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CONCENTRATION DEPENDENCES OF GROUND-STATE AND EXCITED-STATE INTRAMOLECULAR PROTON TRANSFER OF PIROXICAM IN METHANOL

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Abstract—The absorption and fluorescence spectral properties of piroxicam (PRX) in the hydrogen-bonding solvents show the most sensitive dependence on the concentration ranging from 8×10^{-5} to 2×10^{-5} *M*. These are attributed to both the solvent-mediated ground-state intermolecular proton transfer ($GSI_{er}PT$) leading to formation of the ground state anion and the excited-state intramolecular proton transfer ($ESI_{ra}PT$). The concentration dependences of the time-resolved emission kinetics at both room temperature and 77 K have also been investigated. It is shown that in the excited state, the $ESI_{ra}PT$ of PRX is the dominant process to form a keto tautomer at the high concentration, whereas at the low concentration the excited-state conformational change of the anion is an additional process leading to formation of a zwitterion. The $ESI_{ra}PT$ of PRX in the hydrogen-bonding solvent is coupled with the ultrafast excited-state solvent reorganization.

INTRODUCTION

The excited-state intramolecular proton transfer ($ESI_{ra}PT$)† reaction has been an important topic of several photochemical and photophysical studies.[1-5] For the $ESI_{ra}PT$ to take place, there should exist a proton donor and a proton acceptor in the same molecule. The energetic driving force for the $ESI_{ra}PT$ is provided by a change in the acid-base properties of the donor and the acceptor as a consequence of the change in the charge densities at the functional groups in the excited state.[6] The $ESI_{ra}PT$ process induces a large change of the electronic configuration of the molecule, resulting in dual (normal and tautomeric) emission which has been often used to identify the $ESI_{ra}PT$ processes.

When the two functional groups are *ortho* or *peri* to each other as in the case of 3-hydroxyflavone[7] and *o*-hydroxy aromatic acids,[8] the $ESI_{ra}PT$ process takes place along the pre-existing intramolecular hydrogen bond. This $ESI_{ra}PT$ is strongly perturbed even by the trace amount of the hydrogen-bonding solvent.[9] Such perturbation usually results in relative decrement of the tautomeric emission, but it can also induce an anion formation *via* the excited-state intermolecular proton transfer ($ESI_{er}PT$) or the ground-state intermolecular proton transfer ($GSI_{er}PT$) from the hydroxy group to the solvent cage depending on complexation of the solute with solvent.[9,10] Actually the $ESI_{er}PT$ has been observed in matrix isolated 3-hydroxyflavone $(NH_3)_n$, (n=2, 3) complexes while n=1 complex exhibits the $ESI_{ra}PT$.[7] Recently, it has been reported that in a very strong hydrogen-bonding solvent such as formamide, the $GSI_{er}PT$ is possible for 3-hydroxyflavone but the $ESI_{ra}PT$ process is dominant without intervention of the $ESI_{er}PT$.[10] Therefore, this hydrogen-bonding solvent effects on the $ESI_{ra}PT$ do not seem to be universal for all the hydrogen-bonding solvents and $ESI_{ra}PT$ molecules. However, the $ESI_{er}PT$ process also plays a role in the biprotonic phototautomerization observed from the molecule containing two functional groups which cannot form the pre-existing intramolecular hydrogen bond as in 7-azaindole.[11] Despite these advances, there are still many controversial arguments of the effects of the solvent-solute interaction on the excited-state proton transfer and/or ground-state proton transfer in the hydrogen-bonding solvents.

Recently, there have been few investigations on

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† *Abbreviations:* PRX, piroxicam; DMSO, dimethyl sulfoxide; $GSI_{er}PT$, ground-state intermolecular proton transfer; $ESI_{ra}PT$, excited-state intramolecular proton transfer; $ESI_{er}PT$, excited-state intermolecular proton transfer; HMBDC, (4-hydroxy-2-methyl-1,2-H-benzothiazine-1,1-dioxide-3-methyl-carboxylate); TCSPC, time-correlated single photon counting

