Picosecond Dynamics of Stepwise Double Proton-Transfer Reaction in the Excited State of the 2-Aminopyridine/Acetic Acid System†

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The dynamics of the amino−imino double proton-transfer tautomerism reaction of the 2-aminopyridine (2AP)/acetic acid system in hexane have been investigated with steady-state absorption, steady-state fluorescence, and picosecond time-resolved fluorescence spectroscopies. It has been confirmed that the double proton-transfer reaction takes place in the excited state of the double hydrogen-bonded complex of 2AP with acetic acid. The imino tautomer fluorescence shows a rise behavior with a 5 ps time constant at 480 nm, while a decay with a 5 ps time constant has been observed at 360 nm. The rate of this kinetics is reduced to 7 ps with deuterium substitution. From the comparison with the steady-state fluorescence spectra of proton-transferred model compounds, the 5 ps decaying species has been identified as the intermediate in which one proton is transferred from acetic acid to 2AP. It is thus concluded that the 5 ps time constant represents the second proton-transfer process which follows the first proton transfer that is too fast to be detected in the present experiment. The photoexcited double hydrogen-bonded complex of 2AP and acetic acid undergoes a stepwise double proton-transfer reaction within 5 ps in the excited state.

1. Introduction

Proton-transfer reaction, which includes acid−base reactions and the hydrogen atom exchange reactions of water, plays a crucial role in chemistry and biology.[1,2] From the viewpoint of physicochemical studies, excited-state proton transfers have been studied extensively because the reactions can be triggered easily by photoexcitation.[3−6] Among the many known proton-transfer reactions, multi proton-transfer reactions in hydrogen-bonded networks are of particular interest. Typical examples are the proton pump relay in biological systems[2] and the proposed proton relay in water.[7] The minimum unit of the multi-proton-transfer process is a double proton-transfer reaction involving two hydrogen bonds. Thus, the double proton transfer is a good starting point for understanding multi-proton-transfer reactions.

One of the fundamental questions on the double proton transfer is whether it proceeds in a concerted fashion or in a stepwise mechanism.[4] If the reaction occurs through a concerted pathway involving concomitant transfer of both protons, then the reactant tautomer is produced directly after the photoexcitation. If, however, the reaction proceeds in a stepwise mechanism, then a reaction intermediate in which only one proton is transferred will be formed first. As a prototype of the excited-state double proton-transfer reaction, the 7-azaindole (7AI) dimer system has been studied thoroughly, both experimentally[8−16] and theoretically.[17−19] The 7AI dimer system is regarded as a model system for the photoinduced mutation of the DNA base pairs.[8] In protic solvents, proton-transfer reactions are facilitated by strong intermolecular interaction between the solute and the solvent. For example, 7AI[20−24] and 7-hydroxyquinoline (7HQ)[25−30] show phototautomerization only in protic solvents.

Proton-transfer reactions in nitrogen-containing heterocyclic molecules are particularly relevant to biochemical system. Amino−imino tautomerizations facilitated by proton transfer in the heterocyclic molecules have been the subject of a number of studies.[31−37] They are involved in spontaneous mutations in DNA[31−33,35] or in the function of vitamin B1 coenzyme.[34]

In the present study, we investigate the photoinduced tautomerization of 2-aminopyridine (2AP) to 2(1*H*)-pyridineimine (1H2PI). This process is thought to take place in a specific complex between 2AP and acetic acid (ACID).[38,39] This reactive complex is formed by two hydrogen bonds, in a geometry suitable for an excited-state double proton-transfer reaction. The acetic acid molecule acts as a catalyst (see Scheme 1). The relationship between the structure and the fluorescence spectrum of 2AP in various solvents as well as in the gas phase has already been studied.[40−43] In the electronic ground (S_0) state, 2AP itself and its imino-tautomer 1H2PI are in equilibrium. Upon electronic excitation, basicity of the pyridine moiety is expected to increase while the acidity of the amino group increases. It is quite possible that the tautomeric equilibrium is shifted toward the imino-structure when the two forms are in their first excited singlet (S_1) state. Since 2AP does not have an intramoleculer hydrogen bond available for the tautomerization, ACID will act as a catalyst for the excited-state tautomerization.

In this article, we study the photoinduced double proton-transfer reaction in the 2AP/ACID system in detail, with the aim of clarifying the mechanism of the double proton transfer. We first mention briefly steady-state UV absorption spectroscopy of the 2AP/ACID system. Second, we describe steady-state fluorescence spectra of the 2AP/ACID system. The fluorescence spectra of the model compounds for the intermediate and the product of the proton-transfer reaction are also

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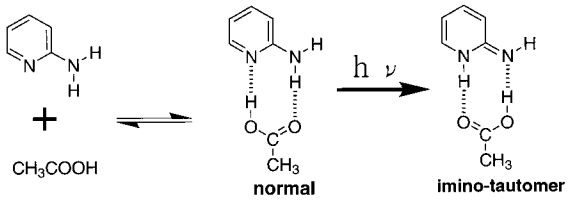
SCHEME 1: Proton-Transfer Tautomerism in the 2-Aminopyridine/Acetic Acid System



discussed (see Figure 1). Third, we report on picosecond time-resolved fluorescence measurements of the intermediate and the product of the excited-state double proton-transfer reaction. Finally, we discuss the mechanism of the proton-transfer reaction of the 2AP/ACID system.

