZW-H₂O cluster, we also fail to find the stationary structures. Thus, hydrogen

TABLE 3: Calculated Electronic Energies and Zero-Point Energies of the Neutral Alanine-(H₂O)₂

MP2/6-311G**	<i>E</i> (Hartree)	ZPE (kcal/mol)	Δ <i>E</i> (kcal/mol)	corresponding alanine
(N2-1)	-475.36308	101.27	0	(N-1)
(N2-2)	-475.35654	100.37	3.21	(N-1)
(N2-3)	-475.35505	100.78	4.55	(N-1)
(N2-4)	-475.35866	101.13	2.53	(N-2)
(N2-5)	-475.35866	101.13	2.64	(N-2)
(N2-6)	-475.35833	101.14	2.86	(N-3)

bonding with a single water molecule does not produce stable AlaZW in the gas phase.

The first AlaZW-(H₂O) cluster we find to be stationary is for *n* = 2, indicating that at least two water molecules are needed to produce a stable zwitterion-water cluster. This observation is similar to the case of glycine-water clusters studied by Jensen and Gordon.³¹ We obtain four stationary structures for the AlaZW-(H₂O)₂ cluster. Of these, the two lowest energy structures have been predicted to be unstable toward isomerization to the corresponding neutral cluster, as discussed in our previous work,⁴⁵ because the barrier height from the zwitterion clusters was computed to be smaller than the difference in the

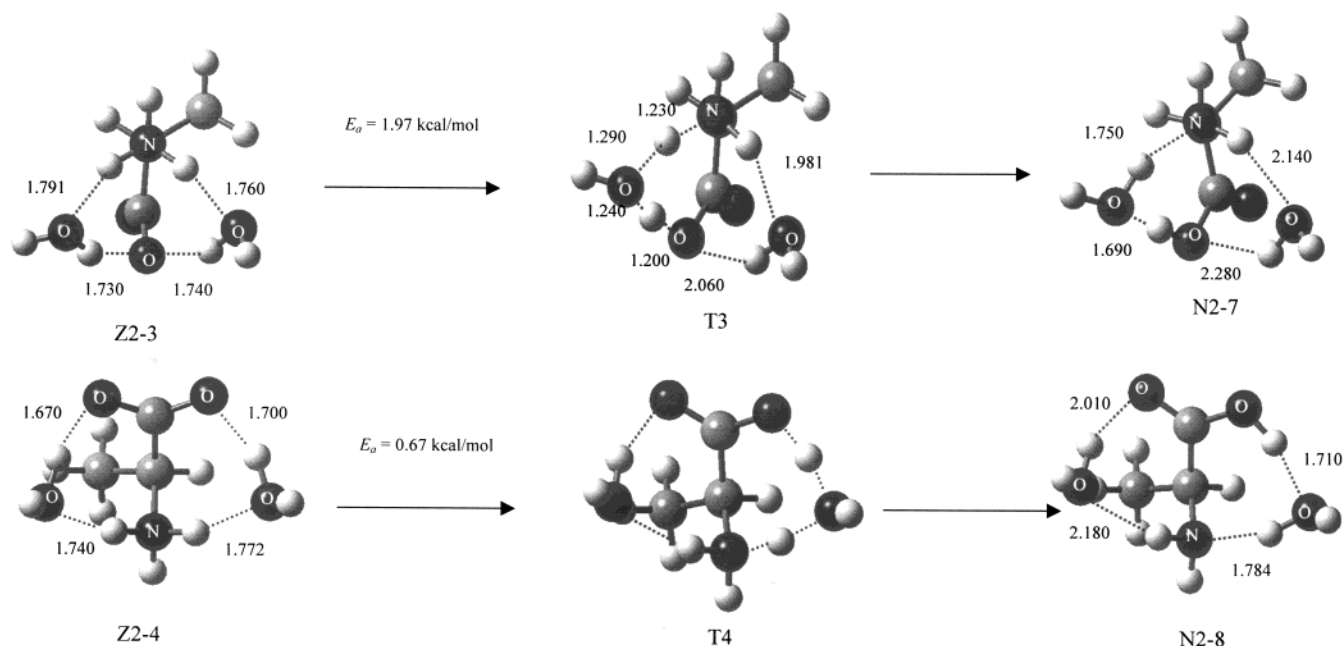


Figure 4. Concerted double proton transfer pathways from the alanine zwitterion-(H₂O)₂ cluster (Z2-2) to the neutral alanine-(H₂O)₂ cluster (N2) (bond distances in Å).

