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Barrierless Proton Transfer within Short Protonated Peptides in the Presence of Water Bridges. A Density Functional Theory Study

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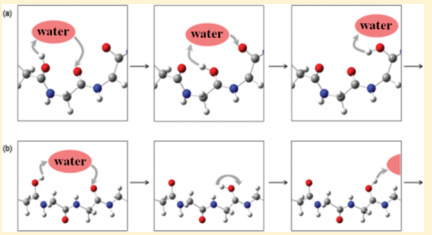
Supporting Information

ABSTRACT: We have used density functional theory at the B3LYP/6-31++G(d,p) level of theory to investigate proton transfer in protonated N^2-acetyl-N^1-methylglycinamide and *N*-acetylglycyl-N^1-methylglycinamide with multiwater assistance and to determine the structures and energies of the most important minima and transition states corresponding to the proton-transfer pathways. We propose mechanisms for proton transfer between adjacent and nonadjacent carbonyl oxygen atoms with water bridge assistance. The presence of a two-water bridge connected to the two carbonyl oxygen atoms provides a proton-transfer mechanism having such a low-barrier that the excess proton is almost freely mobile.



INTRODUCTION

Proton transfer along peptides is a critical biological process. The role of hydrogen bonding in these processes has been identified in the mechanisms of many biological processes,[1] especially enzymatic reactions and bioenergetic proton translocations.[2−4] Since Grutthus proposed his famous concept of highly mobile proton transfer through water about 200 years ago,[5] the role played by water molecules during proton transfer has attracted much interest.[6−11] At present, we have quite a good understanding of the structures of protonated water clusters,[6,7] which are described using two main basic models: the Eigen cation[8] $[H^+(H_2O)_4]$ and the Zundel cation[9] $(H_2O\cdots H^+\cdots OH_2)$. In the Eigen cation, the hydronium core H_3O^+ ion is bound to three water molecules; in the Zundel cation, the proton is shared between two water molecules. Interconversion between Eigen and Zundel cation leads to proton transfer in water.[10] For peptides in water, a series of water chains presumably function as a bridge connecting the sites of a peptide, allowing the proton to be pumped from one site to another.[11]

Protonation on the amide nitrogen atom weakens an amide bond, enhancing the rate of fragmentation of the peptide; in contrast, protonation on the amide oxygen atom strengthens the amide bond relative to that of its neutral counterpart. Previous calculations[12,13] have revealed that protonation on the amide nitrogen atom is thermodynamically disfavored compared with protonation at other sites, such as the amide oxygen atom of the N-terminal amino group. Another theoretical study[14] of protonated glycylglycylglycine revealed that protonation on the N-terminal nitrogen atom produces an isomer that is approximately 1 kcal mol^{-1} higher in free energy than the isomers resulting from protonation on the carbonyl oxygen atom of the N-terminal residue, where hydrogen bonding to the N-terminal nitrogen atom is stabilizing. Several studies have focused on proton migration between the oxygen and nitrogen atoms of peptide bonds.[14−17] Notably, a study of proton migration in protonated glycylglycylglycine revealed that an intramolecular proton transfer from an oxygen atom to a nitrogen atom of an amide bond has a high energy barrier of 39.1 kcal mol^{-1},[14] implying that the only interactions that are important for proton transfer between peptide bonds are those involving oxygen atoms, particularly the carbonyl oxygen atoms. A study of protonated *N*-formylglycylglycinamide revealed that proton transfer along the backbone of the peptide chain most likely occurs through proton hopping between the oxygen atoms of adjacent amide bonds, with the rate-determining step being an internal rotation-type transition of the protonated C═O$^+$—H group between two adjacent carbonyl groups (C═O$^+$—H···O═C), with an energy barrier of 16.5 kcal mol^{-1}.[16] To describe proton transfer accurately, a particularly long peptide chain should be used as a model; the flexibility of such a chain would, however, lead to a very complicated reaction potential energy surface and time-consuming calculations. The unblocked terminal amino group of a peptide will either interact directly with the proton or, at least, have a strong influence on the interactions of the proton with other parts of the molecule. Therefore, we chose the model peptides N^2-acetyl-N^1-methylglycinamide (diamide)[18] and *N*-acetylglycyl-N^1-methylglycinamide (triamide)[19] to investigate proton transfer along peptides.