the role of the hydrogen-bonding solvent in the $ESI_{ra}PT$ in molecules having more than three functional groups. For example, in the equilibrium between lactim and lactam form, the multiple fluorescence was observed. Depending on the inter- and intramolecular hydrogen bonding and proton transfer, each fluorescence components were assigned to the different ground-state structured species.[12] In these points of view, it would be interesting to examine piroxicam (PRX) (4-hydroxy-2-methyl-N-pyridyl-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide), a non-steroidal antiinflammatory agent, which has four different functional groups as the proton donor and/or the proten acceptor.[13] We have recently found that both PRX and its skeletal analog, HMBDC (4-hydroxy-2-methyl-1,2-H-benzothiazine-1,1-dioxide-3-methyl-carboxylate), in aprotic nonpolar solvents exhibt the $ESI_{ra}PT$ from the hydroxyl group of the benzothiazine ring to the *ortho*-carbonyl group of the amide (see Fig. 1).[14] Both molecules show very large Stokes-shifed tautomeric emissions with a peak around 480 nm as the consequence of the $ESI_{ra}PT$. However, in the protic polar solvent such as methanol, weak normal emissions around 400 nm have been observed with the tautomeric emissions suppressed. These results suggest that the intermolecular hydrogen bonding between the solute and the solvent plays an important role in preventing the $ESI_{ra}PT$. However, it is not yet certain whether such specific solute-solvent interaction should induce the $ESI_{er}PT$ and/or $GSI_{er}PT$ even though PRX is acidic in the ground state and even more acidic in the excited state. Indeed, the pK_a of hydroxy group of PRX has been observed to be low in the ground state (3.3) and even lower in the excited state (-1.0).[15] Furthermore, there still remains a possibility of the involvement of the pyridyl group in the proton transfer processes, because the pyridyl nitrogen is observed to be highly basic in contrast to the acidic hydroxyl group.[15] With these questions in mind, we have attempted to investigate the concentration dependences on the spectral properties of PRX in the hydrogen-bonding solvents, since the $ESI_{ra}PT$ mechanism and the $GSI_{er}PT$ in some molecules are known to be sensitive to the dilution with solvents.[9,16] In this paper, we report the absorption and fluorescence spectra indicating that mostly the $ESI_{ra}PT$ occurs in methanol solution of PRX with-

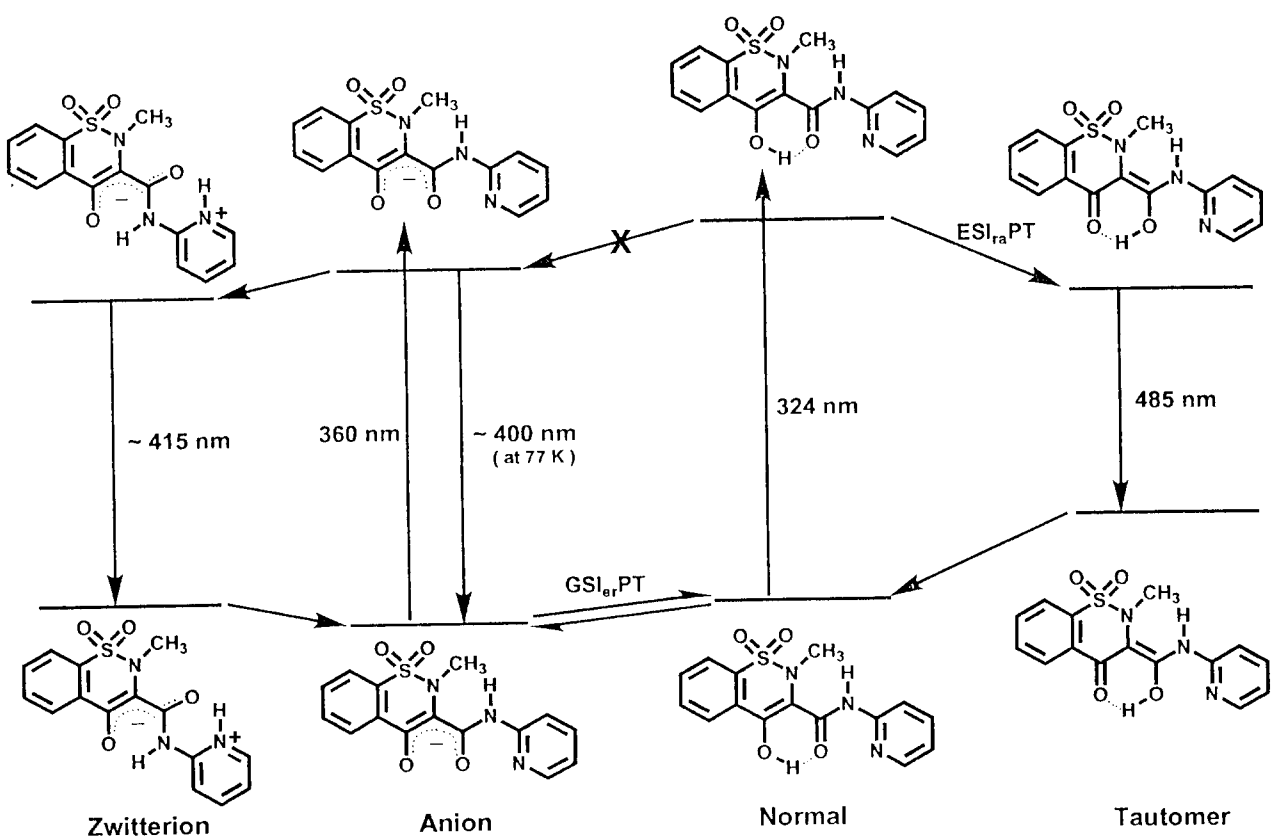


Figure 1. Proton transfers observed in PRX in hydrogen-bonding solvent.

out the ESIerPT at the higher concentration. But upon dilution the GSIerPT becomes feasible to form an anion which can undergo an excited-state conformational change into a zwitterion on the picosecond time scale. The picosecond emission kinetics indicate that the excited-state proton trnasfer is coupled with the excited-state solvent reorganization which is very fast even in rigid glass solution at 77 K (*ca.* 100 ps).