2. Experimental Section

A. Picosecond Time-Resolved Fluorescence Spectroscopy. The experimental setup for the picosecond time-resolved fluorescence spectroscopy is essentially the same as that described previously in detail.[44,45] In short, the third harmonic of a regeneratively amplified mode-locked Ti:sapphire laser (Clark-MXR RA1, 280 nm, 300 fs, 1 kHz, 3−4 mW) was used as the pump light source to photoexcite the sample. The pump pulses were focused on a thin-film jet stream of the sample solution. The collected fluorescence signals, whose polarization was 54.7° (magic angle) to the excitation polarization, were dispersed with an astigmatism-corrected spectrograph (Chromex 500, 100 grooves/mm), and detected with a streak camera (Hamamatsu C2909, modified). The time and spectral profiles of the signals were recorded simultaneously on a CCD detector (Hamamatsu C3077) as a two-dimensional image. The time-resolution of the system was 9 ps (fwhm of the instrumental response function). One streak image covered the spectral range of 90 nm. Time profile of the excitation laser pulse was recorded simultaneously with the fluorescence signals in the same streak image. This assured the exact determination of $t = 0$. Typical exposure time for obtaining one streak image was 10 s. Several streak images were averaged for achieving a high S/N ratio. The wavelength dependence of the sensitivity was calibrated with 2AP fluorescence in the 1 N H_2SO_4 water solution.[46] However, even before the calibration, the sensitivity variation over the observed spectral region was smooth and flat.

B. Steady-State Absorption and Fluorescence Spectroscopy. Steady-state absorption spectra were recorded on a Hitachi U-3500 UV−Vis spectrophotometer. Samples were placed in a 0.1 cm quartz cell. Fluorescence and fluorescence excitation spectra were recorded by a Hitachi F-650-60 spectrofluorometer.

C. Sample Preparation. 2AP was purchased from KANTO Chemical Co., Inc. It was recrystallized several times from HPLC grade hexane and was subsequently dried in vacuo before use. 2-(Dimethylamino)pyridine (2DAP) was purchased from Tokyo Kasei Kogyo Co., Ltd. and was purified by vacuum distillation for three times. Acetic acid (atomic absorption spectroscopic grade) was purchased from KANTO Chemical Co., Inc. and was used without further purification. No fluorescence from acetic acid was detected in the wavelength region of interest. Acetic acid-d_4 (isotopic purity 99.9%) was purchased from Aldrich Inc. Hexane (HPLC grade) was purchased from Wako Pure Chemical Industries, Ltd. and dried further with molecular sieves. Spectroscopic grade hexane was also used, but there was no difference observed between the two types of solvents. Water was used as solvent of aqueous solutions after being distilled twice and deionized. Sulfuric acid (spectroscopic grade) was purchased from KANTO Chemical Co., Inc. and was used without further purification.

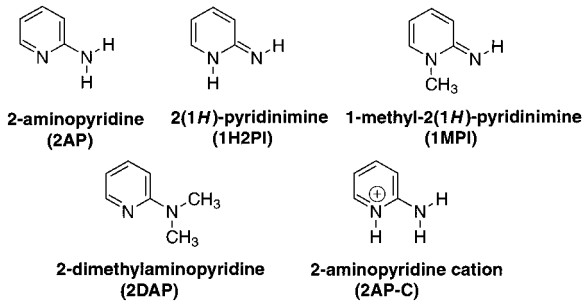


Figure 1. Molecular structures of 2AP and related compounds; 2AP 2(1*H*)-pyridineimine (1H2PI), 1-methyl-2(1*H*)-pyridinimine (1MPI), 2-(dimethylamino)pyridine (2DAP), and 2-aminopyridine−cation (2AP−C).

D. Synthesis of NH_2-Deuterated 2AP (2AP−ND_2) and Imino-Tautomer Model Compound (1-Methyl-2(1*H*)-pyridinimine, 1MPI). The imino-tautomer model compound, 1-methyl-2(1*H*)-pyridinimine (1MPI), was synthesized and purified with the methods already published.[47,48] In short, the mixture of 2AP and methyl iodide was kept at 70 °C for about 3 h. After the reaction was complete, the mixture was dried in vacuo and was recrystallized from ethanol. The obtained salt was dissolved in a 1 mol dm^{-3} sodium hydroxide solution and the solution was stirred for 15 min. The free base was extracted with ether and was dried over sodium sulfate. After the solvent was removed, the sample was distilled for three times for purification. 1MPI was obtained as a yellow liquid. The product and its purity were confirmed by absorption, fluorescence, and ^{1}H NMR (DMSO-d_6) spectroscopies. 2AP−ND_2 was obtained by refluxing a mixture of 0.01 mol of 2AP and 3 mol of D_2O (isotopic purity 99.9%) for 60 min under the N_2 atmosphere and subsequent vacuum evaporation. Typically five cycles of reflux and vacuum evaporation were repeated. The ^{1}H NMR spectra (500 MHz) of the product were measured with a Bruker DRX-500 spectrometer and compared with the literature. The isotopic purity was 91%.

3. Results and Discussion

3.1. Establishment of Double Proton-Transfer Reaction. In this section, we describe the results of steady-state UV−Vis absorption and fluorescence spectroscopies and fluorescence lifetime measurement of the 2AP/ACID system in hexane. These results have been already reported.[38,39] However, we present these data here because it would be impossible to interpret and discuss the following picosecond time-resolved fluorescence spectra without the detailed knowledge of the steady-state results.