The hypothetical proton transfer along the hydrogen-bonding network in bacteriorhodopsin is strongly affected by the protein−water environment.[20−23] Garczarek et al. found that intraprotein

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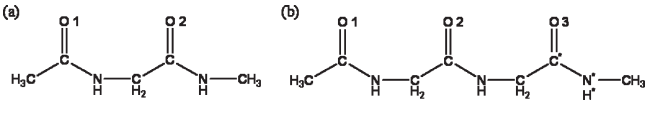

Figure 1. Structures of the peptides (a) N^2-acetyl-N^1-methylglycinamide (diamide) and (b) *N*-acetylglycyl-N^1-methylglycinamide (triamide).

water molecules are essential for biological functions and, through measurement of time-resolved Fourier transform infrared difference spectra, proposed a mechanism for proton exchange between second-shell water molecules and the protein.[20,21] Several theoretical and experimental studies have increased our understanding of the interactions of biomolecules with water clusters.[17−27] During proton-transfer processes, water molecules behave as both the solvent and as "hydrogen bridges"; they give or accept protons to enhance long-range proton transfer.[28,29] Nevertheless, the complicated set of interactions between biomolecules and water molecules makes the study of proton transfer between heterogeneous peptides ambiguous in theory. The geometries of the water clusters and of the peptide itself undergo reorientation to a considerable degree during proton migration because of the flexibility of their hydrogen bonds and the low energy barriers for the rotational motions of the peptides. Thus, we wished to ideally clarify the mechanism of proton transfer along these model peptides and between heterogeneous peptides with multiwater assistance. In this study, we performed density functional theory (DFT) calculations to study proton migrations between the oxygen atoms of the carbonyl groups in protonated N^2-acetyl-N^1-methylglycinamide and *N*-acetylglycyl-N^1-methylglycinamide associated with one, two, or three water molecules through water-bridged connected by hydrogen bonds. We used the former model peptide to study the mechanism of proton transfer between two adjacent carbonyl oxygen atoms with water bridge assistance, thereby mimicking proton transfer along the peptide backbone; we used the latter model peptide to study the mechanism of proton transfer between two nonadjacent carbonyl oxygen atoms with water bridge assistance, thereby imitating both proton transfer within peptides and proton transfer between heterogeneous peptides.

COMPUTATIONAL DETAILS

All geometry optimizations and frequency determinations were performed using the Gaussian 03 package of programs[30] at the B3LYP computational level,[31] with a 6-31+G** basis set used throughout this study, as has been used in previous studies.[18,19] The minima [number of imaginary frequencies, (NIMAG) = 0] and transition states (NIMAG = 1) were confirmed through calculations of harmonic vibrational frequencies, which were also used to obtain zero-point vibrational energies (ZPE).

Figure 1 displays the structures of the model peptides, which had been proposed by Kulhanek et al., and are well representative as real protein.[18,19] In this study, N^2-Acetyl-N^1-methylglycinamide (diamide) was used to investigate proton transfer between adjacent carbonyl oxygen atoms; *N*-acetylglycyl-N^1-methylglycinamide (triamide) was used to study proton transfer between nonadjacent carbonyl oxygen atoms. This diamide and triamide, which lack carboxyl and amino termini, are objectively considered as resembling the central fragments of long peptides. Additionally, the triamide is the smallest unit featuring nonadjacent oxygen atoms. Although a real peptide can fold in such a way that any two sites can be located in close proximity, herein the oxygen atoms O1 and O3 on the triamide are considered to be nonadjacent sites. This study was concerned primarily with the role of the assistant water molecules,