MATERIALS AND METHODS

PRX was a generous gift from Yu Han Pharmaceutical Co. (Korea) and purified by recrystallizing three times from methanol to produce bright white crystals. Its purity was checked by thin layer chromatography (chloroform: methanol:25% ammonia; 80:15:5, as a mobile phase)[17] and the melting point (198-200°C). The skeletal analog of PRX, HMBDC was synthesized and purified by the methods described in a reference.[14] All the solvents used were spectroscopic grades (Merck Co.).

The absorption spectra were recorded on a Beckmann UV-5260 spectrophotometer. Steady-state fluorescence measurements were made on a SLM-AMINCO 4800 spectrofluorometer which makes it possible to obtain corrected spectra by using Rhodamine B as a quantum counter.[18] The oxygen-free samples were prepared by four freeze-pump-thaw cycles from the vacuum line under *ca.* 10^{-4} torr. Measurements of the fluorescence at 77 K were performed by using a quartz liquid nitrogen optical dewar.

Fluorescence decay times of PRX were measured by the time-correlated single photon counting (TCSPC) method as described in the earlier work.[19] The excitation source is a mode-locked Ar ion laser (Coherent; Innova 200) pumping a dual-jet dye laser (Coherent; Model 701-1). The cavity-dumped dye laser has 1 ps pulse width and average power of *ca.* 50 mW at 3.8 MHz dumping rate. To excite the sample, the dye laser pulse was frequency-doubled by a β-BBO crystal to generate 302 nm pulses. All the standard electronics for the TCSPC were purchased EG&G Ortec and Tennelec. The temporal instrument response function was measured by detection of the scattered laser pulse and it had a 60 ps FWHM when a Hamamatsu photomultiplier tube (R2809U) was used. Under optimum conditions this allows a time limit of about 20 ps after deconvolution.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of PRX in various solvents were measured at different concentrations between 10^{-4} and 10^{-6} *M*. The absorption spectra of PRX in nonpolar and/or non-hydrogen-bonding polar solvents (*e.g.* cyclohexane, n-hexane, acetonitrile) did not show any wavelength-shift except hypochromic effects as the concentration changed, indicating that no dimer-monomer equilibrium is possible in this concentration range. However, the absorption spectrum of PRX in methanol shows the bathochromic effect as well as the hypochromic effect upon decrease of its concentration, as shown in Fig. 2. The absorption maximum (324 nm) at the higher concentration (8×10^{-5} *M*) is shifted to longer wavelength (360 nm) as the concentration is lowered to 2×10^{-5} *M*, exhibiting the isosbestic point at 344 nm. This indicates that two species of PRX exist in equilibrium in the ground state. Such a concentration dependence of the absorption spectrum was also observed in other hydrogen-bonding polar solvents such as ethanol and dimethyl sulfoxide (DMSO) (data not shown). These results imply that the intermolecular hydrogen-bonding plays an important role in forming of the two species.

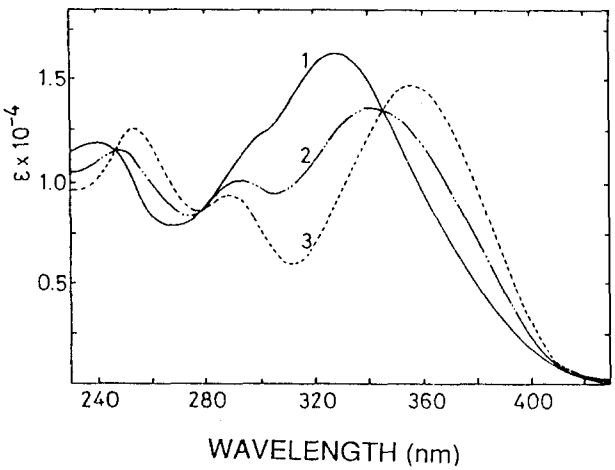


Figure 2. Absorption spectra of PRX in methanol measured as a function of concentration ; (1) 8×10^{-5}, (2) 4×10^{-5}, (3) 2×10^{-5} *M*.

