Systematic Study of the Tautomerism of Uracil Induced by Proton Transfer. Exploration of Water Stabilization and Mutagenicity

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To systematically investigate all the possible tautomerisms from uracil (U) and its enol form (U*) induced by proton transfer, we describe a study of structural tautomer interconversion in the gas phase, in a continuum solvent, and in a microhydrated environment with 1 or 2 explicit water molecules, using density functional theory (DFT) calculations by means of the B3LYP exchange and correlation functions. A total of 62 geometries including 25 transition states were optimized, and the geometrical parameters have been discussed. Some rules of the configuration variation in tautomerization were summarized. The relative stabilities of all the tautomers were established. When a proton transfers from the di-keto form to the keto−enol form, water molecules in different regions show absolutely opposite effects: some assist, whereas others hinder the tautomerization. However, when a proton transfers from the keto−enol form to the di-enol form, water molecules in different regions show similar effects: the Gibbs free energy always increases and the activation energy always decreases. Additionally, some important factors that obviously affect the activation energy and Gibbs free energy were found and discussed in detail. The reasons that water molecules can assist or prevent the proton transfer were given. Furthermore, on the basis of our calculated results, we explain why it is hard to detect the di-enol form of uracil in general experiments.

Introduction

Uracil is a pyrimidine base and a constituent of nucleotides and, as such, is one member of the base pair AU. Generally, the keto form of uracil exists as the main form in the double helix.[1] However, the enol form of uracil can also be generated by proton-transfer reaction. Such a tautomer may cause base mispairing, which has been proven to be one of the origins of gene mutation.[2−8]

As is well-known, nucleic acids are exposed to water in a number of biological structures. Water acts both as a proton acceptor and as a proton donor, and it can affect the structural features that are necessary for the biological functions of nucleic acids. During the past few years, interest in understanding the intricacies of the interactions between U/U* and water has led to theoretical investigations on the following aspects: to study the interaction with the water molecule in different sites of U,[9−20] to increase the number of water molecules up to 15 in different regions of U,[21−23] to study the interaction in the cube model,[24] to study the interaction between anion-U and water,[25−28] to study the interaction between protonated or deprotonated uracil and water,[29−30] to study the uracil−water interaction using dynamic theoretical analysis,[31−33] to study the enol forms of uracil and their interactions with water,[34−38] and to study the interaction using the CPCM model.[39] Besides these, the interaction between uracil and other molecules haas also attracted much interest,[40−42] especially its pairing with other bases.[43−45] These calculations have been performed at the semiempirical AM 1,[34] HF,[20,24−28,38,41−44] MP2,[9,11,15,16,23,25,26,28,41−45] MPBT,[28] and B3LYP[10,12−14,17−19,21,22,24,29,37,39,40,44] levels of theory.

These works have enhanced our perceptions on the complicated interactions between U/U* and water molecules. Although proton transfer is the primary origin to generate the enol form of uracil, this tautomerization process has not been well-understood. Previous papers dealing with the proton-transfer process mainly focused on the assisting role of water for other bases such guanine,[46−48] cytosine,[49] and adenine.[50−51] In our earlier paper, we showed that the explicit water molecules do not always assist the tautomerization process of uracil.[52] Such a phenomenon has brought to light a new mechanism of A−T to G−C mutation.[53] However, only one enol form of uracil was investigated in these papers.[52−53] In fact, U contains a row of alternating C=O and N−H groups, and U* contains C−O−H, C=O, C=N, and N−H, which provide a wide range of possible hydrogen-bonding arrangements for the water molecule. Until now, twelve enol forms of uracil have been reported.[34−38] Do water molecules have similar stabilization and mutagenicity effects on other tautomers of uracil? Are there any differences in the ability of proton transfer between these tautomers? What are the primary factors influencing the proton transfer? The present work will address these questions and focus on the following aspects: (i) investigation of the proton-transfer process in all possible ways and determination of the transfer tunnel with minimum energy, and (ii) investigation of the role of water molecules in the proton-transfer process for all of the tautomers and determination of the origin of the phenomena induced by water molecules. To have a systematic understand of the proton-transfer process of uracil, we will investigate this problem (a) in the gas phase, (b) in a solvent by adopting the self-consistent isodensity polarized continuum (SCI−PCM) model, and (c) in a microhydrated environment with water molecules.

Computational Methods

As shown in the Introduction, most of the pioneering works were performed at the B3LYP and MP2 levels of theory. Gaigeot et al. has shown in detail that B3LYP and MP2 gave

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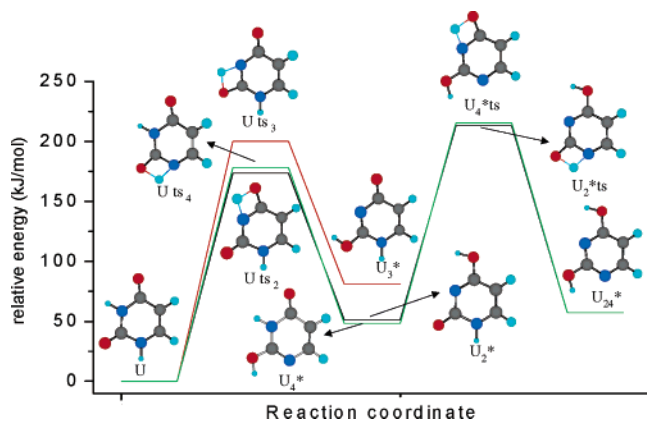
Figure 1. The reaction coordinate of isolated U.

U-W*i*-W*j* (i,i=1,2,3,4, i≠ j)

Figure 2. Preferential sites of water molecules in the vicinity of U/U*. S1, S2, S3, and S4 mean the favorable regions for water molecules. Because the skeleton of U* is the same as that of U, the sketch map of U*−W*i*−W*j* is omitted.

similar results as far as the geometrical and vibrational features of nucleic-acid bases were concerned.[12,54] As we know, DFT is an excellent compromise between computational cost and reasonable results. Therefore, in this paper, B3LYP [55,56] was adopted and a considerably large basis set, 6-311++G**, was used. Geometric configuration optimization, energy, and frequency calculation, as well as zero-point energy (ZPE) correction, have been performed at this level of theory.