zero-point energy (that of the zwitterion cluster is larger than that of the transition state). Thus, they may spontaneously transform to the neutral alanine-(H₂O)₂ cluster by transfer of a single proton and may not be observed experimentally. The kinetically stable conformers (Z2-3 and Z2-4) we obtain for the AlaZW-(H₂O)₂ cluster are depicted in Figure 4. For the conformer (Z2-3), we have predicted that the two water molecules binding to the zwitterion by hydrogen bonding may prevent the proton at the ammonium group from moving freely to the carboxylate group, providing a small reaction barrier (3.84 kcal/mol, ZPE-corrected by MP2/6-31++G** theory) and kinetic stability for the zwitterionic cluster. We carry out similar analysis for the zwitterion conformer (Z2-4) and find that the barrier height from (Z2-4) is calculated to be about 4.31 kcal/mol (0.64 kcal/mol, when corrected for ZPE) by the BLYP/6-31++G** theory. This finding clearly indicates that the higher energy conformer (Z2-4) may be kinetically stable, at least in a very low-temperature environment.

The detailed mechanism of the isomerization (proton transfer) process between the neutral and zwitterion clusters would be highly interesting with regard to the role of solvent both in the cluster and the solution. Several groups have extensively studied the proton-transfer process of glycine, taking advantage of PCM type approximations. For example, Tunon et al.¹⁴ employed B3LYP/6-31G** and the SCRF method to study the *direct* proton transfer in the neutral/zwitterion isomerization of glycine. The role of solvent (water) studied by the PCM type approximation is (by definition) *indirect*, in the sense that the solvent may only affect the dynamics by changing the structures of the reactants, the transition state, and the products. Therefore, no studies have proposed a proton transfer mechanism in which the solvent molecules are directly involved. For the case of alanine, Tortonda et al.¹³ employed the ellipsoidal cavity model for the water continuum and calculated that the AlaZW is of lower energy (by 1.15 kcal/mol) than neutral alanine in aqueous solution in good agreement with the experimental observation. To our best knowledge, calculations for the (gas phase) alanine-H₂O cluster by the same group¹² are the only report in which the direct participation of the water molecule in the isomerization

process was mentioned for alanine-water clusters. The intermolecular proton-transfer mechanism they obtained by the Hartree-Fock level of theory, however, was not reproduced in the MP2 calculations, and thus, they concluded that the alanine-H₂O complex is not a stationary point, in agreement with our calculations.

Therefore, we calculate the dynamic pathway of isomerization from (Z2-4) both by BLYP/6-31++G** and by MP2/6-31++G**, and we find that the mechanism of this process is *concerted double proton transfer* via the binding water molecules, as depicted in Figure 4, along with the similar pathway for (Z2-3). The role of the binding water molecules are *direct* in this case, because one of them accepts a proton from the ammonium group of alanine and the other donates a proton to the carboxylate, in addition to the proton transfer between them. We believe that the concerted double proton transfer via the binding water molecules demonstrated here may serve as an excellent example of the *solvent-mediated chemical reaction*, which has not been systematically studied for biochemical reactions. Thus, this latter prediction may be highly intriguing to observe experimentally.

We have tried to find any mechanism of stepwise proton movement through intermediate structure containing the hydronium cation H₃O⁺, but failed to find one. Thus, it seems that the double proton transfer is the only feasible mechanism for transformation between the AlaZW-(H₂O)₂ and the corresponding neutral clusters.

The mechanism of concerted double proton-transfer found in this work for the dynamic pathways between the neutral alanine-(H₂O)₂ and the AlaZW-(H₂O)₂ clusters is in high contrast with the serial proton hopping mechanism proposed⁴⁶ for ion mobility of acidic water or for the rate-determining step in the reaction of human carbonic anhydrase II.⁴⁷ We believe that the absence of the stepwise proton hopping mechanism is due to the small number of water molecules in the clusters. When there exist more water molecules binding to neutral alanine and/or to AlaZW, the clusters would become more similar to pure water clusters, and thus, this latter mechanism may also contribute or may even become a dominant one.

