Excited-State Double Proton Transfer Dynamics of Model DNA Base Pairs: 7-Hydroxyquinoline Dimers

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The excited-state double proton transfer of model DNA base pairs, 7-hydroxyquinoline dimers, in benzene has been investigated using picosecond time-resolved fluorescence spectroscopy. Upon excitation, whereas singly hydrogen-bonded noncyclic dimers do not go through tautomerization within the relaxation time of 1400 ps, doubly hydrogen-bonded cyclic dimers undergo excited-state double proton transfer on the time scale of 25 ps to form tautomeric dimers, which subsequently undergo a conformational change in 180 ps to produce singly hydrogen-bonded tautomers. The rate constant of the double proton transfer reaction is temperature-independent, showing a large kinetic isotope effect of 5.2, suggesting that the rate is governed mostly by tunneling.

1. Introduction

Proton transfers have been widely studied because they play important roles in a variety of biological and chemical phenomena.[1−3] In particular, tautomerization involving hydrogen (H) bonds has been attracting considerable attention.[4−6] Since the discovery of the double helix structure of DNA by Watson and Crick in 1953,[7] the proton-translocating tautomerization of DNA base pairs has been suggested to cause point mutations.[8,9] Thus, photoinduced proton-transfer reactions are often considered to be helpful for understanding the causes of mutagenesis in DNA. In this regard, a number of researchers have investigated the excited-state double proton transfer (ESDPT) of 7-azaindole (7AI) dimers both in the gas phase and in the condensed phase[8−14] because they are structurally similar to H-bonded DNA base pairs. Although many aspects of their ESDPT have been revealed by previous studies, some details are not clear yet. For example, whether the transfer process of two hydrogen atoms in the ESDPT of 7AI dimers occurs through the concerted manner or in the stepwise manner has been discussed extensively,[10,11] but results are still in controversy.[12] To elucidate the ESDPT mechanisms of DNA base pairs more precisely, it is desirable to study a new kind of model DNA base pairs.

7-Hydroxyquinoline (7HQ) having both a photoacidic enolic group and a photobasic imino group is an amphoteric aromatic molecule and is an interesting system to study proton-transfer reactions.[15−18] Especially, due to structural similarity between 7AI and 7HQ, 7HQ molecules are also known to form dimers in an aprotic solvent.[19−23] Therefore, the study of 7HQ dimers is expected to shed light on the proton-transfer mechanisms of DNA base pairs. Chou et al. have carried out nanosecond lifetime measurements and theoretical calculations on the ESDPT of 7HQ dimers.[20] Although they provided important knowledge on the reaction, the ESDPT mechanism of 7HQ dimers was not verified because of their limited temporal resolution. In this paper, we have studied the ESDPT mechanism of 7HQ dimers in benzene using picosecond time-resolved fluorescence spectroscopy. 7HQ dimers exist in two different conformers in the ground state (Figure 1): doubly H-bonded cyclic dimers (**Nd**) and singly H-bonded noncyclic dimers (**Ns**). Upon excitation, **Nd** conformers undergo ESDPT to form doubly H-bonded tautomeric dimers (**Td**), which go through a conformational change in the excited state to produce entropically favorable singly H-bonded tautomers (**Ts**). Not only the ESDPT rate of **Nd** is temperature-independent but also its kinetic isotope effect is very large, indicating that **Nd** goes through ESDPT via tunneling.

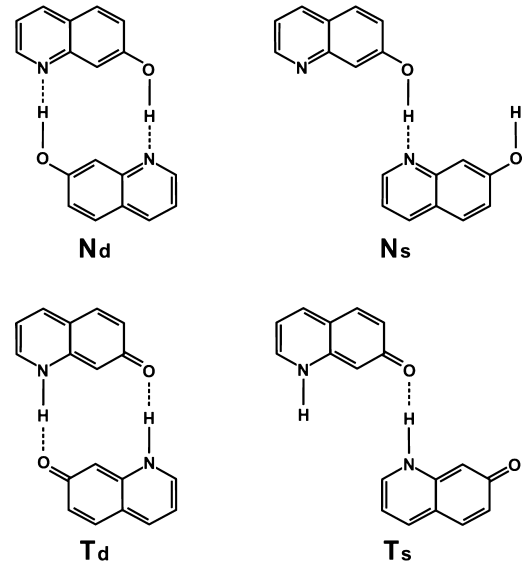

Figure 1. Conformers (**Nd** and **Ns**) of 7HQ dimers and their prototropic tautomers (**Td** and **Ts**) present possibly in benzene.