The computed stationary points have been characterized as minima or transition states by diagonalizing the Hessian matrix and analyzing the vibrational normal modes. In this way, the stationary points can be classified as minima if no imaginary frequencies are shown or as transition states if only one imaginary frequency is obtained.[57,58] The particular nature of the transition states has been determined by analyzing the motion described by the eigenvector associated with the imaginary frequency.

To explore the tautomer interconversion in aqueous solution, the SCI−PCM model has been used.[59] The dielectric constant applied for water is 78.39. The isodensity value of 0.0004 for the SCI−PCM model is used as recommended by Wiberg and Rablen.[60]

The effect of the basis set superposition error (BSSE)[61] was not taken into consideration in this manuscript, because it was trivial in the uracil−water system[30,37] and a considerably large basis set was used in this paper. Our former study also showed that when the interacting water molecules were less than two, the BSSE has little effect on our results.[52]

The binding energy of U/U* to water molecules has been determined by[21,62]

$$\Delta E_{BE}^{MW} = E_{M-n(W)} - E_M - \sum E_{n(bW)}$$

Here, W and bW denote free water and the best cluster-containing water molecules, respectively. A negative value of ΔE_{BE}^{MW} indicates that the molecule can interact favorably with the corresponding most-stable $(H_2O)_n$ cluster.[62]

All calculations have been performed with the *Gaussian 98* suite of programs.[63]

Results and Discussion

Because there are two O and two N in uracil, to distinguish them, we use $N_{(H)}$ and $O_{(H)}$ to represent the one which donates a proton and accepts a proton, respectively. Furthermore, in the discussion, when we use U*, it represents $U_2*/U_3*/U_4*/U_{24}*$ (Figure 1) synchronously. U means the di-keto form of uracil. U_2*, U_3*, and U_4* mean the keto−enol form. $U_{24}*$ means the di-enol form.
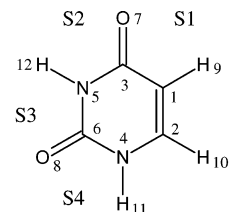
Kryachko et al. have reported that uracil has 12 different enol-form tautomers.[36,37] Four of them are generated by the proton-transfer process, and the others are generated by the proton rotational process. Although only one-third are directly related to the proton-transfer process, such a transition is the first step for uracil to tautomerize. More specifically, the proton rotational process is based on the occurrence of proton transfer for uracil. Hence, in this paper, our study mainly focuses on the proton-transfer process and the corresponding tautomers generated in this process.

1. The Proton-Transfer Process of Isolated U/U* in the Gas Phase. There are five possible tunnels to transfer a proton: proton transfer (1) from N5 to O7 of U, U → U_2*; (2) from N5 to O8 of U, U → U_3*; (3) from N4 to O8 of U, U → U_4*; (4) from N4 to O8 of U_2*, $U_2* → U_{24}*$; (5) from N5 to O7 of U_4*, $U_4* → U_{24}*$ (Figures 1−2). The optimized geometrical parameters of these stationary points are listed in Table 1. The geometry optimizations of uracil show good agreement with not only theoretical results (MP2/6-311G*[11] and CPMD simulations[31]) but also the experimental results (X-ray[64] and electron diffraction[65]). It is interesting to find that, in all these tautomerizing processes, the skeleton of uracil shows a regular change: two bonds of the pyrimidine ring are lengthened, and the other four are shortened (Table 1). Moreover, the lengthening bonds are those opposite the transferring proton at all times. For the two C−O bonds, the one that accepts a proton is lengthened, whereas the other is shortened.

Although single-proton transfer happens in all these tautomerizing processes, there exists a considerable difference in the ability for a proton to transfer (Figure 1). Clearly, U → U_3* possesses the highest barrier. At the same time, U_3* is the most unstable of these tautomers generated by proton transfer. Although the barrier of U → U_4* is higher than that of U → U_2*, U_4* is more stable than U_2*. Namely, generating U_2* is dynamically more favored; however, it will generate more U_4* from the perspective of thermodynamics. The barriers of U_2* → $U_{24}*$ and U_4* → $U_{24}*$ are lower than those of the other three. The reason may be that U_2*, U_4*, and $U_{24}*$ are thermodynamically unstable, and the tautomerism among them becomes easy. On the whole, the results clearly show that, in the gas phase, the di-keto form is more stable than all the enol forms.

As mentioned already, although the skeleton of uracil shows a regular change in all the tautomerizations, the abilities for a single proton to transfer from N5 to O7 and O8 and from N11 to O8 are considerably different. Why? We find that it is mainly related to the distance of proton transfer and the variations of the C−O$_{(H)}$ bond as well as the N$_{(H)}$−C−O$_{(H)}$ angle in the tautomerism. The proton-transfer distance and the related activation energy obtained by our calculation are plotted in Figure 3. As we can see, as the proton-transfer distance increases, the activation energy increases correspondingly. It indicates that the farther the proton moves to get to the transition

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TABLE 1: Geometrical Parameters of Isolate U and Its Tautomers[a]

	U	Uts2	U2*	Uts3	U3*	Uts4	U4*	U2*ts2	U4*ts4	U24*
C1=C2	1.347	1.363	1.358	1.343	1.344	1.362	1.360	1.382	1.383	1.383
C1−C3	1.458	1.422	1.428	1.484	1.474	1.452	1.447	1.412	1.406	1.400
C2−N4	1.375	1.359	1.355	1.396	1.383	1.363	1.347	1.336	1.351	1.341
C3−N5	1.412	1.352	1.303	1.400	1.413	1.440	1.423	1.330	1.368	1.326
N4−C6	1.393	1.429	1.424	1.349	1.364	1.340	1.297	1.373	1.325	1.331
N5−C6	1.383	1.369	1.378	1.330	1.283	1.340	1.352	1.334	1.331	1.332
C3=O7	1.215	1.278	1.342	1.213	1.216	1.213	1.216	1.338	1.279	1.342
C6=O8	1.212	1.209	1.212	1.279	1.345	1.277	1.340	1.278	1.334	1.342
N$_{(H)}$−H		1.326		1.360		1.331		1.289	1.296	
O$_{(H)}$−H		1.333		1.318		1.340		1.366	1.371	
N5−C3−O7	120.3	105.8	118.1				119.8		105.2	117.5
N5−C6−O8	124.3			108.5	121.7					
N4−C6−O8	122.8		119.2			107.6	121.1	105.2		117.3