III.5. Effects of Water Continuum on the Isomerization between AlaZW-(H₂O)_n and Neutral Alanine-(H₂O)_n (*n* = 1 and 2) Clusters: A Model of Solvent-Mediated Chemical Reaction in Aqueous Solution. It is quite obvious that the continuum approximation for the solvent, in which the solvent is represented by a few macroscopic properties such as the dielectric constant, is not very efficient for treating the solution phase where the interactions between specific functional groups in the solute and the solvent molecules largely determine properties. This would be the case especially for the solvent-mediated isomerization between neutral alanine and AlaZW discussed above. On the other hand, it is also evident that the large number of solvent molecules cannot be treated on the molecular level by quantum chemical methods and that thermodynamic properties of amino acids such as the relative stability of neutral vs zwitterionic form and the energy barrier for *intramolecular* proton transfer process in aqueous solution phase may be treated by regarding very large number of solvent molecules as continuum media. Investigators have studied the effects of the solvent continuum on the relative stability (thermodynamic and/or kinetic) of conformers both of neutral and zwitterionic form by employing a variety of continuum model. For example, Tortonda et al.¹² have studied the effects of continuum solvent on the stability of glycine and found that, although the trans form of glycine is the global minimum structure of neutral glycine in the gas phase, the cis form of glycine is the lowest in the aqueous solution phase. They also calculated that the zwitterionic alanine is of lower energy than the neutral cis form of glycine by 1.15 kcal/mol in aqueous solution by employing the MP2/6-31+G** level of theory and the ellipsoidal cavity model, with the energy barrier of 1.39 kcal/mol from the cis neutral alanine to the transition state. Although qualitatively correct, these calculated values seem to be smaller than the experimental estimations.

One possible way to improve the calculations may be to employ the discrete/solvation (the supramolecular/continuum) model,^{49–53} in which several solvent molecules directly interacting with the specific part(s) of the solute are treated explicitly by quantum chemical methods while other numerous solvent molecules are approximated as continuum. By adopting this model, one may examine the solute–solvent interactions on the molecular level while retaining the effects of infinite number of solvent molecules of secondary influence. This approach was adopted, for example, by Kassab et al.³⁰ to obtain the calculated the free energy change and the energy barrier for transformation between neutral and zwitterionic glycine in aqueous solution. They have found that the calculated results from this approach (glycine interacting with three water molecules plus continuum water) agree with the experimental observations⁴⁸ much better than the (glycine + continuum water) approximations. Zgierski et al.⁴⁹ and Leszynski et al.⁵⁰ also employed this approach to examine the proton-transfer paths in the isomerization of glycine and guanine molecules in aqueous solution, respectively.

Therefore, we also try this method in this study to study the dynamics of the aqueous solution of alanine by modeling it as “the alanine-water cluster immersed in the continuum water”, although to our best knowledge no experimental observations have been reported yet for the thermodynamics or kinetics of alanine in aqueous solution. The SCRF method would be useful in this regard, and we employ this method for the alanine-(H₂O)₂ plus water continuum model to study the effects of water continuum. Tomasi’s polarized continuum model (PCM), which is the most popular method for treating this kind of problem, is

used here. We employ the dielectric PCM (D-PCM)^{54–56} and the integral equation formalism of PCM (IEF-PCM)^{57–59} versions.

Adopting this discrete/continuum approach, we examine the effects of water continuum on the isomerization of neutral/zwitterionic alanine-(H₂O)_n (*n* = 1 and 2) cluster. As already discussed above, two water molecules, need to bind to produce stable AlaZW in the gas phase. We find, on the other hand, that the AlaZW-H₂O cluster may also be stable in the presence of water continuum. Their structures and relative energies are presented in Figure 5. We find that the relative energies of the conformers depend on the methods employed, although the energy differences are small. The conformer (Z1P-1) is the most stable structure obtained by the B3PW91/6-31++G(d,p) method combined with IEF-PCM, but (Z1P-3) is more stable than (Z1P-1) when employing the BLYP/6-31++G(d,p) method for the discrete part. The water molecule binds to the carboxylate group in (Z1P-2) and (Z1P-3) and the difference is only in the dihedral angle ∠(H–N–C–C). The binding water molecule bridges the ammonium and carboxylate groups of the alanine moiety in (Z1P-1). We find that AlaZW-H₂O is more stable (Gibbs energy lower by 4.63 kcal/mol) than the neutral alanine-H₂O cluster in the presence of water continuum, indicating that alanine would be observed as zwitterionic rather than the neutral form. The AlaZW-H₂O cluster (Z1P-1) is calculated to isomerize to the neutral alanine-H₂O cluster (N1P-2). In the isomerization process, the proton migrates from ammonium to the carboxylate group through water by the concerted double proton-transfer mechanism, as depicted in Figure 6. This isomerization mechanism may be considered as a model of solvent-mediated proton-transfer process in aqueous solution.