2. Experimental Methods

7HQ (≥99%) was used as purchased from Acros. Benzene (≥99.9%), purchased from Sigma-Aldrich, was distilled once and stored over molecular sieves of 4 Å prior to use. The protic ^{1}H atom of 7HQ was exchanged with a ^{2}H atom by dissolving 7HQ in CH_3O^2H (isotopic purity ≥99.5%), received from Sigma-Aldrich, and subsequently removing the solvent completely in a vacuum. Absorption spectra were measured with a

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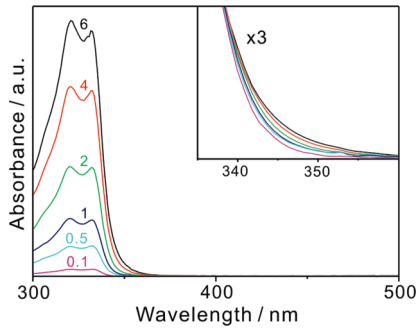

Figure 2. Absorption spectra of 7HQ in benzene. The concentrations of 7HQ are indicated inside in units of 10^{-4} M. Inset: The magnified view of the absorption spectra normalized at 333 nm.

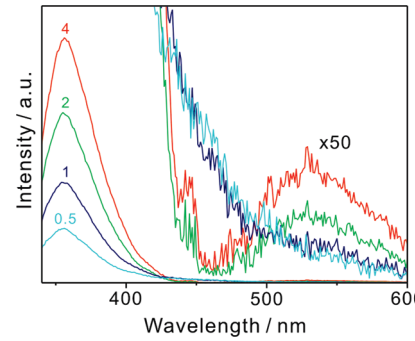

Figure 3. Emission spectra, with excitation at 330 nm, of 7HQ in benzene. The concentrations of 7HQ are indicated inside in units of 10^{-4} M.

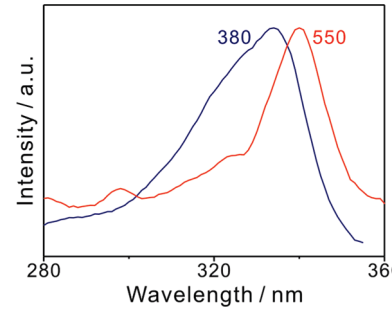

Figure 4. Excitation spectra of 0.4 mM 7HQ in benzene. Monitored emission wavelengths are indicated inside in units of nm.

UV/vis spectrometer (Scnico, S-3100). Emission spectra were obtained using a home-built fluorometer consisting of a Xe lamp of 75 W (Acton Research, PD438) with a monochromator of 0.15 m (Acton Research, Spectrapro-150) and a photomutiplier tube (Hamamatsu, R928) attached to a monochromator of 0.30 m (Acton Research, Spectrapro-300). A mode-locked Nd:YAG laser of 25 ps (Quantel, Pizzicato) and a streak camera of 10 ps (Hamamatsu, C2830) attached to a CCD detector (Princeton Instruments, RTE128H) were employed for the excitation and the detection of fluorescence kinetic profiles, respectively. Samples were excited with third-harmonic pulses (355 nm), and emission wavelengths were selected by combining band-pass filters and cutoff filters. Fluorescence kinetic constants were extracted by fitting kinetic profiles to computer-simulated exponential curves convoluted with instrumental response functions. Sample temperatures were controlled using a refrigerated bath circulator (Jeio Tech, RC-10V).