[a] Geometrical parameters calculated in solvent (water) using SCI−PCM model are listed in Supporting Information.
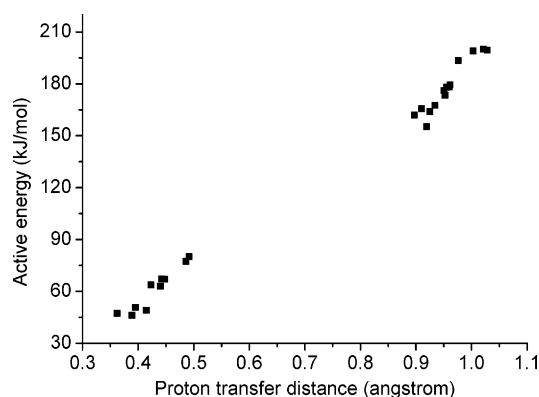

Figure 3. Activation energy as a function of proton-transfer distance. The corresponding values and the proton-transfer processes represented by these points are listed in Table 4.

states, the harder the proton transfer becomes. However, the relation between transfer distance and activation energy is not strictly a direct ratio. There exist other subordinate factors that affect the activation energy. We also studied the energy change induced by the corresponding configuration variation (Table 2). It reveals that the changes of the C−O$_{(H)}$ bond and the N$_{(H)}$−C−O$_{(H)}$ angle also have important contributions in the activation energy. For the change of the uracil skeleton, only those bonds lengthened or shortened more than 0.02 Å show obvious contributions.

2. The Proton-Transfer Process in a Solvent (SCI−PCM Model). In fact, many structural features that are necessary for the biological functions of nucleic acids depend on the interactions with surrounding water. For this reason, we also investigated the role of water in the tautomerism process. We use two methods to deal with this problem: (1) investigation of proton transfer in a cavity surrounded by a continuum solvent (water); SCI−PCM model was used; and (2) investigation of proton transfer in a microhydrated environment with one or two explicit water molecules. The first point is discussed in this section, and the second point will be considered in the next section.

The geometrical parameters of all the optimized stationary points using the SCI−PCM model are listed in the Supporting Information. In comparison with the configurations calculated in the gas phase, the bonds located at the right-hand side of uracil and its tautomers (such as C1−C2, C1−C3, and C2−N4) are shortened, and the C−O bonds are lengthened. Other bonds (such as C3−N5, N4−C6, and N5−C6) show irregular variation. If we compare the configuration variations in all these tautomerization processes, we find the changes of the uracil skeleton follow the same rule as that obtained in the gas phase:

two bonds of the pyrimidine ring are lengthened, and four are shortened.

It is evident that various tautomers have quite different dipole moments, presented in Table 3. The smallest one belongs to U$_{24}$*, and the biggest one belongs to U$_3$*. It is known that a larger value of the dipole moment indicates more stabilization when the complex is exposed to a polar solvent like water. Hence, we can predict that U$_3$* will become much more stable, whereas U$_{24}$* will become quite unstable in solvent surroundings.

As we can see from Table 4 (part B), when we compared with the results calculated in the gas phase (Table 4, part A), solvent has different effects on the stabilities of the tautomers. The existence of solvent increases the stability of U$_2$* and U$_3$*. On the other hand, it decreases the stability of U$_4$* and U$_{24}$*. At the same time, solvent greatly increases the barrier from U to U$_4$*, whereas its effects on other transfer barriers are much smaller. As a result, in the gas phase, U$_4$* is the most stable enol-form tautomer. However, in solvent, the most stable one is U$_2$*.

3. The Proton-Transfer Process in a Microhydrated Environment. In this section, the proton-transfer processes are investigated in a microhydrated environment with one or two explicit water molecules in the vicinity of U/U*. Four favorable regions S1, S2, S3, and S4 for water molecules (Figure 2) are taken into consideration. We adopt W1, W2, W3, and W4 to represent a single water molecule in S1, S2, S3, and S4, respectively. It is worth noticing that if water acts both as a proton acceptor and as a proton donor, the relevant structure is energetically favored over the alternative double-donor or double-acceptor hydrogen bonding.[37,66] Therefore, we consider water as an H-bond acceptor and donor via the interactions occurring through its oxygen or hydrogen atoms, respectively.

As the results in Table 5 show, although there are considerable differences in the binding energies of U or U* and water molecules, the negative values of ΔE_e^{MW} indicate that water can interact favorably with U or U* in the selected regions (S1, S2, S3, and S4). The relative stability of U + 1H$_2$O is U−W4 > U−W2 > U−W3 > U−W1, which is in agreement with the values obtained at MP2/6-311++G**.[28] For U*, the relative stability of U* + 1H$_2$O is U$_2$*−W2 > U$_2$*−W4 > U$_2$*−W3 > U$_2$*−W1, U$_4$*−W4 > U$_4$*−W2 > U$_4$*−W1 > U$_4$*−W3, U$_{24}$*−W2 > U$_{24}$*−W4 > U$_{24}$*−W3 ≈ U$_{24}$*−W1. It indicates that, when water is in different regions, some changes of the stability of U* happen in the tautomerizing process. It is clear that W1 and W3 always show the smallest binding energy with U$_2$*/U$_4$*/U$_{24}$*. This is mainly because W1 and W3 act only as H-bond donors when interacting with U$_2$*/U$_4$*/U$_{24}$* (Figure 5).

TABLE 2: Energy Change (kJ/mol) Induced by Configuration Variation^a

	U ts2		U ts3		U ts4		U2*ts		U4*ts	
	ΔR	ΔE	ΔR	ΔE	ΔR	ΔE	ΔR	ΔE	ΔR	ΔE
C1=C2	0.016	0.84	−0.004	0.06	0.015	0.74	0.024	1.75	0.023	2.62
C1−C3	−0.036	4.77	0.026	2.16	−0.006	0.13	−0.016	0.88	−0.041	4.72
C2−N4	−0.016	0.70	0.021	1.20	−0.012	0.39	−0.019	1.07	−0.023	1.60
C3−N5	−0.060	9.76	−0.012	0.38	0.028	1.75	0.027	2.41	−0.055	7.67
N4−C6	0.036	2.91	−0.044	5.14	−0.053	7.55	−0.051	5.74	0.028	2.60
N5−C6	−0.014	0.48	−0.053	7.01	−0.043	4.55	−0.044	4.51	−0.021	1.49
C3−O7	0.063	12.74	−0.002	0.01	−0.002	0.01	−0.004	0.03	0.063	12.60
C6−O8	−0.003	0.04	0.067	14.60	0.065	13.80	0.066	13.84	−0.006	0.10
N$_{(H)}$−C−O$_{(H)}$	−14.5	44.71	−15.8	52.98	−15.2	49.47	−14.0	44.29	−14.6	44.36

^{a} ΔE was calculated by changing the corresponding reactant configuration according to the transition states.