The structures and the isomerization reaction of the neutral/zwitterionic alanine-(H₂O)₂ cluster discussed above in section III-4 are also expected to be influenced by the presence of continuum water. Thus, we carry out similar supramolecule/continuum model calculations for the alanine-(H₂O)₂ cluster/water continuum system. The structures of the zwitterionic alanine-(H₂O)₂ clusters in the presence of the water continuum are given Figure 7. The structure (Z2P-1), which corresponds to the gas phase (Z2-3) in Figure 4, are found not to isomerize to the neutral cluster by the effects of the water continuum. It seems that as the isomerization proceeds, the bond between the carboxylate group and the carbon center rotates toward the water molecule located along the proton-transfer pathway. This change may weaken the hydrogen bonding with the other water molecule, increasing the interactions with the ammonium group. Thus, the water molecule favors the bonding with ammonium in a linear fashion, and the double proton transfer may not occur.

The other conformer of the zwitterionic alanine-(H₂O)₂ cluster of slightly higher Gibbs energy (by 0.01 kcal/mol) in the presence of water continuum is (Z2P-2), also depicted in Figure 7. In this conformer, one of the two water molecules is located significantly farther from the alanine moiety than the other. As can be seen in Figure 7 that water molecule does not participate in the concerted double proton transfer pathway. The distance between the hydrogen atom of that water molecule and the oxygen-of the carboxylate group is calculated to be 3.397 Å, far larger than any normal bond distance. Thus, this water molecule cannot be considered as forming hydrogen bond with the ammonium group in any sense, and it may rather be regarded to be “solvated” by the water continuum (on the other hand, in the gas-phase clusters (Z2-3) or (Z2-4) in Figure 4 the two water molecules are located more or less symmetrically with respect to the alanine moiety, even though only one of them play role