3. Results and Discussion

Figure 2 shows that the lowest absorption band of 7HQ in benzene grows around 330 nm and gains the vibronic structure as the concentration of 7HQ increases. The concentration increase does not result in perceivable absorption around 410 nm, suggesting that proton-transferred 7HQ tautomers do not form in the ground state. 7AI molecules at high concentrations are known to undergo self-association to produce dimers in nonpolar aprotic solvents.[9−11] Indeed, absorption spectra normalized at 333 nm show the growth of absorption in the region of 340−350 nm with the concentration increase of 7HQ (inset of Figure 2). This indicates that 7HQ molecules in benzene at sufficiently high concentrations aggregate together to form dimers or higher oligomers, showing the increment of absorption in the region of 340−350 nm due to the self-association of 7HQ molecules by H bonding. Referring to concentration-dependent absorption spectra, Chou et al.[20] have reported that 7HQ dimers of **Nd** or **Ns** are dominant among possible aggregates in benzene. The values of K_a and ΔG for the formation of 7HQ dimers have been reported to be 1.2×10^3 M^{-1} and -4.2 kcal mol^{-1}, respectively, in benzene at room temperature. Thus, we consider that 7HQ monomers cannot be excited at 355 nm, where our samples have been excited for the measurements of fluorescence kinetic profiles.

The emission spectra of Figure 3 show that the excitation of 7HQ in benzene at 330 nm gives rise to a prominent normal fluorescence band having the maximum at 355 nm. Only the ultraviolet (UV) normal fluorescence band of 7HQ was observed at low 7HQ concentrations below 0.1 mM. However, magnified visible emission spectra show that a new fluorescence band around 530 nm appears at high concentrations of 7HQ above 0.2 mM. The emission spectra of 7AI at high concentrations in nonpolar aprotic solvents also show a visible fluorescence band, which is known to arise from tautomers generated by the ESDPT of 7AI dimers.[9−11] Thus, the new fluorescence band of 7HQ around 530 nm in Figure 3 indicates that the ESDPT of 7HQ dimers are also operative in benzene to produce proton-transferred tautomers. Moreover, we consider that the UV fluorescence band is emitted compositely from 7HQ monomers and 7HQ aggregates, which cannot undergo ESDPT within their excited-state lifetimes.[20]

Figure 4 shows that the excitation spectrum of the tautomer fluorescence band monitored at 550 nm is shifted to the red by 6 nm from the excitation spectrum of the normal fluorescence band. This bathochromic shift is due to the H bonding of 7HQ dimers, and it indicates that 7HQ dimers are spectrally quite different from 7HQ monomers. Upon absorption of photons, 7HQ dimers formed via intermolecular H bonds can undergo ESDPT to form tautomers.

The fluorescence kinetic profile of 0.4 mM 7HQ in benzene, monitored at 420 nm, shows biexponential decay times composed of 25 ps (59%) and 1400 ps (41%) at 300 K (Figure 5a and Table 1). However, Chou et al.[20] reported that the fluorescence of concentrated-7HQ monitored at 365 nm had a biexponential decay profile of 0.36 and 1.2 ns, assigning the fast decay component to emission from monomers and the slow decay component to emission from 7HQ-associated species. They could not resolve our fast decay component of 25 ps because of their limited temporal resolution (~200 ps). In addition, the decay component of 0.36 ns was not observed in our fluorescence kinetic profile since our samples were excited at 355 nm to exclude the excitation of 7HQ monomers. Considering that the ESDPT of 7HQ dimers is operative to produce tautomers (Figure 3), we have attributed the fast component of 25 ps to the ESDPT of **Nd**, although a concomitant rise component is not resolvable in the fluorescence kinetic profile monitored at 550 nm. The kinetic profile of

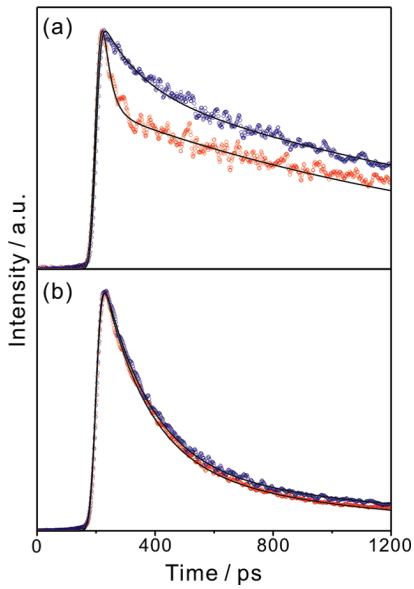

Figure 5. Fluorescence kinetic profiles of 0.4 mM 7^1HQ (red) and 7^2HQ (blue), where the protic-hydrogen isotopes of 7HQ are ^{1}H and ^{2}H respectively, in benzene at 300 K. Samples were excited at 355 nm and monitored at 420 nm (a) and 550 nm (b). Solid lines are best-fitted curves to extract kinetic constants.