TABLE 3: Dipole Moments (in debye) of Uracil Tautomers

	U	Uts2	U2*	U ts 3	U3*	U ts 4	U4*	U2*ts2	U4*ts4	U24*
μ^a	4.58	5.40	4.99	6.16	6.60	2.54	3.33	3.61	2.64	1.31
μ^b	6.10	7.22	6.65	8.41	9.28	3.30	4.67	4.71	3.54	1.56

^{a} Values calculated in gas phase. ^{b} Values calculated in solvent (water) using SCI−PCM model.

TABLE 4: Free Energy Changes (kJ/mol) and Activation Energy (kJ/mol) of Isolated Uracil or Uracil−Water Tautomerism

tautomerism	ΔG	$\Delta(\Delta G)^a$	H transfer^b	$\Delta^{\neq}G$
Proton Transfer of Isolate U				
U → U2*	51.33	0.00	0.952	173.41
U → U3*	81.10	0.00	1.021	200.05
U → U4*	48.41	0.00	0.955	178.02
U2* → U24*	5.87	0.00	0.897	161.85
U4* → *U24*	9.12	0.00	0.910	165.65
In Solvent (Water) Using SCI−PCM Model				
U−R → U2*−R	48.45	−2.88	0.950	176.07
U−R → U3*−R	76.24	−4.68	1.003	198.98
U−R → U4*−R	57.57	9.16	0.976	193.53
U2*−R → U24*−R	29.42	23.55	0.934	167.68
U4*−R → U24*−R	20.30	11.18	0.925	163.97
With Water in Assisting Region				
U−W2 → U2*−W2	40.49	−10.84	0.423	63.85
U−W3 → U3*−W3	63.40	−17.70	0.486	77.37
U−W4 → U4*−W4	43.82	−4.59	0.440	63.05
U2*−W4 → U24*−W4^c	14.08	8.21	0.362	47.26
U4*−W2 → U24*−W2^c	10.06	0.94	0.389	46.21
With Water in Protective Region				
U−W1 → U2*−W1	58.36	7.03	0.961	179.45
U−W4 → U3*−W4	87.11	6.01	1.028	199.39
U−W3 → U4*−W3	54.41	6.00	0.960	178.18
U2*−W3 → U24*−W3^c	9.22	3.35	0.904	153.83
U4*−W1 → U24*−W1^c	19.55	10.43	0.919	155.18
With Water Molecules in Both Regions				
U−W1−W2 → U2*−W1−W2	46.19	−5.41	0.448	67.01
U−W3−W4 → U3*−W3−W4	66.59	−14.51	0.492	80.11
U−W3−W4 → U4*−W3−W4	46.85	−1.56	0.442	67.08
U2*−W3−W4 → U24*−W3−W4	19.80	13.93	0.395	50.79
U4*−W1−W2 → U24*−W1−W2	16.24	7.12	0.415	49.10

^{a} $\Delta(\Delta G) = \Delta G_{(U−X \rightarrow Um*−X)} - \Delta G_{(U \rightarrow Um*)}$, ($m$=1−4; X means R or explicit water molecules) ^{b} H transfer distance was defined by the method presented in the Supporting Information. ^{c} These tautomerizing process are exceptions for water stabilization and mutagenicity for uracil (see section 4). The assisting or protective regions in this case only represent the position in reference to the located water molecule and do not mean the role of water.

Many pioneering papers on the interaction between water and U/U* have taken water molecules in region S1 into consideration.[11,16,19,21−25,28,37] However, whether water in S1 could act as a weak H-bond acceptor is still a controversy. Recent CP−MD (Car−Parrinello molecular dynamics, an ab initio MD method) results contributed by Gaigeot et al. argued against the

TABLE 5: Binding Energies (kJ/mol) between U/U* and Water Molecules^a

compound	ΔE_{BE}^{MW}	compound	ΔE_{BE}^{MW}	compound	ΔE_{BE}^{MW}
U−W2	−30.85	U−W1	−25.34	U−W1−W2	−52.78
U2*−W2	−44.00	U2*−W1	−11.61	U2*−W1−W2	−58.46
U−W3	−28.05	U−W4	−35.18	U−W3−W4	−63.88
U3*−W3	−48.93	U3*−W4	−25.51	U3*−W3−W4	−79.94
U4*−W4	−41.53	U4*−W3	−18.09	U4*−W3−W4	−64.79
U2*−W4	−40.76	U2*−W3	−22.36	U2*−W3−W4	−61.34
U24*−W4	−34.65	U24*−W3	−10.77	U24*−W3−W4	−49.11
U4*−W2	−37.08	U4*−W1	−22.08	U4*−W1−W2	−58.34
U24*−W2	−38.14	U24*−W1	−10.35	U24*−W1−W2	−52.14

^{a} Values were corrected with ZPE.

existence of the C1−H9···O(W1) weak hydrogen bond in region S1.[31,32] Because CP−MD describes a more realistic model system, we do not take the C1−H9···O(W1) hydrogen bond into consideration in this work, and W1 only hydrogen bonds to C3−O7.

3.1. Water Assists the Proton Transfer. Our previous paper has shown that water molecules located in region S2 can assist the proton transfer from U to U$_2$*.[46] The effect water molecules in region S3/S4 have on the proton transfer from U to U$_3$*/U$_4$* and the differences will be discussed in this section.

A water molecule has been located in regions S2, S3, and S4, and the proton-transferring processes from U to U$_2$*/U$_3$*/U$_4$* are investigated (Figure 4). As we can see, in these processes, water acts as an H-bond acceptor and donor simultaneously, and there are two protons taking part in the transfer: water accepts the hydrogen atom from the N$_{(H)}$−H bond of U/U*; at the same time, it donates its hydrogen atom to U/U*, which is accepted by O$_{(H)}$. The changes in the uracil skeleton follow the same rule as that obtained in the gas phase and in the SCI−PCM model: two bonds of the pyrimidine ring are lengthened, and the other four are shortened. In comparison with isolated U/U*, when interacting with water, the C−O$_{(H)}$ bonds of the reactant/transition state (TS) are lengthened, whereas in product, they are shortened.