We first study the 2AP/ACID system in the ground state. Figure 2a shows the UV absorption spectra of 2AP with various concentrations of ACID in hexane at room temperature. In the spectra, an absorption maximum appears at 289 nm. This band is predominantly due to the 2AP monomer. When the concentration of ACID is increased, the absorption maximum is shifted by 10 nm to 299 nm.[38] Then a shoulder grows in the region of 310−350 nm. Isosbestic points are observed at 230 and 291 nm.

In 2-(dimethylamino)pyridine (2DAP), two hydrogen atoms of 2AP are replaced with two methyl groups. This molecule is a model compound for the amino form because the amino−imino tautomerization is prohibited due to its lack of hydrogens of the amino group. Figure 2b-1 and -2 show the UV absorption

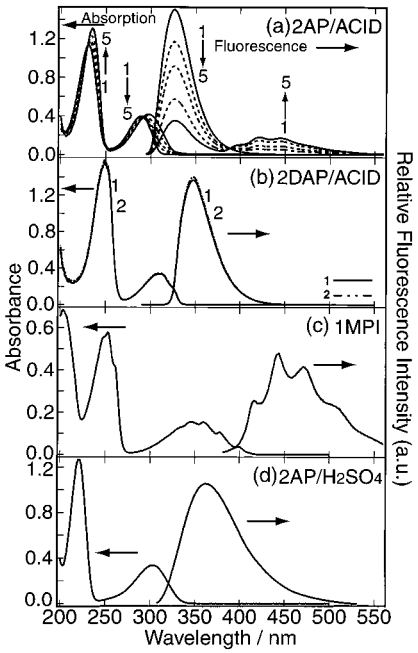


Figure 2. (a): UV absorption spectra (left axis) and fluorescence spectra (right axis, excitation at 280 nm) of the 2AP/ACID system in hexane. Concentration of 2AP; 1.0×10^{-3} mol dm^{-3}, concentration of ACID/mol dm^{-3}; (1) 0, (2) 5.0×10^{-4}, (3) 1.0×10^{-3}, (4) 5.0×10^{-3}, (5) 1.0×10^{-2}. (b)1 and 2: UV absorption and fluorescence spectra (excitation at 280 nm) of the 2DAP/ACID system in hexane. Concentration of 2DAP; 1.0×10^{-3} mol dm^{-3}, concentration of ACID/mol dm^{-3}; 0 and 5.0×10^{-3}. (c): UV absorption and fluorescence spectra (excitation at 330 nm) of 1MPI in hexane. Concentration of 1MPI; 1.0×10^{-3} mol dm^{-3} (d): UV absorption and fluorescence spectra (excitation at 280 nm) of 2AP with H_2SO_4 in water. Concentration of 2AP; 1.0×10^{-3} mol dm^{-3}, concentration of sulfuric acid; 0.5 mol dm^{-3}.

spectra of 2DAP with and without ACID in hexane. The UV absorption band from 2DAP is observed at 309 nm. Although 2DAP has an absorption band in a similar spectral position with 2AP, it dose not show an ACID-dependent spectral shift as observed for 2AP. Because dimethylamino group of 2DAP dose not have hydrogens, this group unlike 2AP, cannot form a hydrogen bond with ACID. This finding suggests strongly that the shift of the absorption band for the 2AP/ACID system is caused by the hydrogen bond including the amino group. It also suggests that formation of a hydrogen bond between the ring nitrogen and ACID has little effect on the electronic structure of 2AP.

A model compound for the imino tautomer of 2AP is 1MPI in which the imino structure is stabilized with an *N*-methyl group. UV absorption spectrum of 1MPI is shown at Figure 2c. The spectrum shows a vibronic structure in the 345 nm region.[49] The UV spectrum of 1MPI is quite different from that of 2AP/ACID. We thus conclude that the imino tautomer is not predominantly formed in the ground state of 2AP.

We investigate the 2AP/ACID system in the excited state by steady-state fluorescence. Figure 2a shows the fluorescence spectra of 2AP with various concentrations of ACID in hexane at room temperature. Because the excitation wavelength (280 nm) is close to the isosbestic point (291 nm), both 2AP and the 2AP/ACID complex absorb the excitation light. Therefore, two fluorescence components, one from 2AP and the other from the 2AP/ACID complex, are expected in this spectral region. At any concentration, with and without the presence of ACID, an emission maximum is observed at 325 nm (the F1 band). Since this band is observed without ACID, it is assigned to the

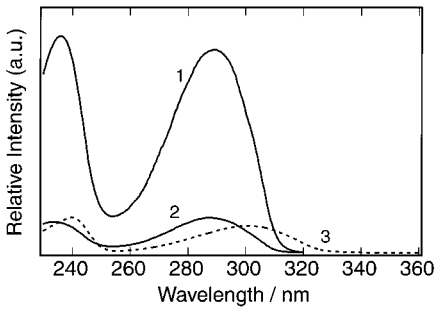


Figure 3. Fluorescence excitation spectrum of 2AP (1.0×10^{-3} mol dm^{-3}) monitored at 325 nm (1) and those of 2AP (1.0×10^{-3} mol dm^{-3})/ACID(1.0×10^{-3} mol dm^{-3}) monitored at 325 nm (2) and at 480 nm (3).

fluorescence from 2AP monomer that does not form a hydrogen-bond complex with ACID. The shape of this emission band does not depend on the ACID concentration. When ACID is added, distinct dual fluorescence features are observed. In addition to the F1 band at 325 nm, another band (the F2 band) appears at 420 nm. This band shows a large Stokes shift of 9300 cm^{-1} and owns a characteristic vibrational structure. The series of concentration-dependent spectra reveal an isoemissive point at 387 nm.[49]