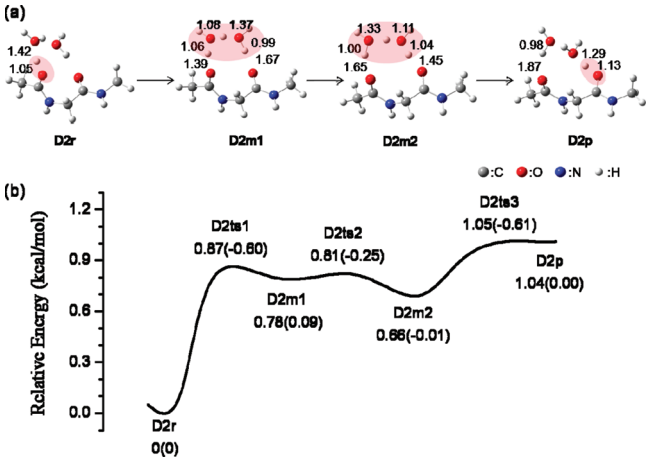

Figure 2. (a) Local minima structures and (b) potential energy surface (values in parentheses are corrected for the ZPE) for proton transfer within a heterogeneous diamide in the presence of a two-water-molecule bridge.

inserted as a bridge between two adjacent or nonadjacent oxygen atoms. In the starting arrangements, the water bridge was placed in position from which favorable hydrogen bonds could be formed between two carbonyl oxygen atoms which a proton has located at carbonyl oxygen atom. However, the real global minima of the water clusters of the amides were not sought; only the obvious and representative structures describing the paths of proton transfer along the peptide with assistant water molecules are described herein. To calculate the potential energy surface of water-assisted proton transfer between two carbonyl oxygen atoms, the distance between the proton and carbonyl oxygen atom was chosen as the driven internal coordinate, with the step size from 0.01 to 0.1 Å. The structure with the maximum energy was then considered to be a trial transition state and submitted to the full transition-state optimization as described above.

RESULTS AND DISCUSSION

The assistant water in heterogeneous water−peptide complex for proton transfer has been considered with the function that peptide exchanges excess protons with the water cluster, which connects two sites of peptide, water cluster can obtain a proton from peptide and release another proton back to peptide which leads the proton migrating along peptide.[17,18] To further explore the nature of proton transfer mechanisms in peptide, we used the model peptides, as shown in Figure 1, with a water bridge between the amide carbonyl oxygen atoms.

A. Proton Transfer Between Adjacent Carbonyl Oxygen Atoms. From a study of one-water-molecule-assisted proton transfer on N^2-acetyl-N^1-methylglycinamide, Kulhanek et al.[18] reported that proton transfer from O1 to O2 (presented in Figure 1a), mediated through a water molecule by exchanging a proton, has a rate-determining Gibbs energy barrier of 7.6 kcal mol^{-1}. In this present study, we considered two-water-molecule-assisted proton transfer on the model diamide. In Figure 2a, the initial state is D2r—the reactant comprising the protonated diamide bound to two water molecules. An excess proton in D2r is located to the left of O1 with a distance of 1.05 Å; the water bridge connects O1 and O2 of the diamide. The proton is transferred in three steps from O1 to O2 through a two-water-molecule bridge: D2r → D2m1, D2m1 → D2m2, and D2m2 → D2p. This process involves shifting protons along the network of

Table 1. Relative Electronic Energies (ΔE), Sums of Electronic and Thermal Enthalpies (ΔH), and Sums of Electronic and Thermal Free Energies (ΔG) for Proton-Transfer Reactions along the Diamide[a]