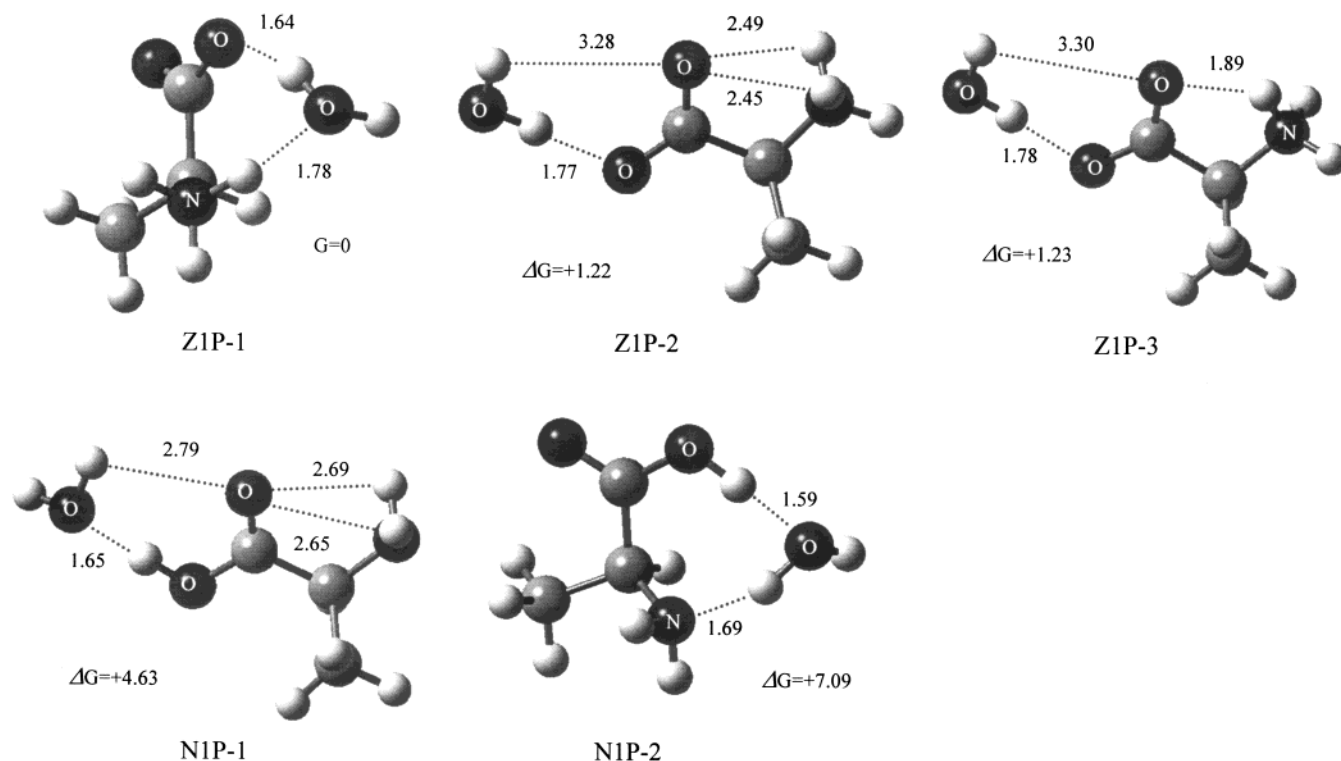


Figure 5. Lowest energy conformers of alanine - (H_2O) cluster in the presence of water continuum (B3PW91/6-31++G(d,p) combined with IEF-PCM).

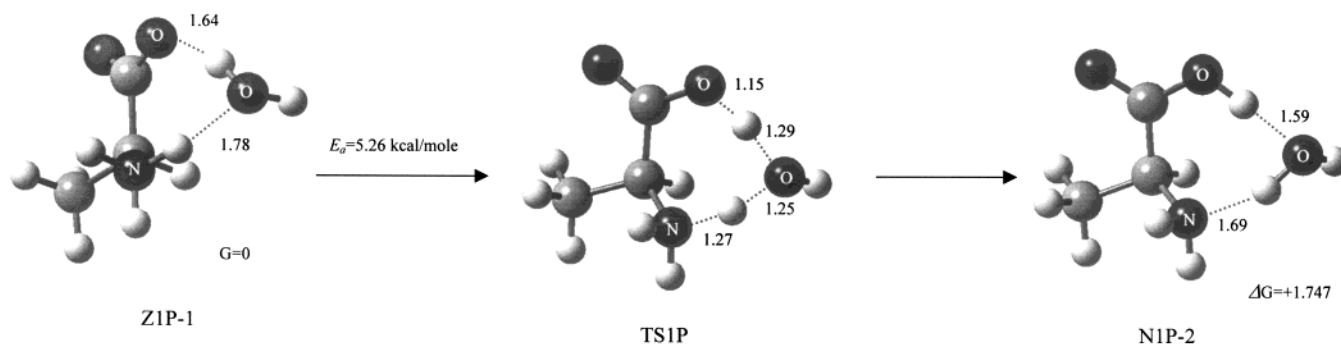


Figure 6. Isomerization of neutral/zwitterionic alanine-(H_2O) cluster in the presence of water continuum. (BLYP/6-31++G(d,p) combined with IEF-PCM).

in the proton transfer process). This latter observation is clearly the difference that the presence of water continuum makes on the structure of the alanine-(H_2O)₂ water cluster. The isomerization reaction of (Z2P-2) to the corresponding neutral alanine-(H_2O)₂ cluster is also shown in Figure 6. We find that the reaction occurs by the concerted double proton-transfer mechanism in the presence of the water continuum as in the case of gas-phase neutral/zwitterionic alanine-(H_2O)₂ clusters. The reaction barrier from (N2P-2) is calculated to be 6.71 kcal/mol. We find that the cluster AlaZW-(H_2O)₂ (Z2P-2) is more stable (Gibbs energy lower by 4.23 kcal/mol) than the neutral alanine-(H_2O)₂ cluster (N2P-2) in the presence of water continuum.