As mentioned earlier, amino−imino tautomerism is prohibited for 2DAP. This molecule serves as a good model for the amino form of 2AP. Figure 2b-1and -2 show the fluorescence spectra of 2DAP in hexane with the ACID of 0 (trace 1) and 5×10^{-3} mol dm^{-3} (trace 2) at room temperature. A structureless fluorescence band is observed at 346 nm. The fluorescence spectrum of 2DAP is similar to that of the 2AP monomer. A relatively small red shift from 2AP is explained by the perturbation of methylation at the amino group. The fact that the 2DAP spectrum is not affected by the presence of ACID is consistent with the above conclusion that the F1 band is due to the 2AP monomer. Because the F1 band is observed at any concentration of ACID, there should be free 2AP monomers present in the 2AP/ACID system.

The assignment of each emitting species can be reinforced by measuring fluorescence excitation spectra. Fluorescence excitation spectra of 2AP as well as the 2AP/ACID mixture are shown in Figure 3. When monitored at the region of the F1 band (325 nm), the excitation spectrum of 2AP (Figure 3−1) is the same as that of the 2AP/ACID mixture (Figure 3−2). They exhibit a maximum at 289 nm. However, the excitation spectra of the 2AP/ACID mixture monitored at the long-wavelength region of the F2 band (at 480 nm) show a maximum at 302 nm (Figure 3−3), red shifted by 13 nm compared with the excitation spectra monitored at the F1 band region. Two spectral components which cause the ACID concentration-dependent change in the UV absorption spectra (Figure 2a) are now clearly separated in these excitation spectra.

The fluorescence excitation spectra show that the dual fluorescence originates from different ground-state species, most probably the 2AP monomer and the 2AP/ACID hydrogen-bonded complex. It is strongly suggested that the initial state of the F1 band transition is the 2AP monomer. It is also obvious that the F2 band is associated with the 2AP-ACID hydrogen-bonded complex. Due to the high acidity of the carboxylic proton ($pK_a = 4.75$) and a drastic increase of the basicity in the excited pyridine nitrogen ($pK_a^* = 8.95$ [40]), it seems possible that the F2 band emission comes from the tautomer in which a single proton is transferred from the ACID carboxylic group to

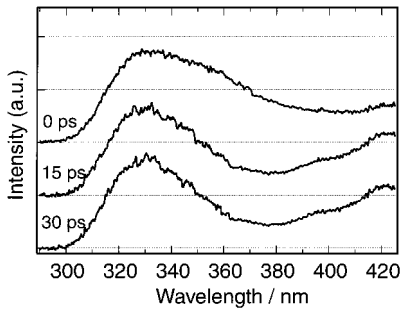


Figure 4. Picosecond time-resolved emission spectra of the 2AP/ACID system in hexane. Spectra at 0, 15, and 30 ps after excitation are shown.

the 2AP pyridine ring nitrogen. Such a possibility, however, has been eliminated by the following experiments.

It has been known that in 0.5 mol dm^{-3} H_2SO_4 water solution, where the pyridine ring nitrogen is protonated and 2AP exists predominantly as a $2APH^+/HSO_4^-$ ion pair, the emission maximum is observed at 360 nm (see Figure 2(d)).[40,41] This fluorescence peak wavelength is different from the F2 band. The fluorescence spectrum from the protonated 2AP dose not have the characteristic vibrational structure observed for the F2 band. It is not possible to assign the F2 fluorescence component of the 2AP/ACID system to the protonated 2AP cation species. It should be noted that a new band at 370 nm appears in the fluorescence spectrum at much higher concentrations of ACID.[38,39] This band lies within the region where the fluorescence spectrum of the protonated 2AP cation appears, with an excitation peak at 307 nm. The emergence of the new band may be attributed to the protonation of the ring nitrogen atom in the pyridine. Although this fluorescence component is clearly distinguishable from the F2 band, we took much care for keeping the ACID concentration low enough that such luminescence was not observed in the experiments.

For supporting the above conclusion that the F2 fluorescence is not from the protonated 2AP, an imino model compound 1MPI (see Figure 1) was synthesized. This molecule has a similar molecular structure as the imino tautomer of 2AP and does not isomerize to the amino tautomer. In the 2AP/ACID system, the 2AP imino tautomer is formed after two protons are transferred between 2AP and ACID. As shown in Figure 2c, 1MPI exhibits a fluorescence band at 442 nm.[49] This spectral feature is quite similar to the F2 band of the 2AP/ACID complex (Figure 2a). From these results, it is strongly suggested that the F2 band corresponds to the emission from the imino tautomer of 2AP. From the two sets of experiments mentioned above, it is obvious that the F2 band of the 2AP/ACID system originates from a double proton-transferred 2AP imino tautomer and not from a single proton-transferred 2AP cation. These experimental results support strongly that an excited-state double proton-transfer phototautomerism reaction occurs in the 1:1 hydrogen-bond complex of 2AP and ACID. In this reaction, the ACID molecule acts as a bifunctional catalyst.