In the previous studies on the pH dependence of the absorption spectra of PRX in aqueous solution (2×10^{-5} *M*), the absorption maxima of the neutral and anionic species had been observed at 324 and 360 nm, respectively.[15] These two absorption maxima are quite well matched with those of the methanol solution, and the absorption spectrum at the lower-concentration is very similar to that of the chemically generated anion. Actually, when sodium hydroxide (1 m*M*) was added to the methanol solution of 8×10^{-5} *M*, the absorption maximum was shifted from 324 nm to 360 nm. From these observations, we can conclude that in the hydrogen-bonding solvents the neutral PRX exists in equilibrium with an anionic species formed via the solvent-mediated proton transfer (see Fig. 1). These results are in good accordance with the work of Becker *et al.*[16] who had also showed that 5'-nitro derivatives of salicylideneaniline exist in the anionic form with other two species in ethanol or DMSO at the diluted concen-

tration. Furthermore, it is noteworthy that such concentration dependence of the absorption spectrum was not observed for a skeletal precursor of PRX, HMBDC, in alcohol. This may be due to pyridyl amide group of PRX which can produce more resonance contribution to stabilize the anion than the methoxy group in HMBDC. Hence deprotonation of the hydroxyl group of PRX should be facilitated relative to that of HMBDC. Supporting this, the pK_a of the hydroxyl group of HMBDC had been observed to be much higher (6.5) than that of PRX (3.3).[15] At present it is difficult to pin-point unambiguously the reason why the concentration dependence of the GSIerPT is possible even in the diluted and narrow concentration range of the bulk solution. However, one possible explanation can be made on the basis of energetic considerations with regard to a specific solute-solvent interaction. The difference in pK_a values between the ground and the excited states of PRX (3.3 and -1.0, respectively)[15] indicates that the deprotonation energy of PRX in the ground state is about 3 kcal/mol (= 2.3RT ΔpK_a) more endothermic than in the excited state. This endothermicity is much smaller than that of 3-hydroxyflavone (14 kcal/mol) or β-naphthol (20 kcal/mol) which are known to undergo the intermolecular proton transfer in the excited state rather than in the ground state.[7] This endothermicity may be overcome by the specific solute-solvent interaction to form a critical solvent cage at the lower concentration. Such an assumption can be supported by the earlier works reported by the Rentzepis's[20] and the Robinson's[21] groups. They have proposed a water cluster model to interpret the proton transfer from several aromatic alcohols in water/alcohol mixtures. They have pointed out that the energy required for hydration of a proton decreases as the size of the cluster increases so that the proton affinity increases. The energy required to form the proton accepting cluster in water or methanol has been estimated to be 3-4 kcal/mol. This energy is good enough to overcome the endothermicity for the deprotonation of PRX in the ground state.

Fluorescence spectra

Fig. 3 shows the fluorescence emission spectra of PRX in methanol measured by exciting at the isosbestic wavelength as a function of concentration at room temperature. The emission spectrum at the high concentration (8×10^{-5} *M*) shows the emission maximum at 485 nm with a very weak shoulder around 400 nm. As the concentration decreases, the intensity of the short wavelength band around 400 nm increases slightly and extends to the long wavelength. The weak emission around 400 nm has been also observed in the basic aqueous solution whereas it is not observed in non-hydrogen-bonding solvents. The concentration dependence of the fluorescence spectral change is in parallel with that of the absorption spectral change, indicatig that the 400 nm and 485 nm emissions are attributed to the excited anionic or neutral (keto tautomer) species, respectively (see Fig. 1).

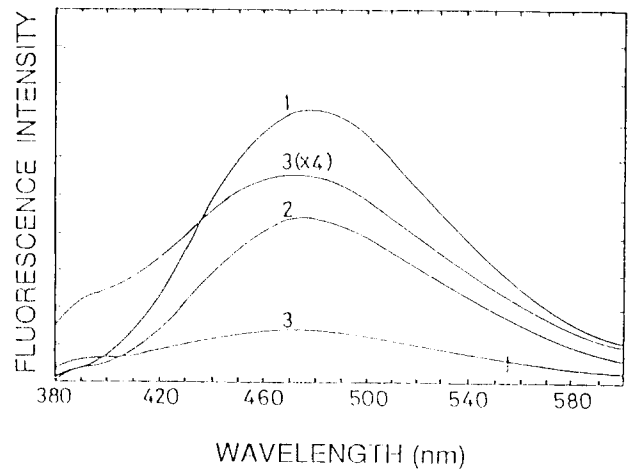


Figure 3. Fluorescence emission spectra of PRX measured at room temperature as a function of concen tration in methanol (1) 8×10^{-5}, (2) 4×10^{-5}, (3) 2×10^{-5} *M*. The excitation was made at the isosbestic point (344 nm). The dotted spectrum was obtained by excitating at 360 nm. The spectral intensities are normalized unless otherwise indicated.