	ΔE (ZPE)	ΔH	ΔG	barrier (ZPE)
		Reaction D2		
D2r	0 (0)	0	0	
D2ts1	0.87 (−0.60)	−1.41	0.32	0.87 (−0.60)
D2m1	0.78 (0.09)	−0.38	0.39	
D2ts2	0.81 (−0.25)	−1.25	1.03	0.03(−0.34)
D2m2	0.66 (−0.01)	−0.48	0.16	
D2ts3	1.06 (−0.61)	−1.34	0.22	1.60(−0.60)
D2p	1.04 (0.004)	−0.27	0.36	
		Reaction D3		
D3r	0 (0)	0	0	
D3ts1	0.27 (−0.73)	−1.47	0.43	0.27 (−0.73)
D3m1	0.24 (−0.20)	−0.61	0.27	
D3ts2	0.32 (−0.69)	−1.57	0.31	0.08 (−0.49)
D3m2	0.30 (−0.21)	−0.64	−0.07	
D3ts3	2.75 (1.51)	0.79	2.89	2.45 (1.72)
D3p	2.40 (2.62)	2.41	3.72	

[a] Energy unit in kcal/mol; Numbers in parentheses are the relative energies with ZPE correction.

hydrogen bonds. Proton transfers are the concerted processes of the breaking and making of O−H bonds, which correspond to heterolytically dissociating and re-forming individual H_2O molecules, in interchange with the making and breaking of the associated hydrogen bonds.[5] Our calculations demonstrated that a Zundel-like cation appeared in the local minima, in agreement with previous studies.[20−22] In D2m1, the proton is closer to O1, whereas in D2m2 the proton is closer to O2 (see Figure 2a). In the final product D2p, the proton is shared by the water dimer and O2; the H···O2 distance is 1.13 Å. Specifically, proton transfer occurs from the left side of O1 to the left side of O2. This finding implies that the excess proton on O2 can undergo continuous transfer to the next oxygen atom through another water-dimer bridge with similar procedure. The previous proposed mechanism[16] via the internal rotation of the C═O$^+$—H bond is unfavorable for continuous transfer, which is a high barrier process.

Figure 2b displays the potential energy surface for proton transfer, mediated through a two-water-molecule bridge, between two adjacent carbonyl oxygen atoms in the diamide. Table 1 lists all the calculated energetic data for water-assisted proton transfer along the model diamide. After ZPE corrections (the values in parentheses), the energies of D2r, D2m1, D2m2, and D2p are very close, and the proton transfer is barrierless. Therefore, we conclude that the proton is freely mobile between the adjacent carbonyl oxygen atoms when assisted by the two-water-molecule bridge.

Figure 3a illustrates the proton transfer scheme with the assistance of a three-water-molecule bridge. Again, three steps exist from the left side of O1 to the left side of O2: D3r → D3m1, D3m1 → D3m2, and D3m2 → D3p. Figure 3b presents the potential energy surface for this proton-transfer process. With ZPE correction, D3m1 and D3m2 are more stable than D3r by ca. 0.2 kcal mol^{-1}. The reactions from D3r to D3m1 and from D3m1 to D3m2 are both barrierless, but that from D3m2 to D3p

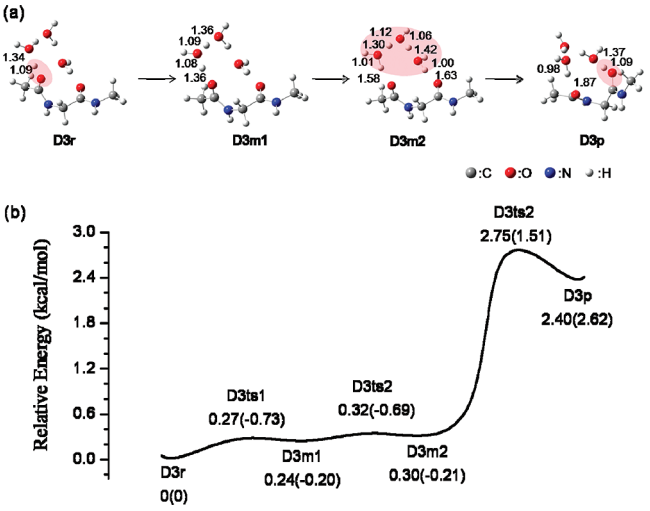

Figure 3. (a) Local minima structures and (b) potential energy surface (values in parentheses are corrected for the ZPE) for proton transfer within a heterogeneous diamide in the presence of a three-water-molecule bridge.

possesses a barrier of 1.72 kcal mol^{-1} and involves considerable backbone distortion.