From the steady-state fluorescence experiments, it has been shown that the F1 band (325 nm) corresponds to the emission from the photoexcited 2AP monomer. Time dependence of this emission was measured with a picosecond time-resolved fluorescence spectrometer. Obtained time-resolved fluorescence spectra are shown in Figure 4. In the figure, the F1 band appears at $t = 0$ ps and decays in nanosecond time scale. The fluorescence decay curve from 2AP in hexane, monitored at 325 nm, is shown in Figure 5a. The recorded decay curve is fit well with a single-exponential function with a lifetime of 1.28 ± 0.04 ns, which agrees well with the reported value of

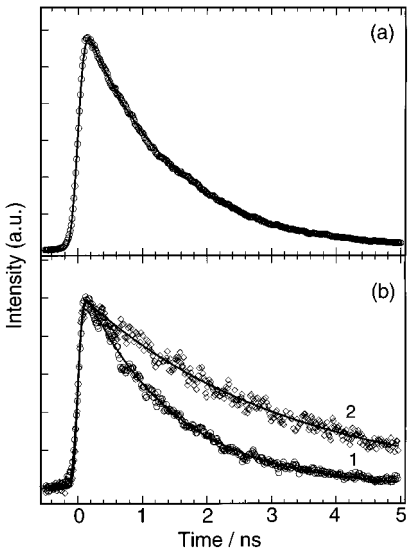


Figure 5. (a): Time-dependent fluorescence of 2AP (1.0×10^{-3} mol dm^{-3}) in hexane monitored at 325 nm ($\tau = 1.28 \pm 0.04$ ns), (b) 1: time-dependent fluorescence of the 2AP (1.0×10^{-3} mol dm^{-3})/ACID (1.0×10^{-3} mol dm^{-3}) system in hexane monitored at 325 nm ($\tau = 1.41 \pm 0.08$ ns), and (b) 2: monitored at 480 nm ($\tau = 3.26 \pm 0.12$ ns). All curves fit assuming a single-exponential decay.

1.1 ns.[39] The decay kinetics was independent of the monitored emission wavelength. In the 2AP/ACID system, the F1 emission free from the interference of the F2 band was monitored at 325 nm. The obtained decay curves are fit by a single-exponential function with a lifetime of 1.41 ± 0.08 ns (Figure 5b-1). The F1 band lifetime of the 2AP/ACID system is close to that of the 2AP monomer and it dose not depend on the concentration of ACID. Fluorescence decay kinetics at 480 nm was measured for the F2 band as well. The results are shown in Figure 5b-2. The obtained decay curve is explained well with a single-exponential decay having a lifetime of 3.26 ± 0.12 ns. The obtained lifetime, once again, agrees well with a reported value of 3.2 ns.[39] In the spectral region between 350 and 400 nm, fluorescence signals from the F1 band and those from the F2 band overlap with each other. In this region, the obtained decay curves in several different wavelengths were explained by only two components with $\tau \sim 1.41$ ns and $\tau \sim 3.26$ ns. These values are, within experimental errors, identical with the lifetimes of the F1 (310 nm) and the F2 (420 nm) bands. It is unambiguously concluded that there are two emitting species with different lifetimes observed in the entire spectral region of 300 to 500 nm and that the excited tautomerized 2AP/ACID complex is not in equilibrium with the free 2AP excited state.

On the basis of the experimental results from steady-state UV−Vis absorption and fluorescence spectroscopies and picosecond time-resolved fluorescence spectroscopy, we conclude that the 2AP/ACID system in hexane undergoes a double proton-transfer reaction after the photoexcitation. In the ground state, 2AP in the 2AP/ACID system has two forms, the free 2AP monomer and the 2AP-ACID hydrogen-bonded complex. When the monomer is photoexcited, it emits fluorescence at 325 nm (the F1 band) with a lifetime of 1.4 ns. When the hydrogen-bonded complex is photoexcited, the imino-tautomer of 2AP is formed after the double proton-transfer reaction between 2AP and ACID. The imino-tautomer product emits fluorescence at 480 nm (the F2 band) with a lifetime of 3.2 ns. In the excited states, the free 2AP and the imino-tautomer are not in equilibrium. A detailed mechanism of the excited-state double proton-transfer reaction will be discussed in the following section.

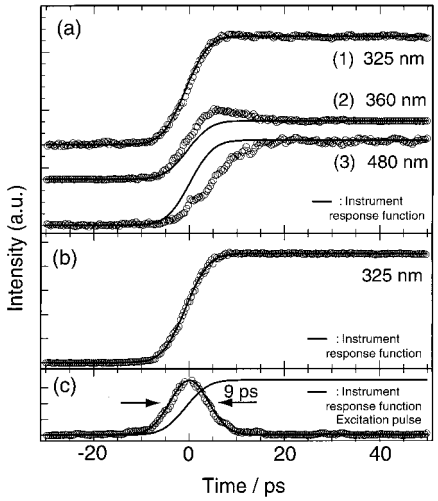


Figure 6. (a): Picosecond fluorescence signals obtained from the 2AP (1×10^{-3} mol dm^{-3})/ACID (1×10^{-3} mol dm^{-3}) system in hexane for three different monitoring wavelengths. (b): Picosecond fluorescence signals obtained from 2AP (1×10^{-3} mol dm^{-3}) without ACID. (c): The instrumental response function obtained from the excitation pulse. The open circles are experimental data points and the solid curves are instrumental response functions. The excitation wavelength is 280 nm.

3.2. Stepwise Mechanism of Double Proton-Transfer Reaction. In the excited-state double proton-transfer reaction of the 2AP/ACID system, one proton is transferred from ACID to the pyridine nitrogen of 2AP, and another proton is transferred from the amino group of 2AP to ACID. As stated in the Introduction, the fundamental problem on this reaction is to elucidate whether it occurs in a concerted fashion or in a stepwise mechanism. Direct evidence of the problem can be obtained from picosecond time-resolved fluorescence spectroscopy by identifying the possible reaction intermediate which exists solely in the stepwise mechanism.