III.6. Effects of Water Continuum on Bare Alanine: A Model of Amino Acid in Aqueous Solution. Finally, we examine the effects of continuum water on the structure and the reaction of bare alanine itself to see whether the presence of water continuum could produce the experimentally observed properties of alanine. We focus especially on whether the relative thermodynamic stability of neutral/zwitterionic alanine or of the conformers of neutral alanine in the solution phase may change from the prediction for the gas phase. As discussed

above, the relative stability of the alanine conformers (N-2) and (N-3) may be significantly altered in the gas phase by the presence of a binding water molecule. Thus, it will be very intriguing to see how it would be affected by the water continuum to predict the lowest-energy conformer of alanine in aqueous solution phase. We have tried to employ the BLYP/PCM method for the alanine plus water continuum system but found that the method does not produce the converged results. After extensive testing of various discrete/continuum models, we find that combining the B3PW91/6-31++G** method for the discrete part (alanine) and the IEFPCM for the water continuum (B3PW91/IEFPCM) may successfully give the structures of alanine conformers in the presence of water continuum, as shown in Figure 8. Several important observations are to be noticed, concerning the effects of water continuum on the properties of neutral and zwitterionic alanine. First of all, we find that AlaZW becomes a stationary structure ((ZP-1) in Figure 8) in the presence of water continuum, in contrast to the case of gas-phase alanine. Then, the immediate question will be whether the discrete/continuum model calculations may affect the relative thermodynamic stability of neutral and

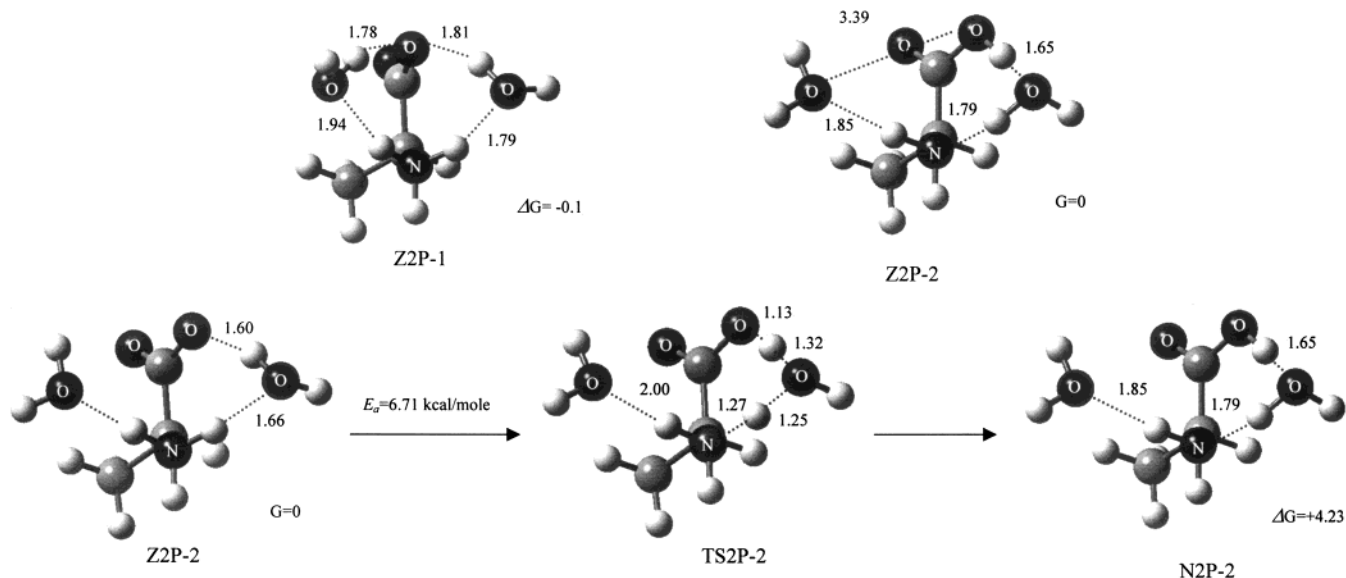


Figure 7. Structures and isomerization of neutral/zwitterionic alanine-(H₂O)₂ cluster in the presence of water continuum. (BLYP/6-31++G(d,p) combined with D-PCM).