The 485 nm emission band at the higher concentration exhibits large Stokes shift (*ca.* 9,000 cm^{-1}) as observed in nonpolar aprotic solvents, and it is attributable to an keto tautomer formed *via* the ESIaPT as reported in the previous paper.[14] Then, it is interesting to note that the 475 nm emission is blue-shifted by about 10 nm as the concentration decreases (Fig. 3). This small but perceptible shift implies that there could exist another excited-state conformer in addition to the keto tautomer (see Fig. 1). Fig. 4 shows the fluorescence excitation spectra of PRX in methanol solution measured by monitoring the 475 nm emission with higher intensity than the 400 nm emission. It is shown that the excitation maximum wavelengths depend on the concentration, and differ significantly from the absorption maximum wavelengths of both neutral and anionic species. These results confirm that the long-wavelength emissions are not directly originated from the initially excited state of the neutral species. The initially excited neutral species undergoes the rapid

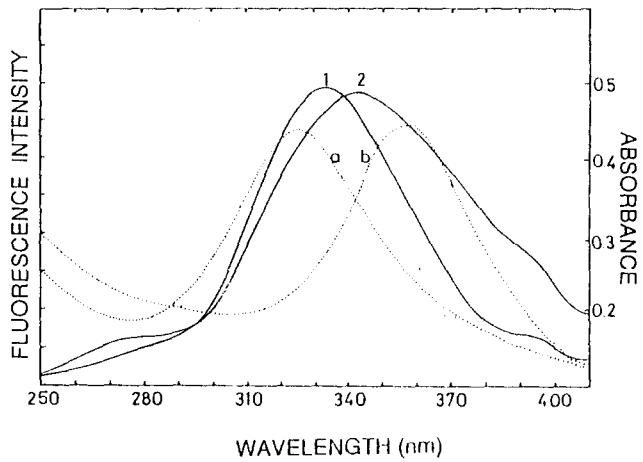


Figure 4. Fluorescence excitation spectra of PRX in methanol at different concentrations at room temperature with emission wavelength at 475 nm; (1) 8×10^{-5}, and (2) 2×10^{-5} *M*. The dotted spectra represent the absorption spectra of (a) the neutral and (b) anionic species. Intensities of the excitation spectra are normalized.

$ESI_{fa}PT$ process as stated above to result in the formation of a keto tautomer. This should be the reason why the normal emission is too weak to be observed. Also the emission around 400 nm does not seem to be directly originated from the initially excited state of the anionic species and the $ESI_{er}PT$ from the neutral species is not likely, since the excitation spectrum at the lower concentration is different from the corresponding absorption spectrum. Considering these results, we believe that the blue shift of the 485 nm emission is due to some other excited conformer which is formed upon excitation of the anionic species formed *via* the $GSI_{er}PT$ at the lower concentration. The excited-state anion would subsequently change on the picosecond time scale to give the conformer such as zwitterion as depicted in Fig. 1. Recently, the ^{13}C NMR study[22] has demonstrated the presence of the zwitterion of PRX in the polar hydrogen-bonding solvents at low temperature. Also the crystallographic study[23] has proved that the PRX molecules can be crystallyzed in the zwitterionic form in the hydrogen-bonding solvent. Thus, our results along with the earlier works have drawn conclusion that the hydrogen-bonding solvent not only interacts with the hydroxyl group of PRX to facilitate the deprotonation but also interacts with the pyridyl group to protonate the nitrogen especially in the excited state because the pyridyl nitrogen is observed to be highly basic in contrast to the acidic hydroxyl group.[15] This should be the reason why the zwitterion can be formed in the excited state. Additionally, this conclusion is supported by the concentration independence of the fluorescence spectrum of HMBDC which has no pyridyl group in contrast to the case of PRX (data not shown).