TABLE 1: Fluorescence Kinetic Constants of 0.4 mM 7HQ in Benzene at Various Temperatures

isotope[a]	temperature (K)	decay time (ps) at 420 nm	decay time (ps) at 550 nm
^{1}H	279	25 (74%)[b] + 1200 (26%)	140 (80%) + 800 (20%)
	285	25 (68%) + 1200 (32%)	150 (80%) + 900 (20%)
	293	25 (64%) + 1300 (36%)	140 (77%) + 800 (23%)
	300	25 (59%) + 1400 (41%)	150 (75%) + 800 (25%)
^{2}H	279	130 (25%) + 1600 (75%)	150 (70%) + 1300 (30%)
	285	130 (26%) + 1600 (74%)	130 (72%) + 1100 (28%)
	293	130 (27%) + 1700 (73%)	140 (73%) + 1100 (27%)
	300	130 (28%) + 1700 (72%)	160 (77%) + 1100 (23%)

[a] Isotope of protic hydrogen. [b] Initial amplitude percentage of each component.

tautomeric fluorescence at 550 nm shows biexponential decay times of 150 ps (75%) and 800 ps (25%) at 300 K (Figure 5b and Table 1). Since the decay time of 150 ps is remarkably fast and the full width at the half-maximum of the temporal response function is about 30 ps, we consider that a rise of 25 ps at 550 nm is marked by the fast decay component and the slow instrumental response. To elucidate the ESDPT mechanism of 7HQ dimers in benzene at 300 K, we suggest that there exist two different conformers of 7HQ normal dimers in the ground state (Figure 1). One conformer has a singly H-bonded noncyclic structure (**Ns**)[20,23] and cannot go through ESDPT within the relaxation time of 1400 ps, whereas the other conformer has a doubly H-bonded cyclic structure (**Nd**) and undergoes ESDPT on the time scale of 25 ps (k_{PT}^{-1}) to become doubly H-bonded tautomeric dimers (**Td**). According to a theoretical calculation in the ground state,[23] the molecular plane of **Td** as well as that of **Nd** is highly nonplanar and the relative energy of **Td** is 21.5 kcal mol^{-1} higher than that of **Nd**. We suggest that **Td** subsequently undergoes a conformational change in the excited state to produce a singly H-bonded tautomeric dimer (**Ts**), which is entropically favorable. Thus, the observed rate constant of (150 ps)$^{-1}$ at 550 nm originates compositely from the conformational variation and the relaxation of **Td**. Assuming that the relaxation time of **Td** is the same as that of **Ts** (800 ps), we can calculate that **Td** undergoes a conformational change in 180 ps to transform into **Ts**.

The kinetic isotope effect (KIE) of k_{PT} is defined as the ratio of $k_{PT}(7^1HQ)$ to $k_{PT}(7^2HQ)$. The exchange of protic ^{1}H atoms with ^{2}H atoms has revealed that the KIE of ESDPT is as large as 5.2, implying that the rate-determining step is mainly a tunneling process. In case of 7AI dimers, the important role of tunneling in ESDPT has been discussed extensively.[9−11] Temperature-independent KIEs have been reported in enzymatic proton transfers[24,25] and solvent-mediated proton transfers.[4,15−17] Usually, configurational optimization is a prerequisite to efficient proton tunneling, and solvent fluctuation plays a crucial role in the formation of the optimal precursor configuration.[4,15−17] Solvent fluctuation decreases with temperature decrease because solvent motions are reduced at low temperature. Accordingly, if the solvent reorganization is required to form optimized angles and proper H-bond distances for tunneling, the overall rate constant of proton transfer would decrease slightly as temperature decreases, and a little activation energy for solvent reorganization would be obtained from an Arrhenius plot. However, Table 1 shows that k_{PT}, as well as KIE, remains invariant regardless of temperature in the region of 279−300 K, suggesting that the ESDPT of 7HQ dimers are not affected by solvent reorganization. In other words, invariant k_{PT} values regardless of temperature for both dimers of 7^1HQ and 7^2HQ indicate that the rate of ESDPT is determined entirely by a tunneling process. In addition, temperature-independent k_{PT} naturally brings about invariant KIE. If any other factors induced by solvent fluctuation besides tunneling, such as heavy-atom motions, are involved in the rate-determining step, KIE depends on temperature because solvent viscosity having a decisive effect on solvent fluctuation tends to decrease with temperature increase. Thus, 7HQ dimers of a cyclic form having double H bonds already at the ground state undergo ESDPT facilely via tunneling.