As we can see from Table 4 (part C), a water molecule in S2, S3, and S4 can decrease the activation energy and Gibbs free energy in the tautomerization from U to U$_2$*/U$_3$*/U$_4$* simultaneously. Namely, it makes the proton transfer easier on both thermodynamics and dynamics. The order of the assisting effect of water for proton transfer is W3 > W2 > W4. In comparison with isolated U/U*, a water molecule existing in the assisting region has changed the clusters' stabilities and made U$_2$* more stable than U$_4$*.

3.2. Water Prevents the Proton Transfer. In this section, a single water molecule is located in S1/S3/S4, and the proton-transfer processes from U to U$_2$*/U$_4$*/U$_3$* are investigated (Figure 5). Quite similar to that in the gas phase, the SCI−PCM model, and water in assisting regions, two bonds of the

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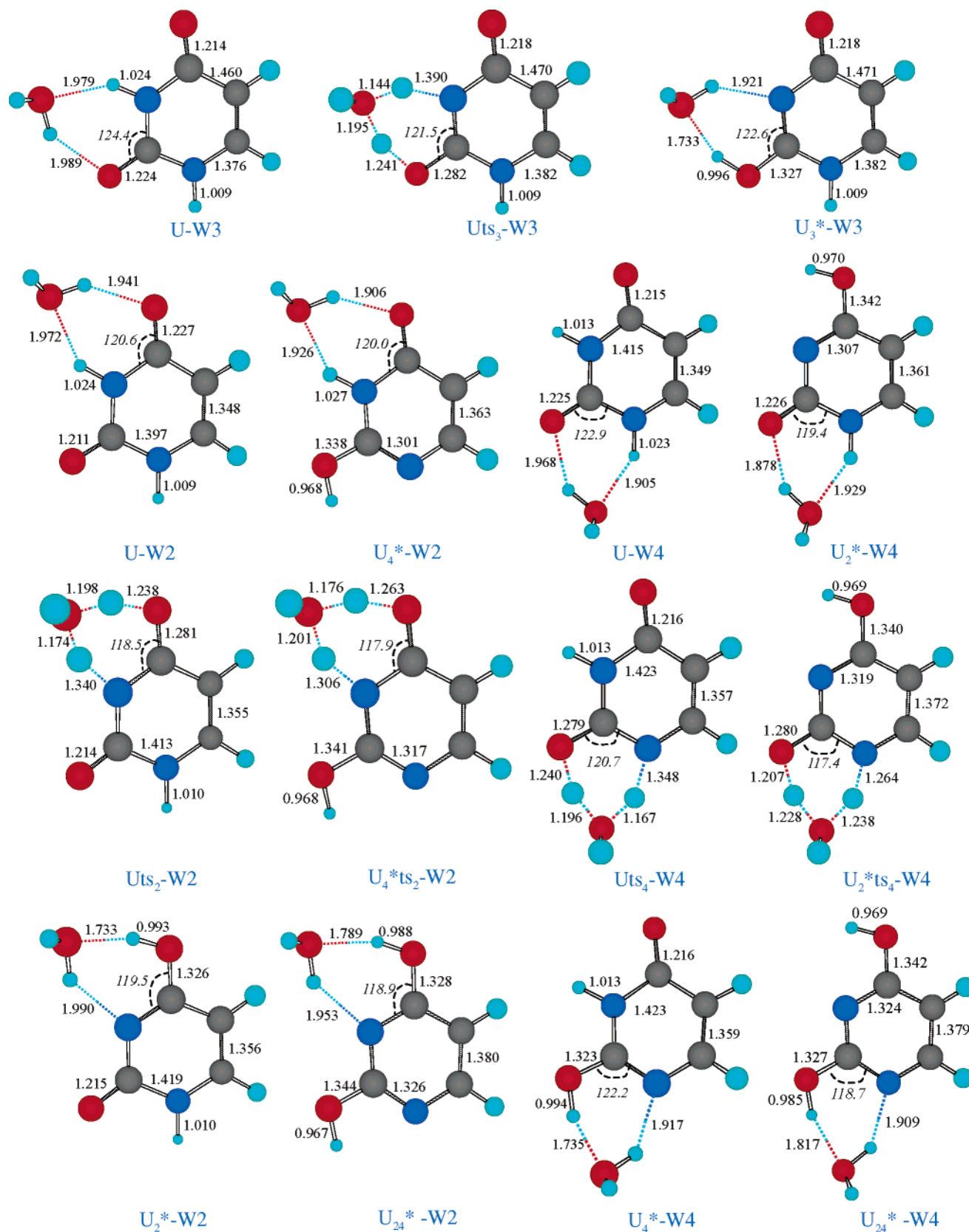*J. Phys. Chem. B, Vol. 109, No. 12, 2005* **5939**

Figure 4. Optimized structures with the water molecule in the regions where water molecules can assist proton transfer ("assisting region" in brief). The number values shown refer to computed bond distances and intramolecular angles.

pyrimidine ring are lengthened and four are shortened in the transition. In comparison with isolated U/U₂*/U₄*, the C−O(H) bonds of the reactant/TS/product with water in the protective region are all lengthened, unlike the transitions with assisting water.