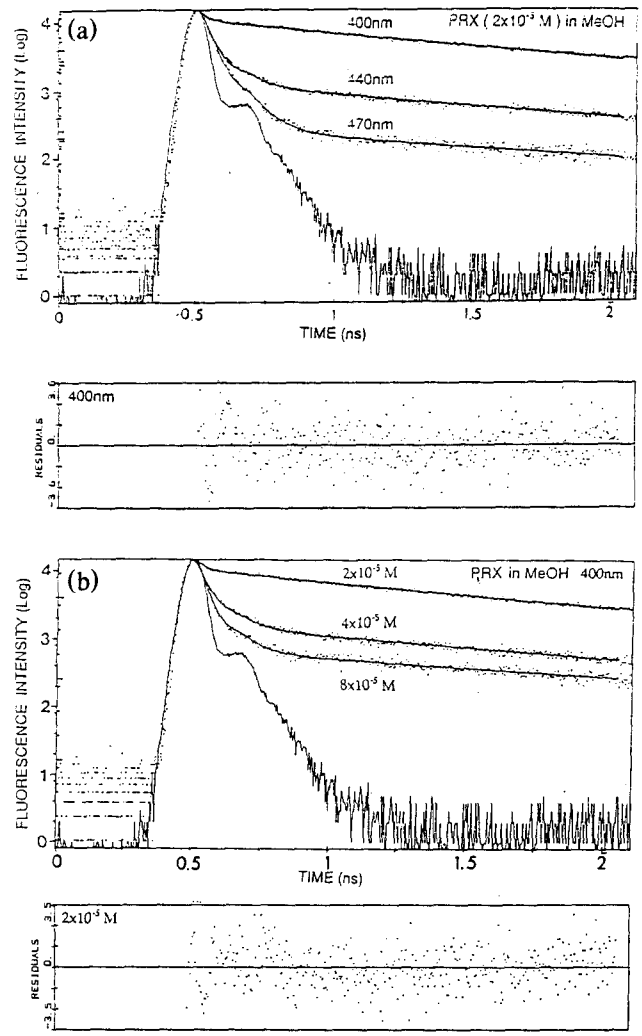


Figure 5 (a) Observed fluorescence decay profiles of PRX in methanol (2×10^{-5} *M*) at room temperature, monitored at 400, 440 and 470 nm; (b) Observed fluorescence decay profiles of PRX in methanol at different concentrations at room temperature, monitored at 400 nm. The excitation wavelength was 302 nm.

Fluorescence decay kinetics

To further support our model of the concentration effects on the photophysical processes in PRX, we have measured the concentration dependences of fluorescence decay behaviors of PRX in methanol at room temperature by using the time-resolved fluorescence apparatus based on picosecond laser. Fig. 5a shows the typical fluorescence decay profile of PRX in methanol at the lower concentration (2×10^{-5} *M*) at room temperature, monitored at different wavelengths. The fluorescence decay profile consists of two decay components with the

Table 1. Fluorescence lifetimes of PRX in methanol at different concentrations at room temperature, monitored at various wavelengths.*

Conc.	Monitoring wavelength(nm)			
	400	440	470	490
2×10^{-5} *M*	31 ps(0.85) 1380 ps(0.15)	29 ps(0.96) 1330 ps(0.04)	33 ps(0.99) 1400 ps(0.01)	330 ps(1.00) –
4×10^{-5} *M*	33 ps(0.94) 1460 ps(0.06)	35 ps(1.00) –	32 ps(1.00) –	32 ps(1.00) –
8×10^{-5} *M*	30 ps(0.98) 1430 ps(0.02)	28 ps(1.00) –	34 ps(1.00) –	32 ps(1.00) –

* measurement error limit : ±5%

lifetimes of about 30 and 1400 ps, respectively. These results at various monitoring wavelengths are listed in Table 1. The amplitudes of the two decay components are dependent on the monitoring wavelengths, though little change is observed in the lifetime values within experimental error. The amplitude of the short-lived component increases while that of the long-lived component decreases as the monitoring wavelength increases. This supports again that the steady-state emission is originated from the two excited-state conformers. At the higher concentration these two decay components are also clearly resolved in the fluorescence decay profile monitored at 400 nm (Fig. 5b) but the amplitude of the long-lived component is much reduced relative to that at the lower concentration (Table 1). Since the amplitude of the long-lived component increases as the concentration decreases in parallel with the concentration dependence of the steady-state spectral change, it could be assigned to the anionic species. However, this possibility is not likely since the fluorescence excitation spectrum is different from either the absorption spectrum of neutral or anionic species as discussed above. Additionally, in the alkaline methanol solution (methanol+0.1 m*M* NaOH) the fluorescence decay curve of the anionic PRX monitored at 400 nm exhibits the decay time of *ca.* 2.4 ns with the rise time of *ca.* 28 ps (Fig. 6a). This indicates that the decay component is not directly attributed to the excited anion but attributed to some other conformer produced from the excited anion *via* a certain relaxation which takes place in *ca.* 28 ps. Also the decay time is close to the lifetime of the long-lived component measured in pure methanol, even though its value is longer than that in pure methanol. Therefore, these results support that the long-lived component of the PRX emission in pure methanol should be originated from the fluorescence of the zwitterion formed *via* the rapid excited-state conformational change of the ground-state