The proton affinity (PA) of the three-water-molecule bridge is greater than that of the carbonyl oxygen atom in the diamide (see the Supporting Information (Table S1) and a previous report from Zhang et al.[32]). Therefore, unlike the case with the two-water-molecule bridge, in which the proton is close to the carbonyl oxygen atom, the proton prefers to be located at the water cluster in the case of the three-water-molecule bridge. Similar to the situation with the two-water-molecule bridge, the excess proton in the case with the three-water bridge can migrate continuously from the left side of the carbonyl oxygen atom to the left side of the adjacent carbonyl oxygen atom along the peptide. Figure S1 in the Supporting Information presents detailed structures of the intermediates in these reactions.

B. Proton Transfer Between Nonadjacent Carbonyl Oxygen Atoms. In addition to examining proton transfer along adjacent amide bonds, we also used the modeled triamide to investigate proton transfer along nonadjacent amide bonds. Kulhanek et al.[19] proposed a mechanism for proton transfer between nonadjacent carbonyl oxygen atoms (from O1 to O3, see Figure 1b) through a process involving backbone distortion without internal rotation; the rate-determining step in this case also featured an energy barrier of 8.3 kcal mol^{-1}. Herein, we propose a different proton-transfer mechanism with a water-bridged assistance.

One-Water-Molecule Bridge. Figure 4a displays T1r, the structure of the triamide in which the excess proton is located at O1 with a one-water-molecule bridge connecting O1 and O3, which we used to investigate the proton-transfer reaction. The proton migration from O1 to O3 is a one-step reaction; because the PA of the water molecule is less than that of a carbonyl oxygen atom, the proton prefers to reside at the carbonyl oxygen atom rather than on the water molecule. The product, T1p, is slightly more stable than T1r by 0.44 kcal mol^{-1} (Figure 4b). When treated with ZPE correction, both the forward and backward reactions are barrierless. Table 2 lists all the calculated energetic data for the water-assisted proton transfer along the modeled triamide.

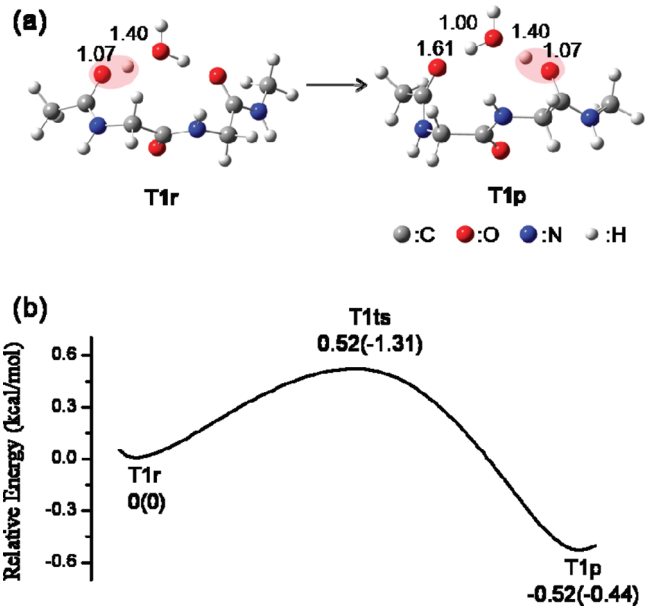

Figure 4. (a) Local minima structures and (b) potential energy surface (values in parentheses are corrected for the ZPE) for proton transfer within a heterogeneous triamide in the presence of a one-water-molecule bridge.