It is noticeable that, in these cases, water molecules do not directly participate in the proton transfer: this is another different point to the complexes with assisting water. Namely, the hydrogen atom of water does not transfer. However, water molecules in S1/S3/S4 have changed the stability of U/U*. As
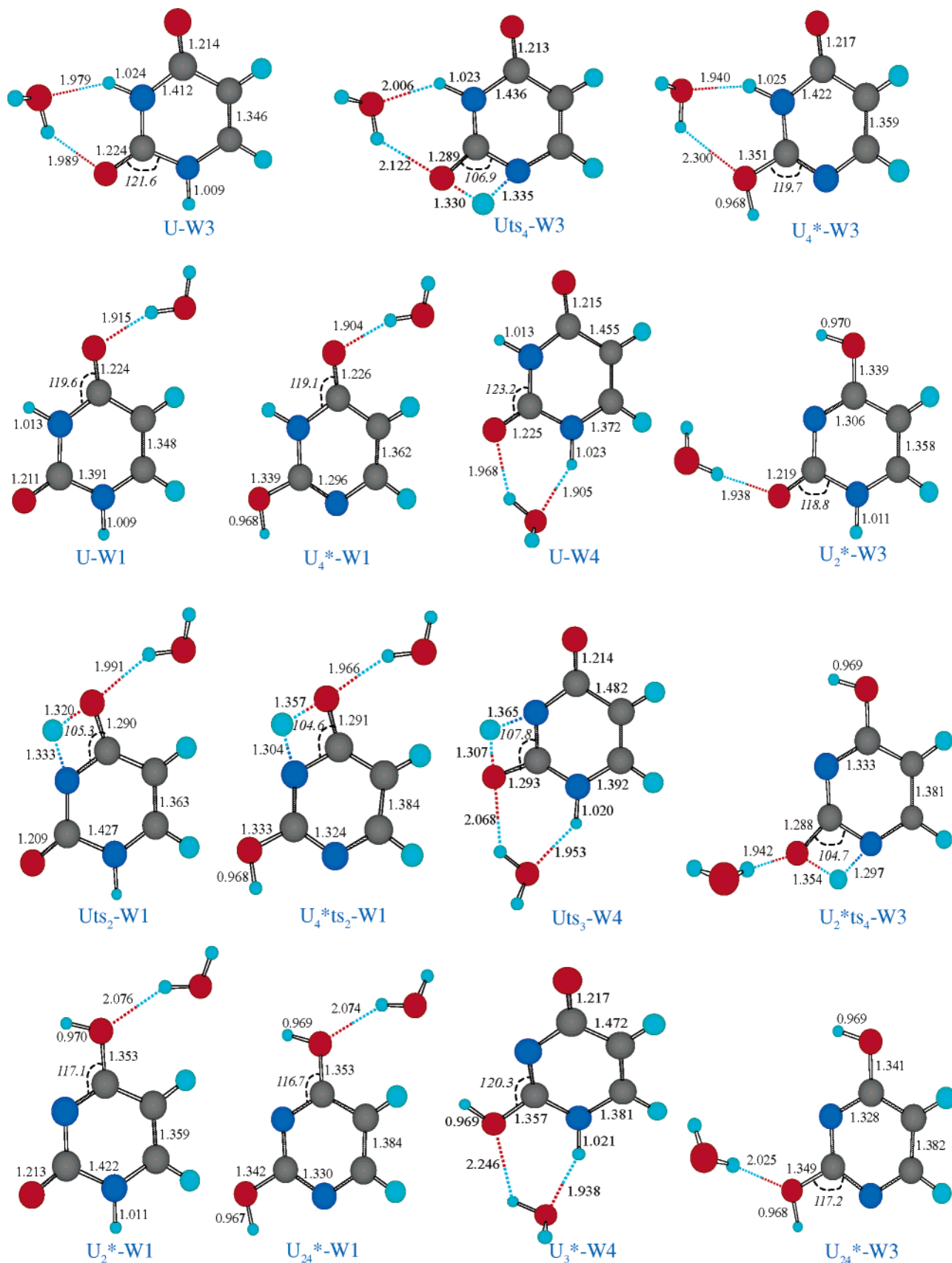
Figure 5. Optimized structures with the water molecule in the regions where water molecules can prevent proton transfer ("protective region" in brief).

shown in Table 5, there is a decrease in the binding energy as U tautomerizes to U_2^*/U_3^*/U_4^* with water in protective regions. As a result, the Gibbs free energies of these proton-transfer processes with water protection are increased (Table 4, part D).

Unlike water in the assisting region which can decrease the activation energy and the Gibbs free energy simultaneously, in comparison with the isolated tautomerizing process from U to U*, water in the protective region only increases the activation

energy in U$-$W1 $\rightarrow$ U$_2$*$-$W1 and almost has no effect on U$-$W4 $\rightarrow$ U$_3$*$-$W4, U$-$W3 $\rightarrow$ U$_4$*$-$W3.

Unexpectedly, we found that the proton transfer from U$_2$*/U$_4$* to U$_{24}$* with a single water in the assisting or protective region showed a completely different rule compared with those from U to U$_2$*/U$_3$*/U$_4$*. We will discuss this exception in section 4.

3.3. Proton Transfer with Water in Both Regions where Water Molecules Assist Proton Transfer and the Region where They Prevent Proton Transfer. In this section, water molecules have been located in both regions where water molecules assist proton transfer and the region where they prevent proton transfer. The proton-transfer processes from U to U$_2$*/U$_4$*/U$_3$* from U$_2$*/U$_4$* to U$_{24}$* are investigated (Figure 6). In these processes, water in the assisting region acts as an H-bond acceptor and donor simultaneously and takes part in the transfer, which will shorten the proton-transfer distances. The changes of C$-$O$_{(H)}$ bonds are similar to what has been observed in section 3.2, and the changes of N$-$C$-$O$_{(H)}$ are similar to what has been observed in section 3.1. As we have discussed, the proton-transfer distance and the changes of C$-$O$_{(H)}$ and N$-$C$-$O$_{(H)}$ play the most important roles in deciding the activation energy. Hence, we can expect a compositive effect induced by water in both the assisting region and the protective region.

However, it should be noted that the energy variation in the tautomerism with water in both the assisting region and the protective region cannot be considered as a simple superposition of that water in the assisting and protective regions separately. It can be seen that water molecules in the assisting region have bigger effect than those in the protective region. However, the protective effect induced by water molecules in the protective region could never be ignored. In comparison with the transition of the clusters with water only in the assisting region, the introduction of a protective water molecule greatly increase the Gibbs free energy and the activation energy (Table 4, part E). This comparison highlights the important role of the protective water molecule. Furthermore, it is interesting to find that, compared with water in the protective region only, water in the assisting region can enhance the protective role of water in the protective region. As we can tell from part D of Table 4, in comparison with isolated U$_2$* $\rightarrow$ U$_{24}$* and U$_4$* $\rightarrow$ U$_{24}$*, single water in the protective region decrease the activation energy of U$_2$*$-$W3 $\rightarrow$ U$_{24}$*$-$W3 and U$_4$*$-$W1 $\rightarrow$ U$_{24}$*$-$W1. However, when introducing water in the assisting region, compared with the transition with water in the assisting region only, water in the protective region can increase the activation energy from U$_2$*/U$_4$* to U$_{24}$* (Table 4, part E). It indicates that the transition process is a collaborative result of all the impacts induced by the water molecules in different regions.