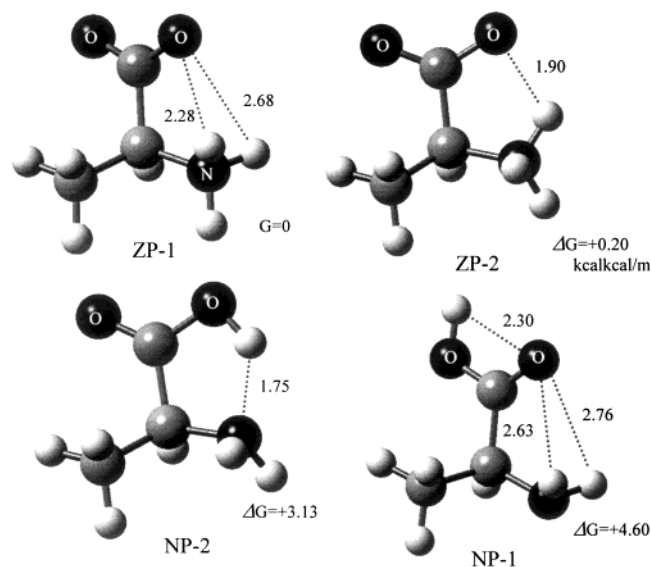


Figure 8. Lowest energy conformers of alanine in the presence of water continuum (B3PW91/6-31++G(d,p) combined with IEF-PCM).

zwitterionic alanine in aqueous solution (AlaZW is observed experimentally to be much more stable in aqueous solution). We calculate that the free-energy difference ($G(\text{ZP-1}) - G(\text{NP-2})$) between AlaZW and the lowest-energy neutral alanine (NP-2) is -3.13 kcal/mol by the B3PW91/IEFPCM method. To our best knowledge, no experimental values are known for this property yet. However, assuming that the free energy difference for the alanine system is similar to that⁴⁸ (-7.27 kcal/mol) of neutral/zwitterionic glycine in aqueous solution, the calculated value seems to be small. The structures of the gas phase neutral alanine conformers are also found to change significantly in the presence of continuum water. For example, the alanine conformers (N-2) and (N-3) exhibiting different dihedral angles in the gas phase (see Figure 1) are calculated to be of very similar structures and energy by the influence of continuum water (NP-2 in Figure 8). It is extremely interesting to observe that the conformer (NP-1) corresponding to lowest energy-gas phase conformer (N-1) with the OH and NH₂ groups in trans positions now lies higher in energy (by 1.42 kcal/mol) than the conformers (NP-2), which correspond to the higher energy gas

phase conformers (N-2) and (N-3). This finding clearly indicates that the thermodynamic stability of gas-phase alanine conformers would be different from that in the solution phase.

IV. Conclusions

Our present results clearly have shown that the solvent molecules interacting with the functional group(s) in the solute may profoundly affect the structures, the thermodynamic properties, and the reactivity of alanine both in the gas-phase clusters and in aqueous solution phase, providing an excellent example of the influence of microsolvation on the structures and reactions of biomolecules. We have shown that the binding water molecules may alter the relative thermodynamic stability of the neutral and zwitterionic alanine conformers in the gas-phase clusters. Treating an infinite number of water molecules by the PCM type approximations in discrete/continuum methodology has been shown to provide results that are in qualitative agreements with the experimental observations for neutral and zwitterionic alanine in aqueous solution: for example, AlaZW is calculated to be more stable than neutral alanine in the solution phase in contrast to the gas phase.

We have also presented systematic study for the reaction pathways between neutral and zwitterionic alanine both in the gas-phase clusters and in solution by carrying out the IRC analysis, as an example of the solvent-mediated chemical reaction. We have demonstrated that the solvent molecule(s) may profoundly influence the reaction mechanism. The AlaZW-(H₂O)₂ clusters may isomerize to the neutral form either by direct proton transfer or by the concerted double proton-transfer mechanism, depending on the conformation. We believe that the present work will obtain fundamental importance in this highly interesting subject, especially in view of recent experimental advances in the separation and reaction of conformers in the gas phase, as beautifully demonstrated by several experimental groups.^{15,16,60}

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