The temperature-dependent dynamic behavior of the ESDPT of 7^1HQ dimers shows that the amplitude percentage of the fast components becomes large as temperature decreases (Table 1). This indicates that the more fraction of 7HQ dimers exist as **Nd**, which can transform into **Td**, at low temperatures. Nagai et al. have carried out ab initio calculations for the stable structures of 7HQ dimers in the ground state to report that the energy of a doubly H-bonded dimer (**Nd**) is higher than that of a singly H-bonded dimer (**Ns**) by 0.40 kcal mol^{-1}.[23] Because two factors—ΔH and ΔS—of association are responsible for the formation of 7HQ dimers, we consider that **Nd** is favorable in terms of ΔH due to one additional H bond, whereas **Ns** is advantageous in terms of ΔS because of higher degrees of freedom. Thus, the equilibrium is expected to move toward **Ns** at high temperatures because the contribution of ΔS becomes large. On the other hand, the amplitude percentage of the fast component monitored at 420 nm is much smaller in the ESDPT of 7^2HQ dimers than in the ESDPT of 7^1HQ dimers. The electronic structures of H bonds with ^{1}H are not changed compared to those with ^{2}H when nuclei are held at fixed positions. However, the enthalpy of formation as well as the geometry of H bonds depends on not only the electronic structures but also the vibrational component that is sensitive to isotopic substitution.[26] Thermodynamically, H bonds with ^{1}H are known to be a little stronger than H bonds with ^{2}H.[26] For example, the formation constants of cyclic 7HQ·$(H_2O)_2$ in diethyl ether are reported to be 11 M^{-2} with ^{1}H and 3.3 M^{-2} with ^{2}H.[16,17] In addition, medium-strength H bonds with ^{1}H are shorter than those with ^{2}H by about 0.05 Å.[26] This was

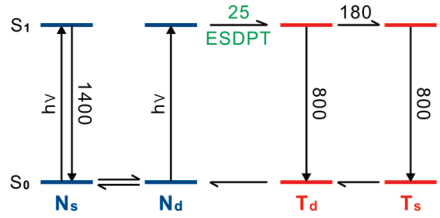

Figure 6. Schematically presented proposed reaction mechanism for the proton-transfer and relaxation processes of excited 7HQ dimers in benzene at 300 K. The numbers are shown in units of picoseconds.

evidenced by the NMR studies of acetic-acid cyclic dimers.[27] Although these differences are relatively small at high temperatures, we consider that those factors cannot be ignored in the ESDPT of 7HQ dimers because **Nd** is nonplanar and highly unstable.

4. Conclusions

Figure 6 summarizes our proposed reaction mechanism of the excited-state double proton transfer (ESDPT) of 7HQ dimers in benzene at 300 K. Two conformers of 7HQ dimers exist in the ground state. A singly hydrogen (H)-bonded noncyclic dimer (**Ns**) cannot undergo ESDPT within the lifetime of 1400 ps, whereas a doubly H-bonded cyclic dimer (**Nd**) transforms into a tautomeric dimer (**Td**) within 25 ps upon absorption of a photon. We also suggest that **Td** successively undergoes a conformational change in 180 ps to become an entropically favorable singly H-bonded tautomer (**Ts**), which relaxes on the time scale of 800 ps. The temperature-independent rate and a large kinetic isotope effect (5.2) of ESDPT imply that intrinsic proton transfer via tunneling mainly determines the rate of the overall proton-transfer reaction of excited **Nd**.

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