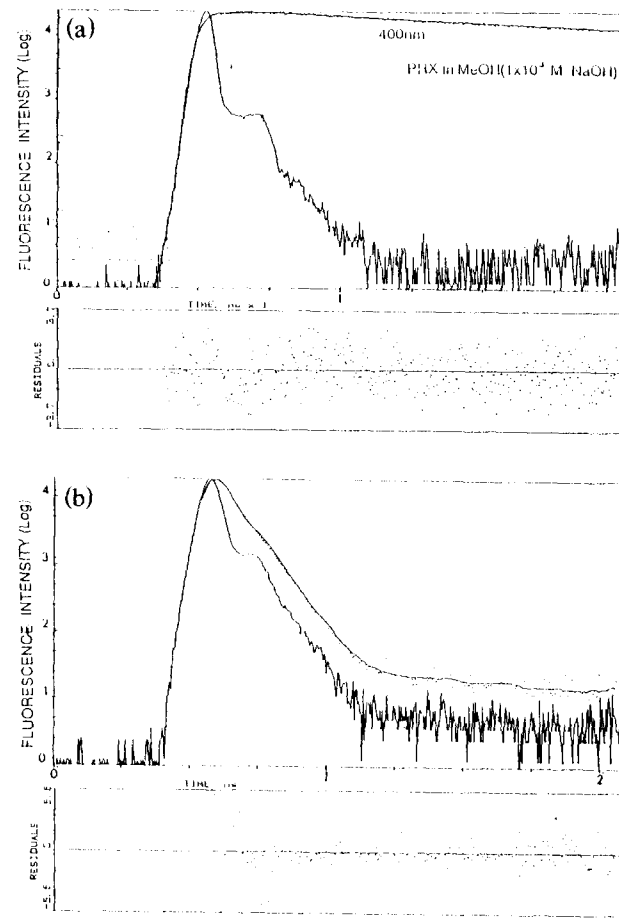


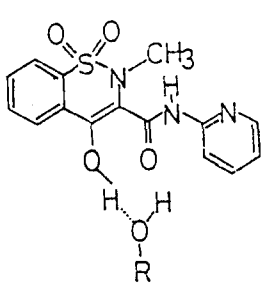
Figure 6. (a) Observed fluorescence decay profile of PRX in alkaline methanol solution (1 m*M* NaOH in methanol) at room temperature, monitored at 400 nm. (b) Observed fluorescence decay profile of PRX in n-hexane (8×10^{-5} *M*) at room temperature, monitored at 470 nm. The excitation wavelength was 302 nm.

PRX anion as assigned from the steady-state spectral results. The reason why the decay time of the zwitterion in the alkaline methanol solution is larger than in pure methanol may be that the viscosity of the alkaline methanol is increased by the electrostatic interacton between the Na^+ ion and the polar methanol as in the case of water affected by the Na^+ ion.[24] It can the same reason that the rise of the zwitterionic fluorescence could be observed in the alkaline methanol while it is not observed in the pure methanol.

In contrast to the long-lived component (*ca.* 1.4 ns), the amplitude of the short-lived component (*ca.* 32 ps) increases as the concentration and the wavelength increase. In addition, the 470 nm fluorescence decay of PRX in non-hydrogen-bonding solvent such as n-hexane or toluene (Fig. 6b) was measured to be single exponential with a lifetime of about 40-60 ps which is close to that of the short-lived component measured in methanol. The fast rise of

the 470 nm fluorescence of PRX in n-hexane is about 20 ps as shown in Fig. 6b. This is in good agreement with the transient absorption rise in toluene,[25] indicating that the lifetime of the normal fluorescence is about 20 ps. Thus, these observations suggest that the short-lived component is attributed to the tautomer fluorescence of the neutral molecule.

It is interesting to note that the rise of the long-wavelength emission was not observed in methanol in contrast to the significant observation of the rise in toluene, indicating that the rate of the ESIπPT is faster in the hydrogen-bonding solvent than in nonpolar solvent such as toluene (*ca.* 20 ps). This implies that the intermolecular hydrogen bonding plays an important role in the ESIπPT process, being in good accordance with the observation for 3-hydroxyflavone reported by Harris and his coworkers.[26] As in the case of 3-hydroxyflavone, the intermolecular hydrogen bonding between PRX and the solvent would result in complexation as depicted in structure I. Such a complex is assumed to undergo solvent rearrangement upon excitation to form a cyclically hydrogen-bonded conformer as depicted in structure II which then the dual ESIπPT occurs to form the enol tautomer. The hydrogen bonds in the PRX solvated complex are considered to be of higher frequency than the intramolecular hydrogen bond of the unsolvated molecule in the nonpolar solvent, leading to the rapid dual excited-state proton transfer.



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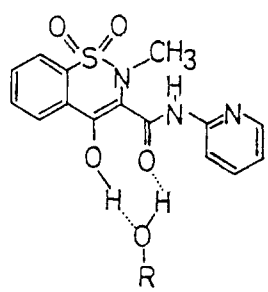

II

In order to further confirm the interpretation of the results obtained at room temperature, we also measured the fluorescence decay in methanol glass at 77 K. The fluorescence decay monitored at 400 nm consists of three decay components as shown in Fig. 7 and Table 2. The first lifetime component of about 4.5 ns has the largest amplitude which further increases as the monotoring wavelength and the concentration increase as in the case of the short-lived component at room temperature, indicating that the first component is attributed to the tautomer emission. However, it is noteworthy that the lifetime of