Table 2. Relative Electronic Energies (ΔE), Sums of Electronic and Thermal Enthalpies (ΔH), and Sums of Electronic and Thermal Free Energies (ΔG) for Proton-Transfer Reactions along the Triamide[a]

	ΔE (ZPE)	ΔH	ΔG	barrier (ZPE)
		Reaction T1		
T1r	0 (0)	0	0	
T1ts	0.52 (−1.31)	−1.81	−0.18	0.52 (−1.31)
T1p	−0.53 (−0.44)	−0.41	0.07	
		Reaction T2		
T2r	0 (0)	0	0	
T2ts	1.87 (−0.54)	−1.18	0.31	1.87 (−0.54)
T2m	1.69 (0.13)	−0.14	0.52	
T2ts2	1.79 (0.24)	−0.60	1.83	0.10 (0.11)
T2p	−3.36 (−2.22)	−2.60	−0.57	
		Reaction T2d		
T2r	0 (0)	0	0	
T2dts1	0.10 (−0.01)	−0.56	1.62	0.10 (−0.01)
T2dm1	−0.44 (0.08)	−0.23	1.20	
T2dts2	1.05 (−0.57)	−1.45	1.00	1.49 (−0.65)
T2dm2	0.953 (−0.63)	−0.88	−0.66	
T2dts3	0.954 (−0.77)	−1.56	0.61	0.00$_1$ (−0.14)
T2p	−3.36 (−2.22)	−2.60	−0.57	

[a] Energy unit in kcal/mol; Numbers in parentheses are relative energies with ZPE correction.

Two-Water-Molecule Bridge. Here, we consider two possible pathways for proton transfer between nonadjacent carbonyl oxygen atoms of the modeled triamide with the assistance of a two-water-molecule bridge. The first (Figure 5a) is direct proton transfer via the two-water-molecule bridge, in which the proton

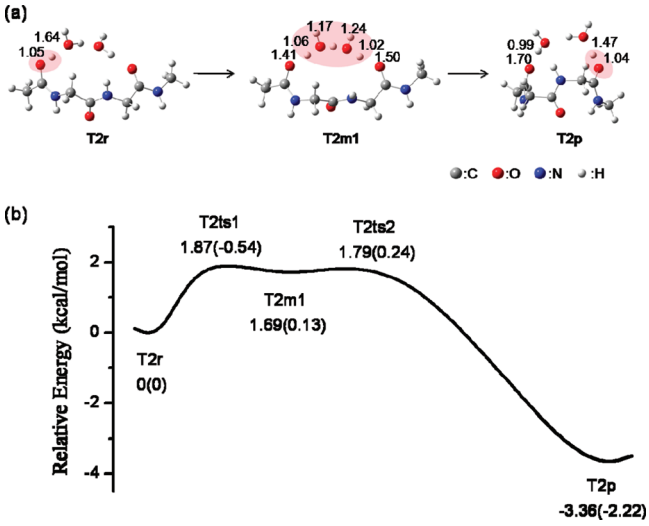

Figure 5. (a) Local minima structures and (b) potential energy surface (values in parentheses are corrected for the ZPE) for the two-step proton transfer within a heterogeneous triamide in the presence of a two-water-molecule bridge.

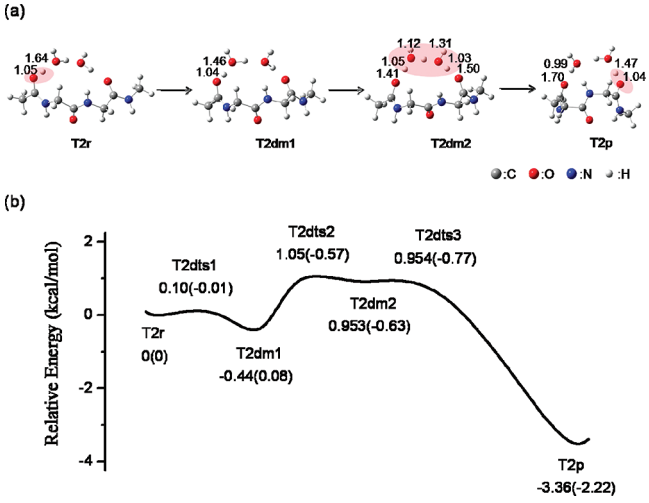

Figure 6. (a) Local minima structures and (b) potential energy surface (value in parentheses are corrected for the ZPE) for three-step proton transfer within a heterogeneous triamide in the presence of a two-water-molecule bridge.