For clarifying the mechanism of the double proton transfer, we measured the time-resolved fluorescence signals of 2AP/ACID in hexane solutions at three wavelengths, 325 nm for the F1 band, 480 nm for the F2 band, and 360 nm for the possible intermediate. In Figure 6, observed fluorescence intensities are plotted with open circles while the instrumental response function in its time integrated form is shown with solid curves. As shown in Figure 6a, observed fluorescence behaviors varies depending on the monitored wavelength. When detected at the F1 band region of 325 nm (Figure 6a-1), the fluorescence signal rises with the instrumental response function, followed by a nanosecond decay, which appears to be constant in the picosecond time scale. This represents the temporal behavior of the 2AP monomer fluorescence. At a longer wavelength of 360 nm (Figure 6a-2), however, the fluorescence signal shows a rapid decay immediately after the photoexcitation, although a large contribution from the 2AP monomer component is still present. At the longest wavelength of 480 nm, a long-lived fluorescence component with a finite rise time is observed. In Figure 6a -3, it is clear that the instrumental response function does not match the rise of the fluorescence signal. This slow-rising component is assignable to the fluorescence from the excited imino-tautomer (after the double proton transfer) because the fluorescence was detected at the wavelength of the F2 band. However, the fluorescence component with a decay time constant of several picoseconds, observed at 360 nm, is explained not by the 2AP monomer (1.4 ns) nor by the imino-tautomer (3.2 ns). A transient species other than the 2AP monomer or the imino-tautomer contributes to the time-resolved fluorescence signal observed at 360 nm.

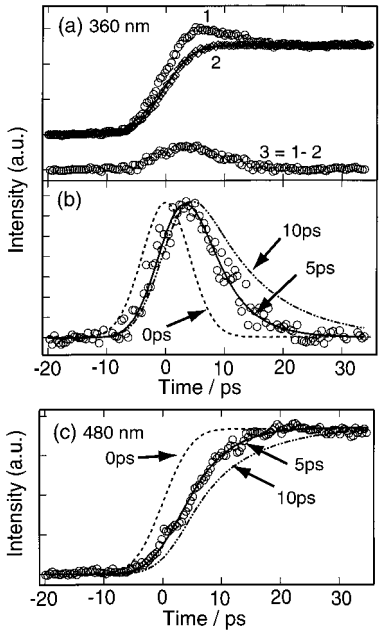


Figure 7. (a) 1: Fluorescence signal of the 2AP/ACID system at 360 nm. (a) 2: Fluorescence signal of 2AP at 360 nm and the normalized instrument response function. (a) 3: The fluorescence difference signal (a)1 − (a)2. (b): Comparison between the decay component (a)3 and several calculated single-exponential decay curves. (c): The fluorescence signal at 480 nm and several calculated single-exponential rise curves.

Now, temporal changes of the fluorescence signals at 360 and 480 nm are analyzed quantitatively. In Figure 7a, fluorescence signals, detected at 360 nm, are compared between 2AP and the 2AP/ACID mixture. As shown in Figure 7a-2, the fluorescence signal from 2AP (without ACID) dose not have a fast-decaying component observed for the 2AP/ACID mixture (Figure 7a-1). From the nanosecond fluorescence lifetime measurement, it has been concluded that both 2AP and the 2AP/ACID mixture have fluorescence components decaying with a lifetime of 1.2 ns or longer. These long-lived components should appear as a flat line in Figure 6a. Because the fast-decaying component from the 2AP/ACID mixture vanishes at $t = 20$ ps, the offset level at 20 ps represents the contribution from the long-lived species to the fluorescence signals. Thus, we normalize the two sets of fluorescence signals at 20 ps and calculate the difference between the normalized traces. The 2AP monomer component is eliminated in this process and only the fast-decaying component is extracted. The result is shown in Figure 7a-3. This procedure enables us to perform a fitting analysis with a reduced number of parameters, focusing only on the decay component. We fit the obtained kinetic trace (Figure 7a-3) with a single-exponential decay curve convoluted with the instrumental response function. The results are shown in Figure 7b. The best fit is obtained when the decay lifetime is $\tau = 5 \pm 1$ ps. Expected kinetics for lifetimes of 0 and 10 ps are also shown in this figure. It should be noted that the rise time for this fluorescence component was not detected with the instrumental response time of our apparatus (9 ps).

For clarifying the 5 ps decaying component observed at 360 nm, transient fluorescence spectrum of the 2AP/ACID mixture at $t = 0$ ps is reconstructed for the 290−425 nm spectral region from three separate streak images. Each image covers 90 nm. The fluorescence signals within the time period of 15 ps are integrated for obtaining the transient spectrum. The result is

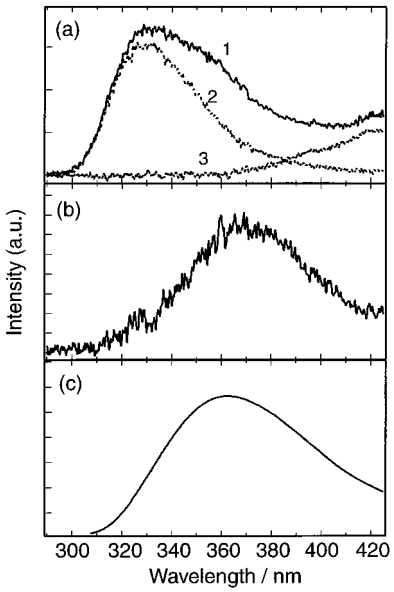


Figure 8. (a) 1: The time-resolved fluorescence spectrum of the 2AP/ACID system at 0 ps. (a) 2: The normalized time-resolved fluorescence spectrum of 2AP at 0 ps. (a) 3: The normalized time-resolved fluorescence spectrum of the imino tautomer at 0 ps. (b): The difference time-resolved fluorescence spectrum at 0 ps (a)1 − [(a)2 + (a)3]. (c): The steady-state fluorescence spectrum of 2AP with H_2SO_4 in water (280 nm excitation).