3.4. Why Does Water in Different Regions Have Different Impacts on Proton Transfer? On the whole, a water molecule in S2, S3, or S4 can assist proton transfer from U to U$_2$*/U$_3$*/U$_4$*, while a water molecule in S1, S3, or S4 can prevent proton transfer from U to U$_2$*/U$_4$*/U$_3$*. In our earlier paper, to explain the water stabilization and the mutagenicity for U $\rightarrow$ U$_2$*, we have employed natural bond orbital (NBO) analyses and molecular orbital analyses.[52] In this manuscript, on the basis of the investigations of all the possible proton-transfer processes, besides our earlier analyses, some new explanations are put forward.

First, for water in the assisting region, (1) the water located in these regions increases the partial electron density surrounding C$-$O$_{(H)}$ in U significantly, which makes the O$_{(H)}$ of U susceptible to attack by the proton. In detail, the atomic charge

of O$_{(H)}$ has been increased by 0.033, 0.041, and 0.048 for U$-$W2, U$-$W3, and U$-$W4, respectively. (2) It can be seen that there is a hexahydric ring in the transition state (Uts2$-$W2, Uts3$-$W3, and Uts4$-$W4), in which water acts as a bridge. Just this bridge makes the proton move a shorter distance to get to the TS. In comparison with those in the gas phase, water molecules in the assisting region have shortened the proton-transfer distance by 0.529, 0.535, 0.515, 0.525, and 0.521 Å (Table 4). As we have discussed, such small proton-transfer distances will correspond to low activation energies (Figure 3). (3) As mentioned already, the value of the N$-$C$-$O$_{(H)}$ angle undergoes a large change (about 15°) in the tautomerization of isolated U to U*, which increases the activation energy considerably. However, when there are water molecules in assisting region, the N$-$C$-$O$_{(H)}$ angle only undergoes a small change (about 3°), which will decrease the activation energy greatly. (4) In comparison with the keto form, the enol form is more favorable to bind with water in the assisting region, which makes the stability of the enol form increase.

Second, for water in protective region, (1) because the formation of the hydrogen bond C$-$O$_{(H)}$$\cdots$H$-$O (W) makes the electron cloud around O$_{(H)}$ shift toward water in the protective region, the proton must a move longer distance to interact with O$_{(H)}$ (Table 4). As we have discussed, the farther the proton transfer is, the higher the barrier is. (2) In comparison with the keto form, the enol form is less favorable to bind with water in the protective region, which decreases the stability of the enol form. (3) Water in the assisting region can collaboratively enhance the protective ability of water in the protective region.

4. An Exception for Water Stabilization and Mutagenicity for Uracil. Why It Is Hard to Observe the Di-Enol Form in Experimentation. As mentioned already, water molecules in different regions can assist or prevent proton transfer from the keto form of uracil (U) to its keto$-$enol form (U$_2$*/U$_3$*/U$_4$*). However, for proton transfer from the keto$-$enol form to the di-enol form (U$_{24}$*), wherever the water molecule is located, in assisting regions (Figure 4) or protective regions (Figure 5), the Gibbs free energies are always increased and the activation energy are always decreased. That is to say, water located in the assisting region that can assist proton transfer from the keto form to the keto$-$enol form loses its ability to decrease the Gibbs free energy in the proton transfer from the keto$-$enol form (U$_2$*/U$_4$*) to the di-enol form (U$_{24}$*). The water located in the protective region loses its ability to increase the activation energy in the proton transfer from U$_2$*/U$_4$* to U$_{24}$*. Why does this happen? This could be explained by the extremely small dipole moments of the di-enol form U$_{24}$* (Table 3), which makes the interaction between U$_{24}$* and water unstable.

Until now, we can explain why Tsuchiya et al. did not obtain any sign of the di-enol form (U$_{24}$*) in their experiment. Tsuchiya et al. analyzed the n, π* states of six tautomers of uracil (including the di-keto, keto$-$enol, and di-enol forms) and thought it was possible to detect the keto$-$enol or di-enol tautomer by observing the fluorescence from n, π*. At last, although they successfully detected the keto$-$enol form for the fist time, the di-enol tautomer was not detected.[67] We think the reasons are as follows: (1) According to our calculation results using the SCI$-$PCM model, because of the small dipole moments of U$_{24}$*, when U$_{24}$* is exposed to a polar solvent like water, U$_{24}$* will become very unstable. (2) As mentioned in this section, there is the exception of water stabilization and mutagenicity for uracil: water molecules in different regions always enhance the Gibbs free energies. As a result, the enhanced Gibbs free energies prevent population of the di-enol
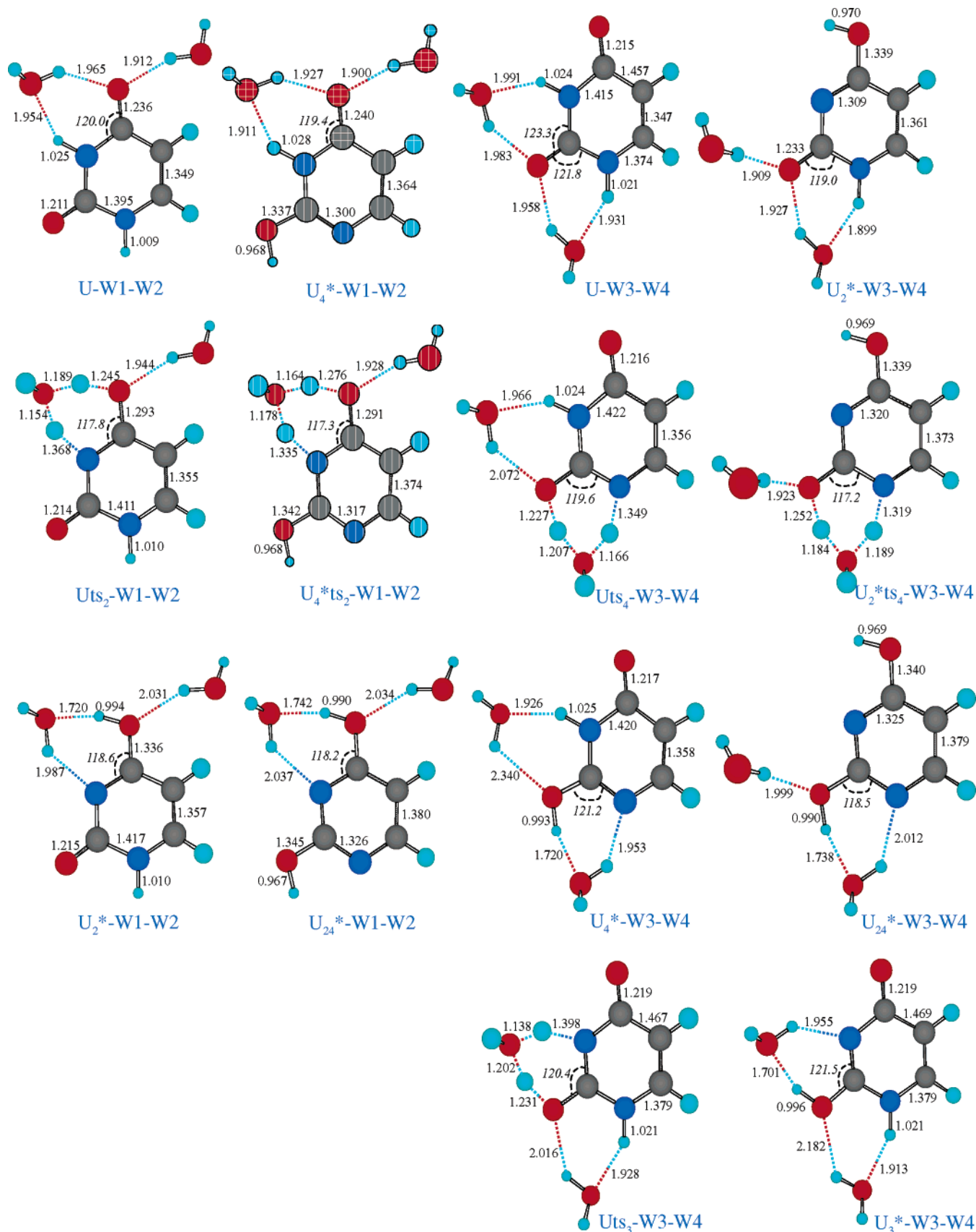
Figure 6. Optimized structures with the water molecule in both assisting region and protective region.