transfers from O1 to the water cluster (T2r → T2m1), which then transports a different proton to O3 (T2m1 → T2p). The second (Figure 6a) is caused by internal rotation of the triamide backbone to another minimum (T2r → T2dm1), proton transfer from O1 to the water cluster (T2dm1 → T2dm2), and transport of a different proton to O3 (T2dm2 → T2p). The location of the proton in the initial state, T2r, is to the right of O1 (Figure 5a); in the final state, T2p, it is positioned to the left of O3 (Figure 6a). Thus, the proton is localized between these two carbonyl groups, and further transfer is unfavorable if considering only such a proton-transfer mechanism. Because the PA of a water dimer is similar to that of a carbonyl oxygen atom, both the intermediates (for proton migration to the water cluster) T2m1 and T2dm2 have Zundel-like cation structures. The energy of the final state (T2p) is lower than those of the other minima because of the formation of the intramolecular N*H*···O2 hydrogen bond.

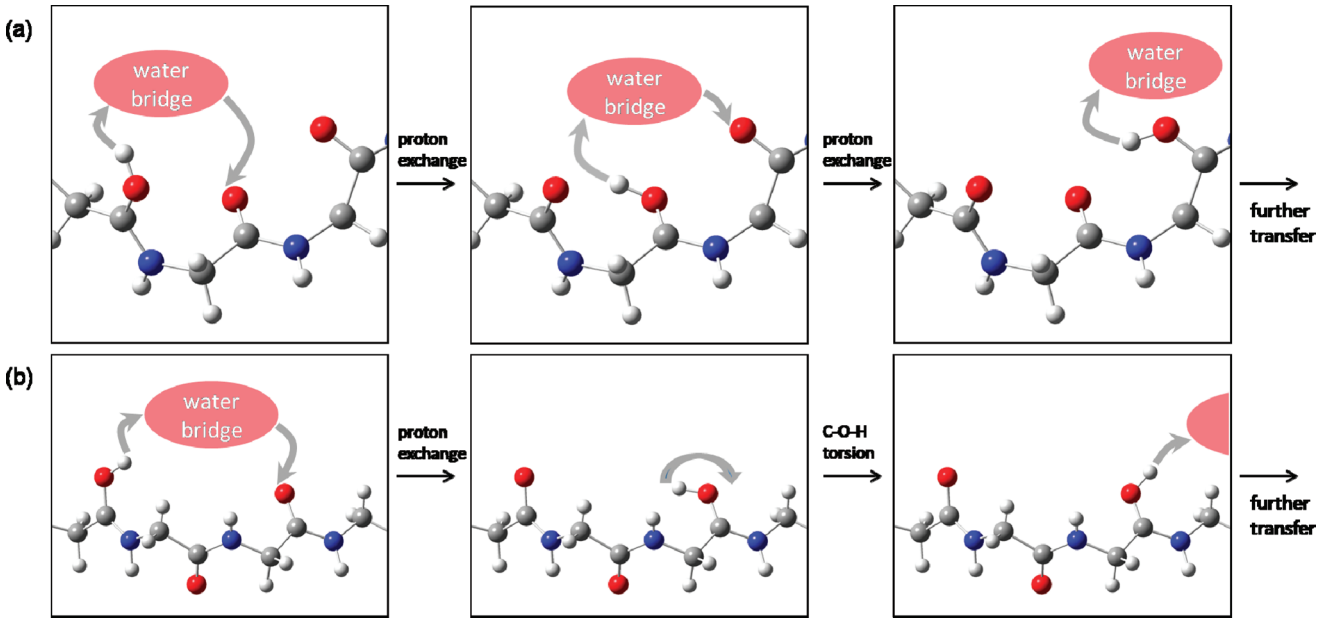

Figure 7. Proton-transfer pathways: (a) continuous mechanism, in which the proton migrates in a stepwise manner between adjacent carbonyl oxygen atoms; (b) regional mechanism, in which the proton is transferred locally, for a distance by a water bridge, between nonadjacent carbonyl oxygen atoms.