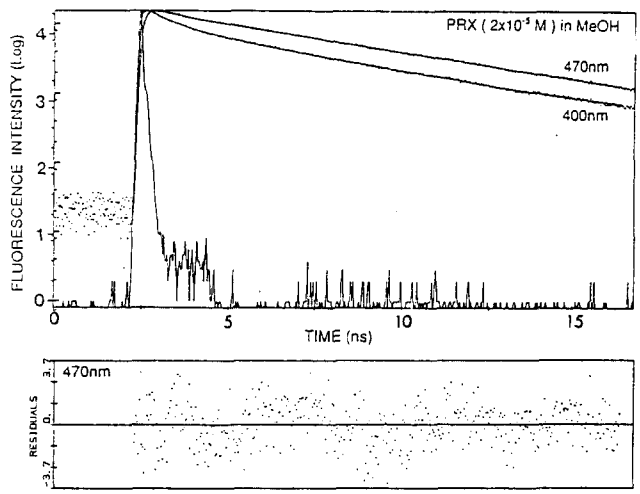

Figure 7. Observed fluorescence decay profiles of PRX in methanol (2×10^{-5} *M*) at 77 K, monitored at 400 and 470 nm. The excitation wavelength was 302 nm.

Table 2. Fluorescence lifetimes of PRX in methanol at different concentrations at 77 K, monitored at various wavelengths.*

Conc.	Monitoring wavelength(nm)			
	400	440	470	490
2×10^{-5} *M*	41 ns(0.51)	4.9 ns(0.88)	4.7 ns(0.90)	4.7 ns(0.86)
	0.7 ns(0.41)	0.5 ns(0.12)	0.7 ns(0.10)	0.7 ns(0.14)
	16±3 ns(0.08)†	–	–	–
4×10^{-5} *M*	4.4 ps(0.74)	4.5 ns(0.85)	4.6 ns(0.85)	4.7 ns(0.85)
	0.6 ns(0.24)	0.7 ns(0.13)	0.6 ns(0.15)	0.7 ns(0.15)
	16±3 ns(0.02)†	16±3 ns(0.02)†	–	–
8×10^{-5} *M*	4.4 ps(0.80)	4.5 ns(0.84)	4.5 ns(0.86)	4.6 ns(0.86)
	0.5 ns(0.17)	0.6 ns(0.16)	0.5 ns(0.14)	0.5 ns(0.14)
	16±3 ns(0.03)†	–	–	–

* measurement error limit : ±5%
†uncertainty of ±3 ns was estimated on uncertainties in the fits to data and error propagation in the deconvolutions.

the second component (about 0.6 ns) is even shorter than that of the long-lived component at room temperature. Thus, the origin of this component should be different from that of the long-lived component at room temperature. This may be attributed to the anion fluorescence which has very low fluorescence quantum yield at room temperature, since the amplitude of this component decreases as the concentration increases. Then, the third component has the lifetime of 16±3 ns and its amplitude decreased as the concentration increases. Thus, it corresponds to the long-lived component at room temperature which was assigned as the zwitterionic emission above. The drastic suppress of the amplitude of the zwitterionic emission is due to the inhibition of the

conformational change upon freezing of the solution.

The amplitude of the first component is also relatively lower than that of the short-lived component at room temperature. This supports that the fast excited-state solvent reorganization should be involved in the $ESI_{ra}PT$ as discussed above. In other words, the inhibition of the $ESI_{ra}PT$ should be due to the inhibition of the fast solvent reorganization at 77 K. This is in good agreement with the observation that the long-wavelength emission band of PRX in methanol was observed to be blue-shifted by about 10 nm upon freezing (data not shown). The blue shift in the emission band is generally observed when the solvent reorganization of a high dielectric and strongly interacting solvent is frozen.[27] In parallel with this observation, the rise time of about 100 ps was clearly observed for the 470 nm emission of 2×10^{-5} *M* PRX in the methanol glass at 77 K as shown in Fig. 7, while no rise time was observed at room temperature.

CONCLUSION

We have demonstrated that the excited-state proton transfer and the ground-state proton transfer processes of PRX are very sensitive to the variation in concentration of PRX in the polar hydrogen-bonding solvents. At the high concentration (8×10^{-5} *M*) the $ESI_{ra}PT$ occurs predominantly on the picosecond-time scale (< 20 ps), resulting in the formation of the enol tautomer. However, at the low concentration (2×10^{-5} *M*) the $ESI_{ra}PT$ is still dominant but the $GSI_{er}PT$ is also feasible to form an anionic species which can undergo the conformational change upon excitation to form the zwitterion. No $ESI_{er}PT$ was observed. The $ESI_{ra}PT$ is coupled with the fast excited-state solvent-reorganization which is observed to be 100 ps at 77 K. Such concentration dependences of the excited-state proton transfer and the ground-state proton transfer of PRX in the hydrogen-bonding solvent may be due to changes in a specific solute-solvent interaction occurring upon variation of concentration. Further investigations of the ps-transient absorption spectra along with the matrix-isolation studies are now in progress in our lab in order to get the exact insight into how the solvation is related with the excited-state proton transfer and the conformational change in PRX.

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