form. (3) The di-enol form is generated by the keto−enol form via proton transfer, and there exists a considerable barrier between them. Because the keto−enol form itself is a minority

in the equilibrium reaction, the di-enol form must be much more scarce. Hence, it is hard to the detect di-enol form of uracil ($U_{24}*$) by the general method.

Tautomerism of Uracil Induced by Proton Transfer

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Conclusions

To systematically explore the proton-transfer process of uracil as well as the roles water molecules play in these processes, and also explain the relative phenomenon, all the possible proton-transfer processes of U/U* have been investigated. Altogether, 62 structures are studied theoretically, including the di-keto, keto−enol, and di-enol forms of uracil, with and without explicit water interaction, in the gas phase and in solvent. Among these, 25 transition states of the related proton-transfer processes have also been studied. On the basis of the results obtained from our calculations, the following can be stated.

(1) For proton transfer from the di-keto form to the keto−enol form, a water molecule in S2, S3, or S4 can assist proton transfer from U to $U_2^*/U_3^*/U_4^*$, while a water molecule in S1, S3, or S4 can prevent proton transfer from U to $U_2^*/U_4^*/U_3^*$.

(2) For the latter proton transfer from the keto−enol form to the di-enol form, water molecules in different regions show similar effects: always increasing the Gibbs free energies and decreasing the activation energy. Furthermore, proton transfer from the keto−enol form to the di-enol form in solvent (water, using SCI−PCM model) is also a process with Gibbs free energies increased. On this basis, we explain why the di-enol form of uracil is hard to detect.

(3) An interesting rule was summarized: longer proton-transfer distance corresponds to higher activation energy. The more the $N_{(H)}-C-O_{(H)}$ angle distorts, the higher the activation energy is. A large variation of the $C-O_{(H)}$ bond is also responsible for the increased activation energy.

(4) Because the mutagenic and protective role of water for uracil was first put forward by us in an earlier paper,[52] it is very important to understand why water molecules in different regions have different effects on the proton-transfer processt. In an earlier paper, we had explained this phenomenon from NBO and molecular orbital analyses. In this manuscript, more reasons are given.

(5) It is also interesting to find that in all situations including in the gas phase, in solvent, and in a microhydrated environment, two bonds of the pyrimidine ring are lengthened and four of them are shortened in the proton-transfer processes. Furthermore, the lengthening and shortening positions are related to the type of proton transfer.

In another work, we used formamide as a model and investigated proton transfer with water molecules at the B3LYP and quadratic configuration interaction with single and double excitation (QCISD) levels of theory. The calculation results also indicated the assisting and protective roles played by water for the model molecule.[68] Can we predict the mutagenic and protective roles of water for other molecules? The appearance of the exception of proton transfer from the keto−enol form to the di-enol form makes it becomes hard to say. However, we are well-supported in our belief that the mutagenic and protective roles of water in the proton-transfer process should be widely evident in other molecules. Such phenomena of water molecules in different regions of the host molecules having stabilizing or mutagenic effects on the proton-transfer process should necessarily be considered when we study proton transfer in aqueous solution, especially when we want to gain accurate results about this process. Such phenomena may provide an incentive for the future development of the research on the proton-transfer process and related gene mutation.

It should be noticeable that temperature plays a role in proton transfer. Our research in this field is in process. The initial results indicated that water stabilization and mutagenicity are affected by temperature.[69] Finally, it should be mentioned that a quantum chemistry study, such as the present one or some previous ones,[16−30,33−37,52] gave us a static picture of the interaction of the uracil−water system. The dynamics at 300 K of water molecules around uracil completed by Gaigeot et al. have given a different view of the first hydration shell of uracil.[31,32] We believe that a dynamic theoretical analysis (such as first-principles molecular dynamics simulations) of the proton-transfer process of the uracil−water system will give us a deeper understanding of water stabilization and mutagenicity for uracil.

Supporting Information Available: (1) The definition of proton-transfer distance. (2) Geometrical parameters calculated in solvent (water) using SCI−PCM model. (3) The Z-MATRIX of calculated results. These materials are available free of charge via the Internet at http://pubs.acs.org.

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