shown in Figure 8a-1. Similarly, the transient fluorescence spectrum of 2AP without ACID at 0 ps is obtained. The spectrum is shown in Figure 8a-2 where intensity is normalized to trace 8a-1 in the 290−310 nm. The transient fluorescence spectrum of the imino-tautomer (after the double proton transfer) at 0 ps was also calculated. This spectrum is obtained by subtracting the fluorescence spectrum of 2AP at 30 ps from the fluorescence spectrum of the 2AP/ACID system at 30 ps. The fluorescence spectrum of the 2AP/ACID system at 30 ps consists of the 2AP monomer spectrum and the imino-tautomer spectrum. A coefficient for the subtraction was determined so that the subtracted value became zero in the luminescence area from the 2AP monomer. This criterion is rationalized by steady-state fluorescence and the fluorescence lifetime measurements mentioned earlier. The resultant spectrum of the imino-tautomer is shown at Figure 8a-3. The transient fluorescence spectrum of the 5 ps decaying component was obtained by subtracting the fluorescence spectrum of 2AP (without ACID, trace 2 in Figure 8a) and that of the imino-tautomer (trace 3) from the spectrum of the 2AP/ACID complex (trace 1). The obtained spectrum is shown in Figure 8b. This spectrum has a feature with a maximum at 370 nm. To account for the obtained spectrum, the following three possibilities are considered.

First, it is possible that the obtained spectrum is luminescence from the photoexcited 2AP cation that is already formed in the ground state. This possibility, however, is excluded on the basis of the following reasons. In the steady-state fluorescence measurements, an isoemissive point was observed (Figure 2a). This means that, under the present experimental conditions, there should be no additional spectral component other than the 2AP monomer and the imino-tautomer unless the third component has very small fluorescence intensity after averaged over time. We produced the 2AP cation in the 2AP/ACID system by intentionally increasing the ACID concentration to a much higher level than the usual condition. It was found that the fluorescence lifetime of the 2AP cation was about 6 ns. Observed 5 ps component is not explained by the fluorescence from the ground-state 2AP cation.

Second, the 5 ps fluorescence species can be the hydrogen-bonded complex immediately after excitation, before the proton transfer. This assignment, however, is less likely for the following reason. The fluorescence excitation spectrum of the hydrogen-bonded complexes of 2AP and ACID has a maximum at 302 nm, as shown in Figure 3-3. If the amount of the Stokes shift for the hydrogen-bonded complex is similar to that of the 2AP monomer (3800 cm^{-1}), the fluorescence of the hydrogen-bonded complex will appear at 340 nm. However, the obtained spectrum (Figure 8b) has a maximum at around 370 nm. This corresponds the Stokes shift of 6100 cm^{-1}. If the fluorescence comes from the hydrogen-bonded complex immediately after the photoexcitation, the observed larger Stokes shift has to be explained properly.

Third, we consider the possibility that the spectrum of the 5 ps component (Figure 8b) represents fluorescence from a photochemical reaction intermediate in which only one of the two protons is transferred. To examine this possibility, we measured the steady-state fluorescence spectrum of 2AP in the water solution of 0.5 mol dm^{-3} sulfuric acid. It has been known that, under this condition, 2AP is protonated.[40,41] Protonated 2AP is a good model compound for a single proton-transferred 2AP/ACID complex. The steady-state fluorescence spectrum of this protonated 2AP is shown in Figure 8c. Agreement between the fluorescence spectrum of the 5 ps decaying component (Figure 8b) and that of the protonated 2AP (Figure 8c) is surprisingly well. From these experimental data, it is most probably that the fluorescent species with a 5 ps lifetime is an excited-state ion-pair intermediate in which one proton of ACID is transferred to the ring nitrogen of 2AP.

The rise of the imino tautomeric component should also reflect the double proton-transfer reaction dynamics. Observed rise kinetics at 480 nm was fit with a single-exponential rise function convoluted with the instrumental response function. The results are shown in Figure 7c. The best fit was obtained when the time constant for the rise was $\tau = 5 \pm 1$ ps. The rise time of 5 ps matches the reported $\tau = 8 \pm 12$ ps for the tautomer formation of the 2AP/trimethylacetic acid sysytem.[39] The agreement between the decaying lifetime of the 360 nm transient and rise time of the tautomer fluorescence indicates that the species observed at 360 nm is the precursor of the imino tautomerized complex.