Table 2 lists the energetic data of the local minima and transition states. All the energies—including the electronic energies, enthalpies, and free energies—of the minima and maxima are very close. The potential energy surfaces in Figures 5b and 6b reveal that the reactions of these two pathways are either barrierless or possess very low barriers, presumably because the PAs of the carbonyl oxygen atom and water dimer are close, causing the proton to be freely mobile among the O1 atom, the water dimer, and the O3 atom. For the T2r → T2p reaction, the location of proton in the initial state, T2r, is to the right of O1 (Figure 5a); in the final state, T2p, it is to the left of O3 (Figure 6a). Thus, the proton is localized between these two nonadjacent carbonyl groups; further migration is unfavorable if considering only such a proton-transfer mechanism.

C. Proton-Transfer Mechanism. The proton-transfer mechanism proposed previously[18,19] features rate-determining barriers of ca. 7−8 kcal mol^{-1}; in contrast, our present investigation suggests that regardless of whether the proton transfer occurs between adjacent or nonadjacent carbonyl oxygen atoms, the calculated barriers are low—or even barrierless—with water assistance. Indeed, the concept of highly mobile proton transfer through water is even reported along the peptide.[5] Figure 7 summarizes the possible proton-transfer pathways. In the presence of an assisting water bridge, the proton can be transported through the middle fragment of peptide between adjacent or nonadjacent carbonyl oxygen atoms. We propose two mechanisms involving assisting water bridges: (i) a continuous mechanism (Figure 7a), in which proton transfer occurs between adjacent carbonyl oxygen atoms in a stepwise manner; and (ii) a regional mechanism (Figure 7b), in which proton transfer occurs between nonadjacent carbonyl oxygen atoms, but with the proton being transported only locally for a distance by the water bridge—continued transfer must occur via peptide backbone distortion or in combination with the continuous mechanism. Of course, we have deduced this proton-transfer mechanism based on a simplified peptide model in which the PAs of the carbonyl oxygen atom and water-dimer bridge are close. The mechanism for a real peptide would probably be more complicated, because the carbonyl oxygen atoms would have different PAs as a result of the presence of various side chains, resulting in localization of the proton on high-PA sites, making proton transfer a higher energy barrier process.

CONCLUSION

We have used DFT to investigate the mechanisms of proton transfer along a model diamide and triamide featuring water bridges. We propose two possible proton-transfer mechanisms: a continuous mechanism, in which proton transfer occurs between adjacent carbonyl oxygen atoms with the assistance of a two-water-molecule bridge, and a regional mechanism, in which proton transfer occurs between nonadjacent carbonyl oxygen atoms with water-bridge assistance. Our calculations reveal that proton transfer between the carbonyl oxygen atoms on the diamide is almost barrierless when assisted by a two-water-molecule bridge, and that the proton-transfer barrier along the model triamide is also low with one- or two-water-molecule bridge assistance. The location of the proton is dependent on the length of the water bridge. For the case of a one-water-molecule bridge, the proton is located at the carbonyl oxygen atom, because the water monomer has a lower PA. Water bridges featuring two or more water molecules, however, have similar PAs as a carbonyl oxygen atom, allowing the proton to move almost freely between the water cluster and the amide units. Our calculations reveal that Zundel-like cations ($H_5O_2^+$) can be observed in the local minima.

ASSOCIATED CONTENT

Supporting Information. Table S1 lists the calculated PAs of multiwater clusters (1−5 water molecules) and model peptides, diamide, and triamide. Figures S1 and S2 provide the structures of local minima and maxima, respectively, reported herein. Figure S3 and S4 illustrate the potential energy surface of for proton transfer within a heterogeneous triamide in the

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presence of a two-water-molecule bridge surrounded by water monomer and dimer, respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

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