We also studied the effect of isotopic substitution on this double proton-transfer reaction. Picosecond time-resolved fluorescence spectra of the deuterated 2AP−ND_2/ACID-d_4 system were measured under the same experimental conditions as the normal species. The results are shown in Figure 9. In the figure, we compare time-resolved fluorescence signals obtained from the normal 2AP/ACID system and its deuterated analogues. The fast-decaying fluorescence component observed at 360 nm has a lifetime of 5 ps in the 2AP/ACID system, as mentioned above. For the deuterated analogue, the lifetime is changed to 7 ± 1 ps (Figure 9a). The rise of the tautomer fluorescence also shows an isotope effect. As shown in Figure 9b, the 5 ps rise time of the normal species, once again, changes to 7 ± 1 ps upon the deuteration. We repeated the same measurement five times and the results were reproducible. The observed effect of the deuterium substitution on the reaction kinetics is consistent with our previous assignment that the 5 ps component corresponds to actual translocation of a proton. The second match, at 7 ps, of the decaying lifetime at 360 nm and rise time at 480 nm supports strongly that the 5 ps species is a precursor for the

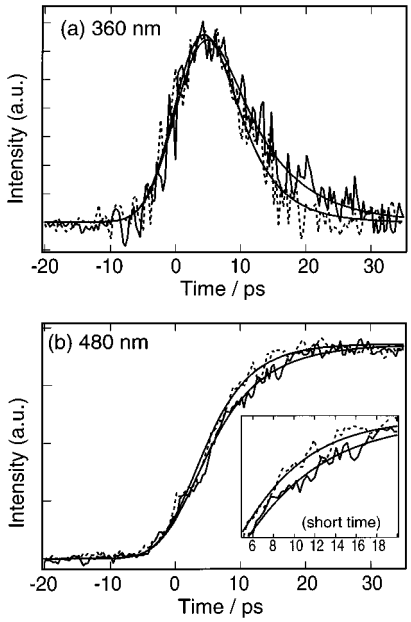


Figure 9. Effect of deuterium substitution on the picosecond fluorescence signals of the 2AP/ACID system in hexane, detected at 360 nm (a) and at 480 nm (b). The excitation wavelength is 280 nm. The normal 2AP/ACID system (- - -) and the 2AP−ND_2/ACID-d_4 system (−). The rise curves in an expanded scale are shown in the inset.

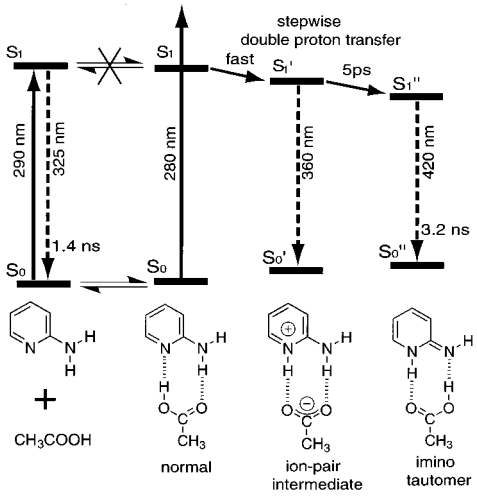


Figure 10. Schematic energy diagram illustrating the dynamics of the excited-state double proton-transfer reaction of the 2AP/ACID system.

imino-tautomer. The monomeric and tautomeric fluorescence lifetimes of the deuterated analogues were also measured in the nanosecond range. On the deuteration, the tautomeric fluorescence lifetime changed from 3.2 ns to 3.67 ± 0.11 ns, whereas the monomeric fluorescence lifetime remained unchanged.

The excited-state double proton-transfer reaction of the 2AP/ACID system in hexane clarified in the present study is summarized in Figure 10. Before the photoexcitation, the 2AP monomer and the hydrogen-bonded complex between 2AP and ACID are in equilibrium. When the hydrogen-bonded 2AP−ACID complex is photoexcited, one proton is transferred from the OH group of ACID to the ring nitrogen of 2AP, forming an ion-pair of the protonated 2AP cation and the ACID anion. Because the 2AP−ACID hydrogen-bonded fluorescence is not observed, this first reaction should occur in much shorter time scale than the time resolution of our apparatus. The ion-pair intermediate, showing a fluorescence band at 370 nm, decays with a lifetime of 5 ps. At the same time, the imino-tautomer in which the second proton is transferred from the protonated 2AP to ACID is formed with a rise time of 5ps. Thus, the excited-state double proton-transfer reaction of the 2AP/ACID system proceeds in 5ps with a stepwise mechanism. The excited state of the imino-tautomer species relaxes to the ground state in 3.2 ns.

The excited-state tautomerization reactions of 7AI/alcohol[20−24] and 7HQ/alcohol [25−30] systems are well-known to proceed via double proton-transfer mediated by a protic catalyst. They resemble the present 2AP/ACID system in that the double proton transfer takes place within a hydrogen-bonded complex. Kohtani et al. reported that the proton-transfer rate in the 7HQ/alcohol system showed a correlation with the acidity of alcohols.[30] They suggested that the reaction was initially triggered by the transfer of an alcoholic proton to the hydrogen-bonded ring nitrogen of 7HQ. However, they did not detect the ion pair of the protonated 7HQ cation and its counterion of alkoxide, reaching a conclusion that the reaction proceeds in a "quasi-concerted" fashion. Petrich and co-workers suggested that the excited-state double proton-transfer reaction in the 7AI/alcohol system proceeded in a concerted way, based on the observed deuteriation effects on fluorescence decay kinetics.[23] Lack of the observation of the ion-pair intermediate, however, does not necessarily mean a concerted mechanism. It is not possible to make a clear distinction experimentally between a stepwise mechanism with a very short-lived intermediate and a truely concerted one.

The present study has proved a stepwise mechanism for the 2AP/ACID system, with the ion-pair intermediate being unambiguously identified by its fluorescence spectrum. It gives a firm ground for future studies on the nature of the intermediate both experimentally and theoretically.

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